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African swine fever in wild boar. Evaluation of its epidemiology, surveillance and control integrating participatory epidemiology.

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1 List of publications used

1.1 African swine fever in wild boar

1.1.1 The epidemiology of ASF in wild boar

1. **SCHULZ, K.**, STAUBACH, C. & BLOME, S. 2017. African and classical swine fever: similarities, differences and epidemiological consequences. Veterinary Research, 48, 84. https://doi.org/10.1186/s13567-017-0490-x
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1.2 Participatory epidemiology

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(Own Contribution 30%; Impact Factor 2020: 2.67)

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(Own Contribution 30%; Impact Factor 2020: 3.12)

2 General introduction

African swine fever (ASF) is a viral disease caused by ASF virus (ASFV). It is a large enveloped double-stranded DNA virus, which belongs to the *Asfarviridae* family (Alonso et al., 2018). The disease only affects suid species; however, in the presence of suitable habitats, ticks (*Ornithodoros* spp.) can constitute competent vectors (Costard et al., 2013, Sanchez-Vizcaino et al., 2015). The disease was first described 100 years ago. In 1921, Montgomery (1921) reported the detection of a new virus that caused several outbreaks in pigs in British East Africa. The virus was called East ASFV and unsurprisingly, the main characteristics of the disease were very similar to the characteristics of the disease we today call ASF. The main clinical symptoms observed in extensive experimental studies were lethargy, fever and inappetence. Also, the high case/fatality ratio that is typical for ASF was already described at the time and confirmed by many experimental and observational studies in the following years (Gabriel et al., 2011, Blome et al., 2012, Guinat et al., 2014, Pietschmann et al., 2015, Gallardo et al., 2017). Until now, at least 24 ASFV genotypes have so far been identified (Quembo et al., 2018).

In parts of Africa, the original sylvatic cycle involving bush pigs and warthogs as healthy virus carriers and ticks as vectors was described to support the maintenance of ASFV circulation predominantly (Montgomery, 1921, Heuschele and Coggins, 1969, Plowright et al., 1969). In addition, a tick-pig cycle, where infected ticks transmit the virus into domestic pigs, and a domestic cycle, in which no vectors or reservoirs are involved, were described (Costard et al., 2013).

In 1957, ASFV isolates of genotype I crossed the African borders for the first time emerging in Portugal (Sanchez-Cordon et al., 2019). The country managed to eliminate the disease, until it emerged again in 1960 (Wardley et al., 1983). Unfortunately, the virus spread to Spain this time and became endemic on the Iberian Peninsula (Wilkinson, 1984). At the beginning, the epidemic in Spain was mainly driven by the broad distribution of small backyard holdings, where pigs were usually kept outdoors. The presence of *Ornithodoros erraticus* ticks in some parts of Spain complicated the control of the disease even further. However, the increasing industrialization of pig production led to a raising political interest to combat the disease. Thus, an eradication program was established that included intensive surveillance measures, controlled livestock movements and regionalization (Bech-Nielsen et al., 1995, Arias et al., 2002). In the following years, also other European and South American countries suffered from local ASF outbreaks, e.g. Malta, the Netherlands, Belgium, the Dominican Republic and Brazil (Wilkinson et al., 1980, Wilson and Diaz, 1981, Terpstra and Wensvoort, 1986, Biront et al., 1987, Lyra and Freitas, 2015). The involvement of vectors and feral pigs, the partly low biosecurity measures in small, private pig holdings and the lack of a curative treatment or even a vaccine, made the control of ASF a major challenge. Despite these challenges, ASF could be eliminated in all countries outside the African continent except for the Italian island of Sardinia, where the virus has been circulating since 1978 (Mur et al., 2016).

In 2007, however, another transcontinental virus spread occurred. This time, the ASFV isolate belonged to genotype II and was probably introduced through the harbour of Poti, a city located at the Black Sea in Georgia (Rowlands et al., 2008). Since then, the virus has continuously spread across the Eurasian continent, affecting several countries in the European Union (EU) and more recently China, the largest pig producing country worldwide (Gavier-Widen et al., 2015, Wang et al., 2018). In contrast to the previous incursion into Europe, the current epidemic is characterized by the involvement of wild boar (*Sus scrofa*). The virus can be transmitted between domestic pigs and wild boar, either directly by wild boar-pig contact or indirectly by contaminated material like food waste, clothes and vehicles (Gogin et al., 2013, European Food Safety Authority, 2018).

The control of ASF in domestic pigs is strictly regulated. Following the Commission Implementing Decision 2014/709/EU, all pigs in affected holdings must be culled and thus, the risk of further disease spread is reduced to a minimum. The surveillance of ASF in domestic pig holdings is straightforward and regulated by the World Organisation for Animal Health (2019). However, as indicated by the almost 15 years, in which the current ASF epidemic is constantly expanding, both disease control and surveillance are much more complicated as soon as any wild pig species is involved (Gortazar et al., 2016). By now, wild boar are native to almost the entire European continent and the population is constantly growing (Massei et al., 2015). They are not only animals with a regular reproduction cycle, which is almost independent from seasonal influences in regions with abundant resources for the wild boar, but also robust, adaptable and clever. In addition, due to mild winters and the lack of natural predators, natural mortality is low, supporting population growth (Okarma et al., 1995, Bieber and Ruf, 2005, Keuling et al., 2013, Massei et al., 2015). Consequently, it is obvious that the susceptibility of wild boar to ASF makes the disease control challenging. In several countries, disease introduction was first recognized in the wild boar population, emphasizing the major role of this species in the current epidemic (Oļševskis et al., 2016, Smietanka et al., 2016, Nurmoja et al., 2017b, Linden et al., 2019, Mačiulskis et al., 2020, Sauter-Louis et al., 2020).

Surveillance and control measures of wildlife diseases are generally implemented by hunters. They are regularly engaged in hunting activities, familiar with the environmental circumstances and usually have a profound knowledge of the local wild boar population. While hunters seem to be the people suited best in supporting surveillance activities and control measures, their support is highly dependent on their willingness to cooperate (Schulz et al., 2016). Including hunters actively in the process of the design, the implementation and the evaluation of measures might help to increase their willingness to support the control of ASF in wild boar. Participatory epidemiology (PE), which originates from social science, is one way to increase the influence of affected groups of persons. Thus, they may be motivated to participate in the crucial processes of diseases surveillance and control and by doing so to solve problems, from which they and their livelihoods are directly affected (Chambers, 1994, Mariner et al., 2011, Catley et al., 2012).

The aim of the present thesis is to investigate the epidemiology of ASF in wild boar, its surveillance and control. Furthermore, the role of participatory methods in veterinary epidemiology and their specific potential to improve the surveillance and control of ASF in wild boar will be assessed. Moreover, the potential of PE to complement conventional epidemiological methods will be discussed. The long-term aim is to support the successful eradication of ASF in wild boar, but also to stimulate the integration of PE in the design, the implementation and the evaluation of disease surveillance and control in general.

The thesis is divided into two main topics. In the first part, the current epidemiological course of ASF in different countries is analysed. Challenges in the surveillance and the control of the disease and knowledge gaps, which impede successful disease control, are identified. Weaknesses in the system are revealed and their identification is used to define starting points for the integration of participatory methods. Thus, in the second part, the potential of PE is evaluated including its limitations and potential pitfalls. Using different participatory methods, the perceptions of hunters regarding ASF in wild boar are investigated and opportunities to increase the effectiveness of surveillance and control of ASF in wild boar identified.

3 African swine fever in wild boar

3.1 Scientific background

The control of animal diseases, in which wildlife plays a significant role in the maintenance of the disease, is much more complicated than the control of diseases only affecting livestock (Gortazar et al., 2007, Gortazar et al., 2016). Wildlife species can constitute reservoir host, whereby the specific definitions of reservoir hosts differ (Ashford, 1997, Haydon et al., 2002). However, following the concepts of Haydon et al. (2002), a reservoir can maintain pathogen circulation and thus pose a risk for disease transmission to the target population. In case of ASF, domestic pigs can be seen as target hosts, which are of interest regarding the control of the disease. Wild boar can be defined as reservoir hosts, as ASFV can persist within their population, assuming a sufficiently large population density (Haydon et al., 2002). Consequently, as long as ASFV circulates in the wild boar population, the risk of ASFV introduction into domestic pig holdings is constantly present, thus continuously threatening the pig economy. Due to this threat, the control of ASF in wild boar is of huge socioeconomic and political interest. In previous outbreaks of ASF in domestic pigs, diseased wild boar in the close vicinity were often determined as potential source for virus introduction (Gogin et al., 2013, Oļševskis et al., 2016, Nurmoja et al., 2018), although the unambiguous identification of the source of disease introduction often turned out to be difficult. The essential role of wild boar in the current ASF epidemic, which started in Georgia in 2007, resulted in the definition of a new, and thus a fourth, transmission cycle (Chenais et al., 2018). The high tenacity of the virus in the environment, particularly at low temperatures, supports the definition of the new wild boar–habitat cycle (European Food Safety Authority, 2010). Carlson et al. (2020) found that ASFV can survive in sand or potting soil for two weeks, pointing out the potentially major role of carcasses of infected wild boar and their surroundings, e.g. the so-called decomposition island, in the maintenance and the spread of ASF in the wild boar population. The crucial role of wild boar carcasses in ASFV transmission was also confirmed by Pepin et al. (2020). While Probst et al. (2017) observed no intra-species scavenging in their study, they hypothesised that the regular contacts of wild boar with their dead conspecifics pose a risk of ASFV transmission. A recent study proofed the long survival of infectious ASFV in different wild boar tissues at 4 °C or colder (Fischer et al., 2020), emphasizing the increased probability that a wild boar sniffing around an infected wild boar carcass and the surrounding soil might get infected with ASFV. Within the scientific community, it is undisputed that removal of wild boar carcasses is one of the most important measures in the control of ASF (European Food Safety Authority et al., 2018).

3.2 Contributions

3.2.1 The epidemiology of ASF in wild boar

Although an incredible amount of knowledge regarding ASF has been gathered within the scientific community over the last decades, several epidemiological mechanisms are still poorly understood. However, for a successful disease control, it is vital to understand the virus, the host and their epidemiological interactions. Consistent effort has to be made to close existing knowledge gaps, which hamper the implementation of effective control measures. With regard to ASF and the essential role of wild boar in the maintenance of ASF in a country, the transmission dynamics between and within wild boar populations are a key factor, which is still under scientific debate. This is aggravated by decades of intensive, so far unsuccessful, search for an effective vaccine (Gavier-Widen et al., 2020). Particularly at the beginning of the current ASF epidemic, it was obvious that stakeholder and scientists compared ASF with classical swine fever (CSF). Experiences from past CSF epidemics in Europe supported the design of surveillance and control measures for ASF in wild boar. Thus, the question arose whether the similarities of the two diseases and the viruses causing them really go beyond that of the name.

- Contribution **(Publication 1)**: SCHULZ, K., STAUBACH, C. & BLOME, S. 2017. African and classical swine fever: similarities, differences and epidemiological consequences. Veterinary Research, 48. https://doi.org/10.1186/s13567-017-0490-x

In addition to the necessity to differentiate between the epidemiological features of ASF and CSF to ensure the design of targeted control measures for ASF, it is of utmost importance to understand the mechanisms of ASFV spread. For decades, ASF was called a very contagious disease (Montgomery, 1921, Costard et al., 2013, Barongo et al., 2015). However, recent experiments and field observations suggested that it is by far more complicated. Transmission rate, spreading speed and infectiousness are important characteristics, which need to be understood for successful control of infectious diseases. A comprehensive narrative literature review was conducted to define these characteristics more precisely and thus to support the epidemiological evaluation of ASF epidemics and outbreaks.

- Contribution **(Publication 2)**: SCHULZ, K., CONRATHS, F. J., BLOME, S., STAUBACH, C. & SAUTER-LOUIS, C. 2019. African Swine Fever: Fast and Furious or Slow and Steady? Viruses-Basel, 11. 866. https://doi.org/10.3390/v11090866

Summarizing the current knowledge regarding ASF in wild boar including pathology, immunology and epidemiology, an interdisciplinary review was published. Thus, it was possible to further and very precisely identify existing knowledge gaps and research priorities.

- Contribution **(Publication 3)**: SAUTER-LOUIS, C., CONRATHS, F. J., PROBST, C., BLOHM, U., SCHULZ, K., SEHL, J., FISCHER, M., FORTH, J. H., ZANI, L., DEPNER,

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3.2.1.1 African and classical swine fever: similarities, differences and epidemiological consequences

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Highlights

- The differences between ASF and CSF are caused through different viruses
- The differences between the two diseases dominate
- ASF spreads more slowly than CSF
- Surveillance and control measures for ASF should not be adopted one to one from the ones for CSF

Schulz *et al. Vet Res (2017) 48:84*
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VETERINARY RESEARCH

REVIEW

Open Access

African and classical swine fever: similarities, differences and epidemiological consequences

Katja Schulz[1*], Christoph Staubach[1] and Sandra Blome[2]

Abstract

For the global pig industry, classical (CSF) and African swine fever (ASF) outbreaks are a constantly feared threat. Except for Sardinia, ASF was eradicated in Europe in the late 1990s, which led to a research focus on CSF because this disease continued to be present. However, ASF remerged in eastern Europe in 2007 and the interest in the disease, its control and epidemiology increased tremendously. The similar names and the same susceptible species suggest a similarity of the two viral diseases, a related biological behaviour and, correspondingly, similar epidemiological features. However, there are several essential differences between both diseases, which need to be considered for the design of control or preventive measures. In the present review, we aimed to collate differences and similarities of the two diseases that impact epidemiology and thus the necessary control actions. Our objective was to discuss critically, if and to which extent the current knowledge can be transferred from one disease to the other and where new findings should lead to a critical review of measures relating to the prevention, control and surveillance of ASF and CSF. Another intention was to identify research gaps, which need to be closed to increase the chances of a successful eradication of ASF and therefore for a decrease of the economic threat for pig holdings and the international trade.

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1 Introduction

Similar names suggest similar disease characteristics for African and classical swine fever (ASF and CSF). In fact, ASF was even thought to be caused by the same virus as CSF [1] before Montgomery [2] described it as an independent disease entity in Kenya. Yet, despite the similar clinical signs and some shared pathogenic characteristics, the two diseases are caused by completely different viruses [1, 3, 4].

Nonetheless, both diseases are frequently mentioned at the same time or compared to each other, especially when it comes to epidemiology and disease control. They are both listed by the World Organization for Animal Health (OIE). Diseases found on this list are of considerable international interest and subject to specific regulations [5]. ASF as well as CSF are viral diseases affecting pigs (*Suidae*) exclusively. In the case of an outbreak, both diseases may generally entail substantial economic consequences for the affected country or region, particularly in western European countries with a considerable pig industry [6–10].

Up to very recently, most central and eastern European countries had mainly experience with CSF, and in many cases, control strategies for ASF were copied from CSF-contingency plans of the past. However, the recent developments of the ASF epidemics in the Baltic EU Member States and in Poland showed that the disease dynamics did not follow the expected pattern and several open questions remain [11, 12]. The disease neither died out nor spreads with high speed as predicted [13]. So far, the affected countries encounter new cases every week and the situation is out of control in the wild boar population. In this review, our focus was put on similarities and differences of the two viral diseases and the subsequent epidemiological consequences. Due to the particular difficulties to control the diseases in the wild boar population and the constant threat, the presence of the virus in wild boar poses to domestic pig holdings, we focused on the epidemiology in wild boar. By including the latest available scientific findings, this review may help to improve our understanding of the epidemiology of CSF and ASF and thus to optimize prevention and control measures. Furthermore, existing uncertainties were identified and thereby new research can be inspired.

2 Virus

2.1 ASF

2.1.1 Virus taxonomy and morphology

The ASF virus (ASFV) is a large enveloped double-stranded deoxyribonucleic acid (DNA) virus and the only DNA arbovirus (arthropod borne) known so far. The virus belongs to the *Asfarviridae* family; genus *Asfivirus* [14]. The genome consists of a linear double-stranded DNA molecule of 170–190 kbp with terminal inverted repetitions and hairpin loops [15]. The viral genome codes for more than 50 structural proteins and several non-structural proteins. ASFV molecular polymorphism has been investigated by partial sequencing of the gene encoding the major capsid protein p72, and 22 distinct genotypes were defined [16]. Recently, an additional genotype was described by Gallardo et al. [17]. Additional sequence information is gathered through partial sequencing of the B602L gene (CVR) or the gene encoding p54. The virus strains involved in the current eastern European outbreaks belong to genotype II and are highly identical. They show so far only very minor differences. The virus strains circulating on Sardinia are of genotype I and also showed only minor variability, even after decades. In the study of Frączyk et al. [18], they identified genetic variability within genes related to evasion of host immune system. According to Frączyk et al. [18] this could help tracing the direction of ASFV isolates molecular evolution. However, studies, identifying further new genetic markers are clearly needed that allow higher resolution molecular epidemiology and thus outbreak tracing.

Table 1 Characteristics of the four manifestations of an infection with the African swine fever virus

	Peracute form	Acute form	Subacute form	Chronic form
Virulence	High	High/moderate	Moderate	Low
Clinical signs	High fever, appetite loss, lethargy, hyperpnoe	High fever, appetite loss, lethargy, gastro-intestinal signs	See acute form but less pronounced	Respiratory signs, lameness
Pathology	Erythema	Erythema, petechial haemorrhages in several organs, lung oedema, abortion	Erythema, petechial haemorrhages in several organs, haemorrhagic lymph nodes, abortion	Arthritis, necrotic skin, pneumonia, pericarditis, abortion
Mortality	High	High	Variable	Low

Partly adapted from Sanchez-Vizcaino et al. [20]

2.1.2 Clinical signs and pathology

The occurrence and the manifestation of clinical signs depend on different factors. Decisive factors can for example be the virulence of the virus strain, the infection route and dose and the constitution of the affected animal. The incubation period is described to be 2–7 days [19]. According to Sanchez-Vizcaino et al. [20] it can be 5–15 days. Peracute, acute, subacute and chronic form of disease can be distinguished [20]. The ASFV strains causing the outbreaks in eastern Europe are highly virulent and the clinical courses are usually acute and lethal [17, 21]. Experimentally infected wild boar showed also a very high mortality, independently of sex or age [21, 22]. This does not preclude very unspecific courses that can almost go unnoticed. Some characteristics of the different disease forms are outlined in Table 1.

As described in Table 1, mortality may vary according to the virulence of the ASF virus. Infections with high virulent virus strains usually lead to 90–100% mortality.

2.1.3 Immune response and vaccination

Pigs recovering from ASFV infection are usually protected against homologues challenge, but cross-protection against heterologous strains is often missing. Generally, the existence of an antibody-mediated protection, i.e. virus neutralization, is controversially discussed. It is possible to confer a certain level of protection by passive transfer of hyperimmune sera [23]. However, several authors suggest the complete absence of neutralizing antibodies [24], others found that antibodies could reduce virus titers or neutralize ASF virus to a certain extent in vitro [25–27].

It has been reported that animals surviving ASF can become long-term carriers [28, 29]. This may have a tremendous impact in wild boar populations. So far, it is not clear how many of the survivors may act as carriers and how long they remain infectious. Evidence exists indicating that at least not all animals become long-term carriers [30].

While the role of antibodies is controversially discussed, cytotoxic T-cell responses seem to play a major role in mediating antiviral protection. It was demonstrated that depletion of CD8+ cells leads to abrogation of protection [31].

Safe and efficacious vaccines against ASF do so far not exist, although several approaches have been pursued to develop immunization protocols [32]. Thus, a control strategy in both domestic pigs and wild boar has to rely on veterinary hygiene.

2.2 CSF

2.2.1 Virus taxonomy and morphology

The agent causing CSF is a small, positive single-stranded, enveloped RNA virus. The CSF virus (CSFV) belongs to the genus *Pestivirus* within the Flaviviridae family [33]. The genome consists of approximately 12.3 kb and includes one large open reading frame (ORF) flanked by two non-translated regions (NTRs) [34–36]. The viral genome codes for eleven viral proteins, four structural and seven non-structural (NS) proteins. In detail, the core (C) protein along with three envelope glycoproteins (E1, E2, and Erns) constitutes the virion, and Npro, p7, NS2-3, NS4A, NS4B, NS5A, and NS5B are NS proteins [37, 38].

CSFV strains can be assigned to three distinct genotypes with three to four subtypes [39–41]. This classification is based on the nucleotide sequences of fragments of the 5′-non-translated region (5′-NTR), and of the region encoding the glycoprotein E2 [39, 42]. Different subtypes show a particular geographical distribution and genetic typing is used to understand both gross and molecular epidemiology [39, 43, 44]. Recent European strains belong to genotype 2, especially subtypes 2.1 and 2.3. Most often, these virus strains are moderately virulent.

2.2.2 Clinical signs and pathology

Also for CSF, the course of disease depends on several factors like viral virulence, virus dose, health status and particularly the age of the affected animal. Three different courses of infection are known, namely the acute, chronic and prenatal form. The latter can lead to the so called "late onset" form [7, 45]. The incubation period is in the range of 4–10 days. The acute form of CSF manifests often in fever, respiratory and gastro-intestinal signs, lethargy, and inappetence. The acute lethal form can be accompanied by severe hemorrhagic or neurological signs. Mortality in piglets can be very high, whereas older animals can withstand an infection and develop a life-long immunity [46].

The chronic form is caused by viruses with a lower virulence and usually effects unspecific symptoms like runting, secondary infections of both respiratory and gastro-intestinal tract, skin lesions, and, in the case of sows, reduced fertility. Sometimes, animals can show an initial recovery, however after several months all animals succumb to infection and die. During the whole time of infection, the affected animals shed large amounts of virus [46, 47]. This course can play an important role in the maintenance of virus transmission.

The outcome of transplacental infection depends on the stage of gestation. In early pregnancy, CSFV infection usually causes abortion, still birth, mummification or malformation [47]. However, infections in the 2nd and 3rd month of pregnancy may lead to the development of persistently infected piglets. These piglets are immunotolerant towards the causative virus strain and may be born healthy. However, they usually runt and develop the so-called late onset form of CSF. Also, these animals constantly shed virus until they eventually die [45, 47, 48].

Regarding the pathology of acute forms, lymph nodes, spleen and kidneys as well as other organs may be edematous and hemorrhagic. Moreover, spleen infarctions and necrotic regions in the tonsils are sometimes found. In animals dying due to the chronic form of CSF, the typical hemorrhages are usually missing, while necrotic lesions in the gastrointestinal tract are more common [47]. Secondary infections may dominate the pathological lesions. The same is true for the late-onset form [49].

2.2.3 Immune response and vaccination

Protection against CSFV upon vaccination or an overcome infection is mediated by both humoral and cellular immune responses. Animals that have recovered from field virus infection and animals vaccinated with a conventional live-attenuated vaccine develop antibodies against the structural proteins E2 and Erns as well as the non-structural protein NS3 [50–52]. Especially the E2 antibodies are able to neutralize CSFV and antibody titers can be determined using cell culture-based neutralization assays [53]. Measurable titers are usually found between days 14 and 21 post infection and persist probably lifelong. Moreover, antibodies are transferred by immune sows to their offspring via colostrum. These antibodies have a half-life of roughly 12–14 days and are able to passively protect suckling piglets for a couple of weeks [54]. Beside humoral responses, cell-mediated immunity plays an important role in early protection upon vaccination and in beneficial immune responses upon field virus infection.

Safe and efficacious vaccines exist for both intramuscular vaccination of domestic pigs and oral vaccination of wild boar [55]. The latter have proven that they can be an important tool for CSF eradication from affected wild boar populations [56].

3 Epidemiology

3.1 ASF

3.1.1 Transmission and contagiosity

Three main transmission cycles are described for ASF [57]. A distinction is made between the sylvatic cycle, the tick-pig cycle, and the domestic cycle. The sylvatic cycle refers to the circulation between the African wild suid population and soft ticks. This cycle can be seen in African countries where ASF and ticks of the genus *Ornithodoros* are endemic. The tick-pig cycle is present in Africa and played a role on the Iberian Peninsula, where ticks infested pig pens and shelters. In the domestic cycle, direct or indirect transmission occurs between domestic pigs. The same applies to transmission among wild boar in the sylvatic cycle in eastern Europe [57, 58]. Direct contact between infected and susceptible animals is a very effective transmission route, but still depending on the virulence of the virus [28, 59]. Indirect transmission is described through people, vehicles etc. [60]. Although officially banned in most European countries, feeding contaminated meat products or fodder to wild boar or domestic pigs is assumed to play a considerable role in the transmission of ASF [61]. The introduction of the ASF virus from Africa to Portugal in 1957 as well as the introduction into Georgia in 2007 happened most likely through swill feeding of waste from ships at international harbors [62]. ASF virus could be found in boar semen, therefore a transmission through sexual contact or artificial insemination cannot be ruled out [63]. According to Penrith and Vosloo [64] there is no evidence for intrauterine transmission. This is in line with our own unpublished observations.

Ferreira et al. [65] detected viral DNA in air samples and showed a significant association between the detection of virus in feces and in air samples. However, due to the high virus load needed, airborne transmission is not thought to be a major transmission route for ASFV.

Infected animals excrete virus through body fluids like blood, nasal fluid and through feces and urine. However, the amount of virus differs in different fluids. Several studies demonstrated a considerable virus burden in the blood of infected animals, while it was considerably lower in nasal or rectal fluids [22, 58, 66]. Accordingly, contact to infectious blood appears to be the most effective transmission route for ASF [19]. Also, Depner et al. [13] hypothesized that due to the necessary direct contact, the contagiosity of ASF is lower than previously assumed. Results of experimental and field studies support this hypothesis [22, 67]. Following infection studies, the oral infectious dose can vary between 10 000 and 18 000 $TCID_{50}$ (50% tissue culture infective dose) [68].

Virus transmission can be described by the basic reproductive number (R_0), which defines the number of secondary infected animals that result from one infected animal. Existing data about the R_0 value for different ASF virus strains varies considerably in different studies, ranking from 0.5 to 18.0. However, independently of the virus strain, R_0 was generally lower when transmission happened only through indirect contact [22, 58, 59].

3.1.2 Vectors and carriers

In addition to domestic pigs, wild suids play an important role in the transmission pathways of ASF. In Africa, especially warthogs and bush pigs are known as an asymptomatic reservoir for ASFV [60]. Transmission between warthogs has not been described so far: the presence of soft ticks is therefore believed to be necessary for the spread of the disease [69]. The epidemiological role of other African wild suids such as giant forest hogs in the distribution of ASF has not been conclusively evaluated [57]. Many studies demonstrated that the European wild boar is as susceptible to ASF as domestic pigs and can thus act as reservoir under European conditions [69].

As described further above, ASFV is an arbovirus that can replicate in soft ticks. In areas, where ticks of the *Ornithodoros* genus are endemic, they can play an important role in the transmission of the ASFV [57, 58]. There is no indication that birds or rodents from infected farms contracted ASF [58]. These findings could be confirmed by Penrith and Vosloo [64]. Mellor et al. [70] could experimentally transmit ASFV from Stomoxys flies to pigs. For central Europe, there is no evidence that soft ticks could play a role [71]. There is no evidence that *Ornithodoros* spp. occur in this region. Moreover, hard ticks do not seem to play a role either [72].

3.1.3 Tenacity

It is known that the survival time of the virus can be up to 18 months in serum at room temperature. However, the survival time decreases with increasing temperature and can be longer in frozen material. The virus is stable across a wide range of pH-levels; it can resist a pH level between 4 and 13 [73]. Several studies demonstrated that ASFV can stay infective in raw ham or sausage but also in treated meat products for several months. However, it was also shown, that cooking meat kills the virus within few minutes, whereas it can stay infectious at least 1000 days in frozen meat [74–76].

3.2 CSF

3.2.1 Transmission and contagiosity

Virus can be excreted through feces and all body fluids like saliva and urine. Infected animals may excrete large amounts of virus over a relatively long period [77]. Infection usually happens oro-nasally often through direct but potentially also through indirect contact [7, 78, 79]. The infectious dose through oro-nasal infection ranges between 10 $TCID_{50}$ and 80 $TCID_{50}$ [65]. Different indirect transmission routes are described. Indirect contact to wild boar, for example through contact to contaminated hunting material or vehicles could be identified as an important source for virus introduction into commercial pig holdings [80, 81]. Also, indirect transmission through infected feed or garbage (illegal swill feeding) has been suggested as a common source for virus introduction into a naïve population [7, 80]. Movements of persons entail the risk of transmission through contaminated clothes, vehicles or repeatedly used needles [82–84]. Indirect transmission via excretions are described to be rather unlikely [85].

The CSFV is able to cross the placental barrier and consequently to infect fetuses in the uterus [45, 86]. Virus transmission through boar semen has also been reported [87–89]. Transmission via air was suspected in farms where secondary outbreaks without any detectable direct or indirect contact to the originally affected farm have occurred [82]. Potential virus transmission via air could be documented under experimental conditions [15, 90, 91]. Weesendorp et al. [92] and Weesendorp et al. [93] detected CSF virus in a pen where infected pigs had been housed. However, Weesendorp et al. [91] showed that the transmission rate was significantly higher among pigs housed in the same pen then between pigs housed in different pens or via air, which emphasizes the importance of the transmission routes mentioned above.

The R_0 value for CSF virus depends on the number of susceptible animals, on the population density and also on the virulence of the CSF virus [7, 45, 94]. Several studies determined a high R_0 values for within-herd transmission, indicating a high contagiosity when direct contact between the animals is possible [86, 95, 96]. However, Weesendorp et al. [94] showed that direct transmission is highly dependent on the virulence of the virus strain. They found that pigs that had direct contact with animals infected with a low virulent strain did not get infected.

Besides the direct relationship between population density and the R_0, a reduced number of highly susceptible young pigs decreases the chance of disease persistence in a population [7, 97–99]. Stegeman et al. [100] found that the transmission of CSF virus among breeding pigs was clearly lower with a R_0 of 2.9 than in herds of weaned piglets and slaughter pigs.

3.2.2 Vectors and carriers

Although the role of various animal species as potential vectors for CSF has been intensively studied, transmission seems to occur mainly if not exclusively between pigs. Neither arthropods nor rodents or birds could be reliably identified as vectors for the virus [82, 101, 102]. Wild boar constitute an important carrier of CSFV and therefore pose a constant risk to introduce the virus into pig farms [7, 80]. Everett et al. [103] showed in their study that warthogs as well as bushpigs can be infected with CSF virus and can also transmit the disease.

3.2.3 Tenacity

Similar to ASFV, the tenacity of CSFV in the environment depends on a number of factors. Several studies could demonstrate a relationship between ambient temperature and the tenacity of the virus [77, 104–107]. Accordingly, the period of time, the virus remains infectious, decreases with increasing temperature. In the study of Weesendorp et al. [104] it was calculated that virus would remain infectious for a few days in feces and urine at 22 °C. However, at 5 °C infectious virus would remain detectable for several weeks. Botner and Belsham [108] could show that the tenacity of CSF virus in slurry was short when it was heated, but the virus remained infectious for weeks at cool temperatures.

Farez and Morley [107] describe in their study a tenacity of years in meat frozen at −70 °C. They also listed time periods, for which the virus stayed infectious in different meat products, illustrating that these periods may range from 40 days to several years, depending on the treatment. Treatments like salt-cures and smoking do not seem to reduce the infectivity of CSF virus significantly, whereas pasteurization and cooking inactivates the virus [105]. Also, the protein concentration in the matrix influences the tenacity of the virus. The higher the protein concentration, the longer stays the CSF virus infectious [77]. Another factor affecting the stability of the virus is the pH-value [105–107, 109]. It was found that virus is inactivated below a pH-level of 4 and above pH 11 [109].

4 History and today's distribution

4.1 ASF

The first time, when ASF was identified as an independent disease entity, was in Kenya in 1910 [2]. After its first detection, ASF was found to circulate in several African states until it was introduced into Portugal in 1957. After successful eradication in Portugal, the disease was reintroduced in 1960 and spread to several European countries. Before it was finally eradicated in 1995, ASF stayed endemic on the Iberian Peninsula [6, 61, 64]. Since the virus was newly introduced into Sardinia in 1978, ASF has remained endemic in several parts of Sardinia [110]. The disease did not only reach Europe, but also different countries in South and Central America, from where it was successfully eradicated. For many years, ASF could be found endemic only in African states and Sardinia [61]. However, in 2007 ASF was again detected in Europe, namely in Georgia, from where it spread into the neighbor states Armenia, Azerbaijan and the Russian Federation [62, 111]. In 2012 and 2013, also the Ukraine and Belarus reported an ASF outbreak [20]. In 2014, ASF reached the European Union, where outbreaks were confirmed in Lithuania, Latvia, Estonia and Poland [11, 12, 20, 112]. Currently, the virus is still circulating in all four countries with frequent new outbreaks, mainly in wild boar, but occasionally also in domestic pigs (Figure 1). In addition, ASF cases were detected in Moldova for the first time in October 2016 [113].

4.2 CSF

The first official reports about the occurrence of CSF virus originate from Ohio, USA, where the disease was first described in 1833. Between 1860 and 1970 the CSF was widely distributed over the American and the European continents [105]. In 1978, CSF was eradicated in North America [109]. Since then, North America and Australia are officially free from CSF [114]. Mainly due to inadequate reporting and lack of surveillance, the disease situation in Africa remains unclear. However, it is known, that CSF has been endemic in parts of Asia as well as in areas of Central and South America since several years [114]. After devastating outbreaks in the Netherlands and in Germany in the late 1990s and sporadic outbreaks that occurred thereafter, the last outbreaks in Europe were reported in domestic pigs from Latvia in 2014. In wild boar, however, the disease was at least present until 2016 in the latter country [115].

5 Prevention and control measures

5.1 ASF

Currently, no vaccination for ASF is available. To prevent the introduction of ASF, movement restrictions regarding pigs, pork, blood and other products from pigs kept in affected areas as well as potentially contaminated material, vehicles etc. are in place. Following European Commission [116], necessary biosecurity measures are defined, e.g. swill feeding, in commercial pig farms as well as in wild boars, must be prohibited, especially in high risk areas. Direct or indirect contact to wild boar or to any by products has to be avoided. The measures that have to be taken in a case of ASF suspicion or an actual outbreak in the European Union have been specified by European Commission [117]. When an outbreak of ASF in a farm has been confirmed, all pigs of the premise must be culled. In addition, further measures like the safe disposal of all potentially contaminated material, restriction (minimum radius of 3 km) and surveillance (minimum radius of 10 km) zones with movement restrictions for pigs and products of porcine origin have to be set up. Specific regulations have been defined for both zones in European Commission [117].

5.2 CSF

The prevention and control measures regarding CSF in domestic pigs are very similar to the ones described for ASF. Detailed regulations applying for member states of European Commission [118]. However, in the case of specific epidemiological situations, vaccination can be used to control CSF in domestic pigs. Vaccination of

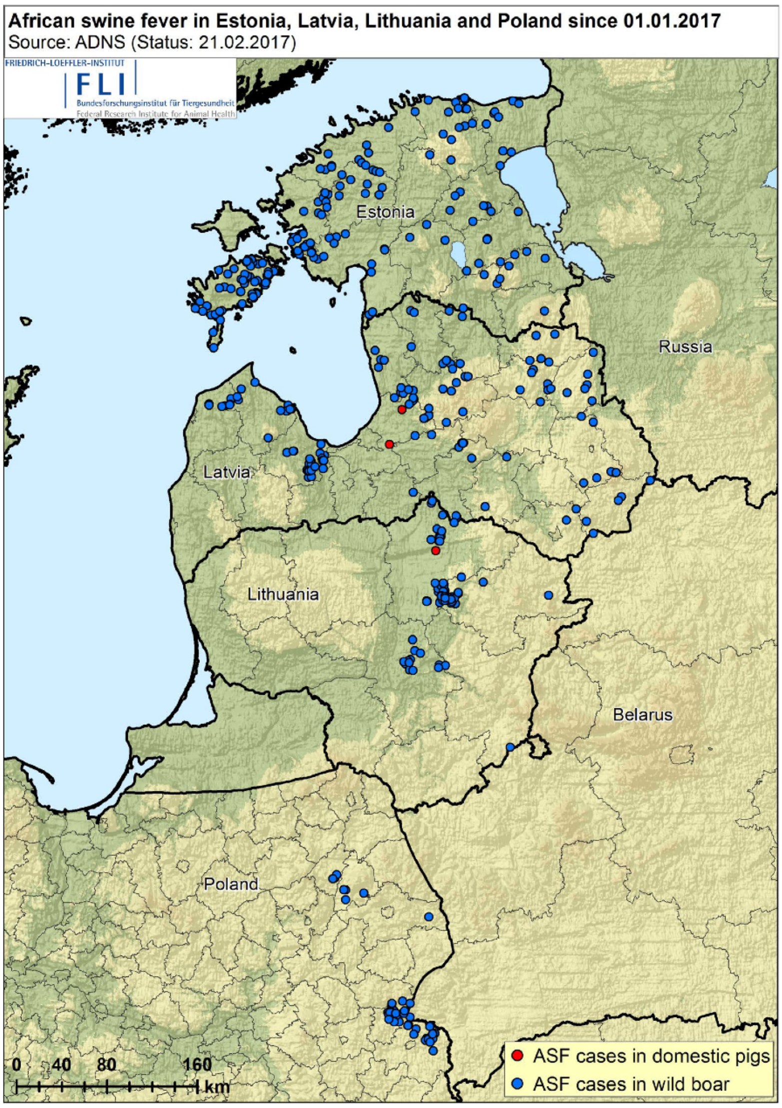

Figure 1 Current distribution of African swine fever cases in domestic pigs and wild boar in the affected countries of the European Union. (Source: ADNS Data, Status 21.2.2017).

wild boar can be also be applied and may represent the method of choice in combination with other elements of surveillance and control [118].

6 Conclusions

Following the introduction of ASF into the Trans-Caucasian countries and the Russian Federation in 2007 and into the European Union in 2014, several countries including Germany sought to set up and update their surveillance and control plans (contingency plans) for the disease.

Especially the countries with previous CSF experience tried to use their CSF contingency plans as a blue print and copied most of the measures that had been found suitable to control CSF.

For the control measures of ASF in wild boar populations, this approach does not seem to be promising as the disease dynamics proved to be too different for the two diseases: Neither self-limitation, which was assumed to occur due to the high virulence of the virus strain circulating in Eastern Europe nor fast spread due to high contagiosity and connected habitats took place [13]. Thus, reconsideration of control and surveillance options is needed.

In this review, we tried to point out major similarities and differences of CSF and ASF with the overall objective to provide background information on disease biology and dynamics that could feed into adapted strategies. Some of the most important similarities/differences are summarized in Table 2.

The similarities mainly concern the range of vertebrate hosts as well as clinical signs and pathomorphological lesions that necessitate swift and reliable diagnostic tools. Both diseases are usually accompanied by a steep increase in mortality when introduced into a naïve population. This gives passive surveillance high impact for the early detection of disease introduction into both domestic pigs and wild boar [119]. With regard to the detection and differentiation of the diseases, molecular tools have been developed and validated that allow both steps in one assay (e.g. [120, 121]). Moreover, both routine sample sets and alternative sample matrices work for both diseases with quite similar performance [122].

Another similarity is the quite high tenacity of the causative agents, especially under cold conditions [74, 76, 105]. Both viruses, ASFV and CSFV, are able to remain infectious for several weeks under adequate climatic conditions (cold environment). Elevated temperatures inactivate both viruses rather quickly. Moreover, both are stable within a wide range of pH-values [73, 109].

Apart from these basic features, which could at least lead to combined passive surveillance approaches in disease free areas that are at risk, several differences exist between ASF and CSF that take effect especially when wild boar populations are concerned.

6.1 Epidemiologically relevant facts concerning CSF

Recent European CSFV strains have shown moderate virulence and an age-dependence of clinical symptoms. This is important for the target population of active surveillance but also disease dynamics as it can be assumed that older animals will survive [119]. Survivors will be safe as they are protected probably livelong from reinfection. Immune sows will confer protection to young piglets via maternally derived antibodies in the colostrum.

In outbreak regions with moderate to high wild boar density, the seroprevalence often rises very quickly and antibody detection is a most valuable tool to characterize the outbreak extent.

Long-term shedders will most probably be present (chronically infected animals and persistently infected piglets after transplacental transmission), but meet increasing population immunity. Shedding is generally high in all se- and excretions and thus, swift spread is likely within a sounder.

Also, CSF has shown potential to become endemic in wild boar populations rather than dying out. This is probably due to the high wild boar density in affected areas in Europe in combination with the above mentioned low/moderate virulence. For this virus, this virulence level could be an optimum for long-term maintenance [123]. However, vaccination exists as an additional tool to eradicate CSF from a wild boar population and most probable, even production and application of a DIVA (differentiation of infected from vaccinated animals) vaccine is feasible [58].

6.2 Epidemiologically relevant facts concerning ASF

Recent European ASFV strains have shown high virulence [124], almost no age-dependence of clinical symptoms and a high case-fatality ratio [19]. The fate of survivors is still not clear as these animals could act as long-term carriers. In fact, survivors will at least be positive for prolonged periods [28, 125]. In the later stages of their infection, mobility can be assumed and thus possible increase in infectious contacts. However, there is also evidence that this is not inevitable [30].

In outbreak regions, the seroprevalence is rising steadily but slowly. It often stays below 10%, even in heavily affected areas. Thus, serology is an important tool to understand and investigate disease dynamics but a difficult target for active surveillance (sample sizes that could detect seropositivity with a sufficiently low prevalence threshold and acceptable confidence can hardly be obtained).

Shedding is generally low in most se- and excretions and thus, blood contact is the main source of infection. Even within groups of animals that have close contact, transmission might be slow and some animals may even go uninfected within a highly affected sounder. Yet, due to the high tenacity of the virus in blood, infectiousness can be assumed for long periods and thus, carcasses and

Table 2 Summary of the most important differences and similarities between African swine fever (ASF) and classical swine fever (CSF)

	ASF	CSF	Both diseases
Virus			
Virus taxonomy and morphology	Large DNA virus	Small RNA virus	
Clinical signs and pathology			Among others high fever, appetite loss, lethargy, erythema, petechiae
Immune response and vaccination	Lack of neutralizing antibodies, no or insufficient cross-protection among strains, protection linked to cytotoxic T-cell responses No vaccination available	Existence of neutralizing antibodies, cross-protection among genotypes, safe and efficacious vaccines available	
Epidemiology			
Transmission and contagiosity			Direct and indirect transmission
	Most effective with blood contact, no evidence for intrauterine transmission	Virus shedding with all se- and excretions, intrauterine transmission and resulting persistent infection of fetuses possible	
Vectors and carriers			Wild boar important reservoir
	Transmission through ticks possible	No transmission through arthropods or rodents described	
Tenacity			Long infectivity in cold environmental temperatures
History and today's distribution	For long time only endemic in Africa and Sardinia since 2007 present in Europe	Long-term epidemics in wild boar over the last decades, sporadic occurrence in domestic pigs; currently no outbreaks in domestic pigs, no cases reported in wild boar	
Prevention and control measures	No vaccination	Effective vaccination	
			High biosecurity, no swill feeding, no contact between domestic pigs and wild boar

blood contaminated fomites can act as long-term source of infection. Transplacental transmission has not been described for ASF [64].

Little is known about the role of maggots or other insect larvae, the fate of carcasses under different conditions, and environmental factors such as so soil underneath a carcass. It could recently be demonstrated that several of these matrices are positive for ASFV genome, but live virus is probably rare or non-existent.

Although not involved in the current situation in Eastern Europe, soft ticks can play a role in ASF transmission. This may add another player and more complexity to the control scenario. It has been proven that tick involvement can have high impact on outbreak duration.

No vaccine exists that could aid control options. Developing a vaccine for the wild boar population would mean to develop a safe and efficacious oral vaccine. So far, there is no such vaccine at the horizon.

Thus, besides the shared common features, the differences between ASF and CSF clearly dominate and entail more serious epidemiological consequences. With regard to surveillance actions, the focus for CSF on piglets is clearly counterproductive for the current ASF situation. For ASF, herd immunity does not play an important role for a long period of time and thus time does not act necessarily as beneficial factor. CSF and ASF have different levels of contagiosity and thus transmission characteristics, for example, the R_0 for ASF is lower than for CSF. However, there is a relatively low number of studies, in which these values were estimated. Moreover, different algorithms, virus strains, diagnostic tools and host characteristics were used, which makes those studies hardly comparable. Nonetheless, experimental as well as field studies refute previous assumptions of a high contagiosity of ASF. Based on the low contagiosity but high tenacity of the virus in carcasses and blood, ASF surveillance has to focus even more on detecting dead individuals to avoid any direct contact and therefore further spread [126].

Regarding ASF, further studies should focus on ASF transmission in the field and on environmental factors, like soil and organisms around wild boar carcasses. Moreover, the role of survivors needs further investigation.

One of the research gaps concerning CSF relates to the final licensing of the available DIVA vaccine. The use of such a vaccine would help to better understand the balance between vaccine induced and natural immunity and thus dynamics of epidemics and their control.

To close these gaps and to deduce appropriate control options, collaboration is needed among research institution of affected and non-affected countries.

Abbreviations

ASF: African swine fever; CSF: classical swine fever; DNA: deoxyribonucleic acid; RNA: ribonucleic acid; $TCID_{50}$: 50% tissue culture infective dose; R_0: basic reproductive number; DIVA: differentiating infected from vaccinated animals.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KS planned and drafted the manuscript. CS and SB provided additional information and contributed to writing the manuscript. All authors read and approved the final manuscript.

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3.2.1.2 African Swine Fever: Fast and Furious or Slow and Steady?

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Highlights

- The spreading speed of ASF is not as fast as originally assumed
- Different virus properties and external factors influence the speed of disease spread
- The disease has a high case/fatality ratio
- The human factor plays a crucial role in the spread of ASF

viruses

MDPI

Review

African Swine Fever: Fast and Furious or Slow and Steady?

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Abstract: Since the introduction of African swine fever (ASF) into Georgia in 2007, the disease has been spreading in an unprecedented way. Many countries that are still free from the disease fear the emergence of ASF in their territory either in domestic pigs or in wild boar. In the past, ASF was often described as being a highly contagious disease with mortality often up to 100%. However, the belief that the disease might enter a naïve population and rapidly affect the entire susceptible population needs to be critically reviewed. The current ASF epidemic in wild boar, but also the course of ASF within outbreaks in domestic pig holdings, suggest a constant, but relatively slow spread. Moreover, the results of several experimental and field studies support the impression that the spread of ASF is not always fast. ASF spread and its speed depend on various factors concerning the host, the virus, and also the environment. Many of these factors and their effects are not fully understood. For this review, we collated published information regarding the spreading speed of ASF and the factors that are deemed to influence the speed of ASF spread and tried to clarify some issues and open questions in this respect.

Keywords: African swine fever; epidemiology; transmission; disease spread; mortality; case fatality ratio

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1. Introduction

The global concern regarding African swine fever (ASF) and the currently circulating ASF virus strains of the genotype II has substantially increased. This is because of the recent spread of the disease within Europe and Asia, where at least Cambodia, China, Mongolia, North Korea, Laos, the Philippines, and Vietnam have been affected so far. Owing to the constant spread of the disease, the expansion of areas in which ASF occurs at least in wild boar, and the increase in the number of affected countries, research and detailed epidemiological analyses are required to close knowledge gaps. By now, several important facts and assumptions are widely accepted, for example, regarding the main transmission pathways and the dominant role of human behavior in the spread of ASF [1–5]. However, there is still little reliable data, but controversial debate, on the transmission rate, the spreading speed of ASF, and the relevant epidemiological terms [6]. Understanding the spread and spatial distribution of a disease, however, is of utmost importance for disease prevention and control [7,8].

After the introduction of ASF into Georgia in 2007, it was initially hypothesized that ASF might either behave like a self-limiting disease and fade out quickly owing to its high mortality and case-fatality ratio or, on the contrary, that ASF might spread rapidly [1,9]. The real situation that emerged falsified both predictions. Currently, at least 14 countries outside Africa are affected by ASF, among them are several member states of the European Union (OIE WAHIS interface, visited online 26th July 2019). In August 2018, China, for which pig production and the consumption of pig products play a significant role, reported the first outbreaks of ASF [10,11]. Two months later, already 33 outbreaks were notified [12]. In May 2019, 134 ASF outbreaks were reported and, within a relatively short time, ASF has spread throughout a large area of the country, suggesting a rapid spread [13]. In addition, presumably through smuggled pork from China, ASF has been transmitted to pigs in other Asian countries including Cambodia, Mongolia, North Korea, Laos, the Philippines, and Vietnam. The epidemic that had its origin presumably in a port in Georgia in 2007 is far from being under control worldwide.

Many research publications and scientific opinions have so far characterized ASF as a highly contagious disease with high mortality [14–19]. However, recent research and the course of disease in affected countries, challenge the belief that ASF is a highly contagious disease [1,2,5,9]. ASF seems to be characterized by a high case-fatality ratio (i.e., most of infected animals die) paired with low to moderate mortality (only a limited proportion of the population becomes infected, at least in wild boar).

We aimed at collecting and summarizing published information on these uncertainties by searching for literature on transmission rates, spreading speed, and mortality in ASF outbreaks in commercial pig holdings, cases in wild boar, and in data from experimental studies.

2. ASF and Its Spreading Speed

The spread of a disease and its speed is very complex. It depends on many different characteristics. They not only include features of the pathogen, but also characteristics of the host, that is, of the susceptible pig or wild boar populations, and of the environment. We reviewed different, partly controversially discussed definitions and descriptions of epidemiological terms related to the spread of ASF, and refined some of these definitions. Thereby, we aimed at ensuring a uniform understanding of the presented terminology. In this process, we reduced the number of terms to those that may be considered as crucial for describing or explaining the spreading speed of ASF. Definitions of more general terms are included in the Supplementary File S1.

2.1. *Basic Reproductive Number (R_0)*

The basic reproductive number R_0 is a measure of the ability of a disease to spread in a population. It is defined as the average number of secondary cases caused by one infectious individual during its entire infectious period in an entirely susceptible population, and constitutes an important quantitative property of an epidemic [20–23]. If the value of R_0 is below 1, this indicates that the disease will disappear from the population, whereas values above 1 suggest that the disease will persist or spread within the population [23]. R_0 is calculated by determining the product of the transmission probability, the average number of contacts, and the duration of infectiousness. This calculation of R_0 is based on the assumption of a homogeneous mixing in the population, that is, all animals in the population are expected to have the same number of contacts [24]. Cross, et al. [25] pointed out that R_0 may be a poor predictor of the course of disease and that other factors, such as transmission within groups or group size, are equally important for predicting the spread of a pathogen.

2.2. *R_0 in ASF Outbreak Situations*

As mentioned before, R_0 is highly dependent on a variety of factors. It depends, for example, on the infectiousness of the affected host, that is, the period it remains infectious, the number of susceptible individuals around the diseased animal, and the virulence of the circulating ASF virus (ASFV) strains or isolates [5].

One of the first published studies, in which R_0 was estimated for ASF on the basis of field data for outbreaks in the Russian Federation, resulted in relatively high R_0 values (8–11) for ASF infections in pig farms [18]. The R_0 for transmission between farms determined in the same study was lower (2–3). In several field and experimental studies, similar values were obtained, whereby the R_0 values for between-farm transmission were always lower than those for within-farm transmission [26,27] (Figure 1). The same applied for the R_0 values in cases of indirect transmission [5] (Figure 2). By contrast, a very high R_0 of 18.0 was determined in an experimental transmission study [28]. Yet, the confidence interval of this estimate was wide (6.90–46.9) (Figure 2), presumably owing to the limited number of experimental animals that can be included in such an experimental setting.

Study	Study type	Transmission	R0
Gulenkin et al. 2011	Within herd	DP to DP	9.8
Korennoy et al. 2017	Within herd	DP to DP	7.5
Summary			**8.7**
Korennoy et al. 2017	Between herd	DP to DP	1.7
Iglesias et al. 2015	Between herd	WB to WB	1.6
Summary			**1.7**

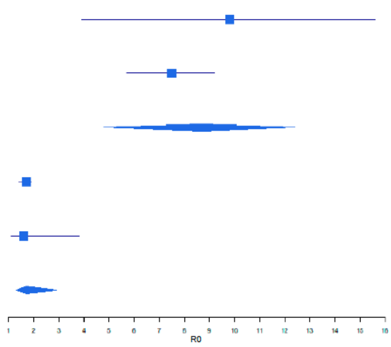

Figure 1. Variation of calculated R_0 for African swine fever (ASF) obtained from ASF field studies. Boxes illustrate the calculated R_0. The lines illustrate the confidence intervals. DP = domestic pig, WB = wild boar.

Study	Study type	Transmission	R0
Ferreira et al. 2013	Direct	DP to DP	18.0
Guinat et al. 2016b	Direct	DP to DP	2.8
Guinat et al. 2016b	Direct	DP to DP	5.3
Pietschmann et al. 2015	Direct	WB to WB	6.1
Pietschmann et al. 2015	Direct	WB to DP	5.0
Summary			**7.4**
Guinat et al. 2016b	Indirect	DP to DP	1.4
Guinat et al. 2016b	Indirect	DP to DP	2.5
Pietschmann et al. 2015	Indirect	WB to DP	0.5
Summary			**1.5**

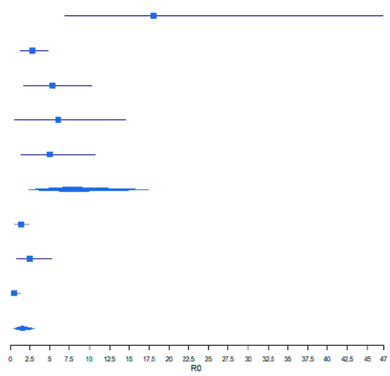

Figure 2. Variation of calculated R_0 for ASF obtained from ASF experimental studies. Boxes illustrate the calculated R_0. The lines illustrate the confidence intervals. DP = domestic pig, WB = wild boar.

A summarizing analysis of published study results shows that the point estimates of R_0 for ASF described in the literature so far cover a broad range from 0.5 to 18.0. This wide variation may inter alia depend on the properties of different virus isolates (e.g., virulence and infectivity), but also on the methods used to determine R_0, which vary considerably [5]. However, regardless of the study setting (field or experimental), the calculated R_0 values for within herd/direct transmission studies seem to be comparable. Likewise, R_0 calculations for between herd/indirect transmission yielded similar values (Figures 1 and 2). These results suggest that the studies included in this analysis obtained reasonably similar R_0 values for ASF, despite the different methods and materials used. Unfortunately, we were unable to investigate whether publication bias may have affected the outcome of this analysis, because most of the available original publications did not contain the required raw data on the size of the study population.

Comparing the R_0 of ASF with R_0 values of other pig diseases, it was found that studies calculating the R_0 of foot and mouth disease, which is described to be highly contagious, often resulted in values higher than 20 [29–31]. Classical swine fever is also described as a highly contagious disease. In several studies, the R_0 showed values similar to that of ASF [32,33]. As in ASF, the values differed between within and between pen infections. In the study of Klinkenberg, et al. [34], the within-pen-R_0 in weaner pigs was as high as 100 (95 percent confidence interval (CI) 54.4–186). However, also in the case of these two diseases, the R_0 was highly dependent on several other factors, like study design, virus strain, and so on.

2.3. Spatio-Temporal Distribution and Disease Spread

The character of transmission and spread of disease cannot be viewed independently of spatial and spatio-temporal vicinity. If individuals that are at risk live close to each other, transmission is more likely as compared to a situation where they are separated through a great distance [7]. The spatial distribution is often only visualized using maps. However, there are several analytical tools such as spatial cluster detection or spatio-temporal modelling, which can be used to include sound spatio-temporal analyses into epidemiological investigations [7,8,35]. Several studies are published describing the use of different models to investigate the spatio-temporal trend of animal diseases [36–41]. Moreover, simulation models are widely used to describe the spatial and temporal spread of animal diseases. They can also be used to analyze patterns of endemic and epidemic diseases or to evaluate the success of control measures [42–51].

2.4. Spatio-Temporal Distribution and Spread of ASF

In this section, we tried to summarize the results of publications that focused mainly on the spreading speed of ASF. The spreading speed highly depends on several factors that were already discussed or will be discussed later; therefore, some of the studies dealing with the speed of ASF spread or transmission rates are included in discussions further above or below.

Modelling the spatial spread of ASF in a wild boar population, Fekede, et al. [52] showed that seasons with temperatures lower than 0 °C support the occurrence of ASF and, therefore, the spread of the disease. In several other studies, an increased detection of ASF-positive wild boar in wintertime was confirmed [3,53,54]. In contrast to wild boar, it was found that the majority of ASF outbreaks in domestic pigs were detected in summer [53–56].

The spatial spread of ASF within the wild boar population in Poland was described as slow [55,57]. Podgorski, et al. [58] hypothesized that this slow spread could be because of the spatial constraints on contacts between wild boar. In their cluster analysis of ASF cases in Russian wild boar, Iglesias, et al. [59] found that individual wild boar cases were spatially associated over a radius of up to 130.79 km and within a maximum of 90 days. The observed spreading patterns were explained through direct and indirect wild boar contact, but may have also been caused by the potential spread of infected wild boar due to increased hunting. In a similar study from Sardinia, Iglesias, Rodriguez, Feliziani, Rolesu and de la Torre [54] identified a maximum spatial association of 25 km between ASF cases in wild boar with an assumed daily movement distance of 2–10 km. In the same study, the maximum distance of spatial association between ASF notifications in domestic pig holdings was 15 km [54]. In a simulation model study, the spreading distance between herds was estimated to be lower than 10 km [60]. However, in the Russian Federation, Korennoy, et al. [61] calculated a mean distance of 156 km between two connected domestic pig outbreaks and an average time period of 7.5 days. Following the statements of the Russian veterinary authorities, Blome, et al. [62] reported already in 2011 about a spreading speed of 350 km per year of ASF within the Russian Federation. These results suggest a rapid spread of the disease. However, in the study of Oļševskis, Guberti, Serzants, Westergaard, Gallardo, Rodze and Depner [2], a slow spread of ASFV within infected pig herds was described. Also, Nurmoja, Mõtus, Kristian, Niine, Schulz, Depner and Viltrop [56] reported a rather slow spread within affected pig herds, suggesting a relatively low infectiousness.

The general terminology for the following terms can be found in the Supplementary File S1.

2.5. Morbidity in ASF Outbreak Situations

Morbidity can be used to characterize the speed of spread of ASF. High morbidity within a defined period requires a certain level of infectivity and a relatively high virulence of the agent. We thus hypothesize that a disease that causes high morbidity is likely to spread with high speed within the target population.

Owing to the high case fatality ratio of ASF, its morbidity values are expected to resemble those for mortality. Infections with a pathogen of low or moderate virulence are likely to result in a higher morbidity, but lower mortality. Therefore, high morbidity was mostly described in experimental studies, which also demonstrated a high mortality [27,63–65]. However, in a few studies, animals that had developed clinical signs after infection, recovered completely, thus suggesting a higher morbidity than the observed mortality [66–68].

2.6. Mortality and Case Fatality Ratio in ASF

Mortality and case fatality ratio are parameters that provide information on circulating ASF viruses and on disease dynamics. However, when interpreting the study results, it should be kept in mind that both terms are frequently confused or incorrectly used as synonyms.

A fast spread of ASF is not necessarily determined by a high case-fatality ratio, as the latter term refers only to the proportion of cases of death among the diseased animals. Likewise, high mortality, which in contrast defines the proportion of death animals among the whole population at risk, does not necessarily correlate with a high speed of ASF spread. Therefore, both terms should always be considered in the context of other parameters such as host-related factors (e.g., age) and properties of the agent (e.g., virulence of the circulating strains). Recent studies showed that the mortality of ASF was lower in older pigs and in animals infected with low-virulent strains [69,70]. Blome, et al. [71] described that the mortality of ASFV ranged from 3% to 100%, depending on the virulence of the virus strain. Mebus [72] found different levels of virulence of ASFV isolates and corresponding values of mortality (from less than 20% to 100%). An experiment with several moderately virulent ASFV isolates led with almost all used strains to moderate mortality. Only the Brazil'78 isolate, though described as moderately virulent, killed all infected pigs, that is, it caused 100% mortality in this experiment [70]. The fact that mortality depends on the virulence of particular ASFV strains or isolates was also confirmed by Vlasova, Varentsova, Shevchenko, Zhukov, Remyga, Gavrilova, Puzankova, Shevtsov, Zinyakov and Gruzdev [64].

Montgomery [73], who first described ASF, found a very high mortality of the disease, both in ASF outbreaks and in experimental infections.

Under the assumption that ASF usually has an extremely high mortality, syndromic surveillance of pig mortality has been proposed as the most appropriate surveillance method for ASF in domestic pig holdings [74]. In the experimental trial of Gallardo, et al. [75], mortality and case fatality ratio both reached the high value of 94.5% when domestic pigs were inoculated with the ASFV Lithuania 2014 genotype II field isolate. Pietschmann, Guinat, Beer, Pronin, Tauscher, Petrov, Keil and Blome [27] used another isolate of genotype II and observed 100% mortality in wild boar (within 17 days post infection) and domestic pigs (within 36 days post infection). Experimental exposure to a highly virulent Caucasian ASF isolate (2008 isolate from Armenia) resulted in 100% mortality [76]. In this study, transmission among wild boar was faster than among domestic pigs. In a subsequent study, Blome, et al. [77] also found 100% mortality in wild boar infected with an ASFV Caucasus isolate. Using the same isolate, Guinat, Reis, Netherton, Goatley, Pfeiffer and Dixon [65] obtained 100% mortality in a trial using domestic pigs. Most of the experimental studies reviewed here are also summarized in a Scientific Opinion of the European Food Safety Authority [78].

Similar to the results of experimental infections, the mortality of ASF was found to be high in field studies. The course of an ASF outbreak in Malta in 1978 indicated a fast spread with a high mortality.

The epidemic resulted in the loss of two-thirds of the pig population of Malta. It was finally decided to slaughter all the remaining pigs on the island [79]. The high virulence of the isolate (Malta/78) was confirmed in an experimental study, which revealed a mortality of 93.3% [80].

When data from ASF outbreaks in Nigeria in 2001 were analyzed, it became evident that the disease had spread very quickly. Although the mortality varied depending on the age of the pigs, it was not lower than 75.9% in any of the age groups [81]. In outbreaks that occurred in Tanzania in 2003 and 2004, mortalities of 82% and 72%, respectively, were recorded [82].

In 2014, Estonia was confronted with the first ASF cases in wild boar. A high mortality was observed in wild boar in the southern part of Estonia, whereas low mortality was found in the northeast of the country [68]. Yet, experimental infections carried out to determine the virulence of the strain that circulated in the northeast of the country showed that the mortality of the isolate was still very high, because 9 of the 10 inoculated wild boar died from ASF infection or became so ill that they had to be euthanized [68].

Recent findings suggest that it may take up to one month until an ASF introduction in a pig herd is detected because of increased mortality, thus indicating a rather moderate mortality for ASFV strains currently circulating in the respective region, in this particular case, in the Russian Federation [6]. A low mortality in the course of an ASF epidemic had already been observed; using an ASF isolate from Cameroon, which had caused high mortality in the field, Ekue, et al. [83] found a very low mortality in their animal experiments. Studies analyzing ASF outbreaks in Malawi suggested a mortality that was significantly lower than 100% in most areas [84].

In outbreaks that occurred in Belgium in 1985, the spread of ASF was described as slow, not only from one pen to another, but even within the same pen. Also, the mortality of the circulating ASF isolate or strain was apparently moderate [85].

When summarizing the results of the literature search regarding mortality, it seems obvious that studies prevail that describe a high mortality of circulating ASFV isolates or strains. It also became apparent that mortality is highly dependent on the virulence of the circulating virus isolate or strain. Moreover, similarly to most pathogens, the properties of the affected host species (in particular age, but also health and feeding condition) seem to play an important role. Therefore, ASF outbreaks are usually, but not always, characterized by a high mortality.

2.7. *Infectiousness and Latent Period of ASF*

Under the specific conditions of ASF, infectiousness describes the period in which an infected animal can transmit the disease. The longer this period lasts, the higher the risk of transmission and, consequently, the risk of ASF spread increases accordingly. The period of infectiousness is highly dependent on the virus strain or isolate. This could be shown in an extensive transmission study, in which a minimum infectious period of 6–7 days was determined for ASF, while the maximum infectious period ranged from 20 to 40 days [28]. The study also showed that the infectiousness also depended on the transmission rates and pathways. In an earlier study, the authors had detected ASFV genome by polymerase chain reaction (PCR) in blood and oropharyngeal fluid even until 70 days post-infection, that is, until the end of the observation period [70]. In addition, infectiousness is defined by the amount of pathogen that is excreted by the animal [20]. Several studies showed that the amount of ASFV is clearly higher in blood then in other excretions [64,65,70,86].

The infectiousness of a pig suffering from hemorrhagic diarrhea due to ASF infection is higher than that of an animal showing only fever or loss of appetite. This difference may be explained by the fact that ASF is extremely efficiently spread through the blood of infected animals. In several transmission studies, which were conducted by Guinat, Gogin, Blome, Keil, Pollin, Pfeiffer and Dixon [5] in domestic pigs and in wild boar, the infectious period lasted from 2 to 14 days, while the latent period of ASFV was found to be 3–6 days. Similar results were obtained by Guinat, Porphyre, Gogin, Dixon, Pfeiffer and Gubbins [6], where the infectious period ranged from 4.5 to 8.3 days and the latent period from 5.8 to 9.7 days. In the study of Gulenkin, Korennoy, Karaulov and Dudnikov [18], the latent period was 15 days. Differences in the estimates may inter alia be the result of variations in the experimental design, different properties of the ASF virus isolates or strains, and the pigs or wild boar used in these studies.

Looking at infectiousness in connection with spreading speed, the ongoing discussion regarding potential ASFV carriers must not be ignored. Sanchez-Vizcaino, et al. [87] stated that animals can develop a chronic form as ASF and become carriers that can infect conspecifics even after a long period. In the study of Gallardo, Soler, Nieto, Cano, Pelayo, Sanchez, Pridotkas, Fernandez-Pinero, Briones and Arias [75], one pig that had contact with infected pigs survived the infection, and it remains unclear if such an animal has the potential to spread ASF. However, in other studies, it was observed that no disease transmission from surviving animals and contact animals took place, suggesting that survivors might play a negligible role in the spread of ASF [66–68].

With regard to the speed of spread, not only the duration of the infectious period is important, but also that of the incubation and latent period [60,88]. When transmission can take place before any clinical signs occur, that is, during the time when the incubation period and the infectious period overlap and the latent period is accordingly shorter than the incubation period, the spread of disease may be faster, as it is unlikely that control measures are applied within this period [88]. However, this applies only to domestic pigs, as the onset of clinical signs in wild boar is difficult to observe, in particular after the initial entry of ASF into a wild boar population.

2.8. *Infectivity of ASFV*

The infectivity of ASFV, usually measured as the 50% lethal dose (LD50) in tissue culture (TCID50), also plays an important role in determining the speed of spread of ASF.

It can be assumed that a virus with low infectivity will spread more slowly than a pathogen with high infectivity. However, the infectivity of ASFV is highly dependent on properties of the virus isolate or strain, in particular on its virulence, on the medium (blood, urine, other body fluids, feces, tissue, and so on), on any potential processing of this material (temperature, pH, storage period, and so on), and on the route of transmission. As already mentioned, transmission through direct blood contact is the most efficient route [27,63,76]. Olesen, Lohse, Boklund, Halasa, Belsham, Rasmussen and Botner [86] showed that the infectivity of ASFV in the environment of the studied pen was low. Correspondingly, transmission through other fomites like urine, feces, or feed appears less effective as compared with direct exposure to the blood of infected animals. However, in the case of hemorrhagic enteritis, feces can play an important role in the transmission of ASF. These assumptions are supported by several studies, which found higher PCR titers in blood than in other excretions [64,65,70]. Direct contact of an uninfected pig to the blood of another pig can be considered as less likely than contact to other excretions (in particular feces and urine) or ASFV-contaminated soil. Thus, the spread of ASF may be slower than that of a disease, where large amounts of infective virus are present in the environment.

2.9. *Contagiousness of ASF*

Regarding the contagiousness of ASF, no information was found in the literature that could add knowledge to that already described in relation to infectiousness.

2.10. *Virulence of ASFV*

Virulence describes, like infectivity, a property of a pathogen. A virus with a moderate or low virulence can still be highly infective [89]. However, it is undisputed that there is a relationship between virulence and infectivity. The effectiveness of different transmission routes might also be influenced by the virulence of a virus strain or isolate. The transmission of ASF was found to be more efficient when a highly virulent ASFV strain was used [90]. McVicar [91] found that the amount of virus in oronasal fluid was higher in pigs infected with highly virulent ASFV strains as compared with infections with an isolate of lower virulence. Thus, the virulence of the circulating ASFV strain has an effect on the spread of ASF and on the speed of spread. By contrast, Guinat, Gogin, Blome, Keil, Pollin, Pfeiffer and

Dixon [5], who summarized the results of several studies, found that the virus load in different body fluids was very similar, regardless of the virulence of the virus strain or isolate used to infect the pigs.

Depending on the virulence of the virus strain or isolate, infection with ASFV can result in only mild clinical symptoms or 100% mortality or anything in between (Table 1).

The examples in Table 1 illustrate the complexity and potential inter-dependency of several parameters regarding virulence and the course of field and experimental infection with different ASFV isolates or strains. When comparing the presented results, the different infection routes should not be neglected (Table 1). However, the course of an ASF epidemic in wild boar may last for a long time, regardless of the virulence of the circulating strain [1,92,93]. In Brazil, where the virus strain circulating in domestic pigs was characterized as low virulent, it took several years until the disease had been eradicated [94]. In this case, the epidemic spread over the entire country and also affected several neighboring countries.

Table 1. Strains and isolates of African swine fever (ASF), their virulence, and course of field and experimental infections.

ASFV Strain or Isolate	Virulence	Course of Disease	
		Field Infection	Experimental Infection/*Route of Infection*
Malta isolate (Malta/78)	Moderate/high	Fast spread in the domestic pig population, which ended in the slaughter of all pigs in Malta within one year [79].	Mild clinical signs in less than 50% of infected pigs, high mortality/*exposure to infected donor pigs* [80].
Brazil'78	Low/moderate	Mild clinical course and decreasing mortality over time in domestic pigs. Wide distribution throughout Brazil for at least eight years [94].	High mortality/*intranasally* [70].
Netherlands'86	Moderate	No information found	Low mortality with a rather subclinical course of disease/*oronasally and through contact* [67,95].
Georgia 2007/1	High	Large scale epidemic, wild boar and domestic pigs affected [96].	Moderate clinical signs, high mortality/*intramuscularly* [65].
Estonian'15/WB, Tartu-14	High	Rapid spread in the wild boar population [37].	100% mortality in experimentally infected domestic pigs. Two survivors in in-contact pigs/*intramuscularly* [66].
Estonian-Ida Viru	High	Only local spread within the wild boar population [37].	Almost 100% mortality (one survivor)/*oronasally* [68].
Armenia'08	*High*	*No information found.*	100% mortality in wild boar and domestic pigs/*oronasally* [27].

2.11. *Tenacity or Resistance of ASFV to Environmental Factors*

Several studies have shown that ASFV is resistant to extremely harsh environmental conditions, and thus can stay infectious for a long time in various matrices. The tenacity of ASFV is particularly high at cold temperatures, for example, in frozen meat, where the virus may stay infectious for several years [16,97,98]. Even at room temperature, substantial infectivity is preserved for months. Dee, et al. [99] found that ASFV stayed infectious for a few days in different feeds and feed ingredients, for example, in moist dog and cat food. It is also known that ASFV can survive putrefaction [78]. Mebus, et al. [100] found a resistance of ASFV across pH-levels ranging from 4 to 13.

3. Discussion

The view that ASF represents a highly contagious disease, spreading very fast and killing all pigs of an affected farm or the whole wild boar population in a region, requires substantial revision. We aimed to clarify a number of aspects in relation to factors that may be important in this respect, particularly those affecting the speed of ASF spread. We identified and described terms that are likely to play a role in the spread of ASF and searched for published information regarding these parameters.

Our findings emphasize the difficulty to define the speed of spread and the parameters relating to it. This is not only because of different definitions of the parameters that may influence the speed of spread, but may also be related to the interdependencies of many parameters on (other) properties of the agent and those related to the host or the environment. A further drawback of the current review is the fact that relatively few, and even less reproduced (and thus presumably reliable), studies concerning the specified parameters were available.

The different properties of the virus isolates and strains, but also the characteristics of the host factors, environmental parameters, and matrix effects, are difficult to separate from each other. Properties are often prone to influence each other and sometimes the definitions for the studied parameters collide if different studies are compared. Furthermore, it appears that some terms are not clearly defined or are used in various, sometime confusing ways in different studies. This refers mainly to the terms mortality and case-fatality ratio, but virulence, infectiousness, and infectivity may also be affected. With regard to mortality and the case-fatality ratio, the size of the susceptible population may be difficult to determine, particularly in wild boar, even if it is defined as the population living in a certain area or belonging to a particular pack of wild boar. On the basis of these parameters, it is often difficult to draw reliable conclusions concerning the true speed of ASF spread.

Depending on the ASFV strain, infection led quickly to 100% mortality, indicating a fast spread within separate epidemiological units [25,57,60,69,93]. Despite the high virulence of the ASFV isolates used for experimental infections and the corresponding high mortality observed, the current course of ASF in Eastern and Central Europe indicates a rather slow spread in the wild boar population over time. The largest amount of ASFV can be found in the blood, which makes direct transmission through exposure to blood of infected animals the most effective way of infection [57,60,62]. However, in other body fluids of infected pigs or wild boar and in the environment, the amount of infective virus is much lower [56,78]. As direct exposure to blood of infected animals is less likely than exposure to other body fluids or contaminated materials in the environment, this may reduce the morbidity, and thus also the speed of ASF spread. However, this might explain the differences in mortality between experimental and field settings, as direct contact to blood is more likely in experimental settings than in the field. Until now, the role of chronically infected animals within the spread of ASF in wild boar is highly disputed [58–60,94]. If animals that have recovered from ASF were still able to spread the disease, this could certainly influence the spreading of ASF and its speed.

Another potential hypothesis regarding the slow spread in the field might be a relatively low virulence of the ASFV circulating in the area. However, for Eastern and Central Europe, this hypothesis is not warranted by the results obtained in experimental studies using the strain circulating in this area, which suggest a high virulence of this ASFV strain [25,56–58,60,69,95].

Moreover, it seems puzzling that ASF is not self-limiting, in particular in view of the difficulties in transmission described above. Some studies demonstrated that ASFV stays infectious for a long time in various tissues [90–92,96–99]. Therefore, it can be assumed that the presence of infectious ASFV represents a risk of exposure of naïve animals and that this risk may be cumulating over time, even if the risk of exposure is relatively low at any given point of time. Correspondingly, the tenacity of ASFV seems to play a major role in the spread of ASF.

In countries where the wild boar population is heavily affected in large areas, the disease has often spread continuously and has led to a significant reduction of the wild boar population [29,85,100]. It is thus important to note that there are several additional factors influencing the spreading speed of ASF in addition to those listed in this review. Several studies mention the population density of wild boar, the density of infected wild boar in the proximity of commercial pig holdings, and the density of pig holdings in a defined area as risk factors for a faster spread of ASF [2,15,48,53]. However, the effect of the population density on the spread of ASF is still disputed, and no population density threshold could be defined so far to stop ASF spread [29,47,84].

In addition, the calculation or estimation of transmission speed is hampered by the behavior and the living habits of wild boar. Wild boar usually live in a pack with regular interactions within the pack, but not between different packs [50]. Although Pfeiffer, Robinson, Stevenson, Stevens, Rogers and Clements [7] stated that transmission is more likely when many animals live closely together, the current knowledge on the behavior of wild boar has to be incorporated into any assessment of ASF disease spread in the field.

When discussing the transmission speed of ASF, the different transmission cycles have to be taken into account [4,101]. Blood of infected pigs is the most efficient medium for ASF transmission [71]. This fact affects every transmission cycle.

The domestic cycle in the form of animal movement patterns, both within and between herds, plays a major role in the spread of ASF among domestic pigs [52,101]. This fact and others suggest, in turn, that biosecurity measures can limit the spread of ASF [2,3]. The hypothesis that high biosecurity standards can decelerate or even prevent the spread of ASF is supported by an increased number of ASF outbreaks in backyard farms with less than 50 pigs, and usually lower biosecurity measures [2,15,102]. Therefore, it can be assumed that the spread of ASF is faster in countries with a high number of small private pig holdings, as can currently be seen in Romania [103]. However, Estonian researchers could show that the biosecurity level had no measurable influence on the risk of an introduction of ASF [56]. Also, in China, it seems that the size of pig holdings has no major influence on the speed of spread of ASF. The number of backyard pig holdings has clearly declined in recent years in China. Despite that households in rural areas keeping pigs do not exceed 20%, a fast spread of ASF throughout the country has been observed [13,52].

Soft ticks of the genus Ornithodoros can also play an important role in the transmission of ASF. This is undoubtedly the case in the tick–pig cycle and in the natural sylvatic cycle of ASF in warthogs in southern Africa, but may also be relevant in other regions, if Ornithodoros ticks are present [5,102] so that it must be expected that these tick vectors can influence the speed of ASF spread. However, there is currently no evidence that ticks play a role in the ASF transmission cycle in Europe [103].

Finally, as mentioned in the introduction, it is of utmost importance to stress that human behavior is without any doubt the most important factor that can facilitate the transmission of ASF over long distances [2,5,47,88,104,105]. In countries with many private backyard holdings, the spread of ASF can be supported by failure of reporting outbreaks or suspect cases and, in the worst case, even by selling sick animals to circumvent problems expected by pig holders if ASF is detected. The influence of the human factor also becomes evident when the introduction of ASF into wild boar in the Czech Republic in 2017 and Belgium in 2018 is taken into consideration. Before these countries were affected, the nearest outbreaks of ASF had been hundreds of kilometers away. Therefore, similar to the foot-and-mouth epidemic in England in 2001 [27], the spatial pattern of disease outbreaks can often not only be explained through distance-dependent transmission, that is, through infected animals in close proximity to each other, but also through the transportation of infected animals or contaminated material over long distances. It is obvious that in scenarios where ASFV is introduced by distance-independent mechanisms, for example, transportation of infectious material in the course of various human activities, the parameters discussed in the context of this review play no or only a minor role.

4. Conclusions

On the basis of the available literature, we propose revising the view that ASF generally has to be referred to as a highly contagious disease. We tried to show that it is not always easy to answer the question raised in the title of this review, because the answer may depend on several epidemiological parameters. ASF is neither generally fast and furious nor is it slow and steady, but the appearance of ASF can be diverse. ASFV strains can vary in their virulence. However, highly virulent strains or isolates, also the one currently circulating in Eastern and Central Europe, which has recently been introduced into China, may be characterized by a low morbidity potentially owing to transmission through materials with a relatively low virus load, leading to slow spread in wild boar populations. Jumps of ASF over long distances are usually the result of human activities, and are thus unpredictable.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4915/11/9/866/s1, Supplementary File S1: Additional definitions.

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3.2.1.3 African Swine Fever in Wild Boar in Europe—A Review

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Highlights

- Wild boar play an important role in the current ASF epidemic
- Neither domestic pigs nor ticks are necessary to maintain virus spread
- ASF control in wild boar is challenging
- Until now, only two countries succeeded in eliminating the disease from the wild boar population

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Review

African Swine Fever in Wild Boar in Europe—A Review

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Abstract: The introduction of genotype II African swine fever (ASF) virus, presumably from Africa into Georgia in 2007, and its continuous spread through Europe and Asia as a panzootic disease of suids, continues to have a huge socio-economic impact. ASF is characterized by hemorrhagic fever leading to a high case/fatality ratio in pigs. In Europe, wild boar are especially affected. This review summarizes the currently available knowledge on ASF in wild boar in Europe. The current ASF panzootic is characterized by self-sustaining cycles of infection in the wild boar population. Spill-over and spill-back events occur from wild boar to domestic pigs and vice versa. The social structure of wild boar populations and the spatial behavior of the animals, a variety of ASF virus (ASFV) transmission mechanisms and persistence in the environment complicate the modeling of the disease. Control measures focus on the detection and removal of wild boar carcasses, in which ASFV can remain infectious for months. Further measures include the reduction in wild boar density and the limitation of wild boar movements through fences. Using these measures, the Czech Republic and Belgium succeeded in eliminating ASF in their territories, while the disease spread in others. So far, no vaccine is available to protect wild boar or domestic pigs reliably against ASF.

Keywords: African swine fever; disease control; wild boar; epidemiological course; diagnostic; clinical picture

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1. Introduction

African swine fever (ASF) is a devastating disease of domestic pigs and wild boar characterized by hemorrhagic fever that can be up to 100% lethal [1]. Despite its limited host range, its socio-economic impact is tremendous. It is therefore notifiable according to international and national regulations usually accompanied by strict control measures. Only members of the *Suidae* family are susceptible to ASF virus (ASFV), which has no zoonotic potential [2].

Over the last decade, ASF has changed from a regional disease of Sub-Saharan Africa to a considerable and tangible threat to pig husbandry, especially in Europe and Asia. After the introduction of genotype II into Georgia in 2007 and the subsequent spread in eastern Europe, the disease found a breeding ground in abundant wild boar populations [3–8]. Based on previous experience on the Iberian Peninsula and Sardinia with genotype I of ASFV, wild boar had so far not been considered a major and long-term reservoir for ASFV [9,10], and self-sustaining infectious cycles in wild boar were not anticipated at the beginning of the epidemic [11]. However, disease dynamics are different under the present conditions, particularly in north-eastern Europe, and long-lasting endemic cycles without any major involvement of domestic pigs were established in affected countries such as the Baltic States or Poland [3–5,7,12–17]. Despite the high virulence of ASFV and considerable case/fatality ratio among wild boar, these cycles remained self-sustaining in many affected countries over several years, while the Czech Republic and Belgium were successful in eliminating introductions of ASF in wild boar [18–20]. In Estonia and Latvia, there are indications that the ASF epidemic among wild boar is subsiding [5,13,14], although the emergence of a limited number of new cases in Estonia illustrates that control measures need to remain in place for a long time.

The first step for assessing risks and planning control measures is gaining knowledge about critical factors in disease transmission and dynamics. In this context, this review summarizes the current knowledge on ASF in wild boar.

2. Clinical Signs and Pathomorphological Lesions

2.1. Clinical Signs

Under field conditions, diseased wild boar often showed disorientation, e.g., by roaming at full daylight, staggering gait, lack of fear towards humans or dogs, and difficulties in breathing. Moribund animals and carcasses of wild boar that succumbed to infection were often found close to water bodies, where they seemed to seek a cold and moist environment, presumably as a reaction to fever [21].

Under experimental conditions, general clinical signs and pathomorphological lesions were quite similar between domestic pigs and wild boar [22–24]. However, for some virus strains with a slightly attenuated phenotype, e.g., “Estonia 2014”, clinical signs appeared more severe in wild boar [25,26].

Upon oro-nasal infection of wild boar with highly virulent ASFV strains, the first clinical signs were observed approximately four days post infection. Typical findings included high fever in all age classes, anorexia, depression, dullness, vomiting, diarrhea, reddening of the skin, respiratory disorders, and ataxia [22,23,25,27–29]. Severe hemorrhagic (epistaxis, bloody diarrhea, skin hemorrhages) and neurological signs were sometimes observed in the final stages of infection [27]. In the acute-lethal course of ASF, most animals died within 7 to 14 days post infection (dpi). However, some animals may survive longer or recover completely [28,30]. Typical findings are depicted in Figure 1.

2.2. Gross Pathological Findings

As common features after oral or oro-nasal infection, inoculated animals revealed swollen, enlarged, edematous and hemorrhagic lymph nodes, lung edema, which could be severe, varying renal cortical petechiae as well as hemorrhagic gastritis in some cases. More specifically, the hepatogastric and renal lymph nodes were generally more severely affected [23,26,28,29]. Occasional findings included a gall bladder wall edema [23,24,28], renal infarction [28], mild pulmonary consolidation [26], arthritis [28] and splenomegaly [23,29]. The latter is frequently mentioned as a characteristic feature of an ASF infection, but was only rarely observed after experimental infections, where humane endpoints are executed.

Skin lesions were less common in wild boar than in domestic pigs. Nevertheless, hematomas or even subcutaneous petechiae have been described in animals inoculated by the intramuscular route using ASFV genotype I strains [31,32], but were less frequently observed after oro-nasal inoculation with genotype II strains [23].

Wild boar that were inoculated via the parenteral route further showed accumulations of fluid in the abdominal and thoracic cavity including hemohydroperitoneum [24,33] and pericardial or pleural effusion [31,32]. Moreover, hemorrhages in the intestinal tract or hemorrhagic enteritis have so far only been mentioned after intramuscular infection with strains of genotype I [24,33].

Figure 1. Clinical signs in wild boar of different age classes. Top row from left to right: hunched-up back and depression (**left**) in a sub-adult boar, severe depression and inappetence in a boar (**center**), severe but unspecific signs, respiratory problems in a sub-adult animal (**right**). Bottom from left to right: severe depression and moribund state in (sub-)adult females (**left**), piglets with high fever and reduced liveliness that later on recovered (**center**), and the same piglets and two adult females showing severe, unspecific signs (**right**).

The development of lesions after ASFV infection has been observed in a single kinetic study investigating the above-mentioned Estonian ASFV strain showing a slightly attenuated phenotype, especially in domestic pigs [26]. Three wild boar were infected experimentally with ASFV "Estonia 2014", sacrificed and necropsied on days 4, 7 and 10 post infection (p.i.). Lesions were generally mild to moderate. Swollen, hemorrhagic lymph nodes mainly affecting the renal and hepatogastric nodes were already observed on day 4 p.i. Renal petechiae occurred on day 10 p.i. in all wild boar, whereas only one animal developed a hemorrhagic gastritis on day 7 p.i. and another one pulmonary consolidation on day 10 p.i.

2.3. Histopathological Findings

Besides macroscopical lesions, histopathological changes were confined to lymph nodes, spleen, tonsil, liver, lung, kidney as well as to brain and male genitals, which appeared inconspicuous during necropsy. Regardless of the virulence of the virus strain used for infection, histopathology confirmed congestion and hemorrhages of affected lymph nodes and showed apoptosis of histiocytes and lymphocytes, referred to as lymphocytolysis [26,29,31]. In addition, the hemolysis of erythrocytes and the thickening of the connective stroma within lymph nodes have been described after infection with the Italian genotype I "Nemi" strain of ASFV [31]. Immunohistochemical antigen staining of lymph nodes showed positive myelomonocytic cells in wild boar infected with ASFV "Belgium 2018/1" and "Estonia 2014" [26,29]. Similar results were observed in lymphoid tissues such as spleen and tonsils, and included apoptosis of lymphocytes and histiocytes [26,29], congestion and hemorrhage [29] and thickening of splenic trabeculae and capsule [31]. Crypt necrosis and abscesses were also recorded. Viral antigen was present in splenic and tonsillar myelomonocytic cells as well as crypt epithelial cells within lesions in animals infected with the "Belgium 2018/1" and "Estonia 2014" ASFV strain [26,29]. Histopathological data from the liver only slightly differed among studies. Subcutaneous infection with the "Nemi" strain led to liver necrosis, periportal lymphocytic and granulocytic infiltration, as well as thickened septa, enlarged sinusoids and centrilobular veins [31]. Wild boar oro-nasally infected with the highly virulent "Belgium 2018/1" strain developed hepatitis, but also showed hepatic angiectasia and congestion [29], whereas both the degeneration and necrosis of Kupffer cells and lymphocytic inflammatory reaction were noticed after infection with the "Estonia 2014" strain [26]. In both groups, viral antigen was detectable in Kupffer cells, sinusoid endothelium and hepatocytes.

Irrespective of the virus strain, ASF infection invariably led to pulmonary inflammation. In detail, interstitial lymphohistiocytic infiltrates [26,29], lymphoid hyperplasia [29], alveolar edema, and hemorrhage [29,31] have been reported. In wild boar infected with the "Nemi" strain, the inflammatory pattern was different: alveoli and bronchi were filled with lymphocytes and cellular debris, and the bronchial epithelium was enlarged, indicating bronchopneumonia rather than interstitial pneumonia observed after infection with the Belgium and Estonian strain. Immunohistochemistry results obtained from the lung showed ASFV antigen-positive macrophages [26,29]. Kidney lesions including degeneration and necrosis of glomerular and tubular cells have only been reported after infection with the highly virulent strains "Nemi" and "Belgium 2018/1" [29,31]. Hemorrhages around vessels and between tubules were especially seen in Nemi strain-infected wild boar [31]. By contrast, infection with the Estonian strain showed rather mild interstitial lymphocytic infiltrates that were not necessarily associated with viral antigen [26]. Immunohistochemistry showed viral antigen in interstitial cells [26], macrophages, endothelium, glomerular and tubular epithelium [29].

An inflammation of the brain, which has not been further characterized, has so far been observed only once after experimental infection with the "Belgium 2018/1" strain [29]. Regardless of the presence of inflammation, viral antigen was detectable in macrophages [29], cerebral and cerebellar glia cells and choroid plexus epithelium [26,29] in wild boar infected with both the "Belgium 2018/1" and the "Estonia 2014" strain. Male reproductive organs have not yet been studied in detail, but were sampled in the study with the "Belgium 2018/1" strain [29]. While macroscopy revealed no changes, histopathological analysis of testis and epididymis showed hemorrhage and congestion, mild interstitial inflammation and single-cell necrosis as well as vasculopathy/vasculitis. Viral antigen was present in macrophages, endothelium, peritubular fibroblasts and to some extent in intraductal apoptotic cells. Accessory sex glands were normal, but revealed some positive macrophages. Although neither gross nor histologic lesions were detectable in bone marrow of ASFV "Estonia 2014"-infected wild boar [26] or salivary glands of animals infected with "ASFV Belgium 2018/1" [29], viral antigen was identified in myeloid cells, megakaryocytes and macrophages.

3. Immunology

As wild boar are part of ecosystems around the world, they are in contact with various pathogens [34]. Their pathogen reservoir is constantly changing and expanding through contact with livestock and humans [35]. Immunity is an important factor in this host–pathogen interaction, also with respect to the outcome of infection in the individual animal and the wild boar population as a whole [36]. Unfortunately, differences in the immunocompetence of the domestic pig and wild boar and thus differences in the immune response to ASFV infection have hardly been studied and are therefore not understood in great detail.

Like in domestic pigs, the ASF virus replicates in wild boar mainly in monocytes and macrophages. However, in vitro studies by Giudice et al. [37], who infected monocytes and macrophages of wild and domestic pigs with the genotype I Sardinian ASFV field strain "22653/14", showed differences between domestic pigs and wild boar. Monocytes of domestic pigs and wild boar were equally susceptible to the virus, but monocytes of wild boar did not respond by producing IL1α, IL1β, IL6, IL10, IL12, and IL18 after infection, while monocytes of domestic pigs did.

In ASFV-immunized domestic pigs, CD8+ T cells play a crucial role in immunity against ASFV. Depletion of CD8+ T cells from these pigs before challenge with highly virulent ASFV resulted in the loss of protection to the level of the non-immunized controls, while the animals with CD8+ T cells survived [38]. Such experiments are missing for wild boar. Recently, however, it could be shown that, after experimental infection with the highly virulent genotype II ASFV strain "Armenia08", CD8+ T cells were activated in wild boar, in contrast to domestic pigs [39]. Interestingly, a loss of perforin expression on all cytotoxic T cells could also be observed in domestic pigs and wild boar on day 5 p.i. These results indicate a cytotoxic reaction of T lymphocytes in wild boar in response to a highly virulent ASFV infection, which is nevertheless not protective. After infection with the moderately virulent strain "Estonia2014", a protective T cell immune response developed, illustrated by T cell activation and sustained proliferation, which apparently reduced mortality significantly [40]. Of particular interest is the more pronounced γδ T cell response in wild boar compared to domestic pigs, as measured by the increasing frequency of CD8+ cytotoxic γδ T cells in wild boar organs. The fact that wild boar became more severely ill despite this response can possibly be explained by a stronger expansion of regulatory T cells (Tregs), which may shorten the pro-inflammatory response [40]. These findings and interpretations are in accord with observations of a lymphohistiocytic interstitial pneumonia even after 10 days p.i., but only in domestic pigs infected with "Estonia2014", which may indicate a prolonged pro-inflammatory response in domestic pigs in contrast to wild boar [26]. Sánchez-Cordón and co-workers conducted experiments in domestic pigs, which showed that Tregs might present a way of viral immune evasion as they were able to inhibit antiviral responses specifically [41]. These observations may indicate that this way of immune evasion is more prominent in wild boar than in domestic pigs.

The few wild boar that survive infection with ASFV usually seroconvert [25,28]. Depending on the ASFV strain and the route of infection, virus-specific antibodies can first be detected 11–20 days p.i. [42]. Experimental infections with highly virulent ASFV strains usually do not lead to measurable antibody titers in serum, because the animals reach the humane endpoint before mounting a measurable antibody response [23,43]. However, reports of PCR-negative, but seropositive, animals increase in the hunting bag of affected regions [44]. Among them are also young animals (<12 month) that seem to have survived the infection and possess ASFV-specific serum antibodies [45]. This age correlation has also been observed in experimental studies with ASFV "Estonia2014": sera obtained from infected piglets contained ASFV-specific antibodies, while adults remained seronegative throughout the experiment [25]. A potential neutralizing effect of virus-specific antibodies is controversially discussed [46,47]. However, studies using domestic pigs revealed that antibodies play a role in protection [48]. On the other hand, it has recently been shown that immunization with the attenuated ASFV strain "OURT88/3" or the vaccine candidates "BeninΔMFG" and "HLJ/-18-7GD" failed to confer long-lasting protection in domestic pigs despite high antibody titers [41,49]. Similar studies with wild boar are lacking.

4. Epidemiology

4.1. Occurrence of ASFV in Europe

Table 1 gives an overview of the introduction of ASF into different European countries in wild boar, and Figure 2 shows the current (as of 8 July 2021) status of ASF in the different European countries in wild boar.

Table 1. African swine fever in wild boar and domestic pigs in Europe.

Country	Region	First Occurrence of ASF in Wild Boar	Presumed or Proven Route of Introduction	Reference	Presumed Main Driver for ASF Epidemic in Wild Boar
Portugal		n.d.		Costard et al., 2009 [50]	Interaction of domestic and free-ranging pigs/ *Ornithodoros* ticks
Spain		n.d.	Spread from Portugal		
Italy	Sardinia	n.d.	Pork products from Portugal	Mannelli et al., 1998 [51]	Interaction of domestic and free-ranging pigs
Georgia		n.d.	Waste from ships	Vepkhvadze et al., 2017 [52]	n.d.
Armenia	North	Oct 2010	Movement of infected pigs and wild boar from Georgia	FAO 2008 [53]; Markosyan et al., 2019 [54]	Interaction of free-ranging domestic pigs and wild pigs
Azerbaijan		n.d.	Pork products from Georgia	FAO, 2008 [53]	n.d.
Russian Federation	Northern Caucasus	Nov 2007	Wild boar movements from Georgia	Gogin et al., 2013 [55]; FAO, 2008 [53]	Wild boar/free-ranging pigs
Belarus		n.d.	n.d.	GF-TADs, 2015 [56]	n.d.
Ukraine	Lugansk (2014)	Jan 2014	Wild boar movements from Russian Federation, 2014	DEFRA, 2014 [57]	Interaction of domestic and free-ranging pigs or backyard holdings
Lithuania		Jan 2014	Wild boar movements from Belarus	State Food and Veterinary Service Lithuania, SCoPAFF, Feb. 2014 [58]	Wild boar
Poland	East	Feb 2014	Wild boar movements from Belarus	Wozniakowski et al., 2016 [8]	Wild boar
	Warsaw	Nov 2017	Human activity	General Vet. Inspectorate, Poand, SCoPAFF, Jan 2018 [59]	Wild boar
	North	Dec 2017	Wild boar movements from Kaliningrad, RF	n.d.	Wild boar
	West	Nov 2019	Human activity (?)	Mazur-Panasiuk et al., 2020 [60]	Wild boar
Latvia	East	June 2014	Wild boar movements from Belarus	Olsevskis et al., 2016 [61]	Wild boar
	North	July 2014	Illegal disposal of contaminated material		
	Madona	Aug 2014	Human activity (?)		
Estonia	South	Sep 2014	Wild boar movements from Latvia	Nurmoja et al., 2017 [4]	Wild boar
	North	Sep 2014	Wild boar movements from RF		
Moldova		May 2019		GF TADs, 2016 [62]	Outbreak in domestic pigs or wild boar movements
Czech Republic	Zlín	June 2017	Illegal disposal of food waste	OIE, 2019 [20]	Wild boar
Hungary		April 2018	Illegal disposal of food waste	EFSA, 2020 [63]	n.d.
Romania	Satu Mare Danube Delta	May 2018 June 2018	Human activity	EFSA, 2020 [63]	Outbreaks in domestic pigs and pig holding structure
Bulgaria		August 2018	Human activity, wild boar movement (from Romania?)	Zani et al., 2019 [64]	Outbreak in domestic pigs or wild boar movements
Belgium	Wallonia	Sep 2018	Illegal disposal of food waste	Linden, 2019 [65]	Wild boar
Slovak Republic		Aug 2019	Wild boar movements from Hungary	EFSA, 2020 [63]	n.d.
Serbia		July 2019	Wild boar movements from Romania / Bulgaria	Reuters, 2020 [66]	n.d.
Germany	Brandenburg and Saxony (Polish Border)	Sept 2020	Wild boar movements from Poland	Sauter-Louis, et al., 2020 [6]	Wild boar

[1] n.d.—no data.

4.1.1. Iberian Peninsula

ASF had been introduced into domestic pigs in Europe on several occasions since 1957. Historically, transmission to and long-term establishment in wild boar was only seen on the Iberian Peninsula (Portugal and Spain) and subsequently in Sardinia (Italy).

Until 1981, only about 6% of the ASF outbreaks in domestic pigs were associated with direct contact to infected wild boar [67]. It was concluded that the disease would not have persisted in wild boar, once it had been eliminated from domestic pigs [10]. This was confirmed after the elimination of ASF from Spain and Portugal. In detail, a survey from 2006 to 2010 suggested that no wild boar in the affected areas were positive, thus indicating that the infection had not maintained itself in the wild boar population long term [68].

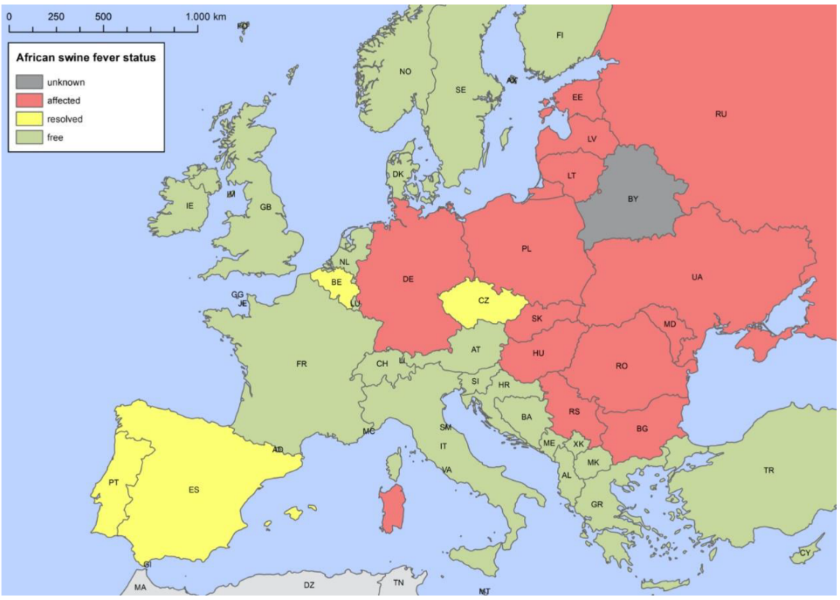

Figure 2. African swine fever status of the different European countries as of 4 August 2021.

4.1.2. Sardinia

ASF had entered Sardinia through contaminated food, similar to the introduction into Portugal. Since its first emergence in 1978, ASF remained endemic in Sardinia, with outbreaks occurring in domestic pigs and wild boar. The ASFV strain circulating in Sardinia belongs to genotype I [69]. In the south of Sardinia, ASF could be eliminated, while elimination attempts failed in the northern, central and eastern regions of the island for a long time [70,71], but the regions seem now to be close to becoming free from ASF [72].

Several risk factors for the persistence of ASF in Sardinia have been identified, such as the number of medium-sized farms, the presence of free-ranging pigs (particularly the local breed called "brado") and the combination of the estimated wild boar density and the mean altitude above sea level [51,70,73,74].

Even after four decades of ASFV presence and circulation on the island, the prevalence in wild boar in Sardinia was found to be very low (1% with the 3rd quartile of 10% for seroprevalence for the time period 2011 to 2016) [71]. This is much lower than the recent prevalence in brado pigs, where antibodies against ASFV were found in more than 50% of the examined animals [74]. When a spatial-temporal analysis was performed, no clusters were identified in wild boar in Sardinia [75]. ASFV-positive wild boar were only found in endemic areas, where outbreaks in domestic pigs occurred [75,76]. Thus, similar to the situation in the Iberian Peninsula, ASF was apparently not able to establish an independent infection cycle in the wild boar population. It has been suggested that the disease would have spontaneously disappeared from the wild boar population, after elimination from domestic pigs [9]. Contacts between wild boar and infected free-ranging domestic pigs and repeated re-introductions into the wild boar population were postulated as necessary for the maintenance of the disease on the island [9,51,70,75,77]. A recent study [78] found direct and indirect interactions between free-ranging domestic pigs and wild boar in an ASF-endemic area of Sardinia, indicating that these contacts facilitated the transmission of ASF on the island. This was also confirmed in a retrospective study, which showed that spatial interactions between wild boar and brado pigs occurred close to pig farms [79].

4.1.3. Russian Federation (RF)

ASF reached the RF through wild boar in November 2007, after introduction into Georgia and notification in the spring of that year, from where it also spread to Armenia, Azerbaijan, Abkhazia and South Ossetia [80,81]. The ASFV circulating in these areas was classified as a strain of genotype II [82]. The first cases of ASF in wild boar were detected in the North Caucasus region. In this area, which is considered to harbor a large wild boar population, transmission and persistence of ASFV in the wild boar population was observed [55,83]. Spill-over into domestic pigs was documented in 2008, when a traditional free-range pig holding was affected. Spill-back into the wild boar population occurred due to low biosecurity in the domestic pig holdings and through illegal disposal of infected material [55,84]. Until 2010, the disease spread within the southern territories of the RF epidemically, both in wild boar and in domestic pigs [11].

In the RF, human behavior played a major role in the spread of ASF [55]. Carcasses of infected pigs, hidden or unsafely disposed of by farmers, especially represented a constant source of introduction of ASFV into the wild boar population [55]. Infection in wild boar then could cause spill-back in domestic pigs due to low biosecurity [85]. In 2011, the spread of disease in the RF was estimated to be 350 km/year (N. Vlasov and D. Kolbasov, cited in [86]).

Spatial analysis of data from the RF obtained between 2007 and 2013 revealed clusters of ASF in wild boar, domestic pigs and overlapping clusters [82]. Similar results were obtained for the time period 2017–2019 [87]. These studies indicate that the virus was able to persist in wild boar without constant reintroduction from domestic pigs in this region. This stands in sharp contrast to the situation that had been observed on the Iberian Peninsula and in Sardinia with the genotype I ASFV.

4.1.4. Baltic States, Poland and Germany

The situation in the Baltic states (Lithuania, Latvia, Estonia), Poland and Germany differs from the situation in the RF in that, in the former, the majority of ASF cases were detected in the wild boar population.

The first introduction of ASFV genotype II occurred in January 2014, when Lithuania reported a case of ASF in wild boar near the border with Belarus. A few weeks later, the first ASF case was recorded in Poland in wild boar, also close to the Belarussian border. In June 2014, the first case of ASF in wild boar was detected in Latvia, again close to the border with Belarus [61]. Estonia followed with cases in wild boar that were detected in September 2014 in the southeast, close to the Latvian border, and in the northeast, close to the border with the RF.

In Lithuania, the disease spread from the border to Belarus in a westerly direction. The number of infected wild boar increased substantially from 2014 to 2018 [88,89]. By 2019, about 86% of the area of Lithuania was affected by ASF [88,90].

In Latvia, the first area affected by ASF was the South-eastern border area with Belarus, followed by an introduction into wild boar in the north of Latvia, close to the border with Estonia, presumably through illegal disposal of offal into the forest [61]. In the meantime, nearly the entire territory of Latvia is affected by ASF [5].

In Estonia, ASF first emerged in two regions, one in the southern part, bordering Latvia, and one in the northeast, close to the border with the RF [4]. In the meantime, ASF has spread throughout Estonia (with the exception of the island of Hiiumaa).

In Poland, until 2016, cases of ASF in wild boar were restricted to a belt of 1–10 km along the border with Belarus [8,91,92]. It is likely that repeated introductions from Belarus into Poland had occurred [15]. From 2014 to 2016, the cases showed a north-south distribution along the border, with an expansion of the affected area during 2017 [93]. In 2017 and 2018, the number of ASF-infected wild boar increased substantially [3,17]. At the same time, new areas were affected around Warsaw and in the north, close to the oblast of Kaliningrad (RF). In November 2019, a new region in western Poland became affected, first in wild boar and subsequently also in domestic pigs.

Ten months later, in September 2020, the neighboring area across the border in Germany recorded the first ASF case in wild boar [6]. Both the vicinity of cases to the German–Polish border and their clustering suggest several independent incursions into Germany [94]. Together with sequence data of the complete viral genome obtained for several ASFV strains, this illustrates that there exists a transboundary epidemic affecting western Poland and Germany.

In the Baltic states and Poland, the disease spread mainly through wild boar migration, but also through human activity. Transmissions over large distances (jumps) were obviously due to human activities (probably improper disposal of contaminated food waste), as described for several countries [61,95–97]. The local spread of ASF among wild boar was not as fast as originally predicted [98]. Depending on the wild boar density, the rate of local spread has been estimated to range between 1 and 2 km/month [99,100]. In Lithuania, it was calculated to be 5 km/month [88]. It should be noted that the spatial-temporal dynamics of ASF in wild boar could not exclusively be explained by the movements of wild boar, i.e., through dispersal and home range sizes [101].

Surveillance for ASF in wild boar is achieved by active and passive surveillance. Several studies have shown that passive surveillance of wild boar carcasses was superior to active surveillance in detecting ASF in wild boar [4,5,7,13–15,17,61]. Data on wild boar killed in traffic accidents are only sparse, and thus there has so far been no statistical evidence that the prevalence of ASF in road-killed wild boar is higher than in wild boar hunted healthy [102]. Nevertheless, wild boar killed in traffic accidents should be sampled in the interest of early ASF detection. At least during the early phases of an epidemic, the prevalence of ASFV-positive dead wild boar (usually determined by PCR, i.e., detection of ASFV genome) is much higher than the seroprevalence (i.e., animals with ASFV-specific antibodies) in hunted wild boar [4,7,14,90,102–104]. In Lithuania, the average prevalence of ASFV-positive wild boar found dead, as determined by PCR, was 65.7% (95% CI: 64.0–67.4%), while the serological prevalence in hunted animals (active surveillance) was only 0.45% (95% CI: 0.39–0.51%) with regional differences [89,90].

In all of these countries, the probability of finding an ASFV-positive animal was much higher in animals found dead in comparison to hunted animals with odds ratios between 69 and 193 [4,7,90].

During recent years, an increasing seroprevalence regarding ASFV of up to 7% was observed in all three countries of the Baltic states [5,14,44,90].

Several factors associated with the detection of the disease in wild boar have been investigated in these countries. Wild boar density was considered crucial for the spread of ASF. In the early years after Poland had become affected, cases of ASF in wild boar occurred mainly in areas with a wild boar population of more than 1 animal/ km^2. It was therefore concluded that reducing the wild boar density to less than this value could reduce the spread of the disease [15]. Polish data on ASF in wild boar between 2014 and 2016 revealed that the probability of finding ASFV-positive wild boar increased with wild boar population density and the proportion of forest in land cover [105].

Data from Estonia and Latvia revealed that young wild boar were more frequently ASFV-positive than older animals [4,14].

Another factor is seasonality. In the Baltic states and Poland, peaks in the detection of ASF in wild boar were observed during summer, but also in late winter (February and March) [8,61]. In Lithuania, the prevalence was higher in autumn than in spring [89] and high in winter [88]. In Estonia, a larger number of samples taken from wild boar found dead were recorded during winter [13]. In Latvia, however, no seasonality was detected in the occurrence of ASF in wild boar [7]. It has so far not been completely clarified whether the observed seasonal effects are due to a higher abundance of wild boar (in summer), increased hunting activity (in winter) or other, so far unknown factors.

In contrast to observations made on the Iberian Peninsula, the numbers of recorded ASF cases in wild boar in the Baltic states and in Poland by far exceeded outbreaks registered in domestic pigs [93]. It is important to note that ASFV was obviously able to persist in the wild boar population in the Baltic states and Poland, regardless of the situation in domestic pigs. This stands in sharp contrast to previous predictions [9], which, however, were based on a different scenario.

4.1.5. Czech Republic and Belgium

The ASF situation in the Czech Republic and in Belgium showed some similarities, but also differences, to that in the Baltic states and Poland. In contrast to multiple incursions into the wild boar population close to the border of neighboring countries, the Czech Republic and Belgium experienced focal or point introductions of ASFV into wild boar populations in regions more than 300 km away from known ASF-affected areas. It should be emphasized that in both countries only wild boar were affected.

The first case of ASF in the Czech Republic was notified in June 2017 in two wild boar carcasses found near the local hospital in Zlín [20,106]. Presumably, the virus was introduced through human activity [20]. Genotyping identified the virus as similar to those variants that were found elsewhere in the eastern and south-eastern parts of the EU (Moldova, 2016; Ukraine, 2012 and 2015; Belarus, 2013) [106].

ASF remained limited to the district of Zlín [106], where a core area of about 58 km^2 was defined [107]. In this core area, 71.7% of the sampled wild boar carcasses were ASFV-positive from June 2017 until April 2018 [106]. Until April 2018, when the last ASFV-positive wild boar was found, a total of 230 ASF cases were detected, of which 212 were in wild boar found dead and 18 in hunted animals [20,106]. This re-emphasizes the important role of passive surveillance in the early detection of ASF [107]. The last ASFV-seropositive wild boar were found in the core area in July and October 2018 [108]. One year after the detection of the last ASFV-positive wild boar, the Czech Republic submitted a self-declaration of the recovery of freedom from ASF to the World Organisation for Animal Health (OIE) [20].

Despite a high wild boar density in the Czech Republic, ASFV spread with a velocity of approximately 0.5 km/month, i.e., more slowly than in the Baltic States and Poland [109].

In the Czech Republic, ASFV-positive carcasses were found in larger distances from roads and forest edges than ASFV-negative carcasses [110]. In contrast to Estonia and Latvia, carcasses of adult animals were more frequently ASFV-positive than those of younger animals (75.4% versus 41.6%) [110].

Due to the fact that a single focal introduction had occurred into the Czech Republic, the basic reproduction number (R0) could be estimated at 1.95 for the first 29 days of the epidemic, i.e., each infected wild boar infected nearly two other wild boar [111]. Such calculations are neither available for the Baltic states nor for Poland.

Similar to the Czech Republic, the introduction of ASF into wild boar in Belgium in 2018 was presumably caused by human activity. The first cases of ASF in wild boar were detected in September 2018 [65,112]. The virus was identified as genotype II and was found to be closely related to ASFV strains from Ukraine, Belarus, Estonia and European Russia [112–114].

Until March 2020, a total of 833 cases of ASF in wild boar had been reported by the Belgian Federal Agency for the Safety of the Food Chain [115,116]. In August 2019, the last fresh ASFV-positive wild boar carcass was detected; thereafter, all positive carcasses found were skeletonized (estimated postmortem interval at least 3–6 months) [115,116]. Since March 2020, no further ASFV-positive wild boar carcasses have been found. Belgium regained its OIE status "free from ASF" in December 2020.

Morelle et al. [21] investigated the deathbed choice of ASFV-positive wild boar carcasses from Belgium, Poland and the Czech Republic. They found that ASFV-positive carcasses were more likely to be found in cool and moist habitats [21].

For Belgium, an R0 of 1.65 has been calculated for the first 11 days in the second wave (starting at day 130 after detection of first case) [111] and a spreading velocity of 0.39 km/week [117]. The disease spread faster in forests than outside [117].

4.1.6. Hungary, Romania, Slovak Republic and Serbia

Hungary, Romania, the Slovak Republic and Serbia reported cases of ASF in wild boar through the Animal Disease Notification System (ADNS) of the EU. However, no scientific publications on the epidemiology of ASF in wild boar in these countries are available.

In April 2018, Hungary notified the first case of ASF in wild boar in the region of Heves, approximately 70 km south of the border to the Slovak Republic. Shortly afterwards, cases in wild boar were reported in the northeast of the country, close to the border with Ukraine. It was suspected that the introduction into the region of Heves was due to illegally imported contaminated pork products [118], whereas the cause of the introduction into the northeast of the country was assumed to be natural movements of wild boar from the Ukraine, where cases had been reported since 2017. Until the end of 2018, a total of 138 ASF cases in wild boar were notified. Until the end of 2020, nearly 6000 cases of ASF in wild boar had been notified, which were mostly localized in the northern and eastern regions of the country.

Romania reported the first case of ASF in wild boar in May 2018, after outbreaks had occurred in domestic pigs in summer 2017 and from January 2018 onwards. The first reported case of ASF in wild boar was found in close vicinity to an outbreak of ASF in a small-holder pig farm. Until the end of 2020, nearly 1800 cases of ASF in wild boar were notified. It must be noted that the ASF situation in Romania is dominated by outbreaks in domestic pig holdings of all sizes, so that the infections in wild boar could actually represent spill-over events from the domestic pig sector [63].

Bulgaria reported its first cases of ASF in wild boar in the northeast of the country in October 2018, after an outbreak of ASF had occurred in domestic pigs in August 2018. Until the end of 2020, a total of 723 cases of ASF in wild boar were notified. The overall epidemiological situation in Bulgaria may not only resemble that in Romania, but is most probably linked to it.

In the Slovak Republic, the first ASF-cases in wild boar were reported in August 2019 in the south of the country, after outbreaks in domestic pigs had been reported close to the border with Hungary in July 2019. While in 2019, a total of 27 cases of ASF in wild boar have been reported, nearly 400 cases were reported in 2020. The affected area is close to a region in the north of Hungary, where ASF was first detected in wild boar in April 2018 and where the disease has since continued to spread.

Serbia reported its first outbreak of ASF in domestic pigs in August 2019 and the first cases of ASF in wild boar close to the border with Bulgaria in January 2020. Until the end of 2020, a total of 69 cases of ASF in wild boar were notified.

4.2. Tenacity of ASFV

In the wild boar habitat, ASFV transmission may occur not only through direct contact with infected conspecifics, but also by indirect contact between susceptible wild boar and carcasses, excretions, food waste as well as contaminated environment, e.g., soil, water, grass or crops (wild boar–habitat cycle) [103,119]. Pepin et al. [120] estimated that over 50% of the transmission events in eastern Poland were related to indirect contact with an infectious carcass.

For indirect ASFV transmission within a wild boar population, virus survival in various matrices plays a crucial role [121], since this can last several months or even years (Table 2). Data concerning the tenacity of ASFV in wild boar organs and tissues that may persist for longer time intervals in the habitat, such as bones, muscle or skin, are scarce. While Fischer et al. [122] could not detect viable virus in bone marrow after one week at room temperature, Kovalenko et al. [123] were able to recover infectious virus from bone marrow stored at 6–8 °C for more than six months. This difference could be due to the detection system, i.e., bioassay with the direct injection of pigs versus macrophage culture. Evidence suggests that muscle and skin/subcutaneous fat originating from wild boar represent long-term reservoirs for infectious ASFV, especially at low temperatures. On the other side, the stability of ASFV in urine and feces seems to be relatively low [122,124].

Virus survival in the soil underneath a carcass strongly depends on the soil type (sand vs. humus-rich soil) with survival times of less than three days to up to two weeks [125,126]. In contaminated water, infectious virus was detectable for at least 14 days [126], while a rapid decrease in infectivity was observed on dry field crops [127].

A study in Lithuania failed to find infectious virus in buried wild boar carcasses that had tested positive for ASFV before burial, but it could detect ASFV genome fragments in the surrounding soil [128].

Table 2. ASFV tenacity in different materials.

	Material	ASFV Stability	Method	Reference
Blood, organs and tissues	Defibrinated blood (RT)	140 days	In vivo assay	Montgomery 1921 [129]
	Blood (−20 °C)	6 years	In vivo assay	De Kock et al., 1940 [130]
	Preserved blood (4 °C)	18 months	In vivo assay	Plowright and Parker 1967 [131]
	Spleen suspension (−20 °C)	105 weeks	In vivo assay	Plowright and Parker 1967 [131]
	Spleen, kidney, lung (−20 °C)	112 days	Virus isolation in macrophages	Mazur-Panasiuk and Woźniakowski 2020 [126]
	Spleen, lung (4 °C)	56 days		
	Kidney (4 °C)	<28 days		
	Spleen, kidney (RT)	7 days		
	Bone marrow (6–8 °C)	>6 months	In vivo assay	Kovalenko et al., 1972 [132]
	Bone marrow, skin (−20 °C)	3 months	Virus isolation in macrophages	Fischer et al., 2020 [122]
	Bone marrow (4 °C)	1 month		
	Bone marrow, muscle (RT)	<7 days		
	Muscle (−20 °C)	>24 months		
	Muscle (4 °C)	3 months		
	Skin (4 °C)	6 months		
	Skin (RT)	3 months		
Feces and urine	Faeces (4 °C)	159 days	In vivo assay	Kovalenko et al., 1972 [132]
	Urine (4 °C)	60 days		
	Faeces, urine (4 °C)	5 days	Virus isolation in macrophages	Olesen et al., 2020 [133]
	Faeces (4 °C and 12 °C)	5 days	Virus isolation in macrophages	Davies et al., 2017 [124]
	Faeces (RT)	3 days		
	Urine (4 °C, 12 °C, RT)	5 days		
Soil, water, field crops and feed	Beach sand (RT)	14 days	Virus isolation in macrophages or cell culture	Carlson et al., 2020 [125]
	Yard soil (RT)	7 days		
	Swamp mud (RT)	3 days		
	Forest soil (RT)	0 days/none		
	Wet soil, leaf litter (4 °C & RT)	<3 days	Virus isolation in macrophages	Mazur-Panasiuk and Woźniakowski 2020 [126]
	Water (−20 °C, 4 °C, 23 °C, 37 °C)	>14 days		
	Water (−16 to −20 °C, 4–6 °C)	≥60 days	Virus isolation in macrophages	Sindryakova et al., 2016 [134]
	Water (RT)	50 days		
	Field crops (drying at RT)	<2h	Virus isolation in macrophages	Fischer et al., 2020 [127]
	Compound feed (−16 to −20 °C)	≥60 days	Virus isolation in macrophages	Sindryakova et al., 2016 [134]
	Compound feed (4-6 °C)	30 days		
	Compound feed (RT)	1 day		
	Compound feed (RT)	≥30 days	Virus isolation in macrophages, in vivo assay	Dee et al., 2018 [135]
	Soy oil cake (RT)	≥30 days		
	Compound feed (RT)	≥30 days	Virus isolation in macrophages	Stoian et al., 2019 [136]
	Soy oil cake (RT)	≥30 days		

4.3. *Transmission of ASFV among Wild Boar*

ASFV is the only known DNA arbovirus of vertebrates [2]. Its natural African vectors are soft ticks of the *Ornithodoros moubata* complex, which acquire the virus through blood-feeding on viremic hosts. They can harbor the virus for up to five years and transmit it vertically, horizontally, venereally and to susceptible suids during feeding [137]. In sub-

Saharan Africa, the virus is transmitted in a sylvatic cycle from ticks to African warthogs (*Phacochoerus africanus*), which remain asymptomatic [138]. The ticks are nidicolous and live inside warthog caves and burrows, but they can also occur in the housings of domestic pigs [139]. In northern Europe, the occurrence of *Ornithodoros* soft ticks has never been reported. However, in southern Europe, different species of *Ornithodoros* soft ticks occur (e.g., *O. erraticus*, *O. maritimus*) and especially on the Iberian Peninsula they were responsible for ASFV infections in domestic pigs [140]. In the Caucasus region, *Ornithodoros* soft tick species have been documented to occur in some affected countries, such as Armenia, Russia and Georgia [141], but their involvement in ASF epidemiology seems unlikely, the more so since wild boar do not use caves, burrows or housings.

Other arthropods were repeatedly discussed as mechanical vectors. However, all arthropods tested so far, including hard tick species indigenous to Europe [142] as well as blowfly larvae [143], could not be incriminated. This is also in agreement with a study from Estonia, where various groups of blood-feeding arthropods were collected in ASFV-affected wild boar habitats but tested negative for the presence of the ASFV genome [144]. However, mechanical transmission on a laboratory scale was reported with the stable fly (*Stomoxys calcitrans*) [145] and two studies from Eastern Europe have reported the detection of a low amount of ASFV genome on insects collected on domestic pig outbreak sites [146,147].

Several incursions of ASFV into European wild boar populations have been mainly attributed to anthropogenic factors, especially to the dissemination of contaminated meat or meat products [92]. When ASFV was first introduced into Georgia in 2007, the first clinical cases were detected near the port of Poti [55]. Although the precise source of virus could not be identified, it has been suspected that ASFV was introduced via ships presumably from east Africa carrying contaminated meat or meat products, and free-ranging pigs acquired infection scavenging on the disposed waste [148]. Human failure to comply with biosecurity requirements has also been suspected to be the initial source of introduction into previously ASFV-free wild boar populations in Poland [149], Hungary [118], the Czech Republic [107] and Belgium [150]. This way of introduction is most likely not true for Germany.

Considering the complexity of parameters, it is not surprising that the epidemiological patterns of ASF vary across different countries and regions. In Sardinia, where ASF has been present for 40 years in domestic pigs and wild boar, the persistence of the disease was related to traditional farming practices [70]. In the Russian Federation, the density of road networks, interactions with the domestic pig population and water bodies have been identified as the main risk factors for disease spread across the southern region of the country between 2007 and 2010 [151]. In the central part of the country, where a secondary endemic zone had formed since 2011, ASF outbreaks in wild boar were attributed to outbreaks in the domestic backyard sector [80].

On the other side, in current outbreaks, the wild boar density seems to be one of the most influential risk factors for the occurrence [152] and the transmission and persistence [153,154] of ASF. In Belgium, disease progression was related to the forest habitat and the wave front velocity was higher within forest areas than in non-forest areas [117]. In Latvia, the persistence of the infection was attributed to wild boar scavenging on carcasses of infected wild boar [61].

These observations have led to the hypothesis that ASF in wild boar is a habitat-borne disease [155] and to the description of the so-called wild boar–habitat cycle, which is self-sustaining and includes wild boar, their habitat and their carcasses [95,156].

To test this hypothesis, in a field study in Germany, 32 wild boar carcasses were exposed to study the behavior of wild boar towards their dead conspecifics. Wild boar were observed rooting in the decomposition islands, sniffing and poking on wild boar carcasses, and chewing on their skeletonized bones [157]. In the Czech Republic, during winter, even scavenging could be filmed [110]. Although it is not yet proven whether such types of interaction are sufficient for ASFV transmission, they support the hypothesis that transmission not only occurs through contact between susceptible and infectious animals, but also through contact between susceptible animals and contaminated material [103]. An age-structured mathematical model based on scenarios in Estonia and Spain further supported the assumption that transmission from infected carcasses to susceptible individuals is a key mechanism in producing disease outbreaks in wild boar [154].

Although it is known that ASFV has a high tenacity, details on the exact transmission routes in the wild boar–habitat cycle are still not known [158,159]. The fast localization and removal of carcasses is considered one of the most important disease control measures in affected regions [152]. However, depending on the weather, vegetation, field and light conditions, finding them can be difficult, and it is estimated that considerable numbers of infected carcasses are not found [12]. Estimating the postmortem interval of the first ASFV-positive carcasses in a previously ASF-free region can assist in estimating the time of disease introduction and the extent of the affected area. Yet, field studies have shown that the decomposition process of wild boar carcasses is highly variable and can take between a few days in summer and several months in winter [160,161].

Given the high stability of ASFV, concern has been expressed regarding scavengers that may spread infectious carcass material in the environment. However, evidence suggests that scavengers represent a minor risk factor for spreading ASFV, at least over large distances. On the contrary, they probably contribute to reducing local virus persistence by metabolizing infected carcasses [162]. However, the transfer of small amounts of ASFV-containing tissues over short distances, e.g., through birds, such as crows or birds of prey, could not be ruled out.

Other matrices that are regarded to play a potential role in the spread of ASFV among wild boar include oral-nasal excretions, blood, meat, offal, feces and urine, soil, insects, fomites, kitchen waste, as well as grass and other fresh vegetables contaminated by virus-containing matter [119].

The prediction of Bosch et al. [163] that the EU countries at the highest relative risk of ASF introduction by natural movements of wild boar were Romania, Slovakia, Finland, the Czech Republic and Germany has mostly been confirmed. However, the prediction that ASFV incursion into France was imminent due to the nation's proximity to Belgium and the movement of wild boar across the border has so far not materialized [164].

Based on data from Poland 2014–2015, it was shown that wild boar contact rates are strongly constrained socially and spatially [101] and that wild boar movements are poor predictors of ASF dynamics in space and time [99]. This has led to the conclusion that the long- and medium-distance spread of ASF (i.e., >30 km) is unlikely to occur due to natural wild boar movements [165]. A recent EFSA report predicted that the natural median spread velocity of ASF in Belgium, the Czech Republic, Estonia, Hungary, Latvia, Lithuania and Poland due to wild boar movements was between 2.9 and 11.7 km/year [63].

4.4. Modeling

As there are many unknowns in the epidemiology of ASF in wild boar, several issues are addressed by modeling to gain a better understanding of the disease dynamics.

Early during the current epidemic in eastern and south-eastern Europe, models focused on attempts to predict the spread of the disease and to assess the risk of introduction into countries that had not been affected so far. These assessments are of great interest for governments to plan preventive measures and prepare for outbreaks, but also for people directly affected by ASF, namely, pig holders and hunters. While there is sufficient data on domestic pigs in terms of abundance, transport routes and transmission routes, information on wild boar is scarce. Some of the early models on disease introduction therefore excluded wild boar. This was also done because it was believed that their expected contribution to the spread of ASF was less important compared to domestic pigs [67,166,167]. Yet, the epidemic situation as it developed since 2014 has shown that the spread of ASF in the wild boar populations in affected countries also matters. In particular, the introduction of ASF via wild boar movement, also across borders, has to be taken into account [163,168]. Several models have therefore been established that address the risk of ASF introduction into unaffected areas through trade and wild boar movements using statistical data fitting approaches [165,169].

The size of the wild boar population in a country is considered a risk factor in many models [153]. However, even in countries with small numbers of wild boar, such as Denmark, simulation models were used to assess the spread of ASF in wild boar and to minimize the risk of transmission to domestic pigs [153].

Other models focused on the presence of wild boar and habitat suitability for wild boar. Some of these model the suitability only, without including specific disease transmission risk [170]. Other models include habitat properties and suitability for wild boar in ASF transmission models. Halasa et al. (2019) applied an agent-based spatio-temporal model to the spread of ASF in wild boar in Denmark, using input parameters taken from the literature [153]. Another model combined the habitat suitability with an agent-based, spatially explicit simulation model that includes multi-source citizen science data on the presence of wild boar [171]. Croft et al. (2020) used a spatial individual-based model with data of a real landscape area in Britain for an isolated wild boar population [172].

Models have shown that the presence of suitable habitats for wild boar is a better predictive factor for the risk of ASF introduction than wild boar density [163,173,174]. Thus, models using environmental data proved to be a useful tool to estimate the risk of ASF introduction into naïve wild boar populations [154].

Several models [120,153,175] found that the presence of carcasses of infected wild boar could explain the transmission observed in real ASF epidemics. Pepin et al. [120] addressed the probability of transmitting ASFV from carcasses to live animals. Using data from eastern Poland and a spatially explicit mechanistic epidemiological model, they estimated that 53–66% of the transmission events may have been due to the presence of infected wild boar carcasses. They concluded that this carcass-based transmission is necessary to maintain the persistence of ASF in the wild boar population [120]. These data are corroborated by the long periods, over which ASFV can remain infectious in carcasses [110,128].

Another output of this model [120] was that carcass-based transmission becomes more important the lower the wild boar density is, as direct contacts are expected to become less frequent. The greater importance of transmission through carcasses of ASFV-infected wild boar as compared to direct transmission between living wild boar is also described by Lange and Thulke [175], who used a spatio-temporally explicit individual-based model [175,176]. This result can be explained by the long time wild boar carcasses remain in the environment, as compared to the relatively short remaining live-time of ASFV-infected animals [27]. Thus, the model showed the best fit to the observed spatio-temporal spreading pattern for the high accessibility of carcasses and a marginal chance of contact frequency of live wild boar to the carcasses [175]. This model was already used in 2015, after ASF occurred for the first time in the Baltic states and Poland, to evaluate different management options for eliminating ASF from the wild boar population [12]. A more recent model also suggested that environmental transmission, e.g., through carcasses of infected wild boar, is the main mechanism for outbreaks in the current epidemic in Europe [154]. These authors also propose that the role of wild boar in an ASF epidemic might be less significant in Spain, where temperatures are higher and wild boar carcasses will decompose faster, as compared to Estonia [154].

ASF in wild boar has proven to be extremely difficult to control [7]. Proposed measures, including massive depopulation in affected areas and the removal of wild boar carcasses, were considered not feasible or, as far as mass depopulation is concerned, unethical. On the other hand, measures that could have a long-term effect on wild boar, and thus on eliminating ASF, may take several years to become effective and have to be applied to larger areas. However, even then, the conclusion of different models is that only a combination of measures, including mass depopulation and carcass removal, are likely to be the most effective and feasible solution [12].

The comparison of control strategies was also the subject of a study conducted by Barongo et al. [177], who applied a model to free-ranging pig populations in resource-poor situations of Africa. They also used the model to determine optimal response times for control measures.

Once control measures are effective, the aim of eliminating the disease from affected areas is often pursued. It is generally accepted that passive surveillance (the testing of all wild boar found dead or shot sick) is more suitable for early detection of the disease than active surveillance (the testing of hunted wild boar) [3,4,7,14,98,102,178]. However, this has been controversially discussed for later phases of the disease events. Therefore, Gervasi et al. [179] used a simulation model to evaluate the efficiency of active and passive surveillance for ASF in wild boar. Only in situations characterized by a low prevalence, a low wild boar population density and a high hunting rate was active surveillance superior to passive surveillance [179].

5. Diagnosis of ASF in Wild Boar

Controlling ASF in wild boar is highly dependent on early warning and thus on a rapid and reliable diagnosis [85]. Due to the international notification requirement, laboratory diagnosis is regulated in recommendations and legal requirements. Methods and protocols can be found in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [180] or, for the European Union, on the website of the European Union Reference Laboratory for ASF (https://asf-referencelab.info/asf/en/procedures-diagnosis/sops, accessed 24 August 2021).

In general, reliable tools for the direct (pathogen) and indirect (antibody) diagnosis of ASF exist that work with appropriate samples from both domestic pigs and wild boar [181]. There is no difference in test performance or the suitability of matrices when testing high-quality samples from either domestic pigs or wild boar as depicted in Figure 3 [182].

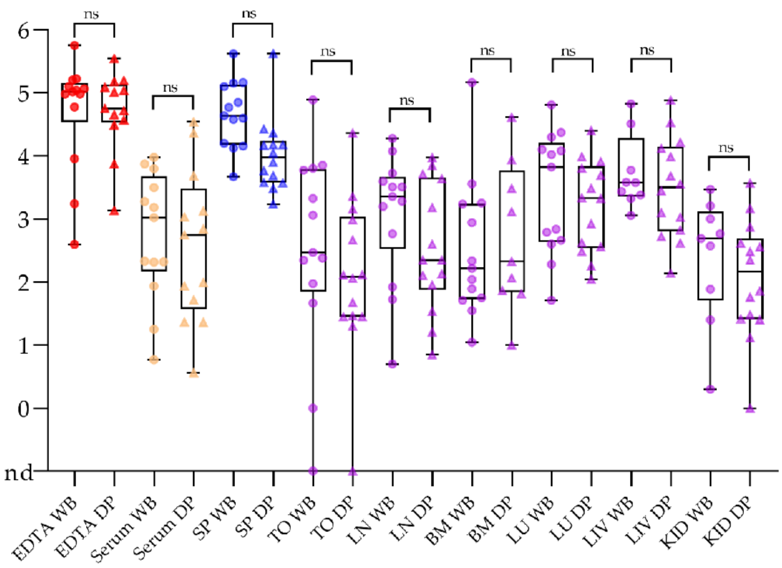

Figure 3. Comparison of sample matrices taken from wild boar (WB; dots) and domestic pigs (DP; triangles). The qPCR results are depicted as log10 genome copy numbers per run. Abbreviations: nd = not detected; SP = spleen, TO = tonsil; LN = lymph node; BM = bone marrow; LU = lung; LIV = liver; KID = kidney; ns = not significant in pairwise comparison. This figure was adapted from Pikalo et al. (2021) doi: 10.3390/pathogens10020177.

5.1. Sample Matrices

Direct detection methods have priority to detect the disease in free areas at risk. For early detection, especially in the pre-clinical phase, EDTA blood and spleen samples are best suited using all types of real-time PCR methods [182,183]. In this phase, serum samples may yield false negative results, especially if pooling is applied [182]. Tonsil, lymph node, bone marrow, lung, liver and kidney samples are also suitable and mentioned in the respective manuals.

In the wild boar context, it has to be kept in mind that the animals to be sampled are obviously sick or dead, so it can be assumed that a significant viral load is present in several organs and tissues [103]. In combination with the high stability of ASFV in wild boar carcasses [122,128], sampling can be facilitated by the use of pragmatic, alternative matrices that minimize the need to open and touch a rotten carcass. Over the last years, dry blood swabs have been validated for passive surveillance under experimental and limited field settings [182–186]. It could be demonstrated that these samples are suitable for the PCR detection of the ASF viral genome and, with a certain limitation, for the detection of antibodies. Recently, state-of-the art flocked swabs and inactivating transport buffers have been tested along the same lines with very promising results, especially for the transport buffer system [182]. Different bloody swabs have been put to practice in the Germany outbreak scenario since September 2020 and performed well [6]. Without the inactivating buffer system, virus isolation is possible over a period of roughly three days (unpublished results). However, for a full characterization of the disease situation, including the generation of virus strains for biological testing and sequencing, additional samples are helpful and should be encouraged. Apart from the above-described swabs, dried filter papers and FTA cards [187–189], fecal samples [142], oral, nasal and rectal swabs [190], meat juice [191], and different rope-based options [192,193] have been assessed as alternative, partly non-invasive matrices. Further matrices such as ear punches have been discussed and showed general suitability for diseased wild boar and domestic pigs [182]. If bloody material is no longer available, bone marrow from femur, humerus, jawbones, ribs, or sternum should be sent in for testing. Interestingly, this matrix performed much better if taken from skeletonized carcasses. In this case, it was superior for the use in shot-gun next-generation sequencing approaches (unpublished results and [6]).

Following the introduction of ASF into a new region, antibody detection becomes a valuable diagnostic tool to monitor disease evolution and potential changes in ASFV virulence. For the detection of antibodies, serum and plasma samples are the first choice mentioned in the respective manuals. However, filter papers [188], the above-mentioned swabs [184], meat juice, fecal material [194], and oropharyngeal fluids [195] could provide additional information if the collection of serum or plasma is not possible.

In general, the choice of sample matrices should be embedded in the overall approach.

5.2. Detection of ASF Virus, ASFV Antigen and Genome

Polymerase chain reaction protocols represent the first line of ASFV detection, and an increasing number of published protocols and fully validated test kits are available [196–208]. Regarding the later, commercial kits with reasonable pricing, the integration of internal control systems and the lack of need for extra consumables are key. As manufacturers are distributed around the globe, performance characteristics are not easy to compare. Within the European Union, some countries have an official licensing process and released products and batches underwent batch release. In Germany, for example, an updated list of licensed kits is included into the German Official collection of methods for notifiable diseases (https://www.fli.de/en/publications/amtliche-methodensammlung/, accessed 24 August 2021). All these tests have been tested at the German national reference laboratory (NRL) with a defined set of experimental samples representing different genotypes (mainly I and II), host species, matrices, and infection status [209]. The licensed kits are (as of March 2021): INgene q PPA (Ingenasa, Madrid, Spain), virotype ASFV and virotype ASFV 2.0 (Indical Bioscience, Leipzig, Germany), ID Gene ASF Duplex (IDvet, Grabels, France), RealPCR

ASFV (IDEXX, Westbrook, ME, USA), SwineFever combi (gerbion, Kornwestheim, Germany), virella ASFV seqc (gerbion, Kornwestheim, Germany), ViroReal Kit ASF Virus (Ingenetix, Vienna, Austria), Kylt ASF Real-Time PCR (Anicon, Emstek, Germany), VetMAX African Swine Fever Virus Detection Kit (Thermo Fisher Scientific, Waltham, MA, USA), and the ADIAVET ASFV FAST TIME Kit (BioX, Rochefort, Belgium). Some of these commercial PCR tests and three routinely used automated extraction methods have been compared at the German NRL in more detail (Schlottau, unpublished). Nucleic acids were extracted from wild boar and domestic pig samples using the NucleoMag® VET (Macherey–Nagel, Düren, Germany), MagAttract Virus M48 (Qiagen, Hilden, Germany), and MagMAX™ CORE (Thermo Fisher Scientific, workflow C) kits on the KingFisher extraction platform (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. In general, all kits were suitable and yielded reliable results in downstream applications. Recently, data were published on the comparison of seven commercially available PCR kits and three polymerase reaction mixes [210]. Here, the following kits were included: virotype ASFV 2.0 PCR kit, (Indical Bioscience, Leipzig, Germany), Adiavet ASFV Fast Time (Adiagen, Rochefort, Belgium), Bio-T kit ASFV (Biosellal, Dardilly, France), VetMax ASFV Detection kit (Thermo Fisher Scientific, Waltham, MA, USA), RealPCR ASFV DNA Test, (IDEXX, Westbrook, ME, USA), VetAlert ASF PCR Test Kit (Tetracore, Rockeville, MD, USA), and the ID Gene™ African Swine Fever Duplex (IDvet, Grabels, France). In brief, the diagnostic sensitivity and specificity was tested on 300 well-characterized wild boar samples collected in Belgium during the 2018–2019 outbreak. This study confirmed that all commercial kits and two out of three Taq polymerases (AgPath-ID™ One-Step RT-PCR Reagents, Applied Biosystems, Waltham, MA, USA, and TaqPathTM 1-Step Multiplex Master Mix, Thermo Fisher Scientific, Waltham, MA, USA) are suitable for ASFV detection in diagnostic laboratories. This is in line with the experience described above and confirms the suitability of commercial kits for a rapid and user-friendly ASFV diagnosis. Apart from traditional gel-based and real-time PCR assays, different alternatives including isothermal amplification methods have been designed and evaluated [205,211,212]. So far, they did not replace TaqMan-based real-time PCR protocols.

Virus isolation is the gold-standard confirmatory test and required to obtain isolates for further characterization. Due to biosafety considerations and the requirement for susceptible cultures of primary cells, this technique is usually only applied in reference laboratories according to standard protocols [213]. Routinely, hemadsorption [214] is used as readout (for hemadsorbing strains).

Under resource-limited settings, the use of antigen ELISAs could be an option. They are commercially available, but lack sensitivity [181,215]. The same is true for antigen lateral flow assays, which showed some promising results in experimental settings [182,216], but would failed to detect most confirmed ASF cases from Germany (P. Deutschmann, personal communication).

Screening for ASFV-specific antibodies is usually done with ELISA-based methods. Again, several in-house and commercial tests are available. Most widely used within the European Union are probably the INGEZIM PPA COMPAC (Ingenasa), the ID Screen® African Swine Fever Indirect (IDvet), and the ID Screen African Swine Fever Competition test (IDvet). These test systems use different antigens and they may therefore be used in parallel. Internationally, there are various test systems, whose performance is not easy to assess. It has to be noted that poor-quality serum samples (as they are often obtained from wild boar, particularly animals that were found dead) may affect test specificity. For this reason, confirmatory testing is recommended [181]. This can be achieved using indirect immunoperoxidase tests or immunoblotting.

6. ASF Control in Wild Boar

ASF is explicitly listed in Article 5 of the Animal Health Law of the EU (Regulation 2016/429) and affected Member States of the EU have to implement the respective prevention and control measures. The measures set a minimum standard, and national authorities can implement additional and stricter measures if necessary.

In several studies, the presence of ASF-infected wild boar in close vicinity to domestic pig holdings is described as a main risk factor for ASF outbreaks [55,61,217,218]. Thus, successful ASF control in wild boar is crucial to protect the domestic pig sector from spill-over incidents. In contrast to ASF control in domestic pigs, which is achieved by depopulation of the farm and further measures that include cleansing and disinfection of the affected premises, movement restrictions, establishing restricted zones, etc., the control of ASF in wild boar is challenging [155,219]. There is no standard control strategy that can be applied everywhere in the same way. Moreover, there is no vaccine available currently. Suitable measures must be selected from a variety of options and need to be adapted to the specific epidemiological situation, as well as to environmental and social factors [220].

The prevention of ASF introduction into a non-infected area is the preferred option. Two different scenarios have been described for the introduction of ASFV into a naïve wild boar population [119]: (i) ASF-infected wild boar may introduce the virus in a neighboring region or country by cross-border migration. This has probably been the route of introduction into several European countries [4,6,61,91]. (ii) Due to the high tenacity of ASFV in raw meat products and the environment [122,126,129–131,221], the virus can also be transmitted indirectly by human activity and thus 'jump' over large distances, as observed in the Czech Republic and Belgium [106,107,222]. Since the location, where ASF emerges in the latter scenario, is hardly predictable, only rather general preventive measures can be applied. These usually aim at raising awareness, informing people about the risk of introducing ASFV with pork, pork products, hunting trophies, vehicles, clothes, shoes, equipment, etc., about cleansing and disinfection measures and the safe disposal of waste that may contain ASFV [119,223].

The role of the wild boar density in disease transmission and spread is highly disputed. However, there seems to be consensus among experts that a reduction in wild boar density decreases the risk of ASFV introduction and spread [224,225]. Efforts to reduce the population density usually imply increased hunting efforts, targeted hunting to decrease the number of reproductive females, and the trapping of wild boar followed by culling. Moreover, the use of supporting tools for hunting such as silencers or night vision is discussed and applied in some affected areas [225]. Population reduction through measures such as fertility control, poisoning or involving snipers are not only difficult to implement, but may also be legally and ethically contentious [224,225]. Building fences to protect an ASF-free region against the immigration of ASF-infected wild boar has been attempted. However, wild boar may overcome fences or find gaps or other ways to circumvent them. Limited acceptance by local people is expected [225], which may lead to constant damage or even robbery of the fences and continued efforts to repair, maintain or replace them.

If the efforts to prevent ASFV introduction fail, the choice of control measures mainly depends on the route of introduction and the stage of the epidemic. In any case, early detection is vital. Due to the high case/fatality ratio of ASF, active carcass searches, i.e., enhanced passive surveillance, constitute one of the measures that need to be implemented as soon as possible [4,7,119,224]. Carcasses of wild boar that died of ASF contain large amounts of infectious virus and therefore represent a source of direct and indirect transmission [95,157,162,226]. Removing carcasses of potentially infected wild boar from the environment may thus help to minimize the risk of disease spread and maintenance.

After a single introduction event, successful elimination of ASF from wild boar populations has been demonstrated in the Czech Republic and Belgium [20,227]. Control measures were implemented in different zones around the affected area. Accordingly, fences or systems of layered fences (in Belgium and France, currently also applied in Germany) were built to avoid disease spread. Furthermore, hunting and feeding was banned within the inner zones, at least in the early phase of the epidemic, whereas in the outer areas hunting was intensified [119]. These control measures were implemented at different intensities for at least 10 or 14 months, respectively, before these countries submitted their self-declaration of freedom of ASF to the OIE. Fencing may also be applied to prevent the migration of potentially infected wild boar across national borders [94].

In regions where ASF was introduced by migrating wild boar, disease control is much more challenging. If there is constant infection pressure, e.g., along a border, control measures have to be implemented in larger regions or in several areas simultaneously and over a longer period. While this may be feasible at the beginning of a new epidemic, the longer the disease persists, the more likely it is that involved stakeholders reach their limits. Their acceptance of the control measures and their willingness to support them may decrease [228–230]. ASF control is more difficult in such an endemic disease situation. Most strategies applied in these scenarios refer to hunting, including the ban of driven hunts or promoting targeted hunting, but also passive surveillance and the removal of carcasses from the environment are conducted. However, the effectiveness of these measures has not yet been unambiguously demonstrated [63,96]. In most of the countries that have been affected by ASF in wild boar for several years, a significant reduction in the population density has been observed [7,13,100]. However, the disease is still present in these countries, so they also suffer from the corresponding economic restrictions, even if the ASFV prevalence decreased and no or hardly any cases in domestic pigs occur [3,5,13,16]. In Latvia, for example, several control measures had been implemented (e.g., incentives, targeted hunting, usage of supporting tools), but none of them showed an immediate effect on ASF prevalence [7].

Although most experts agree on the importance of reducing the wild boar population, to minimize disease spread, the implementation of the necessary steps to achieve this goal currently appears to be unsuccessful [219]. Besides the need to have a variety of control options available and to adapt them to the local situation, communication with the involved stakeholders is crucial, not only during the epidemic, but already before, to ensure a good cooperation in case of a disease emergence. Several participatory studies showed that control measures, performing well in models or in theory, were useless without the support of relevant stakeholders [228,229,231].

Generally, in endemic situations, the effort to detect and remove wild boar carcasses has to be maintained. Moreover, the consequent and ongoing reduction in the wild boar population should be aimed for. Furthermore, the awareness of potentially affected stakeholders, such as hunters or farmers, should be kept up. To avoid virus introduction into domestic pig holdings, high-level biosecurity measures should become a matter of course and, ideally, controls of these should be ensured by regular inspections through the competent authority [232].

While the examples of the Czech Republic and Belgium show that it is in principle possible to eliminate ASF from wild boar populations, the control of ASF in wild boar remains a major challenge and there is no one-size-fits-all solution.

7. Conclusions

After its introduction into Georgia in 2007, ASFV genotype II has spread within Europe and Asia. In contrast to previous introductions observed in the 20th century, wild boar now play an important epidemiological role. The disease occurs in self-sustaining infection cycles in wild boar populations without the need for involvement of the tick vector or domestic pigs, while spill-over infections into domestic pigs occur. Control measures include the removal of wild boar carcasses from the environment, reduction in wild boar density and building fences to restrict or avoid wild boar movements. Yet, only two countries succeeded in eliminating ASF from their wild boar population. The disease continues to spread elsewhere.

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3.2.2 ASF in the Baltic States

The “new” ASF epidemic, which started in Georgia in 2007 and subsequently spread through Eastern Europe and Russia, reached the EU in 2014. Lithuania and Poland were first affected, followed by Latvia and Estonia. For all four countries, it was hypothesized that the disease had been introduced through neighboring countries, in which the virus was already circulating (Oļševskis et al., 2016, Smietanka et al., 2016, Nurmoja et al., 2017b, Pautienius et al., 2018). It was postulated that the virus was introduced either through migrating infected wild boar or through contaminated food, which was privately imported. According to disease introduction from the East, in all three Baltic States, the epidemic started in the eastern parts of the country. Interestingly, in all three countries, the epidemic courses resembled one another. Despite intensive control measures and efforts to contain the epidemic, the disease appeared to spread inexorably across the countries. Within 2-3 years, in all three countries large parts of the wild boar population where affected, which subsequently led to a significant reduction of the wild boar populations. Consequently, the ASF spread decelerated and the epidemiological patterns changed. The number of detected wild boar carcasses decreased, whereas the number of animals increased, which were shot apparently healthy and tested only positive for ASFV-specific antibodies.

Due to EU regulations, all hunted wild boar and all wild boar found dead should be tested for ASFV in ASF-affected regions. Accordingly, a large amount of surveillance data has emerged in recent years, which was entered into the CSF / ASF wild boar surveillance database of the EU Reference Laboratory (https://public.surv-wildboar.eu/Default.aspx). The good working relationship with colleagues from the Baltic States facilitated extensive data analyses and thus, it was possible to evaluate the epidemiological course of ASF in all three Baltic States. The aim of these studies was to improve our understanding of the epidemiology of ASF, to identify patterns and thus, to improve and adapt disease control.

In Estonia, the virus was first detected in the south of the country, close to the border to Latvia, where the disease was already present for several months. A few weeks after the first detection in the south, another epidemic cluster appeared in the northwest of Estonia. The epidemiological patterns within these two clusters seemed to differ and the hypotheses arose that the ASF event in the north actually started prior to the one in the south. Thus, surveillance data of these two areas were analyzed and compared. Prevalence estimates were calculated. A hierarchical Bayesian space–time model was used to evaluate the temporal and spatial effects within the two defined study areas. Furthermore, the aim of the study was to identify risk factors for a higher probability to detect an ASF-positive wild boar and to investigate differences within the two geographical areas of interest.

- Contribution **(Publication 4)**: NURMOJA, I., SCHULZ, K., STAUBACH, C., SAUTER-LOUIS, C., DEPNER, K., CONRATHS, F. J. & VILTROP, A. 2017. Development of

African swine fever epidemic among wild boar in Estonia - two different areas in the epidemiological focus. Scientific Reports, 7, 12562. https://doi.org/10.1038/s41598-017-12952-w

Further analyses of Estonian ASF wild boar surveillance data including data from the whole country and from a longer period were performed. The country was divided into an eastern part and a western part. Thus, the epidemiological course in the area where ASF started and in the area where first ASF cases emerged 2 years later could be compared over time. To account for the different laboratory test results and their epidemiological meaning, analyses where done for samples tested positive for ASFV and samples tested only positive for ASFV-specific antibodies. Investigating the effect of ASF control measures and the disease itself on the numbers of wild boar, wild boar population densities were statistically compared over time, including hunting seasons prior to ASF emergence in Estonia.

- Contribution **(Publication 5)**: SCHULZ, K., STAUBACH, C., BLOME, S., VILTROP, A., NURMOJA, I., CONRATHS, F. J. & SAUTER-LOUIS, C. 2019. Analysis of Estonian surveillance in wild boar suggests a decline in the incidence of African swine fever. Scientific Reports, 9, 8490. https://doi.org/10.1038/s41598-019-44890-0

In Latvia, the virus emerged in June 2014, close to the border to Belarus (Oļševskis et al., 2016). Also in this country, the disease slowly spread towards the West over time. Through the years, the number of serologically positive animals increased. Thus, we aimed to investigate the epidemiological course of ASF to understand the disease patterns. Consequently, a similar study was conducted in Latvia. ASF wild boar surveillance data was analyzed for five statistical regions of Latvia and again, investigating the course of disease in more detail, also for the eastern and the western part of the country. Surveillance data was analyzed descriptively, including different age classes. Estimating prevalences per hunting season, the temporal pattern of the disease was evaluated. These comprehensive data analyses were meant to help improving our understanding of the epidemiology of ASF. The results can be used by other countries to learn about the disease, to interpret laboratory test results in an epidemiological context and to increase their preparedness.

- Contribution **(Publication 6)**: OĻŠEVSKIS, E., SCHULZ, K., STAUBACH, C., SERZANTS, M., LAMBERGA, K., PULE, D., OZOLINS, J., CONRATHS, F. J. & SAUTER-LOUIS, C. 2020. African swine fever in Latvian wild boar-A step closer to elimination. Transboundary and Emerging Diseases, 67, 2615-2629. https://doi.org/10.1111/tbed.13611

Lithuania was the first Baltic country that was affected by the epidemic. First ASF cases emerged in Lithuanian wild boar in January 2014 and, similarly to Latvia, were probably linked to the immigration of infected wild boar from Belarus (Pautienius et al., 2018). Similar to the other two Baltic countries, most cases were detected in wild boar, but several domestic pig outbreaks were also recorded (Mačiulskis et al., 2020). To further increase the knowledge about the epidemiology of ASF and its temporal course in wild boar populations, two epidemiological studies were conducted. In the first one, data from 2018 were analyzed as a continuation of the study from Pautienius et al. (2018).

- Contribution **(Publication 7)**: PAUTIENIUS, A., SCHULZ, K., STAUBACH, C., GRIGAS, J., ZAGRABSKAITE, R., BUITKUVIENE, J., STANKEVICIUS, R., STREIMIKYTE, Z., OBERAUSKAS, V., ZIENIUS, D., SALOMSKAS, A., SAUTER-LOUIS, C. & STANKEVICIUS, A. 2020. African swine fever in the Lithuanian wild boar population in 2018: a snapshot. Virology Journal, 17, 148. https://doi.org/10.1186/s12985-020-01422-x

For a second, more comprehensive, study, ASF wild boar surveillance data from 2016-2021 were available. The data were analyzed descriptively, prevalence estimates were calculated and a Bayesian space–time model was applied. Hunting bag data was used to compare population densities over time.

- Contribution **(Publication 8)**: SCHULZ, K., MASIULIS, M., STAUBACH, C., MALAKAUSKAS, A., PRIDOTKAS, G., CONRATHS, F. J. & SAUTER-LOUIS, C. 2021. African Swine Fever and Its Epidemiological Course in Lithuanian Wild Boar. Viruses-Basel, 13, 1276. https://doi.org/10.3390/v13071276

3.2.2.1 Development of African swine fever epidemic among wild boar in Estonia - two different areas in the epidemiological focus

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NURMOJA, I., **SCHULZ, K.**, STAUBACH, C., SAUTER-LOUIS, C., DEPNER, K., CONRATHS, F. J. & VILTROP, A. 2017. Development of African swine fever epidemic among wild boar in Estonia - two different areas in the epidemiological focus. Scientific Reports, 7, 12562. https://doi.org/10.1038/s41598-017-12952-w

Highlights

- The probability to detect a wild boar tested positive for ASFV is higher in young wild boar
- The probability to detect a wild boar tested positive for ASFV is higher in animals found dead
- A high population density supports virus occurrence and spread
- First ASF cases in Estonia probably emerged in the northeast of the country

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Development of African swine fever epidemic among wild boar in Estonia - two different areas in the epidemiological focus

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African swine fever (ASF) in wild boar emerged in Estonia for the first time in September 2014. The first affected region was located in the South of Estonia close to the border with Latvia. It was considered to be epidemiologically connected to the outbreaks in the North of Latvia. About two weeks later, cases were detected in the North of Estonia, close to the Russian border. In the present study, we aimed to investigate the epidemiological courses of the disease in the South and in the North of Estonia. Potential associations between risk factors and the laboratory test results for ASF were examined. A hierarchical Bayesian space–time model was used to analyze the temporal trend of the ASF seroprevalence in the two areas. Young wild boar were statistically significant more likely to be ASF-positive by both, serology and virus detection, than older animals. A statistically significant difference between the two areas in the temporal course of the seroprevalence was found. While the seroprevalence clearly increased in the South, it remained relatively constant in the North. These findings led to the hypothesis that ASF might have been introduced earlier into the North of Estonia then into the South of the country.

African swine fever (ASF) is a notifiable viral pig disease whose emergence usually entails huge economic consequences for the pig industry[1]. In Europe, the disease affects both domestic pigs and European wild boar (*Sus scrofa*). Therefore, an infected wild boar population holds the constant risk to infect domestic pigs and vice versa[2].

Apart from Sardinia, where ASF has been endemic since 1978, Europe was officially free from ASF since 1995[1]. However, ASF was newly introduced into Georgia in 2007. From there the virus spread to neighboring countries such as Armenia, Azerbaijan, the Russian Federation, Ukraine and Belarus.

The spread of the ASF virus p72 genotype II in eastern Europe has involved both domestic pigs and wild boar[3]. In 2011, the virus entered the central part of the Russian Federation, where it is now endemic[3,4]. In addition, several outbreaks in domestic pig were confirmed in Northwest Russia in the region of St. Petersburg between 2009 and 2012, about 160 km away from the Estonian border[4].

In January 2014, the first ASF wild boar case was reported from Lithuania[5]. Subsequently, in the course of the year, Poland as well as Latvia confirmed ASF cases in wild boar[6,7]. Finally, Estonia officially reported the first ASF case in wild boar in September 2014.

The first ASF-positive dead wild boar in Estonia was reported on 2nd September 2014 in Valga county, six km from the Latvian border[8] (Fig. 1). One week later, the virus was detected in wild boar in Viljandi county, which is also bordering Latvia. The outbreaks in the South were most likely epidemiologically connected with the epidemic in the North of Latvia, which had started few weeks before[7]. On 14th September 2014, an ASF-positive wild boar was found in Ida-Viru county, located in the Northeast of Estonia next to the border with the Russian Federation and more than 200 km away from the affected areas in the South[9]. The third county bordering Latvia, Võru county, was found infected by the end of October 2014.

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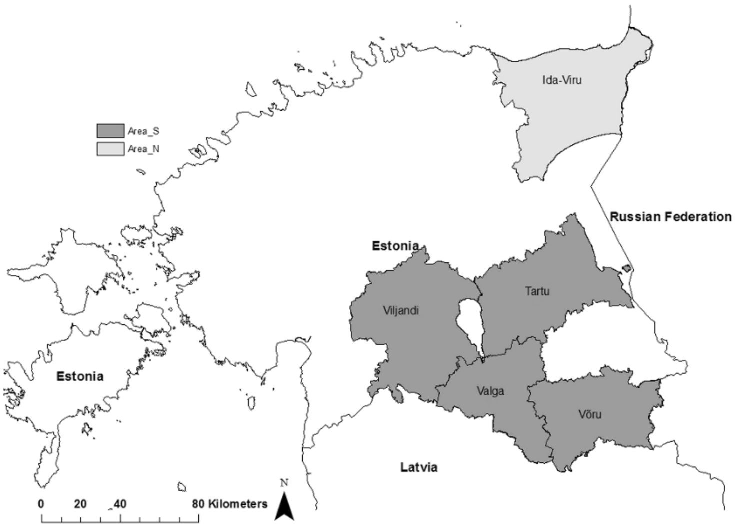

Figure 1. The study areas and the bordering countries in the South and East. Highlighted areas illustrate the four included counties in the South (area S) and the one in the Northeast of Estonia (area N). Map was generated by using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/).

Area	Number of samples	Number of negative samples	Number of positive samples	Averaged prevalence within the study period (%)	95% CI
N	1,174	1,152	22	2.0	1.1–3.0
N1	353	351	2	0.8	0.2–3.5
N2	821	801	20	2.4	1.5–3.7
S	5,841	5,039	802	13.7	12.8–14.6
S1	2,670	2,301	369	13.8	12.5–15.2
S2	3,171	2,738	433	13.7	12.5–14.9

Table 1. ASFV genome positive and -negative wild boar samples, averaged prevalences and 95% confidence intervals (calculated using R) for the study areas (N = study area North, N1 = first 12 months of the study period, N2 = second 13 months of the study period; S = study area South, S1 = first 12 months of the study period, S2 = second 13 months of the study period).

By the end of 2014, 73 infected wild boar had been detected in Estonia; 69 of them in the southern region and four in the Northeast. In the first half of 2015, the disease largely remained in the infected areas. However, in the mid of 2015, it spread to previously uninfected areas. A total of 1,530 ASF cases in wild boar have been officially reported in Estonia until the end of September 2016[10].

There was evidence suggesting that the course of the epidemic differed between the areas in the South and in the Northeast of Estonia. In the Northeast, the proportion of hunted animals that were virologically negative but seropositive was relatively high and almost no findings of dead wild boar were reported, while in the South a high mortality among wild boar was observed. In addition, in the South hunted animals found infected with ASF were mainly virologically positive, but seronegative, while in the North also seropositive wild boar were found[11] (Table 1). Moreover, the spread of the disease in the South appeared to be more rapid as compared to the North, where the infection seemed to remain within one area. We found no obvious factors that may have caused differences in the reporting of fallen or hunted wild boar in these two regions. Hunting practices are similar and the ASF surveillance system as well as the reporting regulations are the same everywhere in Estonia.

In the present study, we aimed to analyze available data and therefore improve our understanding of the epidemiology of ASF and the course of the epidemic in Estonia. We tested potential associations between risk factors such as age, population density and carcass category (i.e. wild boar found dead or hunted) and positive virological or serological laboratory test results as the outcome variable. However, our main aim was to evaluate the apparent epidemiological differences between the infected areas in the North and the South of Estonia. To ensure the comparability of these two areas, we tested the hypothesis that there was a difference in the age of wild boar or in the carcass distribution between the different study areas.

Material and Methods

Study area. Estonia is administratively divided into 15 counties (first level administrative division). The local governance is on municipality level (second level administrative division). Each county comprises of several municipalities (cities or towns and rural municipalities). During the study period 183 rural municipalities existed in Estonia.

We defined two different study areas in Estonia based on county level. The southern region (area S) comprised four counties (50 municipalities), namely Valga (2,044 km^2), Viljandi (3,422 km^2), Võru (2,305 km^2) and Tartu (2,993 km^2), of which the latter is the only one not bordering Latvia. The infected region in the Northeast (area N) bordering the Russian Federation included only one county (21 municipalities), Ida-Viru (3,364 km^2) (Fig. 1).

Sampling and sample analysis. Wild boar were sampled based on the Estonian animal disease control program and included both wild boar found dead and hunted animals. Wild boar found dead, including animals killed in road traffic accidents or shot sick, were sampled in the whole country irrespectively of the ASF status of the area (passive surveillance). However, the sampling scheme of hunted wild boar (active surveillance) changed several times depending on the ASF status of the affected area. These changes were due to updates of European Commission Implementing Decision 2014/709/EU. In practice, in areas where wild boar were affected by ASF (Decision 2014/709/EU, Part II), all hunted wild boar were sampled, whereas in areas at risk of getting infected, but without previous detection of ASF cases (Decision 2014/709/EU, Part I), approx. 2% of hunted wild boar were tested.

From hunted wild boar, blood samples were collected for ASFV genome and antibody detection by hunters immediately after hunting, whereas organ (kidney, spleen, lymph node) or bone marrow samples from animals found dead were collected for virus genome analysis by official veterinarians shortly after detection of the animals had been reported (within 24 hours). Although the quality of samples varied among all sample types, this had no significant impact on the performance of the PCR test. The test result was only reported as valid if correct test performance was confirmed, also by using an appropriate internal control. A total of 30 bone marrow and 57 serum samples were found unfit for PCR testing and therefore excluded.

Real-time PCR (used for virus genome detection), enzyme-linked immunosorbent assay (ELISA) and the indirect immunoperoxydase technique (IPT) (both used for antibody detection) were conducted at the Estonian Veterinary and Food Laboratory the National Reference Laboratory for ASF in Estonia. Real-time PCR was performed according to the protocol published by Tignon, *et al.*[12]. Although specific values for the diagnostic sensitivity and specificity of this protocol have not been published, a high sensitivity and a specificity of almost 100% can be assumed after extensive validation of the method[12,13]. A commercially available blocking ELISA (Ingezim PPA COMPAC, Ingenasa, Madrid, Spain) was used according to the manufacturer's instructions (sensitivity: 98%, specificity: 100%). In the case of an inconclusive ELISA result, the sample was re-tested in the IPT for confirmation. If samples were tested by both ELISA and IPT, the outcome of the IPT was considered as the final result.

For IPT, a protocol provided by the European Union Reference Laboratory for ASF (CISA-INIA, Valdeolmos, Spain) with a sensitivity of 98.2% and specificity from 99.0% to 100% (when used as an individual test), was used. If samples were sent to the European Union Reference Laboratory, this test was also used for the detection of antibodies in organ and bone marrow samples[14,15].

Data. For the analyses, surveillance data from 1st September 2014 until the 30th September 2016 (25 months) were used. In addition, the study period was divided into two parts for the prevalence analyses in each study area (N and S). The virus prevalences and seroprevalences were not only analyzed for entire duration of the study period (25 months), but also separately for the first 12 and the last 13 months. Surveillance data of 2015 and 2016 were extracted from the CSF / ASF wild boar surveillance database of the EU Reference Laboratory (https://public.surv-wildboar.eu/Default.aspx). The data for 2014 were obtained from the database of the Estonian Veterinary and Food Laboratory. It comprised 1,957 data records in total. In the final set, data from counties outside the study area were removed. The data set finally used included information on the place (county and municipality level), year and month of sampling, age (assessed by the hunters) and the origin of wild boar (carcass: hunted or found dead), the virological and serological test results and the population density.

We used wild boar population data provided by the Estonian Environment Agency (Nature department). The data were collected using different methods, such as hunting bag statistics, snow-track counts and hunter estimation[16–18]. Population data were available of the hunting years 2013, 2014 and 2015. The numbers of wild boar were recorded at the end of the according hunting year in the pre-reproductive time (observation dates: march 2014, 2015 and 2016). Data were available as integer numbers per hunting district. A hunting district is defined as an area for big game hunt with a size of at least 5,000 hectares according to the Estonian Hunting Act[19]. To use the data for analyses, we aggregated them at the municipality level. Utilizing the software ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/), the wild boar density per km^2 was calculated based on the estimated number of wild boar per hunting ground. The area of hunting grounds that overlapped with the territories of at least two municipalities, were proportionally attributed to the territory of each municipality. By means of the wild boar density per km^2 and the adapted hunting grounds, the total number of wild boar per municipality was calculated. Finally, wild boar densities were determined for each municipality.

Statistical analyses. All statistical analyses were performed using the software package R (http://www.r-project.org)[20]. We estimated stratified period prevalences over time and space and calculated confidence intervals and odds ratios according to Clopper and Pearson[21]. A p-value of ≤ 0.05 was considered statistically significant.

Area	Number of samples	Number of negative samples	Number of positive samples	Averaged prevalence within the study period (%)	95% CI (%)
N	1,142	1,098	44	3.9	2.8–5.1
N1	338	313	25	7.4	4.8–10.7
N2	804	785	19	2.4	1.4–3.7
S	5,164	4,977	187	3.6	3.1–4.2
S1	2,315	2,281	34	1.5	1.0–2.0
S2	2,849	2,696	153	5.4	4.6–6.3

Table 2. ASF antibody-positive and -negative wild boar samples, averaged prevalences and 95% confidence intervals (calculated using R) for the study areas (N = study area North, N1 = first half of the study period (12 months), N2 = second half of the study period (13 months); S = study area South, S1 = first 12 months of the study period, S2 = second 13 months of the study period).

To test for statistically significant associations between presumed risk factors and a positive virological or serological test results for ASF on the animal level, the Fisher's exact test was performed using the whole data set. Accordingly, the potential association between age and the laboratory test results was investigated. The animals were attributed to the age classes "juvenile" (<1 year) and "adult" (>1 year). Potential associations between the carcass categories ("hunted" or "found dead") and the laboratory test results were also examined. Furthermore, the age distribution within the two carcass categories was analyzed.

When testing for potential associations between the population density and positive ASF laboratory test results, the municipalities as the variable of interest were categorized depending on their test results (0 = only negative test results within the study period, 1 = at least one positive test result within the study period). Since the distribution of the data was not known, the non-parametric Mann-Whitney U test was used for statistical analysis. For this purpose, population densities were averaged over the reported years and assigned to each municipality. Due to lack of knowledge on the distribution of the data, the hypothesis that the population densities differed between the two study areas was also tested using the non-parametric Mann-Whitney U test.

The hypothesis that the age or carcass distribution was different between the study areas was examined using Fisher's exact test. This test was also used to examine potential associations between the study areas and the virological or serological status of wild boar.

Model analyses. To test for a temporal and spatial effect within the two study areas, a hierarchical Bayesian space-time model was used[22,23]. The model was only applied for the seroprevalence. The period for detecting the viral genome in hunted animals is generally short, which is likely to lead to false-negative results, i.e. animals that were ASF-positive, but not at the time of sampling or not in the available sample, have to be regarded as uninfected. Therefore, a stable trend analysis can only be performed with the serological results. The implementation of the model was adapted from the one described by Staubach, *et al.*[22]. Variables identified as statistically significant by univariable analyses were included as fixed effects, whereas space and time were treated as random effects. The analyses were conducted separately for each of the study areas (area N and area S) on municipality level using BayesX 2.0.1 (http://www.uni-goettingen.de/de/bayesx/550513.html). A Markov Chain Monte Carlo algorithm (MCMC) was applied to estimate the parameters of the model. Figures were generated by using the software package R (http://www.r-project.org)[20] and maps created using the software ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/).

Results

Data. After removing data from other counties then the study area, 7,015 data records were available for analyses. Within the study period of 25 months, 7,015 samples had been investigated virologically (Table 1) and 6,306 samples also serologically by ELISA. Only 319 samples were tested by IPT because the method had not yet been implemented in the beginning of the epidemic (Table 2).

Statistical analyses. A statistically significant association between age and the positive laboratory test results was found for both, real-time PCR and serology by ELISA/IPT ($p < 0.001$). Based on the results, the probability to detect an ASFV- or antibody-positive animal was higher in young animals (< 1 year) (real-time PCR: OR = 1.57, 95% CI = 1.35–1.83; serology: OR = 1.89, 95% CI = 1.45–2.47). Also, regarding the carcass category (hunted or found dead), a statistically significant association was found (p-value < 0.001). The probability to find a real-time PCR- or antibody-positive animal was higher in animals found dead (real-time PCR: OR = 69.60, 95% CI = 56.89–85.15; serology: OR = 4.53, 95% CI = 2.83–7.25). No statistically significant difference in the distribution of the two age classes within the carcass categories was detected (p-value = 0.420). In both, hunted wild boar and those found dead, the proportion of old animals was slightly higher (see Supplementary Figure S1).

A significant association was found between the wild boar population density and the test results regarding both ASFV genome detection by real-time PCR and serology (real-time PCR, $p < 0.001$; serology, $p = 0.009$). ASFV-positive municipalities had a higher population density than ASFV-negative ones (Fig. 2).

The age distribution of sampled wild boar was similar in areas S and N (p-value = 0.566) (see Supplementary Figure S2). However, the distribution of wild boar found dead and hunted animals was different (p-value < 0.001); in area S, the proportion of animals found dead was significantly higher than in area N (see Supplementary

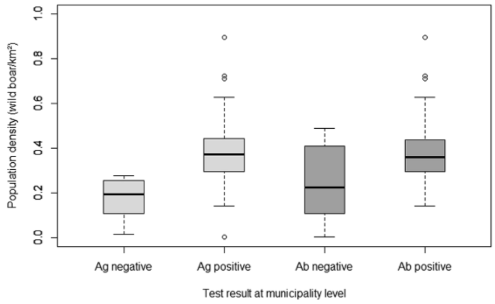

Figure 2. Population density (number of wild boar/km^2) in the municipalities of the study areas stratified by the virological and serological test result at the municipality level. Ag: ASFV genome detection, Ab: antibody detection. Figure was generated by using the software package R (http://www.r-project.org)[20].

Model	Mean	SD	Median (95% BCI)	Mean/St.Dev.*
Constant	−2.735	0.938	−2.687 (−4.678; −0.842)	
Carcass	−0.732	1.292	−0.620 (−3.708; 1.424)	0.567
Age	0.737	0.348	0.741 (0.062; 1.394)	2.122
Population density	−5.713	2.899	−5.573 (−11.841; −0.274)	1.971

Table 3. Parameter estimates obtained from the Bayesian model for three factors in area North (N); BCI: Bayesian credible intervals. DIC:323.82; Deviance:291.558; pD:16.135 *Mean/Std.Dev. >1.96, indicating statistical significance.

Figure S3). In area S, the population density was significantly higher than in area N (p-value < 0.001) (see Supplementary Figure S4).

The prevalence of ASFV genome-positive wild boar was significantly higher in study area S as comped to area N (p-value < 0.001). However, there was no significant difference in the seroprevalence between these areas (p-value = 0.728).

Model analyses. Due to the results of the univariable analyses, namely the significant association between age, carcass category, population density and the serological test results, these factors were included in the hierarchical Bayesian space–time model as fixed effects. In area N, age and population density showed a significant effect on the serological test result, whereas in area S, age and carcass category, but not population density resulted in a significant effect on the test results (Table 3 and Table 4).

The analyses of sample sizes resulting from active surveillance at municipality level showed in both study areas that the sample sizes differed considerably among municipalities and over time (Figs 3 and 4). Spatial analysis on the basis of the Bayesian model confirmed a different trend of the seroprevalences within the two study areas, which was already evident from the raw prevalence data. In area N, the highest prevalences were observed in one municipality in the western part of Ida-Viru county over the entire study period. In 2015 (data of all 12 months were included in the analyses), the prevalences were also higher in municipalities located more east, but in 2014 (data of four months were included) the sample sizes were too small to obtain reliable prevalence estimates for these municipalities. In 2016 (data of nine months were included), the infection expanded also to municipalities located in the South of area N (Fig. 3). In area S, the infection spread over time within the wild boar population. In contrast to area N, the prevalences were high in the municipalities bordering Latvia in 2014 and in the course of the following years, an expansion of the affected areas towards the North occurred (Fig. 4).

In both areas, N and S, the small sample sizes have to be considered when interpreting the results.

The spatial analyses yielded a clear median spatial effect on the logit prevalence per municipality in the North of area N. In the eastern and very southern part of the county, a negative spatial effect was found. The wild boar population density was higher in the western part of area N as compared to the eastern area bordering Russia (Fig. 5).

In area S, the strongest dynamic of infection, shown by a structured spatial effect (Fig. 6), became evident in some of the municipalities bordering Latvia and the ones located further north. Negative spatial effects were seen in the municipalities in the West and the East of the study area (Fig. 6). In area S, the average population density was higher than in area N. In both areas, the population density decreased over time (Figs 5 and 6).

The temporal analyses resulted in a significant difference of the median temporal effect on the logit prevalence between the two study areas. In contrast to area N, where no temporal effect was observed, a significant increasing trend during the whole study period of 25 months was seen in area S (Fig. 7).

Model	Mean	SD	Median (95% BCI)	Mean/St.Dev.*
Constant	−4.370	0.344	−4.371 (−5.081; −3.737)	
Carcass	1.533	0.342	1.544 (0.820; 2.100)	4.480
Age	0.580	0.173	0.579 (0.244; 0.924)	3.357
Population density	0.443	0.604	0.446 (−0.734; 1.600)	0.733

Table 4. Parameter estimates obtained from the Bayesian model for three factors in area South (S); BCI: Bayesian credible intervals. DIC:1344.465; Deviance:1269.215; pD:37.625 *Mean/Std.Dev. > 1.96, indicating statistical significance.

Discussion

When ASF emerged in Estonia in 2014, two different areas, namely in the North and in the South, were affected. Although the events in the South were connected with ASF outbreaks in the North of Latvia[7], only Estonian data were analyzed. Variations in the course of the ASF epidemic in the two areas led to the hypothesis that the events might be independent and differ in their epidemiology. The aim of this study was to test this hypothesis and to describe the epidemiology of the ASF epidemic in wild boar in defined areas of Estonia.

The study area in the South comprised four counties with a total area of 10,764 km^2, whereas the study area in the North consisted only of one county with a size of 3,364 km^2. In the South, not only the area under investigation was bigger but also in that area the wild boar density was higher. Therefore, the number of investigated samples was higher in the South. Confidence intervals therefore need to be considered when interpreting the results. Furthermore, the observed incidence per spatial unit and time step is not a useful estimate of the underlying disease prevalence due to different sample sizes as well as temporal spatial dependencies between neighboring areas. By applying a hierarchical Bayesian space–time model, the extra-sample variation and spatial/temporal correlations in the data were accounted for. The chosen model is suitable to analyze data with gaps and particularly variable sample sizes per spatial and temporal unit[22,23]. To estimate the fitness of the model the deviance information criterion (DIC) was used[24].

It was found that the probability to detect an ASFV genome- or antibody-positive animal was higher in young wild boar. This stands in contrast to the results of experimental studies, where no age-dependent degree of susceptibility could be detected[25,26]. However, recent experiments with a small number of animals showed that young animals survived long enough to develop antibodies, even in the case of acute-lethal courses of ASF. All these animals were also tested PCR positive[27]. Further field and experimental studies are therefore needed for clarification. Statistical analyses resulted also in a higher probability to find virologically and serologically positive animals in wild boar found dead than in hunted wild boar. This is very likely to be due to the high lethality of ASF. These findings once more emphasize the need of an increased effort to support passive surveillance and to encourage hunters to focus on the detection and sampling of dead wild boar[28,29].

The present study demonstrated a statistically significant positive association between population density and the municipality status regarding ASF (by ASFV genome detection or serology). This may be due to the fact that in densely populated regions the transmission rate between wild boar is higher, since it is known that direct contact between wild boar is strongly beneficial for transmission of ASF[30–32].

The findings regarding the association between age, carcass and population density and the serological test results were supported by analyses of virological data and the appropriate result, which showed the same associations. (IPT: specificity approximately 100%)[5]. Only 22 samples originating from 22 animals found dead showed a serologically positive test result, because laboratory routine procedures did not include antibody detection from organ and bone marrow samples. However, the strong association between animals found dead and a positive virological test result still point at the importance of detecting and sampling wild boar found dead[29].

To be able to include the factor population density in the analyses, data had to be transferred from the hunting district level to the municipality level. The applied method certainly led to a slight deviation from true wild boar densities. However, the density data at the hunting district level are mere estimates of hunters, based on their account of the hunting bag. In addition, the population density is subject to constant change. The reliability of these data is therefore always a challenge. The available hunting data originated from the pre-reproductive period before most females give birth. Accordingly, it can be assumed that at another time point of data capture, the number of wild boar per km^2 would be clearly higher.

It was not surprising that the age distribution was the same in the area N and S. This result demonstrates that the population structure was similar in the two areas, which may be due to similar hunting practices. This justifies comparing the results of the laboratory investigations for N and S. The proportion of the sampled animals found dead was significantly higher in area S. The significantly higher average ASFV genome prevalence in area S may be seen as a result of the significantly higher number of animals found dead in study area S and their higher chance to be positive by ASFV genome detection.

The Bayesian model was only applied for serology. Due to the fact that ASFV in wild boar samples is only detectable over a very limited period of time[32] and that no measurable memory effect is available, a trend analyses was not feasible with regard to the results of ASFV genome detection.

The results of the univariable analyses differed slightly from the ones obtained by Bayesian modelling. For the univariable analyses, this may be explained by the inclusion of the whole data set, independently of the study area whereas for the Bayesian model the data were analyzed for area N and area S separately. Also, data were adjusted for space and time in the model. Still, in both areas, the significant association between age and the serological result could be confirmed. In contrast to the univariable analyses, in area S, a significant association was shown between carcass category and serology. This might be due to the higher relative number of animals found dead in

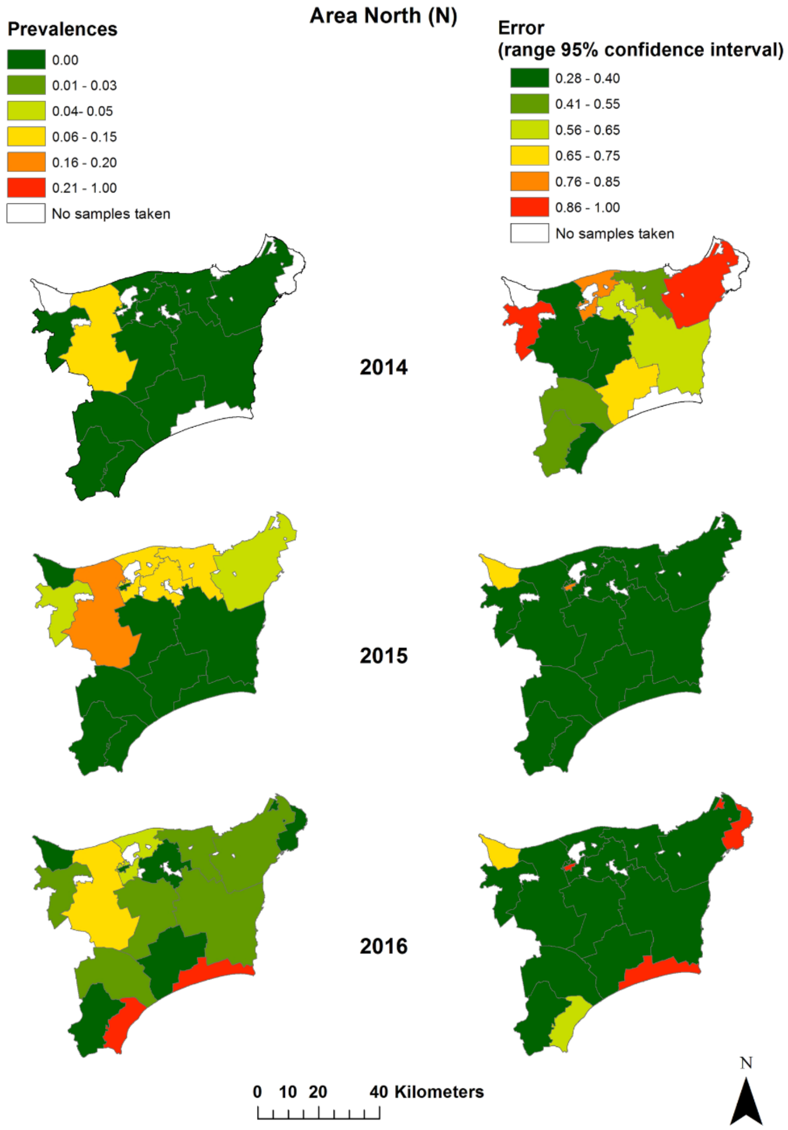

Figure 3. Seroprevalences and 95% confidence intervals for sampled wild boar per municipality in study area N (Ida-Viru county) in 2014 (Sept. – Dec.), 2015 (Jan. – Dec.) and 2016 (Jan. – Sept.). Maps were generated by using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/).

area S and accordingly their greater importance in the epidemics. Population density showed a significant effect on the seroprevalence in area N, which is consistent with the results of the univariable analyses. In area S, population density had no significant effect, which may be explained by the bigger size of study area S as compared to N and the associated heterogeneity of the population densities in the single municipalities.

The spatial effect on the logit prevalence indicates a difference between the respective courses of infection in the two study areas. In area N, the infection seemed to be stable in one area. In contrast, in area S, in 2014 the prevalences were high in the areas bordering Latvia and the infection seemed to move North over time. This

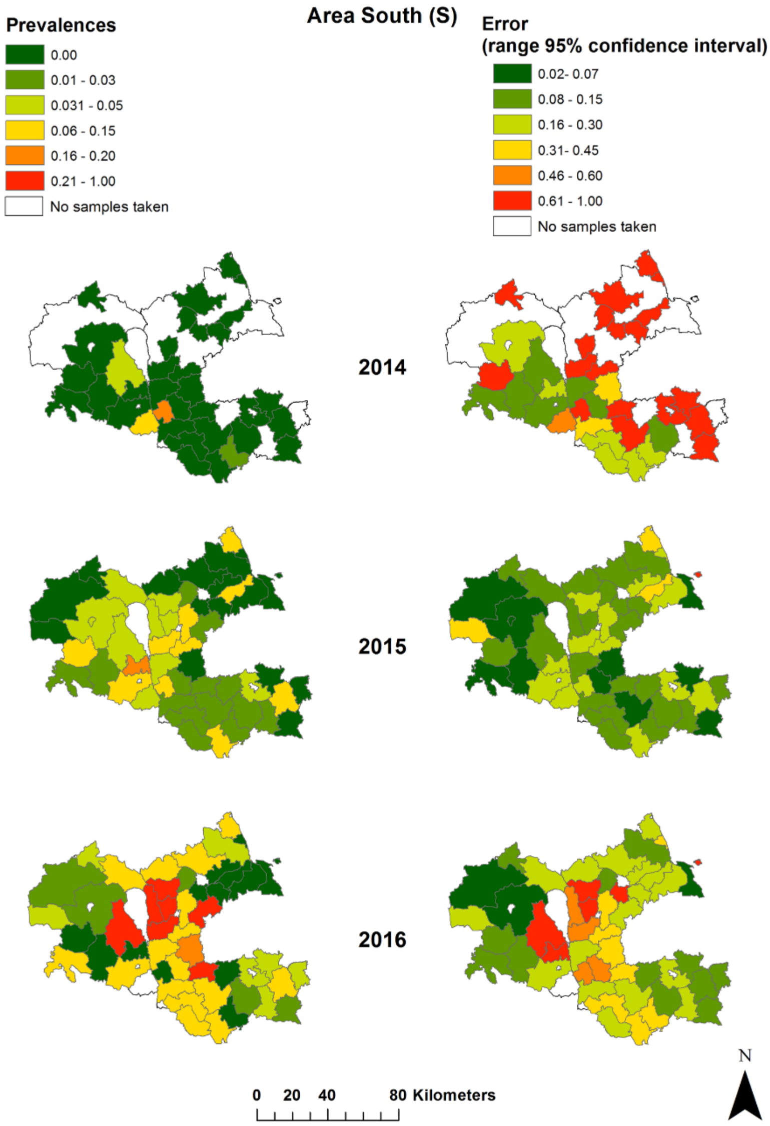

Figure 4. Seroprevalences and 95% confidence intervals for sampled wild boar per municipality in study area S (Viljandi, Tartu, Valga and Voru county) 2014 (Sept. – Dec.), 2015 (Jan. – Dec.) and 2016 (Jan. – Sept.). Maps were generated by using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/).

spread may have been supported by the higher population density in area S, which makes a higher transmission rate likely[30]. Although the prevalence seemed to increase in the center of study area S, the width of the 95% CI was also increasing. This is probably due to the ASF-related decrease of the wild boar population in these municipalities over time and thus to the lower number of investigated samples. The findings of the spatial analysis also support the hypothesis that the infection was already present in area N for a longer period of time, whereas it was still spreading in area S at the time when the study was conducted. Accordingly, since the epidemic in the South did not reach its climax and did not stop spreading, it is impossible to prove these hypotheses at the moment.

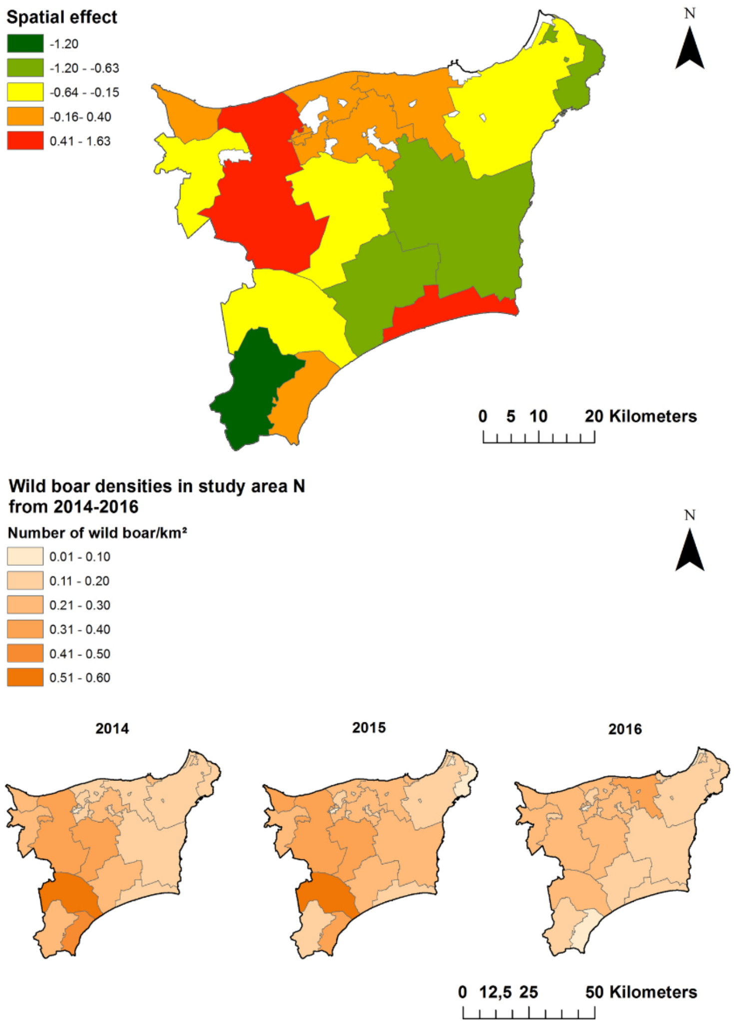

Figure 5. Median-structured spatial effect on the logit prevalence per municipality in study area N (Ida-Viru county) for the study period of 25 months. Maps in the lower row show the population density (number of wild boar/km^2) for each municipality in study area N. Maps were generated by using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/).

However, it would be advisable to re-analyze the situation in the two areas in one or two years again. The incidence of ASF currently seems to level off and no increase of seroprevalence is observed anymore, we expect that the situation in area S will then result in a similar picture as now observed in area N.

Although the average seroprevalence over the study period of 25 months did not differ significantly between the two areas, the temporal trend analysis showed a significant difference in the course of infection. The number of data sets per municipality and per analyzed time point was relatively small, but our data suggest that the trend varied between the two areas, also when on the Bayesian credibility intervals were taken into account.

The increase of the temporal logit prevalence in area S led to the assumption that ASF was newly introduced into that area, that naïve animals got infected and that the proportion of animals developing antibodies subsequently grew. By contrast, no temporal effect was seen in area N. These assumptions were supported by the results of the descriptive analyses. In study area S, the average seroprevalence showed an increase over time, whereas in

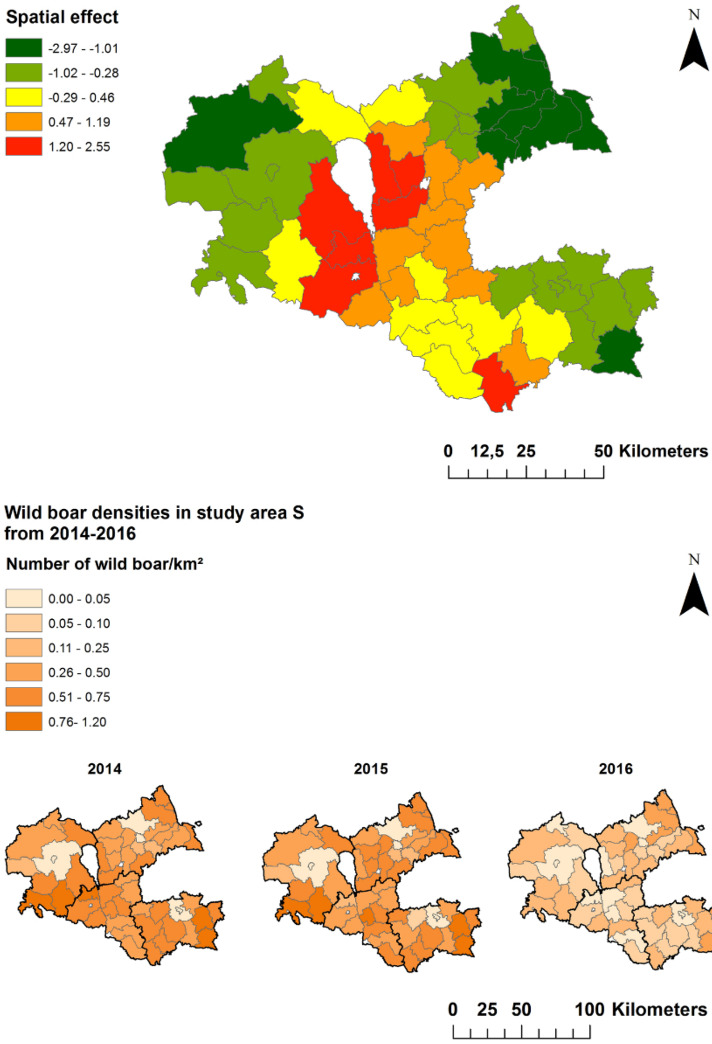

Figure 6. Median-structured spatial effect on the logit prevalence per municipality in study area S (Viljandi, Tartu, Valga and Voru county) for the study period of 25 months. Maps in the lower row show the population density (number of wild boar/km^2) for each municipality in area S. Maps were generated by using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/).

area N the average prevalence of antibody-positive wild boar was even lower in the last 13 months of the study period. We therefore hypothesize that ASF may have been present a longer time period in area N before the start of the study period, i.e. before the first case was officially confirmed. This hypothesis is supported by the fact that several outbreaks had occurred in the St. Petersburg area[4], located only 160 km away from the Estonian border and connected with Ida-Viru county through a highly frequented highway between 2009 and 2012. Furthermore, the very small sample sizes at the beginning of the study period (September 2014) and the ones of 2012, 2013 and of the beginning of 2014, i.e. before ASF was officially detected in Estonia, made an earlier detection virtually impossible. In the study of Nurmoja *et al.*[11], two different hypotheses were formulated. As in the present study, the authors postulated that an undetected epidemic may have occurred in the North of Estonia, which had started earlier. This may explain the different courses of the epidemics in the North and in the South. However, Nurmoja

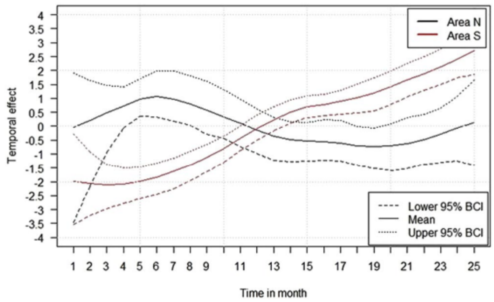

Figure 7. Median temporal effect on the logit prevalence in area North (N) and in area South (S) for the study period of 25 months. 95% Bayesian credible intervals (BCI) are included. Figure was generated by using the software package R (http://www.r-project.org)[20].

et al.[11] also tested the hypothesis that the ASF strain in the North might be less virulent. Although one animal had recovered from an infection with the ASFV strain circulating in the North of Estonia, this virus still proofed to be highly virulent.

Active ASF surveillance in wild boar in Estonia started in 2012. In 2012 and 2013, according to the annual surveillance plan, it was obligatory to investigate serologically 0.5–1% of hunted wild boar, while virological investigations were not performed. In 2012, the total number of investigated wild boar in the whole of Estonia was 122; three samples were taken in area N and 21 in area S. In 2013, the total number of investigated wild boar in Estonia was 279, including six samples from area N and 65 samples from area S. Our analyses showed that even at the beginning of the epidemics in Estonia, the sample sizes in the area bordering Russia in the North were too small to have a reasonable chance of detecting ASF infections. By assuming an unknown population size and perfect specificity, it had been necessary to test at least 66 samples with a negative result to show that ASFV prevalence was below 5%. To detect the virus with a design prevalence of 1% the required sample size would have been over 300 samples (http://epitools.ausvet.com.au/content.php?page=home). When the true sample sizes mentioned above are taken into consideration, it becomes obvious, that the infection would have remained undetected, if it had been present already in 2013 or 2012. However, it must be assumed that a new emergence of ASF in a naïve wild boar population should have led to an increased mortality in wild boar. Such incidences were not reported in the years before the official outbreak in 2014. However, detecting dead wild boar might be difficult in areas with such a low population density as reported for area N[28]. In addition, the population density was even lower in the Eastern part of area N than in the other parts of the area. Accordingly, it might be practically impossible to reach the required sample sizes in areas with such a small wild boar population.

In summary, we studied the epidemiology of ASF in two areas in Estonia. The temporal and spatial differences in the course of the epidemic in the two areas suggest that the first introduction of ASF took place in the Northeast of Estonia and not, as previously assumed, in the South. This first introduction may have happened several months before Estonia was officially declared as affected by ASF.

These findings may initiate a revision and adaptation of current surveillance activities in countries that are at risk of ASF introduction, to prevent an unnoticed introduction of the disease and its spread[29].

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Author Contributions

I.N. provided input to all laboratory data and designed the study. K.S. performed the statistical analyses, designed the study and drafted the manuscript. C.S. conducted the model analyses, designed the study and reviewed the manuscript. K.D. provided input to ASF and reviewed the manuscript. C.S.-L. designed the study and reviewed the manuscript. F.J.C. supported the study with epidemiological hints and carefully reviewed the manuscript. A.V. supervised the study and carefully reviewed the manuscript. All authors read and approved the final manuscript.

Additional Information

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3.2.2.2 Analysis of Estonian surveillance in wild boar suggests a decline in the incidence of African swine fever

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Highlights

- In Estonia, a significant reduction of the estimated wild boar population density was observed over the years of the epidemic
- After 100 days of the survival of an infected wild boar, only antibodies specific for the ASFV are detectable
- Over time, the prevalence of wild boar tested positive for ASFV declined and the number of wild boar tested positive for ASFV-specific antibodies accumulated
- This course of disease suggests a low virus circulation and indicates a potential fade out of ASF

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Analysis of Estonian surveillance in wild boar suggests a decline in the incidence of African swine fever

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African swine fever (ASF) in wild boar populations is difficult to control. In affected areas, samples from all wild boar shot and found dead are investigated. The use of laboratory tests allows estimating the duration of the infection in affected animals. The study aimed to test the hypothesis that the stage of the epidemic in different areas of Estonia can be assessed on the basis of prevalence estimates. ASF surveillance data of Estonian wild boar were used to estimate prevalences and compare them between the East and West of Estonia. The temporal trend of the estimated prevalence of ASF virus positive animals and of the estimated seroprevalence of wild boar showing antibodies against ASFV was analyzed. Due to the potential influence of population density on the course of ASF in wild boar, also population density data (number of wild boar/km^2) were used to investigate the relationship with laboratory test results. In areas, where the epidemic had already lasted for a long time, a small number of new cases emerged recently. The prevalence of samples that were only seropositive was significantly higher in these regions as compared to areas, where the epidemic is in full progress. The observed course of the disease could be the beginning of an ASF endemicity in this region. However, the results may also indicate that ASF has started to subside in the areas that were first affected in Estonia.

African swine fever (ASF) is a hemorrhagic disease of suids caused by a large DNA virus of the Asfarviridae family, African swine fever virus (ASFV)[1]. The virus was introduced into Georgia in 2007. It spread from there affecting both, domestic pigs and wild boar[2]. Until now, ASF emerged also in nine countries of the European Union and in some Asian countries including China, Mongolia, Vietnam and Cambodia (OIE WAHIS interface, visited online 26th. April 2019).

So far, the course of the ASF epidemic in domestic pigs indicates that controlling the disease in farmed animals had been relatively successful in most, but not all countries (e.g. Romania). By contrast, eradicating ASF from an affected wild boar population appears to be difficult[3–5]. Originally, it was hypothesized that ASF in wild boar might either fade out quickly due to the high virulence of the pathogen or it will spread rapidly throughout the whole continent[4]. By now, it is obvious that none of the two scenarios became reality. Nonetheless, there is still the chance that ASF in wild boar might subside due to the increasing herd immunity, developed through the increasing proportion of surviving wild boar[6].

It is known, that also a dense wild boar population may influence the dynamics of ASF. Most experts agree that a low population density reduces the risk of ASF spread[3,7–12]. Nurmoja, *et al.*[7] showed a positive association between population density and the prevalence of ASF in wild boar. Due to the ongoing discussion regarding the potential role of the wild boar population density on the spread of ASF and thus its influence on the sero- and ASFV prevalences, we investigated the potential relationships between the temporal trends of prevalence estimates and the wild boar population.

Considering the known risk factors, the course of the ASF epidemic in Estonia illustrates the challenges to eliminate the disease from a wild boar population. In September 2014, ASF entered Estonia in the Southeast, probably coming from Latvia[7,13]. Further ASF cases were detected in the Northeast, 200 km away from the affected

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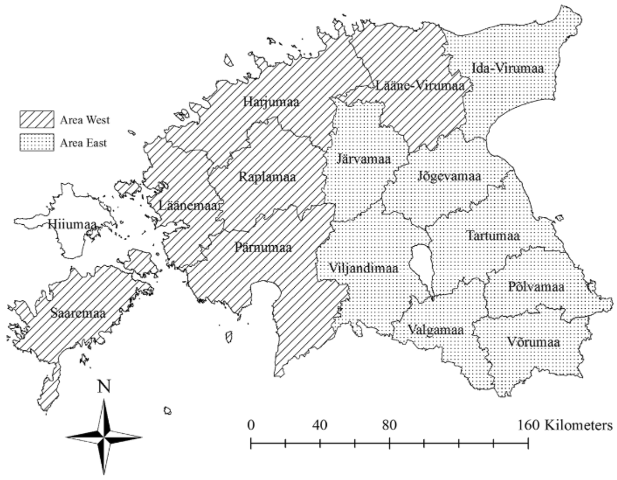

Figure 1. The study areas "East" and "West" in Estonia. The island of Hiiumaa was excluded. Map was generated by using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA).

area in the South and close to the border with the Russian Federation. These cases were considered as epidemiologically independent of the cases in the South of Estonia[7,14]. The disease spread slowly, but inexorably towards the center of the country and reached the western part including the island of Saaremaa in 2016[3,7,15]. Recent surveillance data of 2018 from the whole of Estonia indicated that the number of ASF cases in sampled wild boar has decreased. Initial statistical analyses also pointed at a clear decrease of the number of ASFV-positive wild boar, especially in eastern Estonia, where the epidemic started[3]. An increase of samples that were only seropositive, but ASFV-negative, has also been noticed[3].

We thus aimed to investigate in the present study, whether there is a difference in the surveillance data, namely the laboratory test results, between areas, where ASF emerged in 2014 (i.e. the eastern part of Estonia) and regions, where the epidemic did not start before 2016 (i.e. the western part of Estonia). Correspondingly, we tried to assess the current epidemiological situation based on laboratory test results and tested the hypothesis that a decrease of ASFV positive cases combined with a slight increase of seropositive cases, suggesting an increased herd immunity, might indicate a decline of the incidence of ASF in Estonian wild boar.

Material and Methods

Study area and study period. Estonia was divided into two areas, "East" and "West". Only the Island of Hiiumaa was excluded from the study area because it remained free from ASF so far. The eastern part of Estonia (area "East") consisted of eight counties and the western part of the country (area "West") of six counties (Fig. 1). Area "East" included the counties, where ASF newly emerged in 2014 and that were fully affected by ASF by the end of 2015 and area "West" the counties in which first sporadic cases emerged in the middle of 2015 and the extensive spread started in January 2016.

The sizes of the two study areas were calculated on the basis of hunting grounds to avoid biased results due to cities or large bodies of water. The sizes of the hunting grounds in each county were added up and the total was used for calculations. Therefore, area "East" included a size of 19,611.72 km^2 and area "West" a size of 20,283.23 km^2.

The study period comprised 44 months and lasted from December 2014 to July 2018.

Data. Surveillance data were extracted from the CSF/ASF wild boar surveillance database of the European Union (https://surv-wildboar.eu). The database includes one data record for each sampled wild boar. Each record contains information about the origin of the sample (from animals found dead or hunted apparently healthy), the sampling location, age and gender of the sampled animal and the results of serological and virological laboratory tests (i.e. detection of ASFV by qPCR[16]). For the serological tests, a commercially available ELISA (Ingezim PPA COMPAC, Ingenasa, Madrid, Spain) was used. Further details about the sampling procedures and the laboratory tests have been described elsewhere[7]. Age was categorized by attributing wild boar younger than one year to one group and those older than one year into another group. Data from passive surveillance included samples originating from animals that had been found dead, were shot sick or died in a road traffic accident. All samples that yielded an inconclusive test result for ASFV were excluded from the analyses.

Spatial analyses were based on the administrative reform of municipalities in 2017. Data records from 2014–2017 were assigned to the new administrative units (eight counties for area "East" and six counties for area "West") according to their coordinates using the software ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA (http://www.esri.com/).

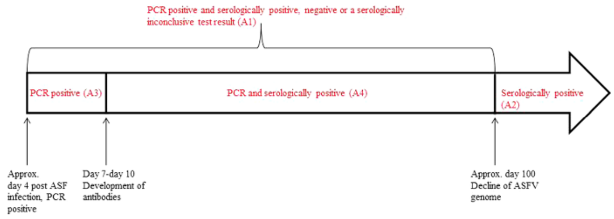

Figure 2. Timescale illustrating the course of laboratory test results for African swine fever.

Wild boar population data were obtained from the Estonian Environment Agency (Nature department) and were based on estimates provided by hunters. Population density data for wild boar in Estonia have previously been described in detail[7].

For the present study, population data estimates were available as integer numbers of wild boar. The respective information was available for six hunting years (April 2012/March 2013 to April 2017/March 2018) and for each county of the study area. The areas (in km^2) of hunting grounds in each county were summed and the estimated wild boar density (wild boar/km^2) in each county was determined based on the calculated size of areas that could function as wild boar habitats (e.g. water bodies and streets were excluded).

Data analyses. The study period was divided into a first half, including data from Dec 2014–Sept 2016 (first 22 months of study period) and a second half consisting of data from Oct 2016–July 2018 (second 22 months of study period).

We analyzed the estimated ASFV prevalence and seroprevalence by grouping the samples as described in the following section.

The estimated prevalence was calculated for all samples that had yielded an ASFV- positive PCR result and either a positive, negative or inconclusive test result for specific antibodies directed against ASFV (designated as group A1, Fig. 2). Prevalences were also calculated for all samples that had tested positive for ASFV-specific antibodies and had yielded an ASFV-negative PCR result (designated as group A2, Fig. 2). We assumed that the former group (A1) represents animals in the active phase of infection and that the latter one (A2) consists of convalescent wild boar. For the sake of completeness, the estimated prevalence for samples that had tested only PCR-positive, but seronegative (group A3, Fig. 2), as well as for samples that had tested positive for ASFV and simultaneously for antibodies against ASFV (group A4, Fig. 2) were calculated separately.

The estimated prevalences based on detection of viral genome by PCR and antibody detection by ELISA (group A1 and group A2, Fig. 2) were first calculated separately for each study area (area "East" and area "West"). Within each study area, the prevalence estimates were compared between the first and the second half of the study period. Fisher's exact test was used to test for statistical significance. A p-value of ≤ 0.05 was regarded as statistically significant and 95% confidence intervals were determined on the basis of Clopper and Pearson[17]. The described analyses were done by using the software package R (http://www.r-project.org)[18].

To investigate the temporal courses of the raw prevalence estimates (groups A1 and A2, Fig. 2) a hierarchical Bayesian space–time model was used. Due to random variation in the estimated prevalence regarding the individual geographical units and time, we adjusted for spatial effects and season. The model was adapted from Staubach, *et al.*[19,20] and analyses were performed applying BayesX 2.0.1 (http://www.uni-goettingen.de/de/bayesx/550513.html) as previously described[7,20,21]. Age and origin of the samples were defined as fixed independent variables and time, space and season were included random factors. The corresponding prevalence constituted the dependent variable. A Markov Chain Monte Carlo algorithm (MCMC) was implemented to obtain parameter estimates. 50,000 iterations were performed and at every 50^{th} iteration, a sample was selected. For the burn-in 1,000 iterations were chosen. A detailed description of the model can be found in the Supplementary Information.

Differences in the population densities (number of wild boar/km^2) between hunting years were calculated for each study area and statistically analyzed using the non-parametric Kruskal-Wallis test. To assign statistically significant differences to the respective hunting years, a Mann–Whitney U test was used for pairwise comparisons. Bonferroni correction was applied to control for the type I-error in multiple testing scenarios[22]. Differences between the two studies areas in individual hunting years were also analyzed using the Mann–Whitney U test. The analyses regarding the population density were performed using the software package R (http://www.r-project.org)[18].

Maps and figures. Maps were generated using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/). Figures were generated using the software package R (http://www.r-project.org)[18].

Results

Data. After exclusion of data sets originating from the island of Hiiumaa (N = 1,255) and all data resulting in an inconclusive virological test (N = 114), a total of 36,456 records were available for analyses. 56.95% of all samples were investigated in the first study half (December 2014 – September 2016). In the area "East", 13,455 samples were examined, of which 10,887 had been investigated in the first half of the study period (December

Study months	Study area	Number of samples tested for ASFV by PCR	Number of PCR-positive samples (A1)	Prevalence (95% confidence interval)	p-value	Number of samples tested for antibodies against ASFV	Number of sero positive samples (A2)	Seroprevalence (95% confidence interval)	p-value
1–22	"East"	10,887	1,497	0.138 (0.131–0.144)	<0.001	9,700	203	0.021 (0.018–0.024)	<0.001
	"West"	9,875	459	0.046 (0.042–0.051)		9,462	55	0.006 (0.004–0.008)	
23–44	"East"	2,568	52	0.020 (0.015–0.026)	<0.001	2,518	165	0.066 (0.056–0.076)	<0.001
	"West"	13,126	889	0.068 (0.063–0.072)		12,600	358	0.028 (0.026–0.031)	

Table 1. Total number of investigated samples (by PCR for ASFV or ELISA for antibodies against ASFV), the numbers of all samples that tested positive for ASFV (irrespective of the serological test result = > group A1) or were seropositive (with a negative ASFV PCR result = > group A2), the calculated prevalence (95% confidence intervals) and the statistical significance of the difference in the prevalences between study area "East" and study area "West" in the first and the second half of the study period.

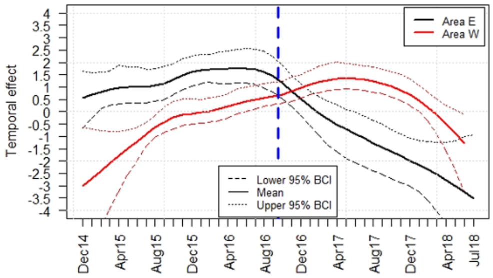

Figure 3. Median temporal effect of all samples from area "East" (E) and area "West" (W) that tested PCR-positive, irrespective of the serological result, on the logit prevalence. 95% Bayesian credible intervals (BCI) are indicated. Figure was generated by using the software package R (http://www.r-project.org).

2014 – September 2016). In the area "West", 23,001 samples were obtained, 9,875 thereof in the first half of the study period (December 2014 – September 2016) (Table 1).

Data analyses. For the first half of the study period (December 2014 – September 2016), analysis yielded a statistically significantly higher estimated prevalence of samples of group A1 (ASFV PCR-positive and seropositive-, seronegative or inconclusive samples) in area "East" compared to area "West" (Table 1; Supplementary Figs S1 and S2). The same applied for the estimated prevalence of samples of group A2 (seropositive samples) (Table 1; Supplementary Figs S3 and S4). By contrast, in the last 22 months of the study period, the estimated prevalence of samples from group A1 was significantly higher in the area "West" than in area "East" (Table 1; Supplementary Figs S1 and S2). In area "East", the estimated prevalence of seropositive wild boar (group A2) was significantly higher than in area "West" in both parts of the study period (Table 1). However, if individual months are considered, the seroprevalence did not show a statistically significant difference between the two study areas in most months (Supplementary Fig. S3).

Additional analysis of the prevalence of samples that were only PCR-positive and serologically negative (group A3) yielded a clear decrease of the estimated prevalence in the East over time (from 8% in month 12 and 20 and 0% in most of the study month of the second half of the study period, Supplementary Fig. S5 and Table S1). In area "East", samples that were PCR and seropositive (group A4) showed a high prevalence in the first half of the study period, whereas a high prevalence was found in area "West" in the second half of the study period (Supplementary Fig. S6 and Table S1).

After adjusting the ASFV prevalence (group A1) or the seroprevalence (group A2) for seasonal effects in the areas "East" and "West" (Supplementary Figs S7 and S8), the temporal course of the prevalence estimates was investigated (Figs 3 and 4). In area "East", the model analyses suggested a decrease in the temporal trend of the logit prevalence of PCR-positive wild boar (group A1) in the last 22 months of the study period. Until August 2016, there was an increase of the logit prevalence of samples from group A1 in area "West". Towards the end of the study period, the logit prevalence also appeared to decrease in area "West". In the last four months of the study period, no significant difference in the logit prevalences between the two areas was detected (Fig. 3). In area "East", the temporal trend of the logit prevalence showed an increase within the first half of the study period for all samples that tested positive exclusively for antibodies against ASFV (group A2). In the second half, the seroprevalence seemed to decrease slightly. By contrast, the logit prevalence of serologically positive wild boar showed a

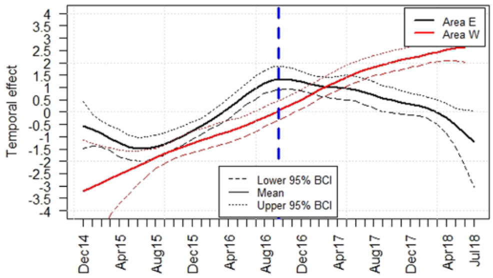

Figure 4. Median temporal effect of all samples from area "East" (E) and area "West" (W) that tested exclusively serologically positive on the logit prevalence. 95% Bayesian credible intervals (BCI) are indicated. Figure was generated by using the software package R (http://www.r-project.org).

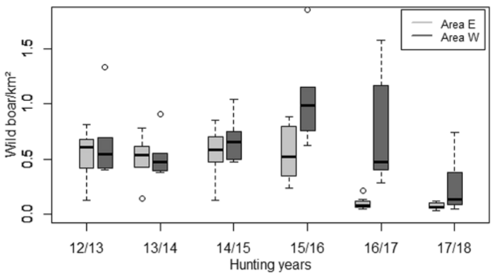

Figure 5. Wild boar population density (number of wild boar/km^2) in the different hunting years for area "East" (E) and area "West" (W). The horizontal line that forms the top of the box illustrates the 75th percentile. The horizontal line that forms the bottom is the 25th percentile. The horizontal line that intersects the box is the median number of wild boar per square kilometer. Whiskers represent maximum and minimum values that are no more than 1.5 times the span of the interquartile range and the open circle represent outlier, which are single value greater or less than the extremes indicated by the whiskers. Figure was generated by using the software package R (http://www.r-project.org).

steady rise over time in area "West". In the last year of the study period, the logit prevalence of seropositive wild boar was significantly higher in area "West" than in area "East" (Fig. 4).

The wild boar population density (number of wild boar/km^2) was calculated for six hunting years for area "East" and area "West". In area "East", the population density appeared to be stable during the hunting years 12/13–15/16 (averaged densities between 0.51 and 0.56 wild boar/km^2). However, a significant decrease from an average of 0.56 wild boar/km^2 to an average of 0.10 wild boar/km^2 was found between the hunting years 15/16 and 16/17 ($p = 0.001$) (Fig. 5). Between 2016/17 und 2017/18, there was only a minor decrease from an average of 0.1 wild boar/km^2 to an average of 0.07 wild boar/km^2 ($p = 0.382$). In area "West", no significant difference in the wild boar population density over the years was found (Fig. 5).

Comparing the population densities between area "East" and "West", there was no significant difference for most of the analyzed hunting years. Only in hunting years 2015/16 and 16/17, the number of wild boar per square kilometer was significantly lower in area "East" ($p = 0.02$ and $p < 0.001$; Fig. 5).

Discussion

The course of ASF in wild boar populations in affected countries appears to be extremely difficult to control so far. It has been hypothesized that eradicating the disease from the wild boar population in some areas may be impossible[4,7,21]. However, Mur, *et al.*[23] suggested that ASF virus may not able to persist only in wild boar populations on the long term, although this hypothesis was made with regard to the ASF epidemic that occurred in Spain in the 1980s and 1990s. There, the wild boar density had been relatively low at that time. There is also recent evidence that ASF may have been brought under control in an affected area near Zlin in the Czech Republic (OIE WAHID interface, visited online 28. February 2019).

The outbreaks in domestic pigs were most probably the result of spill-over of ASF from wild boar to domestic pigs[24]. In Estonia, the last outbreak in a domestic pig farm was reported in September 2017. Direct or indirect (more likely) transmission from infected wild boar into domestic pig holdings seems possible, thus an infected wild boar population still constitutes a major threat to pig holdings and trade. As a consequence, complete eradication of ASF, also from the wild boar population, is desirable[24,25].

The EU legislation (Council Directive 2002/60/EC) intends a 100% sampling of the whole hunting bag (all hunted wild boar) and all wild boar found dead in restricted areas. The hunting bag usually represents about 60–80% of the true population[26,27]. Therefore, the available sample size was higher than in many other published wild life studies[28–30]. The analyses of these comprehensive surveillance data combined with the knowledge of detection probabilities obtained from animal trials open up the possibility to understand disease dynamics and host-virus interactions in a better way[7,11,31,32]. Similar to the study of Nurmoja, *et al.*[7], we used these data to evaluate the temporal trend of ASF within the Estonian wild boar population.

In the study, we aimed at detecting potential differences between the estimated prevalences in wild boar that may have acquired the infection recently and in animals that may be seen as true convalescents.

Firstly, we investigated the prevalence of samples that had yielded a positive PCR result regardless of the serological test result (positive, negative or inconclusive) obtained for the same sample (group A1). We assumed that these were animals that had contracted ASF within the last 100 days before they were sampled (Fig. 2). In doing so, we considered it as irrelevant for the study whether the positive PCR result indicated the presence of infectious virus (4 to 60 days pi) or just the detectability of viral genomes (up to day 100 pi). Under the assumption that ASFV PCR-positive animals have not been infected for more than 100 days, a large proportion of PCR-positive samples in a population indicate an acute epidemic with the emergence of new cases, i.e. a high incidence.

Wild boar that survive an ASFV infection for at least 7 to 10 days will develop antibodies. Subsequently, survivors will first be positive for both viral genome and antibodies for about 90 to 100 days, and then turn to antibody presence alone for an undetermined period (Fig. 2). Experimental studies could show that these animals are usually PCR-negative[6,33]. Following these findings, we secondly looked at the prevalence of samples that had been tested seropositive, but were negative for ASFV by PCR. Despite the fact that it cannot be completely excluded that some seropositive survivors showed intermittent viremia that was not detected at the time point of sampling[31], we assume the survivors are not shedding significant amounts of virus or viral genome. The latter assumption is strengthened by recent studies that showed that survivors did not transmit the disease to naïve sentinels[6,14]. Also, the temporal course of the disease does not indicate a significant virus excretion[3]. However, it is not possible to categorically exclude the existence of carrier animals in the study area, i.e. there might be wild boar that survive the initial phase of acute ASF infection and develop a carrier state. However, if such animals exist, it will be difficult to detect them in the field and the true status of the course of the infection in individual animals remains unknown. The fact that we could detect only nine wild boar that were both ASF-positive by PCR and serology in the area "East" in the second half of the study period, may suggest that such animals are rare in this epidemiological situation. In areas, where seropositive animals dominate the ASF-positive findings, less new incident cases appear to occur, possibly indicating the late phase of the epidemic. However, it has to be emphasized that after the initial peak of serologically positive samples, ASF could still become endemic instead of fading out.

In 2014, ASF emerged in the eastern part of Estonia and spread slowly towards the West, where the broad expansion of ASF started in 2016. Based on this knowledge, the study area was divided into the areas "East" and "West". The study period was also split into two periods for comparison. As expected, the prevalence estimates based on samples of wild boar that tested positive for ASFV by PCR and the ASFV-specific seroprevalence were significantly lower in area "West". This was due to the later onset of disease in the western part of Estonia. The statistically significant differences in the second half of the study period supported the view that the ASF epidemic was in full progress in area "West" during the last 22 months of the study period. The higher seroprevalence in area "East" might indicate the absence of new ASF cases.

These results are also supported by the outcomes of the modelling analyses presented here (Figs 3 and 4). A hierarchical Bayesian space–time model was used to include also temporal, seasonal and spatial effects and to adjust for potential covariates such as age.

A clear temporal trend could be observed, in particular for the serological results. This tendency was also found in the study of Nurmoja, *et al.*[7]. The results of the model analyses indicate that towards the end of the study period, the seroprevalence decreased in area "East" and increased in area "West". These findings were supported by mapping the serological prevalences (Supplementary Fig. S4). In 2018, the samples that tested positive by serology, but were negative for ASFV (group A2) seemed to increase in area "West" and decrease in the "East". It can thus be hypothesized that the western part of Estonia has entered a new phase of the epidemic. The incidence of ASF cases decreases in this area. The decrease of seropositive samples in area "East" and simultaneously the absence of an increase of PCR positive samples at the end of the study period suggest that a reoccurrence of new ASF cases has been lacking so far.

Population density analyses have to be considered with the limitations that usually apply to population estimates in wild life even if generated with different methods. The problems and challenges associated with the use of such data have been a matter of extensive discussion[3,21,34,35]. However, there is no practical alternative to using these data as long as data that are more reliable are not available. Still, any of the results obtained with such data must be interpreted with extreme caution.

In the eastern part of Estonia, the significant decrease of population density (number of wild boar/km^2) from the hunting years 2012/13–15/16 to the hunting years 2016/17 and 2017/18 suggests that the massive occurrence of ASF cases in the wild boar population might have led to a decrease after a while. At the same time, one can conclude that a low population density may lead to a significant reduction of the incidence of ASF cases in wild boar. This is in accord with the study of Nurmoja, *et al.*[7] where a positive association between the population density and the incidence of ASF was demonstrated. Also in the western part of Estonia, the population density (number of wild boar/km^2) has apparently decreased in the hunting year 2017/18. These data suggest that the epidemic had an effect on the population density and that the resulting low density may support the fading of the epidemic. However, the observed decrease in the population density could also be due to the implemented control measures (e.g. intensified hunting, targeted hunting of females and feeding ban). Regardless of the reason for the identified significant decrease, the study therefore demonstrates with real data what others have predicted[3,9,10]. An intensive reduction of the population density prior an emergence of ASF might thus be a huge advantage for controlling the disease in the case of an introduction. However, the question remains, whether it is possible to reduce the population density sufficiently, only by hunting, but perhaps also through other measures (e.g. ban of supplementary feeding, limiting wild boar reproduction, euthanasia with toxic substances, etc.).

The results of the study can raise hope that it may be possible to control ASF in a wild boar population, even in countries, where a large proportion of wild boar population is exposed. It is too early to predict whether an ASF-free status can be reached, but at least this seems no longer impossible. In any case, recording of all ASF-positive cases is of utmost importance, regardless of the type of test result, i.e. ASFV detection by PCR or serology. Yet, it is difficult to assess the temporal course of the epidemic by just looking at the case numbers. With this common practice, the official declaration that a country has gained an ASF-free status requires the complete absence of positive samples. This is hard to reach, particularly because serologically positive animals may be present for several years after virus circulation has stopped. In relation to seropositive wild boar, it should be mentioned that there is a controversial debate regarding potential carrier (i.e. hidden ASFV-positive) status of seropositive animals[6,36,37]. As long as it cannot be ruled out that such animals remain infectious, there might be the danger of a re-emergence of the disease in a previously affected region. Thus, a change of the current official requirements is unlikely, unless it can be proven, that carriers do not exist or that they are not infectious.

In studies regarding the ASF epidemic on the Iberian Peninsula, a possible eradication of ASF in wild boar was already hypothesized[23]. However, a favorable course of the disease still depends on several unknown factors which will need clarification. It is still unknown, how long protective immunity last and which level of herd immunity is sufficient for protection. Also, the role of maternal antibodies for protection against ASFV infection of young wild boar remains in contrast to classical swine fever (CSF) still unknown[38,39]. This is particularly important as wild boar have a high reproduction rate, therefore beget a huge number of potentially susceptible descendants. Furthermore, in contrast to CSF virus, which is not able to remain infectious in the environment for a longer period[38,40], it is generally accepted that ASFV remains stable even under harsh conditions, although reliable figures about the tenacity of the virus are scarce[41,42]. Moreover, it is almost impossible to detect and to remove all dead wild boar from an affected region[43,44]. Therefore, carcasses of infected wild boar might constitute a potential source of reinfection. Probst, *et al.*[45] found that wild boar seem to be interested in the soil around dead conspecifics and suggested therefore an efficient removal of dead wild boar carcasses. This suggestion was also supported by the European Food Safety Authority[3]. ASFV may thus still be present in area "East" despite a few months without or with a very small number of newly detected ASF cases in wild boar, and might cause a re-emergence of the disease at any time. Also, some studies suggest, that an increasing dominance of seropositive results might be the result of a rising number of surviving animals and of a potential attenuation of ASFV in these areas[14,31,37,46]. It is also known from other diseases, e.g. CSF, that the seroprevalence is usually low at the beginning of an epidemic, but that the incidence of seropositive cases increases as the epidemic proceeds, while the number of new cases decreases due to the developed natural immunity and therefore due to the decreasing number of susceptible animals. As soon as herd susceptibility returns again, the numbers of newly infected individuals may increase again[47–49]. In contrast to CSF, the currently observed seroprevalence of ASF is low. This is probably due to the high case-fatality ratio of ASF, which is much higher than that of CSF[38]. Also, in contrast to CSF, there is no vaccination against ASF, which may in the case of CSF have contributed to the increase of the seroprevalence in a CSF-vaccinated wild boar population[38,49].

A favorable course of the disease certainly also depends on the success of implemented control measures, which need to be regularly evaluated and adapted as appropriate, However, not even the best control measures can prevent the new entry of ASF from neighboring regions. The likelihood of a new entry from neighboring countries depends certainly on the current phase of the epidemic in bordering countries, in particular in wild boar, but also on the local surveillance efforts. It is known, that there are still ASFV positive cases in the Russian Federation which is directly bordering Estonia. Finally, the unpredictable human factor, which is known to play a major role in the emergence and spread of ASF[4,13,24,50] can always lead to the re-emergence of ASF, in particular in wild boar.

The present study may open new perspectives with regard to the evaluation of the epidemic status of ASF in wild boar. Comprehensive surveillance and laboratory results can be used to assess the status of an epidemic. Careful analyses of these data may for example help to identify areas with an increased incidence. This knowledge can then be used to adapt control measures accordingly. Follow up studies, investigating virological and environmental factors influencing e.g. immunity or ASF transmission, investigating the course of the epidemic with newly emerging surveillance data and evaluating the implemented control measures for ASF in wild boar in Estonia would be useful to bring the course of ASF in Estonia into context with the course of ASF epidemics in other countries.

In conclusion, the course of ASF in wild boar in Estonia was investigated using the available surveillance data including laboratory test results. The results of the analyses indicate a clear decline of the disease in the East of the country. This temporal course of the disease suggests that there is a chance that ASF will continue to subside in this region.

Data Availability

The original data used for the analyses can be obtained from the author after approval by the responsible institution in Estonia.

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Author Contributions
K.S. designed the study, performed the analyses and drafted the manuscript. C.S. designed the study, supported the statistical and model analyses and edited the manuscript. S.B. supported all issues around the laboratory test results, edited and reviewed the manuscript. A.V. designed the study and supported it with his knowledge about the Estonian epidemic and the Estonian data and reviewed the manuscript. I.N. provided the data, supported the study with her laboratory and data expertise and reviewed the manuscript. F.J.C. supported the epidemiological analyses, co-operated with K.S. in drafting and intensively reviewing the manuscript. C.S.-L. designed and supervised the study and the epidemiological analyses and reviewed the manuscript.

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3.2.2.3 African swine fever in Latvian wild boar-A step closer to elimination

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Highlights

- Surveillance efforts yield significantly more samples from active surveillance
- Most samples originate from wild boar aged between 1-2 years
- A decrease in the sample size can be seen over time emphasising the need to maintain surveillance also in an advanced stage of the epidemic
- The course of ASF in Latvia is comparable to the one in Estonia and suggests a decline of circulating ASFV in the wild boar population

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African swine fever in Latvian wild boar—A step closer to elimination

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Abstract

In 2014, African swine fever (ASF) emerged in Latvia for the first time. The majority of cases appeared in wild boar, but the presence of ASF in these animals constitutes a permanent threat to domestic pig holdings. Recent studies have shown an increase in serologically positive and a decrease in PCR-positive ASF cases in wild boar, possibly indicating a decline of ASF incidence. We aimed to investigate the course of the ASF epidemic in wild boar in Latvia, thus attaining further insights into the ASF epidemiology in this country with the goal of assessing the stage of the epidemic. Latvian ASF surveillance data of wild boar were utilized to estimate the seroprevalence and ASF virus (ASFV) prevalence in the wild boar population. Prevalence estimates were obtained for both the eastern and western part of the country and in addition for the 2014/2015 to 2018/2019 hunting seasons. Moreover, prevalence estimates for three different age classes were calculated. An increase in serologically positive yet PCR-negative wild boar samples from active surveillance was identified over time. When comparing the age groups, wild boar younger than one year displayed the ASFV prevalence to be higher than the seroprevalence, whereas older animals shared higher seroprevalence estimates. These findings support the assumption that only a small proportion of affected animals survive an infection, leading to an accumulation of their numbers over time. As a result, ASF elimination in a country with an infected wild boar population could possibly be achieved, if effective wild boar population management and surveillance is maintained and combined with the detection and removal of wild boar carcasses to reduce the viral load in the environment. In addition, the wild boar population should be kept as small as possible to break the ASFV infection cycle.

KEYWORDS
African swine fever, epidemiology, Latvia, prevalence, serology, wild boar

1 | INTRODUCTION

African swine fever (ASF) is a serious viral disease of domestic pigs and Eurasian wild boar (*Sus scrofa*) caused by African swine fever virus (ASFV) and generally associated with vast socio-economic impact in affected regions (Stahl et al., 2019). After the emergence of ASF in Georgia during 2007, the epidemic reached the first countries of the European Union at the beginning of 2014. In January 2014, the first cases were detected in Lithuania, the southern neighbour of Latvia (Pautienius et al., 2018), followed shortly after (February 2014) by the identification of the first ASF cases in Poland (Smietanka et al., 2016). In Estonia, bordering Latvia in the North, the epidemic started in September 2014 (Nurmoja et al., 2018). Almost three months prior to the Estonian outbreak, Latvia had reported the first cases of ASF in wild boar. It is very likely that the virus was introduced from neighbouring countries (Oļševskis et al., 2016). Until mid-2016, the eastern part of the country was mainly affected. However, in summer 2016, ASF reached the central area of Latvia and continued to spread towards west Latvia. This long-distance jump of ASFV may have been caused by human activities. Since 2016, the disease continued to spread slowly in the local wild boar population. In October 2019, ASF-infected wild boar were present in around 85% of Latvia.

Similar to other affected countries, ASF infection cycle in Latvia is maintained by wild boar (Oļševskis et al., 2016; Pautienius et al., 2018; Pejsak et al., 2018). Before the ASF outbreak, wild boar were the most popular and abundant large game in Latvia, representing nearly 70% of the ungulates hunted per year and playing an important role in trophic cascades. Supplementary feeding, selective shooting and restrictions in the use of hunting rights maintained the population. Concurrently, wild boar had become a conflict species, primarily due to the damage to agriculture and rural infrastructure caused by the animals. Targeted campaigns to reduce the wild boar population nationwide were introduced twice—in the first half of the 1990s and after 2014. Both times, the hunting rates were increased and targeted to females as all age classes of females are known to be highly reproductive and subsequently contribute to a fast increase in population sizes. In the 1990s, the wild boar depopulation measures were rather declarative and formally implemented. However, the numbers of wild boar declined drastically anyway after a harsh winter in 1995/1996 and large outbreaks of classical swine fever (CSF). After the beginning of the 21st century, the population rapidly recovered and reached a new maximum with an estimated population of approx. 74,000 individuals in 2013 (Andersone-Lilley, Balčiauskas, Ozoliņš, Randveer, & Tõnisson, 2010; Kawata, Ozolins, & Baumanis, 2013). At the end of the 2018/2019 hunting season, the estimation comprised only approx. 20,000 wild boar (https://www.zm.gov.lv/public/files/CMS_Static_Page_Doc/00/00/00/85/05/Mezacukasdinamika.jpg).

Due to the transmission potential, ASF-infected wild boar represent a threat for the domestic pig industry. In addition, controlling ASF in wild boar populations has been proven more difficult than eliminating the virus from affected pig farms (Gogin, Gerasimov, Malogolovkin, & Kolbasov, 2013; Nurmoja et al., 2017; Schulz, Oļševskis, et al., 2019).

After 2 years with a growing epidemic, in most countries with comprehensive surveillance data, an increase of ASFV PCR-positive wild boar samples was observed (European Food Safety Authority, Gogin, Richardson, & Gervelmeyer, 2017). Most surveillance samples were retrieved by passive surveillance, that is from animals found dead, shot due to sickness or killed in a road traffic accident (European Food Safety Authority et al., 2017; Nurmoja et al., 2017; Pautienius et al., 2018). However, recent analyses suggest a decline in the incidence of PCR-positive wild boar in some countries, while the prevalence of seropositive wild boar (mainly hunted wild boar showing positive laboratory test results for antibodies against ASFV) increased simultaneously (Schulz, Staubach, et al., 2019). Following the assumptions of Schulz, Staubach, et al. (2019), this may indicate a decline of the ASF epidemic in these countries. A long period of detectability of ASFV antibodies was found in domestic pigs (Penrith et al., 2004; Pujols Romeu, Badiola Saiz, Perez de Rozas, Rosell Bellsola, & Carreras Mauri, 1991), and similar values can be assumed in wild boar. Therefore, it can take several years of surveillance without new virus introduction until no seropositive cases are detected.

The difficulties in controlling ASF in wild boar and the long time period, during which the virus has been circulating in the wild boar population in several countries, emphasize the continuing need of further epidemiological research to understand the course of ASF in wild boar populations (Depner et al., 2016; European Food Safety Authority, 2018, 2020; Gogin et al., 2013). Additionally, ASF continues to spread to further countries, thus increasingly threatening the global pig market. Expanding the knowledge on ASF in wild boar can therefore help to improve control and the effectiveness of surveillance. The detailed research regarding seropositive wild boar and their role in the course of ASF might support the certainty regarding the disease status of a country and thus, in the best case, decrease the economic damage caused by ASF.

The present study aimed to analyse ASFV prevalence and seroprevalence estimates as well as describe the course of ASF in wild boar in Latvia. The results could be compared with those from other countries with the ultimate goal of assessing the epidemiological situation of ASF in wild boar populations in the best possible way.

2 | MATERIALS AND METHODS

2.1 | Study area

According to national legislation (Regulation of the Cabinet of Ministers No 274, 28 April 2004) (https://likumi.lv/doc.php?id=88074), Latvia is divided into six statistical regions: Riga (304 km^2), Pieriga (10,134 km^2), Zemgale (10,732 km^2), Kurzeme (13,606 km^2), Latgale (14,550 km^2) and Vidzeme (15,245 km^2). Because of the high proportion of urban areas, the region of Riga was excluded from the analyses. Due to the slow spread of ASF from the East to the West of Latvia, we divided the country into an

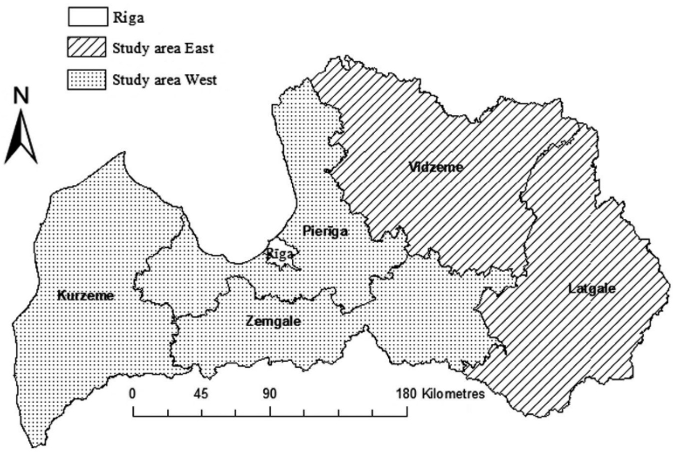

FIGURE 1 The six statistical regions of Latvia grouped into an eastern and western part of Latvia. Riga was excluded from the analysis due to its mainly urban character

eastern (Latgale and Vidzeme) and a western (Pieriga, Zemgale and Kurzeme) section (Figure 1).

2.2 | Data description

Data from five consecutive hunting seasons (2014/2015–2018/2019) were available for the analysis. In Latvia, the hunting year lasts from 1 April to the 31 March of the following year. Surveillance data were used from the CSF/ASF wild boar surveillance database of the European Union (https://surv-wildboar.eu). The structure of the data sets has previously been described (Schulz, Staubach, et al., 2019). In brief, the origin of each sample was recorded, including information on the source, that is active surveillance (hunted wild boar) or passive surveillance (wild boar found dead, involved in a road traffic accident or shot due to sickness). In addition, each data set included information about the age of the sampled animal categorized in three groups (<1 year, 1–2 years, >2 years).

For analyses, all data records containing no clear PCR result (positive or negative) were excluded. Also, data records originating from passive surveillance and tested serologically (i.e. for antibodies against ASFV) were excluded from the final analyses. For age analyses, all data records that lacked information on the age of the respective wild boar were excluded.

In the course of passive surveillance, tissue samples (organs, mainly bone marrow) were taken from animals by veterinary inspectors or state-authorized veterinarians and analysed by PCR.

For active surveillance, blood samples were taken by trained hunters from each hunted wild boar and submitted for the detection of ASFV genome and ASFV antibodies.

Passive surveillance was conducted throughout Latvia starting in June 2014. Active surveillance included the sampling of all hunted wild boar in a radius of 8–20 km around every newly discovered ASF wild boar case. The size of the areas covered by active surveillance progressed correspondingly with the ASFV spread in the wild boar population. The laboratory procedures for detecting ASFV genome via PCR or antibodies via ELISA have been previously described (Schulz, Oļševskis, et al., 2019).

2.3 | Data analysis

PCR-positive samples, regardless of their serological result (i.e. the sample could have shown either a seropositive, a seronegative or no serological test result), were summarized (group 1) as described (Schulz, Staubach, et al., 2019). The prevalence of wild boar showing these test results was calculated based on the total number of animals tested by PCR. In group 1, samples from active and passive surveillance were included.

Following the same procedure, samples that yielded a seropositive but PCR-negative test result were grouped together (group 2). Therefore, the seroprevalence (i.e. prevalence of wild boar with antibodies against ASFV) was calculated based on the total number of animals tested by PCR and ELISA, regardless of the test result. All samples used for the calculations for group 2 originated from active surveillance.

Although the focus was put on analysing and comparing groups 1 and 2, samples with a PCR-positive and simultaneously a seropositive result (group 3) and samples exclusively PCR-positive but seronegative (group 4) were analysed for the sake of completeness. Both groups included samples originated from hunted animals (active surveillance).

As in group 2, the prevalence of wild boar tested PCR-positive and seropositive (group 3) or PCR-positive but seronegative (group 4) were also calculated based on the total number of wild boar that had shown unambiguous PCR and serological test results (i.e. either positive or negative).

For all four groups, prevalence estimates were obtained for each age class (<1 year, 1–2 years and >2 years), for the five study regions, area East and area West, and for all hunting seasons. For group 1, the prevalence depending on the source of the samples, that is active or passive surveillance, was also determined for each area and hunting season. To describe the course of the prevalence in each hunting season and in each study area, confidence intervals were calculated according to Clopper and Pearson (1935). All calculations were done using the software package R (http://www.r-project.org) (R Core Team, 2015), also used to produce the included figures.

3 | RESULTS

3.1 | Data description

After excluding all data records with an inconclusive PCR result (n 251) and data records originating from passive surveillance and with a serological test result (n 423), 60,166 data records were available for analysis (Appendix Table A1). Data records with no age information were removed from the age analyses (n 1,622).

In all areas, most samples originated from animals between 1 and 2 years old. The number of samples resulting from active surveillance was clearly higher than samples from passive surveillance (Figure 2, Appendix Table A1).

The number of samples obtained in the western regions of Latvia was slightly lower than those from the eastern regions, but the differences in samples sizes were not significant. The number of tested wild boar samples from the area East peaked in the 2015/2016 hunting season, whereas the sample size increased in the area West over time and reached a peak in the 2017/2018 hunting season (Figure 2, Appendix Table A1).

3.2 | Data analysis

Group 1: In group 1, all samples with a positive ASFV PCR result were aggregated, regardless of the serological result (negative, positive or not tested). In both the eastern and western areas, the prevalence was highest in the subset of animals younger than 1 year (mean prevalence East: <1 year: 0.048, 1–2 years: 0.033, >2 years: 0.020; mean prevalence West: <1 year: 0.063, 1–2 years: 0.037, >2 years: 0.037) (Figure 3). This was in accordance with the results obtained for each of the five study regions individually (Appendix Table A2).

In both study areas and all hunting seasons, the prevalence estimates for hunted or found dead wild boar showing results in accordance with group 1 were significantly higher (approx. 35 times higher) in wild boar sampled through passive surveillance than those sampled through active surveillance (Figure 4, Appendix Table A6).

Group 2: In group 2, all samples with a seropositive and an ASFV PCR-negative test result were combined. In contrast to group 1, no significant differences in the prevalence estimates between the different age classes were observed, although the mean prevalence of seropositive animals was higher in wild boar older than 2 years (mean prevalence East: <1 year: 0.024, 1–2 years: 0.025, >2 years: 0.035; mean prevalence West: <1 year: 0.020, 1–2 years: 0.016, >2 years: 0.020). However, especially in the East during the last hunting season, the samples with a seropositive result were significantly higher in animals older than 2 years (<1 year: 0.007 (CI: 0.003–0.014), 1–2 years: 0.027 (CI: 0.020–0.036), >2 years: 0.059 (CI: 0.046–0.074)) (Figure 5, Appendix Table A3).

Although the seroprevalence in the East at first increased, a decline was later observed during the 2017/2018 and 2018/2019 hunting

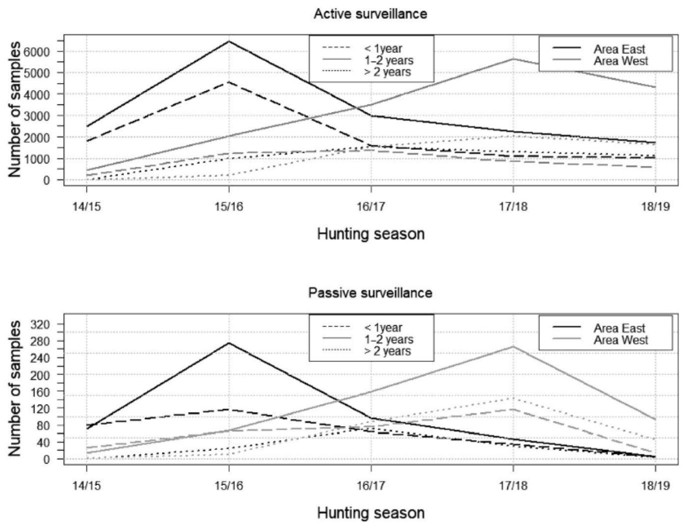

FIGURE 2 Number of samples originating from active or passive surveillance in area East and area West, stratified for three different age classes (<1 year, 1–2 years, >2 years)

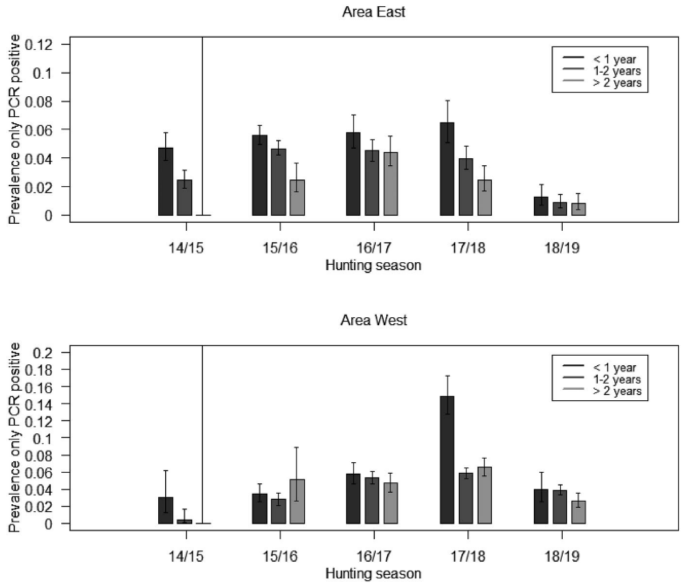

FIGURE 3 Prevalence estimates for ASFV PCR-positive samples, irrespective of their serological test result, stratified by age class, study area East and West and hunting season. The whiskers indicate 95% confidence intervals.

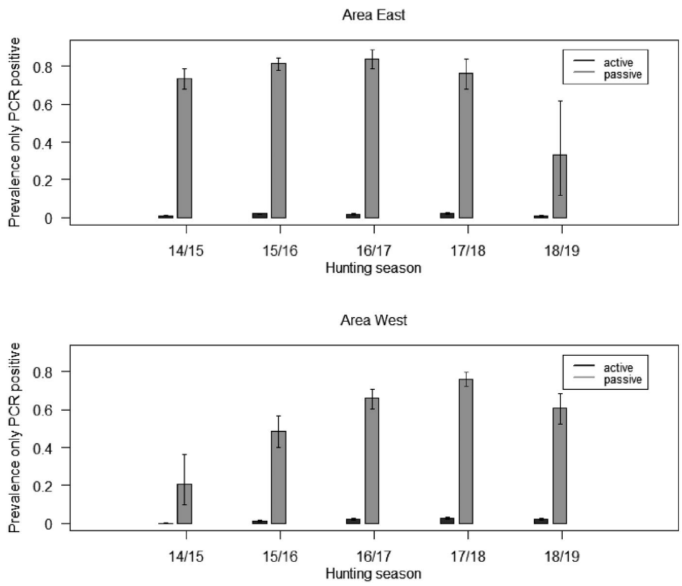

FIGURE 4 Prevalence estimates for ASFV PCR-positive samples, irrespective of their serological test result, stratified by the origin of sample (active or passive surveillance), the study areas East and West, and the five hunting seasons. The whiskers indicate 95% confidence intervals

seasons. This trend was observed in all three age classes. In the West, the average prevalence for each age class was lower than in the eastern area. Nevertheless, an increase over time was also observed, but the highest seroprevalence in the West was not observed before 2018/2019 hunting season in all age classes (Figure 4, Appendix Table A3).

Group 3: In group 3, all samples with a positive ASFV PCR and a seropositive test result were analysed. Generally, the prevalence estimates were very low for hunted wild boar yielding samples from group 3. In areas East and West, the highest prevalence was found in samples obtained from animals younger than 1 year (max.

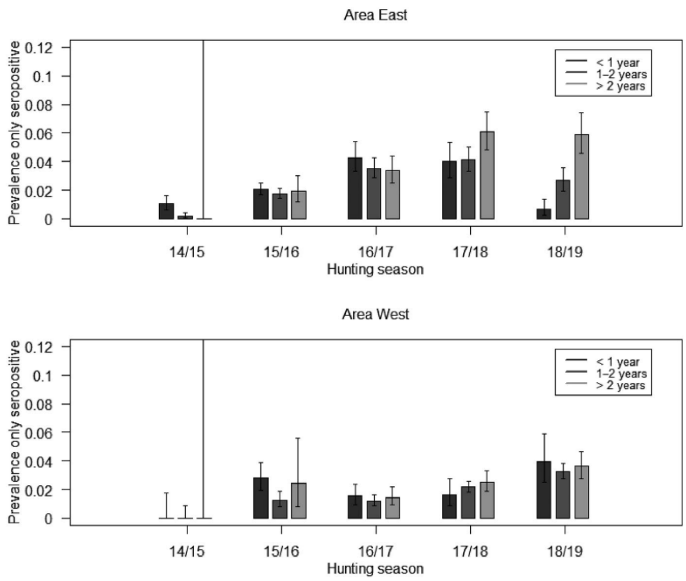

FIGURE 5 Prevalence estimates for ASFV antibody-positive samples, stratified by three different age classes, the study areas East and West, and the five hunting seasons. The whiskers indicate 95% confidence intervals

prevalence 0.025). In both regions, the prevalence estimates were similar. Towards the end of the observation period, that is hunting season 2018/2019, a slight decrease was observed (Appendix Table A4).

Group 4: In group 4, all samples with an ASFV PCR-positive and a seronegative test result were analysed. Corresponding with the previous groups, the prevalence estimates were highest in animals younger than 1 year. This applied particularly to the eastern regions of Latvia (max. prevalence 0.026). Similar to group 3, the differences in the prevalence estimates between the two study areas were not significant. Moreover, the prevalence estimates decreased in both areas over time (Appendix Table A5).

4 | DISCUSSION

The Latvian wild boar population has now suffered from ASF for five consecutive years. During this time, the disease has spread from the eastern part of the country towards the West. For our analyses, the country was therefore divided into two geographically distinct study areas, East and West, to understand the temporal stages of the ASF epidemic. Both areas exhibited a different course of the disease, which therefore had to be taken into account in the epidemiological analysis. Additionally, analysing the data at the regional level allowed us to compare the course of ASF in wild boar in the different regions during the hunting seasons.

When comparing the results for the chosen study areas, the differences in size and any potentially resulting differences in sample numbers had to be taken into consideration. Despite the larger size of area West, the number of samples taken at the beginning of the study period as part of active and passive surveillance was clearly higher in the East (hunting seasons 2014/2015 and 2015/2016). Yet, in the last two hunting seasons, the sample size in the West overtook the number of samples in eastern area. This can be explained by the mandatory intensification of surveillance (particularly of active surveillance) after a region, in this case area West, is affected by ASF. Similar observations were made in other countries (Nurmoja et al., 2017). These findings also illustrate the need to enhance surveillance not only when ASF is present, but before its arrival (Nurmoja et al., 2017). On the other hand, a decrease in sample sizes was seen in area East over time. This might be mainly due to a decrease in the wild boar population density, a possible consequence of ASF, its high case-fatality ratio and increased hunting efforts (Schulz, Oļševskis, et al., 2019; Schulz, Staubach, et al., 2019). In contrast to the reported approx. 74,000 wild boar in 2013 (Andersone-Lilley et al., 2010; Kawata et al., 2013), the estimated wild boar population in Latvia was approximately 20,000 individuals at the end of the hunting season 2018/2019. The decrease in population density and thus also in the number of detectable wild boar carcasses might indicate that the epidemic has reached the deceleration phase as defined in the EU working document 'Strategic approach to the management of African Swine Fever for the EU' (SANTE/7113/2015—Rev 11). However, regardless of the reasons for the decrease in population density, as long as ASF is present, as is still the case in Latvia and in some of its neighbouring countries, effective hunting and surveillance must be maintained (European Food Safety Authority, 2020). These measures should be combined with effective detection and removal of wild boar carcasses, thus reducing the viral load in the environment (European Food Safety Authority, 2018, 2020). Moreover, the wild boar population should be kept as small as possible in order to potentially break the ASFV infection chain (European Food Safety Authority, 2018, 2020; Gavier-Widen et al., 2015; Lange, 2015). Reliable surveillance must be kept in place to detect new entries or further spread of the disease. This applies particularly to passive surveillance, known to represent the most effective way of detecting ASF in wild boar (European Food Safety Authority, 2018; Nurmoja et al., 2017; Schulz, Oļševskis, et al., 2019). Due to the difficulties in finding dead wild boar and the low acceptability among hunters to support passive surveillance (Schulz, Calba, Peyre, Staubach, & Conraths, 2016), the number of samples obtained by passive surveillance was much lower than those received through active surveillance. However, the temporal trend was similar, demonstrating the importance of keeping hunters motivated in regard to supporting passive surveillance. In all regions and hunting seasons, most samples were unsurprisingly obtained from 1- to 2-year-old animals, as this age class represents the typically hunted target group (European Food Safety Authority, 2015; Schulz et al., 2016; Toigo, Servanty, Gaillard, Brandt, & Baubet, 2008).

In the present study, we assumed that samples from ASF-infected wild boar are positive by PCR a few days post-infection, stay positive by PCR and also yield a seropositive test result for up to approximately 100 days and finally remain exclusively seropositive for an undetermined period (Petrov, Forth, Zani, Beer, & Blome, 2018; Schulz, Staubach, et al., 2019; Zani et al., 2018). For domestic pigs, this period was described to last several years (Penrith et al., 2004; Pujols Romeu et al., 1991). Test results were therefore analysed in several groups, taking into account that animals in group 1 were infected within 100 days prior to sampling, whereas animals in group 2 had survived an ASF infection. Due to the decomposition of carcasses, blood samples could often not be taken from animals found dead. Therefore, samples originating from passive surveillance are usually not tested for antibodies against ASFV. Very rarely, fresh samples were obtained from wild boar shot due to sickness or killed in road traffic accidents. These samples were tested for antibodies against ASFV; however, considering the rarity of these events, we excluded all data originating from ASFV antibody testing via passive surveillance, thus avoiding confusion and misinterpretation of the study results.

Based on the grouping of wild boar samples depending on their test results, we aimed to assess the epidemiological stage of the ASF epidemic in Latvia during the five different hunting seasons. While interpreting the study results, it must be acknowledged that factors like wild boar population density or ASF control measures might influence the ASF prevalence estimates in the different hunting seasons (European Food Safety Authority, 2018; Lange, 2015; Smietanka et al., 2016). In Estonia, a decrease of PCR-positive wild boar and an increase of seropositive wild boar over time was observed. At the same time, the population density decreased, possibly due to ASF and the implemented measures to control ASF in wild boar (Schulz, Staubach, et al., 2019). Due to the descriptive character of the study design, these factors and their potential influence were not included. However, also in Latvia, the assumption still stands that the decreasing population density and the long-term effects of control measures influenced the course of ASF (Schulz, Oļševskis, et al., 2019). Moreover, the data were only analysed in connection with the respective hunting season, permitting the comparison of the results from the individual hunting seasons.

Similar to Estonia (Schulz, Staubach, et al., 2019), the results showed after an initial increase as described by the European Food Safety Authority et al. (2017), a decrease in the ASFV prevalence in all wild boar samples over time. Simultaneously, an increase in the seroprevalence in hunted wild boar was detected as previously described by the European Food Safety Authority (2020). Moreover, the differences of the prevalence estimates of group 1 and group 2 between the hunting seasons indicated a time shift between area East and area West, similar to the situation in Estonia. Both prevalence estimates showed a comparable course over time, but particularly the increase in seroprevalence occurred later on in the western part of Latvia. In eastern Latvia, the seroprevalence started to decline in the 2018/2019 hunting season, suggesting the beginning of disease elimination in that area, while the seroprevalence peaked in the West during the same season, indicating the phase of a decreasing epidemic. This course of disease can be explained by the slow spread of ASF from eastern to western parts of Latvia and correspond with the results published by the European Food Safety Authority (2020). The very low prevalence of ASFV in east Latvia and the decrease of the seroprevalence towards the end of the study period are promising findings and may imply the slow disappearance of ASF.

Although western Latvia was affected by ASF from 2016 onwards, the disease had already been circulating for over a year in the East. Analyses of the prevalence estimates for groups 3 and 4, including samples from newly infected hunted animals (infected within the last 100 days prior to sampling), support these results. The prevalence estimates also decreased over time, in the East slightly earlier than in the West. In both areas, the prevalence estimates for these groups were very low and showed the highest values in wild boar younger than one year. In accordance with findings from other researchers, these findings provide no evidence for the presence of long-term virus carriers (Stahl et al., 2019).

ASFV PCR-positive samples (group 1) reached the highest prevalence in wild boar younger than 1 year. This is in line with the finding that the ASFV prevalence showed the highest values in animals between 1 and 2 years, but without a significant difference relative to animals younger than 1 year (European Food Safety Authority, 2015). In addition to the finding that the prevalence of ASFV PCR-positive samples was increased in younger animals, it was obvious that the prevalence of ASFV PCR-positive animals was much higher when the tested samples were obtained by passive surveillance (in comparison with samples received through active surveillance, i.e. hunting of apparently healthy wild boar). Both findings may be explained by the high case-fatality ratio (Schulz, Conraths, Blome, Staubach, & Sauter-Louis, 2019). In addition, these findings once again illustrate the vast difference in the chances of early ASF detection in wild boar found dead (high chance) versus in hunted wild boar (low chance) (European Food Safety Authority, 2018; European Food Safety Authority et al., 2017; Nurmoja et al., 2017; Schulz, Oļševskis, et al., 2019).

The significantly higher values in the seroprevalence estimates (group 2) in hunted animals older than 2 years in the last two hunting seasons of area East might be due to the accumulation of surviving wild boar over time. They simultaneously suggest a decline of the ASF incidence in the eastern part of Latvia (European Food Safety Authority, 2020; Schulz, Staubach, et al., 2019).

In several previous studies, the role of ASF survivors and their potential for spreading ASFV was intensively discussed (European Food Safety Authority, 2015; Gallardo et al., 2018; Petrov et al., 2018; Schulz, Staubach, et al., 2019). However, so far no hard evidence was found to prove the infectiousness of wild boar tested seropositive, but PCR-negative (Stahl et al., 2019). Therefore, this comprehensive description of Latvian surveillance data of the last 5 years provides a glimpse of hope for successful control or even potential elimination of ASF in wild boar, despite the huge difficulties that must be overcome, when combating ASF in these animals. In accordance with the long period of detectability of antibodies found in domestic pigs (Penrith et al., 2004; Pujols Romeu et al., 1991), wild boar most likely follow the same immune response pattern. Therefore, the continuous detection of seropositive animals does not indicate any new virus introduction or circulating virus, but instead the expected findings point towards a subsiding epidemic. Although these findings suggest a potential end of the epidemic, the high efforts to monitor the epidemiological situation of ASF in wild boar continuously have to be maintained. Also, due to the continuous high risk of infection from infected neighbouring countries it is of upmost important, especially at this stage, to cautiously analyse the epidemiological situation of ASF in the wild boar population in Latvia to adjust control measures where necessary and reach final elimination.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

E.O. designed the study, performed the analysis and drafted the manuscript; K.S. supported and finalized the analysis and drafted the manuscript; C.S. supported the analysis and reviewed the manuscript; M.S. supported the design of the study and reviewed the manuscript; K.L. designed the study together with E.O. and supported the drafting of manuscript; D.P. provided and analysed the data; J.O. reviewed the manuscript and provided information on wild boar population status before and after ASF introduction; F.C. carefully reviewed the analyses and the manuscript; and C.S.L. supervised the study and the analyses and reviewed the manuscript.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as no animal experiments were conducted.

DATA AVAILABILITY STATEMENT

The original data used for the analyses can be obtained from the author after approval by the responsible institution in Latvia.

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APPENDIX

TABLE A1 Numbers of samples per study area, hunting season, age class and origin of carcass

	Latgale						Vidzeme					
	<1 year		1–2 years		>2 years		<1 year		1–2 years		>2 years	
	A	P	A	P	A	P	A	P	A	P	A	P
2014–2015	1,260	35	1,649	29	1	0	539	45	829	41	1	0
2015–2016	1,591	29	2,273	59	431	9	2,959	88	4,191	216	556	15
2016–2017	1,004	19	1,194	25	723	21	595	45	1,808	71	822	51
2017–2018	592	15	1,020	20	708	14	490	20	1,219	26	597	15
2018–2019	543	5	816	2	516	3	490	0	908	3	591	1

Pieriga						Zemgale						Kurzeme					
<1 year		1–2 years		>2 years		<1 year		1–2 years		>2 years		<1 year		1–2 years		>2 years	
A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
54	7	158	4	0	1	113	5	192	6	1	0	46	4	74	3	1	0
500	28	852	46	123	7	737	7	1,192	12	82	2	1	17	3	10	0	1
880	40	1,683	59	847	57	378	12	1,126	29	403	7	113	8	683	70	263	24
411	28	1,837	75	728	48	240	34	1,773	50	494	14	209	51	2,042	141	802	81
172	2	844	19	390	20	112	0	787	1	274	2	279	11	2,698	74	968	23

Area East						Area West					
<1 year		1–2 years		>2 years		<1 year		1–2 years		>2 years	
A	P	A	P	A	P	A	P	A	P	A	P
1,799	80	2,478	70	2	0	213	26	424	13	2	1
4,550	117	6,464	275	987	24	1,238	65	2,047	68	205	10
1,599	64	3,002	96	1,545	72	1,371	76	3,492	158	1,513	88
1,082	35	2,239	46	1,305	29	860	118	5,652	266	2,024	143
1,033	5	1,724	5	1,107	4	563	13	4,329	94	1,632	45

Note: A illustrates the number of samples originating from active surveillance (hunting of apparently healthy wild boar) and P from passive surveillance (found dead, shot sick or road traffic accident).

TABLE A2 Prevalence estimates and the 95% confidence interval of wild boar with an ASFV PCR-positive results, irrespective of the serological test result, stratified by region, hunting season and age class

	<1 year			1–2 years			>2 years		
	Prevalence	Lower 95% CI	Upper 95% CI	Prevalence	Lower 95% CI	Upper 95% CI	Prevalence	Lower 95% CI	Upper 95% CI
Latgale									
2014/2015	0.038	0.028	0.050	0.022	0.017	0.028	0.000	0.000	0.975
2015/2016	0.042	0.033	0.053	0.042	0.038	0.047	0.020	0.009	0.038
2016/2017	0.039	0.028	0.053	0.049	0.044	0.055	0.026	0.015	0.040
2017/2018	0.069	0.050	0.092	0.053	0.048	0.058	0.028	0.017	0.042
2018/2019	0.024	0.013	0.040	0.030	0.026	0.035	0.017	0.008	0.033
Vidzeme									
2014/2015	0.068	0.049	0.092	0.043	0.030	0.058	0.000	0.000	0.975
2015/2016	0.063	0.055	0.073	0.055	0.048	0.062	0.028	0.016	0.045
2016/2017	0.088	0.067	0.112	0.057	0.047	0.069	0.060	0.045	0.077
2017/2018	0.059	0.040	0.083	0.039	0.029	0.052	0.021	0.011	0.036
2018/2019	0.000	0.000	0.008	0.002	0.000	0.008	0.000	0.000	0.006
Pieriga									
2014/2015	0.066	0.018	0.159	0.000	0.000	0.023	0.000	0.000	0.975
2015/2016	0.070	0.050	0.095	0.055	0.041	0.072	0.069	0.032	0.127
2016/2017	0.073	0.057	0.092	0.051	0.041	0.062	0.055	0.041	0.072
2017/2018	0.125	0.096	0.160	0.049	0.039	0.059	0.058	0.043	0.077
2018/2019	0.046	0.020	0.089	0.037	0.025	0.052	0.017	0.007	0.035
Zemgale									
2014/2015	0.025	0.005	0.073	0.010	0.001	0.036	0.000	0.000	0.975
2015/2016	0.011	0.005	0.021	0.008	0.004	0.015	0.024	0.003	0.083
2016/2017	0.033	0.018	0.056	0.031	0.022	0.043	0.007	0.002	0.021
2017/2018	0.161	0.119	0.210	0.041	0.032	0.051	0.047	0.031	0.069
2018/2019	0.009	0.000	0.049	0.033	0.022	0.048	0.014	0.004	0.037
Kurzeme									
2014/2015	0.000	0.000	0.071	0.000	0.000	0.047	0.000	0.000	0.975
2015/2016	0.000	0.000	0.185	0.000	0.000	0.247	0.000	0.000	0.975
2016/2017	0.025	0.005	0.071	0.090	0.071	0.113	0.076	0.048	0.113
2017/2018	0.177	0.133	0.229	0.081	0.070	0.093	0.083	0.065	0.103
2018/2019	0.048	0.027	0.080	0.041	0.034	0.049	0.033	0.023	0.046
Area East									
2014/2015	0.047	0.038	0.058	0.025	0.019	0.032	0.000	0.000	0.842
2015/2016	0.056	0.050	0.063	0.047	0.042	0.052	0.025	0.016	0.036
2016/2017	0.058	0.047	0.070	0.045	0.038	0.053	0.044	0.034	0.055
2017/2018	0.064	0.051	0.080	0.039	0.032	0.048	0.025	0.017	0.035
2018/2019	0.013	0.007	0.021	0.009	0.005	0.014	0.008	0.004	0.015
Area West									
2014/2015	0.031	0.012	0.062	0.005	0.001	0.016	0.005	0.000	0.708
2015/2016	0.035	0.026	0.046	0.028	0.021	0.036	0.028	0.026	0.090
2016/2017	0.058	0.046	0.071	0.053	0.046	0.061	0.053	0.037	0.058
2017/2018	0.149	0.127	0.173	0.058	0.052	0.065	0.058	0.055	0.077
2018/2019	0.040	0.025	0.059	0.039	0.033	0.045	0.039	0.019	0.035

TABLE A3 Prevalence estimates of wild boar with an ASFV PCR-negative and a seropositive test results, stratified by region, hunting season and age class

	<1 year			1–2 years			>2 years		
	Prevalence	Lower 95% CI	Upper 95% CI	Prevalence	Lower 95% CI	Upper 95% CI	Prevalence	Lower 95% CI	Upper 95% CI
Latgale									
2014/2015	0.006	0.003	0.013	0.002	0.000	0.005	0.000	0.000	0.975
2015/2016	0.015	0.009	0.022	0.020	0.015	0.026	0.023	0.011	0.042
2016/2017	0.028	0.019	0.040	0.028	0.020	0.040	0.026	0.016	0.041
2017/2018	0.029	0.017	0.046	0.035	0.025	0.049	0.033	0.021	0.048
2018/2019	0.007	0.002	0.019	0.025	0.015	0.038	0.037	0.022	0.057
Vidzeme									
2014/2015	0.021	0.010	0.036	0.001	0.000	0.007	0.000	0.000	0.975
2015/2016	0.024	0.019	0.030	0.017	0.013	0.021	0.016	0.007	0.031
2016/2017	0.068	0.049	0.091	0.040	0.031	0.050	0.040	0.028	0.056
2017/2018	0.053	0.035	0.077	0.046	0.035	0.059	0.094	0.072	0.120
2018/2019	0.006	0.001	0.018	0.029	0.019	0.042	0.078	0.058	0.102
Pieriga									
2014/2015	0.000	0.000	0.066	0.000	0.000	0.023	0.000	0.000	0.066
2015/2016	0.055	0.037	0.079	0.024	0.015	0.037	0.055	0.037	0.079
2016/2017	0.011	0.005	0.021	0.017	0.011	0.024	0.011	0.005	0.021
2017/2018	0.012	0.004	0.028	0.016	0.011	0.023	0.012	0.004	0.028
2018/2019	0.047	0.021	0.091	0.038	0.026	0.053	0.047	0.021	0.091
Zemgale									
2014/2015	0.000	0.000	0.033	0.000	0.000	0.019	0.000	0.000	0.975
2015/2016	0.010	0.004	0.020	0.005	0.002	0.011	0.012	0.000	0.066
2016/2017	0.027	0.013	0.049	0.009	0.004	0.017	0.007	0.002	0.022
2017/2018	0.030	0.012	0.060	0.039	0.030	0.049	0.026	0.014	0.045
2018/2019	0.054	0.020	0.113	0.055	0.040	0.073	0.055	0.031	0.089
Kurzeme									
2014/2015	0.000	0.000	0.079	0.000	0.000	0.049	0.000	0.000	0.975
2015/2016	0.000	0.000	0.975	0.000	0.000	0.708	0.000	0.000	0.975
2016/2017	0.009	0.000	0.049	0.004	0.001	0.013	0.004	0.000	0.021
2017/2018	0.010	0.001	0.034	0.012	0.008	0.018	0.015	0.008	0.026
2018/2019	0.029	0.013	0.056	0.025	0.019	0.031	0.021	0.013	0.032
Area East									
2014/2015	0.011	0.006	0.017	0.002	0.000	0.004	0.000	0.000	0.842
2015/2016	0.021	0.017	0.025	0.018	0.015	0.021	0.019	0.012	0.030
2016/2017	0.043	0.033	0.054	0.035	0.029	0.043	0.034	0.025	0.044
2017/2018	0.040	0.029	0.053	0.041	0.033	0.050	0.061	0.048	0.075
2018/2019	0.007	0.003	0.014	0.027	0.020	0.036	0.059	0.046	0.074
Area West									
2014/2015	0.000	0.000	0.018	0.000	0.000	0.009	0.000	0.000	0.708
2015/2016	0.028	0.019	0.039	0.013	0.008	0.019	0.024	0.008	0.056
2016/2017	0.015	0.010	0.024	0.012	0.009	0.016	0.015	0.009	0.022
2017/2018	0.016	0.009	0.027	0.022	0.018	0.026	0.025	0.019	0.033
2018/2019	0.039	0.025	0.059	0.033	0.028	0.038	0.036	0.028	0.046

TABLE A4 Prevalence estimates of wild boar with an ASFV PCR-positive and a seropositive test results, stratified by region, hunting season and age class

	<1 year			1–2 years			>2 years		
	Prevalence	Lower 95% CI	Upper 95% CI	Prevalence	Lower 95% CI	Upper 95% CI	Prevalence	Lower 95% CI	Upper 95% CI
Latgale									
2014/2015	0.007	0.003	0.014	0.001	0.000	0.003	0.000	0.000	0.975
2015/2016	0.010	0.006	0.016	0.004	0.002	0.008	0.000	0.000	0.009
2016/2017	0.011	0.006	0.020	0.004	0.001	0.010	0.001	0.000	0.008
2017/2018	0.008	0.003	0.020	0.009	0.004	0.017	0.007	0.002	0.016
2018/2019	0.019	0.009	0.034	0.006	0.002	0.014	0.006	0.001	0.017
Vidzeme									
2014/2015	0.002	0.000	0.010	0.000	0.000	0.004	0.000	0.000	0.975
2015/2016	0.016	0.012	0.021	0.004	0.003	0.007	0.000	0.000	0.007
2016/2017	0.014	0.006	0.026	0.007	0.003	0.012	0.004	0.001	0.011
2017/2018	0.014	0.006	0.029	0.012	0.007	0.020	0.005	0.001	0.015
2018/2019	0.000	0.000	0.008	0.001	0.000	0.006	0.000	0.000	0.006
Pieriga									
2014/2015	0.000	0.000	0.066	0.000	0.000	0.023	0.000	0.000	0.975
2015/2016	0.006	0.001	0.018	0.002	0.000	0.009	0.008	0.000	0.045
2016/2017	0.017	0.010	0.028	0.008	0.004	0.013	0.002	0.000	0.009
2017/2018	0.036	0.021	0.059	0.007	0.003	0.011	0.004	0.001	0.012
2018/2019	0.035	0.013	0.075	0.008	0.003	0.017	0.000	0.000	0.009
Zemgale									
2014/2015	0.000	0.000	0.033	0.000	0.000	0.019	0.000	0.000	0.975
2015/2016	0.003	0.000	0.010	0.002	0.000	0.006	0.000	0.000	0.043
2016/2017	0.011	0.003	0.027	0.004	0.001	0.009	0.000	0.000	0.009
2017/2018	0.025	0.009	0.055	0.007	0.004	0.013	0.006	0.001	0.017
2018/2019	0.009	0.000	0.049	0.009	0.004	0.018	0.004	0.000	0.020
Kurzeme									
2014/2015	0.000	0.000	0.079	0.000	0.000	0.049	0.000	0.000	0.975
2015/2016	0.000	0.000	0.975	0.000	0.000	0.708	0.000	0.000	0.975
2016/2017	0.000	0.000	0.033	0.004	0.001	0.013	0.000	0.000	0.014
2017/2018	0.000	0.000	0.018	0.011	0.007	0.016	0.004	0.001	0.011
2018/2019	0.007	0.001	0.026	0.008	0.005	0.012	0.004	0.001	0.011
Area East									
2014/2015	0.006	0.003	0.010	0.000	0.000	0.002	0.000	0.000	0.842
2015/2016	0.014	0.011	0.018	0.004	0.003	0.006	0.000	0.000	0.004
2016/2017	0.012	0.007	0.019	0.006	0.003	0.009	0.003	0.001	0.007
2017/2018	0.011	0.006	0.019	0.011	0.007	0.016	0.006	0.003	0.012
2018/2019	0.010	0.005	0.018	0.003	0.001	0.008	0.003	0.001	0.008
Area West									
2014/2015	0.000	0.000	0.018	0.000	0.000	0.009	0.000	0.000	0.708
2015/2016	0.004	0.001	0.010	0.002	0.001	0.005	0.005	0.000	0.027
2016/2017	0.014	0.008	0.022	0.006	0.004	0.009	0.001	0.000	0.005
2017/2018	0.025	0.015	0.037	0.008	0.006	0.011	0.004	0.002	0.008
2018/2019	0.016	0.007	0.030	0.008	0.006	0.011	0.003	0.001	0.007

TABLE A5 Prevalence estimates of wild boar with an ASF PCR-positive and a seronegative test results, stratified by region, hunting season and age class

	<1 year			1–2 years			>2 years		
	Prevalence	Lower 95% CI	Upper 95% CI	Prevalence	Lower 95% CI	Upper 95% CI	Prevalence	Lower 95% CI	Upper 95% CI
Latgale									
2014/2015	0.009	0.004	0.016	0.005	0.003	0.010	0.000	0.000	0.975
2015/2016	0.017	0.011	0.025	0.007	0.004	0.011	0.005	0.001	0.017
2016/2017	0.011	0.006	0.020	0.008	0.003	0.014	0.003	0.000	0.010
2017/2018	0.039	0.025	0.058	0.013	0.007	0.022	0.010	0.004	0.020
2018/2019	0.004	0.000	0.013	0.010	0.004	0.019	0.008	0.002	0.020
Vidzeme									
2014/2015	0.007	0.002	0.019	0.002	0.000	0.009	0.000	0.000	0.975
2015/2016	0.024	0.019	0.031	0.012	0.009	0.016	0.005	0.001	0.016
2016/2017	0.020	0.011	0.035	0.018	0.012	0.025	0.007	0.003	0.016
2017/2018	0.010	0.003	0.024	0.010	0.005	0.017	0.003	0.000	0.012
2018/2019	0.000	0.000	0.008	0.000	0.000	0.004	0.000	0.000	0.006
Pieriga									
2014/2015	0.000	0.000	0.066	0.000	0.000	0.023	0.000	0.000	0.975
2015/2016	0.035	0.020	0.055	0.018	0.010	0.029	0.016	0.002	0.058
2016/2017	0.017	0.010	0.028	0.020	0.014	0.028	0.009	0.004	0.019
2017/2018	0.036	0.021	0.059	0.016	0.011	0.023	0.011	0.005	0.022
2018/2019	0.006	0.000	0.032	0.021	0.013	0.034	0.003	0.000	0.014
Zemgale									
2014/2015	0.000	0.000	0.033	0.000	0.000	0.019	0.000	0.000	0.975
2015/2016	0.005	0.001	0.014	0.003	0.001	0.009	0.012	0.000	0.066
2016/2017	0.008	0.002	0.023	0.012	0.006	0.020	0.000	0.000	0.009
2017/2018	0.021	0.007	0.049	0.016	0.011	0.023	0.016	0.007	0.032
2018/2019	0.000	0.000	0.032	0.023	0.014	0.036	0.004	0.000	0.020
Kurzeme									
2014/2015	0.000	0.000	0.079	0.000	0.000	0.049	0.000	0.000	0.975
2015/2016	0.000	0.000	0.975	0.000	0.000	0.708	0.000	0.000	0.975
2016/2017	0.018	0.002	0.064	0.029	0.018	0.045	0.034	0.016	0.064
2017/2018	0.014	0.003	0.042	0.022	0.016	0.029	0.013	0.006	0.023
2018/2019	0.011	0.002	0.031	0.015	0.011	0.020	0.017	0.009	0.027
Area East									
2014/2015	0.008	0.005	0.014	0.004	0.002	0.008	0.000	0.000	0.842
2015/2016	0.022	0.018	0.027	0.010	0.008	0.013	0.005	0.002	0.012
2016/2017	0.014	0.009	0.022	0.014	0.010	0.019	0.005	0.002	0.010
2017/2018	0.026	0.017	0.037	0.011	0.007	0.016	0.007	0.003	0.013
2018/2019	0.002	0.000	0.007	0.005	0.002	0.009	0.004	0.001	0.009
Area West									
2014/2015	0.000	0.000	0.018	0.000	0.000	0.009	0.000	0.000	0.708
2015/2016	0.017	0.011	0.026	0.009	0.006	0.015	0.015	0.003	0.042
2016/2017	0.015	0.009	0.023	0.019	0.015	0.025	0.011	0.007	0.018
2017/2018	0.027	0.017	0.040	0.018	0.015	0.022	0.013	0.008	0.019
2018/2019	0.007	0.002	0.018	0.018	0.014	0.022	0.011	0.007	0.017

TABLE A6 Prevalence estimates of wild boar with an ASFV PCR-positive results, irrespective of the serological test result, stratified by region, hunting season and origin of carcass

	A			P		
	Prevalence	Lower 95% CI	Upper 95% CI	Prevalence	Lower 95% CI	Upper 95% CI
Latgale						
2014/2015	0.011	0.007	0.015	0.684	0.567	0.786
2015/2016	0.016	0.013	0.021	0.861	0.781	0.920
2016/2017	0.013	0.009	0.018	0.800	0.687	0.886
2017/2018	0.027	0.021	0.035	0.745	0.610	0.853
2018/2019	0.017	0.012	0.024	0.300	0.067	0.652
Vidzeme						
2014/2015	0.008	0.005	0.013	0.752	0.688	0.810
2015/2016	0.024	0.021	0.028	0.801	0.760	0.838
2016/2017	0.023	0.018	0.028	0.855	0.794	0.904
2017/2018	0.019	0.014	0.025	0.778	0.655	0.873
2018/2019	0.001	0.000	0.003	0.400	0.053	0.853
Pieriga						
2014/2015	0.000	0.000	0.015	0.211	0.061	0.456
2015/2016	0.027	0.020	0.037	0.652	0.546	0.749
2016/2017	0.026	0.021	0.032	0.746	0.673	0.809
2017/2018	0.028	0.022	0.035	0.706	0.629	0.774
2018/2019	0.023	0.016	0.033	0.341	0.201	0.506
Zemgale						
2014/2015	0.000	0.000	0.011	0.417	0.152	0.723
2015/2016	0.006	0.003	0.011	0.483	0.294	0.675
2016/2017	0.013	0.008	0.019	0.593	0.457	0.719
2017/2018	0.026	0.020	0.033	0.796	0.703	0.871
2018/2019	0.025	0.017	0.035	0.750	0.194	0.994
Kurzeme						
2014/2015	0.000	0.000	0.030	0.000	0.000	0.265
2015/2016	0.000	0.000	0.459	0.000	0.000	0.109
2016/2017	0.032	0.022	0.044	0.561	0.465	0.654
2017/2018	0.027	0.022	0.034	0.778	0.724	0.826
2018/2019	0.022	0.018	0.027	0.696	0.602	0.780
Area East						
2014/2015	0.010	0.007	0.013	0.734	0.678	0.785
2015/2016	0.021	0.019	0.024	0.813	0.777	0.845
2016/2017	0.018	0.015	0.022	0.840	0.787	0.883
2017/2018	0.023	0.019	0.028	0.763	0.676	0.836
2018/2019	0.009	0.006	0.012	0.333	0.118	0.616
Area West						
2014/2015	0.000	0.000	0.005	0.209	0.100	0.360
2015/2016	0.015	0.011	0.020	0.484	0.402	0.566
2016/2017	0.023	0.019	0.027	0.658	0.605	0.708
2017/2018	0.027	0.024	0.031	0.759	0.721	0.795
2018/2019	0.023	0.019	0.027	0.605	0.524	0.682

Note: Column A contains the numbers of samples obtained by active surveillance (hunting of apparently healthy wild boar) and P by passive surveillance (found dead, shot sick or road traffic accident).

3.2.2.4 African swine fever in the Lithuanian wild boar population in 2018: a snapshot

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Highlights

- ASFV still circulated within the Lithuanian wild boar population in 2018
- In the centre of Lithuania, higher prevalence estimates were observed
- Prevalence estimates remained stable over the year 2018
- ASFV prevalence estimates showed highest values in wild boar found dead

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Virology Journal

SHORT REPORT Open Access

African swine fever in the Lithuanian wild boar population in 2018: a snapshot

Arnoldas Pautienius[1,5†], Katja Schulz[2*†], Christoph Staubach[2], Juozas Grigas[1,5], Ruta Zagrabskaite[3], Jurate Buitkuviene[3], Rolandas Stankevicius[4], Zaneta Streimikyte[5], Vaidas Oberauskas[1], Dainius Zienius[5], Algirdas Salomskas[6], Carola Sauter-Louis[2] and Arunas Stankevicius[1]

Abstract

The first cases of African swine fever (ASF) were detected in the Lithuanian wild boar population in 2014. Since then, the disease spread slowly through the whole country, affecting both, wild boar and domestic pigs. In the other Baltic states, which both are also affected by ASF since 2014, the recent course of ASF prevalence suggests that the countries might be well under way of disease elimination. In contrast, in Lithuania the epidemic seems to be still in full progress. In the present study, we aimed to extend a previous prevalence study in Lithuania. Looking at ASF virus (ASFV) and seroprevalence estimates of wild boar in all months of 2018 and in all affected municipalities in Lithuania, the course of ASF was evaluated on a temporal and spatial scale. A non-spatial beta-binomial model was used to correct for under- or overestimation of the average prevalence estimates. Within 2018 no big differences between the prevalence estimates were seen over time. Despite of the lower sample size, highest ASFV prevalence estimates were found in dead wild boar, suggesting higher detection rates through passive surveillance than through active surveillance. Accordingly, with the maximum prevalence of 87.5% in May 2018, the ASFV prevalence estimates were very high in wild boar found dead. The number of samples originating from hunted animals (active surveillance) predominated clearly. However, the ASFV prevalence in those animals was lower with a maximum value of 2.1%, emphasizing the high value of passive surveillance. A slight increase of the seroprevalence in hunted wild boar could be seen over time. In the center of Lithuania, a cluster of municipalities with high ASFV and seroprevalence estimates was found. The results of the study indicate that ASFV is still circulating within the Lithuanian wild boar population, constituting a permanent risk of disease transmission into domestic pig holdings. However, additional, more recent data analyses are necessary to re-evaluate the course of ASF in Lithuania and thus, to be able to make a statement about the stage of the ASF epidemic in the country. This is of huge importance for Lithuania for evaluating control measures and their efficacy, but also for neighbouring countries to assess the risk of disease spread from Lithuania.

Keywords: Prevalence, African swine fever virus, Wild boar, Surveillance

Introduction

African swine fever (ASF) emerged in Lithuania in 2014 [1, 2]. First ASF cases occurred in wild boar close to the border to Belarus in January 2014. Six months later, the first ASF outbreaks in domestic pigs were also reported from eastern Lithuania, suggesting disease introduction from Belarus [2]. Since then, the disease has spread through the wild boar population towards the West, affecting almost the whole country by the year 2020 [1, 3]. It is known that an infected wild boar population

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poses a risk for disease introduction into domestic pig farms [4–7], thus threatening the economy of the affected country [8]. Disease surveillance in wild boar is therefore of utmost importance to evaluate the course of the epidemic and thus to assess and, if necessary, to increase the effectiveness of the control measures implemented. Recent studies showed that ASF laboratory test results from wild boar samples and their different prevalence estimates (ASF virus (ASFV) and seroprevalence estimates) follow a similar epidemiological pattern over time depending on the particular stage of the epidemic [1, 9–11]. In case of a longer-lasting epidemic like in Estonia and Latvia, a decrease of ASFV prevalence estimates were observed, whereas the prevalence of wild boar being seropositive increased within both countries [9, 11, 12]. It is hypothesized that this course indicates the decline of circulating ASFV and a country obtaining such results might be on its way of disease elimination [9, 11, 12]. In a previous study, Lithuanian surveillance data from 2014 to 2017 yielded a clear increase in the ASFV prevalence estimates in wild boar found dead (from 20.1% in 2014 to 79.68% in 2017) whereas the average seroprevalence during 2014–2017 was low (0.45%) suggesting that the ASF epidemic in Lithuania was still in full progress by 2017 [1].

The present study aimed to extend this previous study and to gain further insight into the ASF epidemic in the Lithuanian wild boar population. Therefore, ASF surveillance data of 2018 were analysed to highlight recent changes in the epidemiology of ASF and to facilitate further, more comprehensive prevalence studies. In addition, the epidemiological course of ASF in Lithuania should be compared with the courses in the two other Baltic States. Differences in surveillance and control strategies should be identified and potentially adapted.

Materials and methods

ASF surveillance data

ASF wild boar surveillance data originated from the national surveillance program and were provided by the National Food and Veterinary Risk Assessment Institute. The data was obtained from active (hunted wild boar) and passive (wild boar found dead) surveillance and from all areas in Lithuania, which were affected by ASF in wild boar. In these areas, all hunted wild boar and each wild boar found dead were sampled, thus providing the greatest possible sample size. Sample processing is described in detail elsewhere [1]. In brief, blood samples from hunted animals were investigated for ASFV-specific antibodies by using a commercial blocking (Ingezim Compac 1.1. PPA K3, Spain) ELISA. Tissue samples originating from passive and active surveillance were examined for ASFV by PCR. Surveillance data was available for each month of 2018. The 10 counties of Lithuania are divided into 60 municipalities [3]. Analyses were done on municipality level. Aggregated surveillance data of 2018 was available for each of the 37 affected municipalities of Lithuania. The data set included information about the number of wild boars sampled (per month and per municipality), origin of sample (active/passive surveillance) and the type of laboratory investigation.

Prevalence estimations

Similar to previous studies [9, 11, 12], for analyses, samples were grouped depending on their laboratory results.

Prevalence estimates were calculated for samples from hunted animals (active surveillance), which showed the following test results:

1 seropositive and PCR-negative (group 1)
2 seropositive and PCR-positive (group 2)
3 seronegative and PCR-positive (group 3).

In addition, prevalence estimates for wild boar, whose samples came from passive surveillance and were PCR-positive without any investigations for ASF antibodies (group 4) were determined. Thus, samples that were positive for either ASF antibodies or genome or both were considered as positive cases for their respective groups mentioned above.

Prevalence estimates within the different groups were calculated for each month and each municipality. Raw prevalence estimates for each group were calculated by dividing the number of samples showing the appropriate test result (e.g. seropositive and PCR-negative etc.) through the total number of samples that were investigated by ELISA and PCR (in case of passive surveillance, samples were solely examined by PCR) (Additional file 1: Table S1–S8) [9, 11].

Estimated raw prevalence and 95% confidence intervals for each group per municipality and per month were calculated by using the software package R (https://www.r-project.org).

Model analysis

To avoid over- and underestimation of the true prevalence per spatial and time unit due to the heterogeneous sampling effort and population density, a non-spatial beta-binomial model was used. The methodology and software used to calculate the posterior point estimate of the true disease prevalence (corrected prevalence) and 95% confidence intervals for all groups per municipality and for each month of 2018 are described elsewhere [13].

Results

Overall, 11,366 serum samples and 14,441 serum or tissue samples were tested for ASFV-specific antibodies and ASFV DNA, respectively. From the investigated samples, 11,366 samples originated from active and 3,352 samples from passive surveillance. Until the end of 2018, 37 Lithuanian municipalities out of 60 were affected by ASF cases in wild boar. For analyses within groups 1–3, the same number of samples was available since all samples, which were investigated for ASFV and ASFV-specific antibodies were included. Thus, in these groups, the highest number of samples was investigated in January 2018 (n = 1,614), whereas the lowest number of samples was investigated in April 2018 (n = 325) (Additional file 1: Tables S1, S2 and S3). The highest number of samples in 2018 came from the western municipality Telsiu r. sav. (n = 799) and the lowest number from the eastern municipality Svencioniu r. sav. (n = 1) (Additional file 1: Tables S5, S6 and S7).

The number of samples originating from passive surveillance was also highest in January (n = 647), whereas it showed the lowest value in December 2018 (n = 95) (Additional file 1: Table S4). In the central municipality Panevezio r. sav., the highest number of wild boar found dead was sampled within 2018 (n = 359). In the western municipality Kelmes r. sav., only one sample was collected in 2018 (Additional file 1: Table S8).

Temporal analyses

Group 1

In the months January–July 2018, the corrected seroprevalence was slightly higher than the raw seroprevalence. Particularly in April, the biggest difference was found (raw prevalence: 0.0%; CI 0.0–1.1% vs. corrected prevalence: 0.9%; CI 0.4–1.4%). In the remaining months of 2018, the corrected prevalence was lower than the raw prevalence estimates. A slight increase of the seroprevalence was seen from July 2018–October 2018. (Fig. 1; Additional file 1: Table S1).

Group 2

The prevalence of wild boar showing samples being seropositive and positive for ASFV were lower than the prevalence of the previous group with no corrected prevalence being higher than 0.4%. In most of the month, the raw and the corrected prevalence were similar or the corrected prevalence higher, respectively. However, In January, February, September and October, the corrected prevalence was lower than the raw prevalence. No clear differences were found between the months of 2018 for both, the calculated raw and corrected prevalence estimates (Additional file 1: Table S2 and Fig. S1).

Group 3

In group 3, prevalence estimates were calculated from wild boar yielding a seronegative and PCR-positive

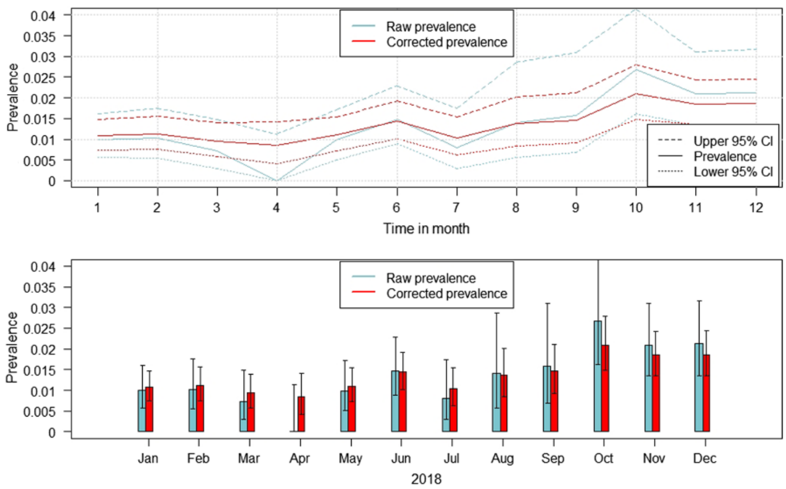

Fig. 1 Estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) of hunted wild boar showing a seropositive and a PCR-negative ASF sample for each month of 2018. The whiskers indicate 95% confidence intervals

test result. In contrast to the two previous groups, in group 3 the highest corrected prevalence was calculated for January (2.1%; CI 1.7–2.7%), even though it was lower than the raw prevalence (2.4%; CI 1.7–3.3%). The prevalence decreased slightly until April, showed higher values in June and July but decreased again until November. In December the prevalence was similar to January (raw prevalence: 2.3%; CI 1.5–3.4% vs. corrected prevalence: 2.0%; CI 1.5–2.6%). In the 3 months with higher prevalence estimates (January, December and July), the corrected prevalence was lower than the raw prevalence whereas in the remaining month, it was slightly higher (Additional file 1: Table S3 and Fig. S2).

Group 4

In group 4, only samples from passive surveillance were included. Thus, prevalence estimates for wild boar yielding a PCR-positive test result and for which no test for ASFV-specific antibodies was done, were calculated. The highest corrected prevalence estimates were found in January (86.2%; CI 83.9–88.4%), in April (86.2%; CI 83.8–88.5%) and in May (87.5%; CI 84.2–90.5%). The corrected prevalence estimates in July, August, September and October were clearly lower with the lowest prevalence in October (29.9%; CI 25.2–34.7%) (Fig. 2, Additional file 1: Table S4).

Spatial analyses

Group 1

For wild boar yielding seropositive and ASFV-negative samples, in most municipalities, the raw estimated and the corrected seroprevalences were similar. Four municipalities in the northern center of Lithuania showed the highest corrected prevalences (Ukmerges r. sav. (13.8%; CI 7.1–27.7%), Panevezio r. sav. (13.2%; CI 6.3–30.5%), Sirvintu r. sav. (21.6%; CI 14.4–32.1%), Anyksciu r. sav. (20.6%; CI 13.0–33.9%). However, in all these municipalities, the sample size was relatively low, which yielded a wide range of the 95% confidence interval. Several municipalities in the western part of the country showed low prevalences but at the same time high sample sizes and therefore narrow ranges of the 95% confidence intervals (Fig. 3, Additional file 1: Table S5).

Group 2

The prevalence of animals being positive for both ASFV and ASF-specific antibodies was also clearly lower than in the remaining groups. The highest value was found in Panevezio r. sav., however, due to the very small sample size, the confidence interval was very wide (raw prevalence: 8.3%; CI 1.8–22.5% vs. corrected prevalence: 3.5%; CI 1.1–6.9%). In 14 municipalities, the raw prevalence was 0%; however, the corrected prevalence yielded slightly higher values. In contrast, in the six municipalities with the highest raw prevalences (Kupiskio r.

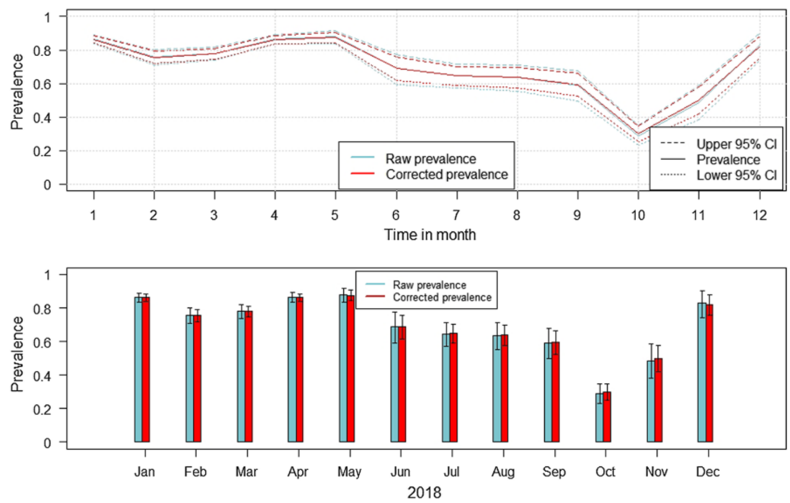

Fig. 2 Estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) of wild boar found dead and showing a PCR-positive ASF sample for each month of 2018. The whiskers indicate 95% confidence intervals

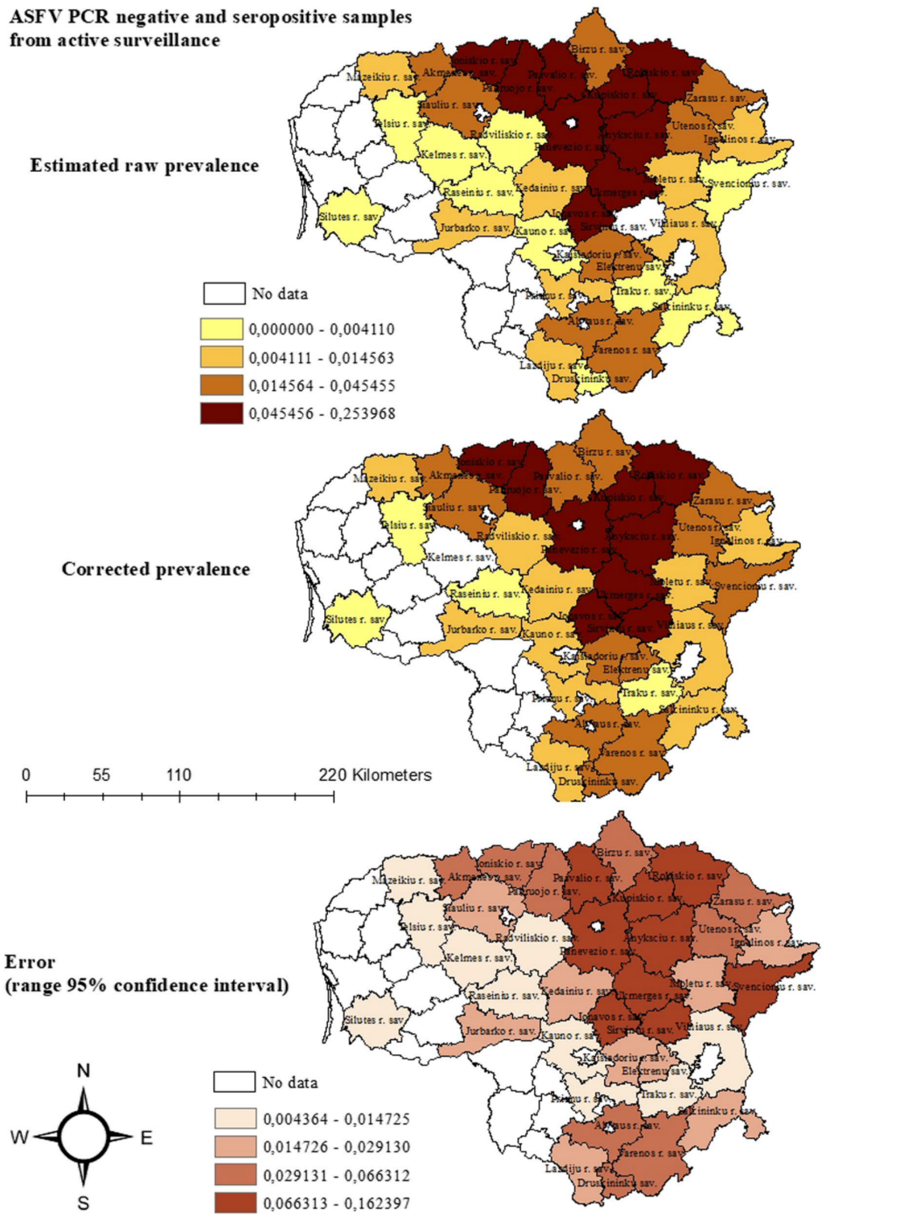

Fig. 3 Estimated raw and corrected prevalence with 95% confidence intervals (calculated using a non-spatial beta-binomial model) of hunted wild boar showing a seropositive ASF sample, but a PCR-negative test result for each ASF-affected municipality of Lithuania

sav., Anyksciu r. sav., Druskininku sav., Pakruojo r. sav., Ukmerges r. sav., Panevezio r. sav.), the corrected prevalences were slightly lower (Additional file 1: Table S6 and Fig. S3).

Group 3

The highest corrected prevalence for hunted wild boar showing a seronegative but PCR-positive test result was found in the municipality Pakruojo r. sav. in the center (5.3%; CI 3.1–8.0%). In contrast to the prevalences of the other groups, the corrected prevalence was also higher in Zarasu r. sav. in the east of the country (3.1%; CI 1.5–5.3%) and in Kedainiu r. sav. in the center (3.4%; CI 2.2–4.7%). The raw and the corrected prevalences were very similar and in cases of a high raw prevalence, the corrected prevalence was slightly lower, whereas in cases of a low raw prevalence, the corrected prevalence was slightly higher (Additional file 1: Table S7 and Fig. S4).

Group 4

Similar to the temporal analysis of this group, also on spatial level, the raw and the corrected prevalences were very high with small confidence intervals. The highest corrected prevalence was found in Panevezio r. sav. (99.1%; CI 98.1–99.7%). The lowest corrected prevalence was found in Svencioniu r. sav. in the east but still with a value of 21.9% (CI 17–27.2%). In the southern municipality Druskininku sav., the raw prevalence was 100% (CI 63.1–100%), however, due to the small sample size, the corrected prevalence was 89% (CI 72.7–98.6%) (Additional file 1: Table S8 and Fig. S5).

Discussion

Lithuania is affected by ASF since 2014 [2]. Several studies suggest that the course of disease within wild boar populations follows similar patterns in different countries [9, 11]. It was found that after a period of approximately 100 days, in which infected animals can be tested positive for ASFV and ASFV-specific antibodies at the same time, surviving animals are seropositive for an unknown period of time [14, 15]. Due to a long half-life of ASFV-specific antibodies, it might well be that this period lasts for several years [16, 17]. In countries in which an increase of seropositive and simultaneously a decrease in ASFV-positive wild boar was found, a decline of ASF incidence and a subsiding stage of the epidemic was hypothesized [9, 11]. Following these observations and as a logical extension of a previous Lithuanian study [1], we aimed to analyse ASF surveillance data in wild boar and thereby to evaluate the course of the disease in 2018.

Taking the assumed role of the individual test results within the course of ASF into account, we divided the samples in different groups depending on the results. To see the difference in the prevalence estimates between hunted animals and those who were found dead, samples were additionally divided depending on their origin (active or passive surveillance). The model was used to take the different conditions in the individual months and municipalities into account. Usually, the main hunting activities take place in the winter months. Thus, for the groups, analysing samples from active surveillance, the corrected prevalence was higher than the raw prevalence in months, in which the hunting effort is usually low (e.g. in April). Therefore, although the calculated prevalence estimates were very low in these months, the prevalence estimates obtained through the model helped to correct for such external conditions and falsified conclusions could be avoided. The same applied for the spatial analyses, where the model took the prevalence estimates of the individual municipalities in the area into account by shrinking the estimates to the global mean, therefore corrected for under- or overestimations due to different sampling behaviour or different population densities.

Between 2016 and 2017, the seroprevalence in Lithuanian wild boar increased clearly [1]. This increase seemed to continue in 2018. However, although a slight increase of the seroprevalence was seen by the end of 2018, these changes were not significant. In contrast, the difference in the mean prevalence of ASFV-positive wild boar between 2017 and 2018 was negligible [1]. Also within 2018, despite of the seasonal fluctuations, no significant change was seen in the ASFV prevalence, particularly not in wild boar samples from hunted wild boar. The higher ASFV prevalence estimates in the summer months corresponds to the seasonal patterns of ASFV, which were found in other countries [12, 18]. Due to a relatively high sample size and a generally low difference within the individual months, the raw and the corrected prevalence estimates were very similar in wild boar samples originating from passive surveillance. These results support results from a previous study with Lithuanian wild boar data [3]. However, in October and November, the prevalence estimates were lower than in the other months. A similar course was seen in Estonia [9]. These results might be due to a lower number of young wild boar being present in these months, which results from the natural reproductive cycle of wild boar. It is assumed that in the field, the case-fatality ratio is higher in wild boar younger than 1 year [10, 19]. However, due to the lack of information on age of the sampled animals in the present data set, no statements were possible regarding the age distribution within the different results.

Both, the raw and the corrected prevalence estimates of wild boar found dead and being PCR-positive for ASFV were much higher than the prevalence estimates of ASFV positive hunted wild boar. Due to the relative high sample size (mean = 279) and the high number of positive samples, the confidence intervals were smaller and so were the differences between the raw and corrected prevalence within the single months (Additional file 1: Table S4). These findings show the higher ASF detection rate in animals found dead and correlate with results from Estonia and Latvia [7, 10, 11, 19, 20]. In addition, they emphasise once more the need to focus on passive surveillance [3, 9, 10, 19, 21, 22]. The high ASFV prevalence estimates in wild boar found dead and the consistent ASFV prevalence estimates over the year suggest that ASFV is still circulating within the Lithuanian wild boar population. By the end of 2018, the prevalence estimates from wild boar showing ASFV- and seropositive test results at the same time hardly differ from the prevalence estimates at the beginning of 2018. These result support the hypothesis that circulating ASFV is still considerably present, as animals showing such a result are very likely to have become infected within the last 3 months [9, 14, 15]. The spatial analyses showed similar patterns. In the central municipalities, ASFV and seroprevalence where high. However, the 95% confidence interval in these areas was very wide, indicating a small sample size and thus a considerable uncertainty regarding the true prevalence. However, all of these municipalities have borders with previously, highly affected areas [1]. Therefore, also the corrected prevalence estimates were clearly higher in the central municipalities than in the neighbouring municipalities. In a previous study, similar results were obtained and high prevalence estimates were mainly found in Anykščiai et al. [3]. A higher wild boar density in these areas is described as a potential cause for the geographical distribution of prevalence estimates [3]. However, in our study, population density was not included in the analyses as well as other risk-factors like different sampling strategies, human activities and other potential factors supporting the spread of ASF. To pinpoint the true reasons for this ASF cluster in the center of Lithuania, further, more comprehensive studies are necessary.

Conclusion

To evaluate the course of ASF and the effectiveness of disease control in Lithuania, a prevalence study regarding ASF in the Lithuanian wild boar population in 2018 was performed. The results of the present study based on 2018 data show that in contrast to similar studies from other countries in the region, ASF was still active in Lithuania in 2018. Therefore, it is of utmost importance to maintain or even to adapt intensive surveillance and control measures and to regularly evaluate the course of ASF on the basis of laboratory data. Further prevalence studies are necessary to assess the current situation of ASF in Lithuania, not only for Lithuania authorities for evaluating the success of control measures but also for neighbouring countries to estimate the risk of disease introduction from Lithuania.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12985-020-01422-x.

Additional file 1. Table S1: Number of wild boar samples, which were investigated by ELISA to detected ASF specific antibodies and by PCR to detect ASF virus genome. Number of samples from active surveillance, which resulted in a seropositive test results for ASF and a negative PCR test result and the estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) including the 95% confidence intervals for each month of 2018. **Table S2**: Number of wild boar samples, which were investigated by ELISA to detected ASF specific antibodies and by PCR to detect ASF virus genome. Number of samples from active surveillance, which resulted in a seropositive test results for ASF and a positive PCR test result and the estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) including the 95% confidence intervals for each month of 2018. **Table S3**: Number of wild boar samples, which were investigated by ELISA to detected ASF specific antibodies and by PCR to detect ASF virus genome. Number of samples from active surveillance, which resulted in a positive PCR but a negative ELISA test result and the estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) including the 95% confidence intervals for each month of 2018. **Table S4**: Number of wild boar samples, which were investigated only by PCR to detect ASF virus genome. Number of samples from passive surveillance, which resulted in a positive PCR test result and the estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) including the 95% confidence intervals for each month of 2018. **Table S5**: Number of wild boar samples, which were investigated by ELISA to detected ASF specific antibodies and by PCR to detect ASF virus genome. Number of samples from active surveillance, which resulted in a seropositive test results for ASF and a negative PCR test result and the estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) including the 95% confidence intervals for each ASF-affected municipality of Lithuania. **Table S6**: Number of wild boar samples, which were investigated by ELISA to detected ASF specific antibodies and by PCR to detect ASF virus genome. Number of samples from active surveillance, which resulted in a seropositive test results for ASF and a positive PCR test result and the estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) including the 95% confidence intervals for each ASF-affected municipality of Lithuania. **Table S7**: Number of wild boar samples, which were investigated by ELISA to detected ASF specific antibodies and by PCR to detect ASF virus genome. Number of samples from active surveillance, which resulted in a positive PCR but a negative ELISA test result and the estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) including the 95% confidence intervals for each ASF-affected municipality of Lithuania. **Table S8**: Number of wild boar samples, which were investigated only by PCR to detect ASF virus genome. Number of samples from passive surveillance, which resulted in a positive PCR test result and the estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) including the 95% confidence intervals for each ASF-affected municipality of Lithuania. **Figure S1**: Estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) of hunted wild boar showing an ELISA and a PCR-positive test result for each month of 2018. The whiskers indicate 95% confidence intervals. **Figure S2**: Estimated raw and corrected prevalence (calculated using a non-spatial beta-binomial model) of hunted wild boar showing an ELISA-negative but a PCR-positive test result for each month of 2018. The whiskers indicate 95% confidence intervals. **Figure S3**: Estimated raw and corrected prevalence with 95% confidence intervals (calculated using a non-spatial beta-binomial model) of hunted wild boar showing a sero- and PCR-positive ASF sample result for each ASF-affected municipality of Lithuania. **Figure S4**: Estimated raw and corrected prevalence with 95% confidence intervals (calculated using a non-spatial beta-binomial model) of hunted wild boar showing a PCR-positive and a seronegative ASF sample result for each ASF-affected municipality of Lithuania. **Figure S5**: Estimated raw and corrected prevalence with 95% confidence intervals (calculated using a non-spatial beta-binomial model) of wild boar found dead showing a PCR-positive ASF sample result for each ASF-affected municipality of Lithuania.

Abbreviations
ASF: African swine fever; ASFV: African swine fever virus.

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Authors' contributions
AP, KS, AST, CS-L and DZ: study concept und design; KS, AP and CS: analyses and interpretation of data; KS and AP drafting of the manuscript; RS, VO, ASA: data collection JB, RZ, JG and ZS: laboratory studies, CS and CS-L: Critical revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data used are available in the Additional file 1.

Ethics approval and consent to participate
Non-experimental research on in this study was conducted in this study in the National Food and Veterinary Risk Assessment Institute that according to Regulation (EC) No 882/2004 has been designated as a National Reference Laboratory for African Swine Fever. All research activities in this study comply with institutional and national guidelines. Additionally, permanent permission to conduct research with animal samples was issued by the Ethics Commission on the Use of Laboratory Animals (Project No. 0241).

Consent to publication
Not applicable.

Competing interest
The authors declare that they have no competing interests.

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Publisher's Note

3.2.2.5 African Swine Fever and Its Epidemiological Course in Lithuanian Wild Boar

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Highlights

- Most samples were taken in 2017, indicating successful awareness campaigns, incentives and a high willingness of hunters to support ASF control
- The wild boar population density decreases in the course of an ASF epidemic
- Most wild boar carcasses were detected in July
- Also in Lithuania, an accumulation of seropositive wild boar was observed over time

viruses

MDPI

Article

African Swine Fever and Its Epidemiological Course in Lithuanian Wild Boar

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Abstract: African swine fever (ASF) has been present in Lithuania since 2014. It is mainly the wild boar population that is affected. Currently, little is known about the epidemiological course of ASF in Lithuania. In the present study, ASF surveillance data from 2016–2021 were analyzed. The numbers of samples taken from hunted wild boar and wild boar found dead per year and month were recorded and the prevalence was estimated for each study month and administrative unit. A Bayesian space–time model was used to calculate the temporal trend of the prevalence estimates. In addition, population data were analyzed on a yearly basis. Most samples were investigated in 2016 and 2017 and originated from hunted animals. Prevalence estimates of ASF virus-positive wild boar decreased from May 2019 onwards. Seroprevalence estimates showed a slight decrease at the same time, but they increased again at the end of the study period. A significant decrease in the population density was observed over time. The results of the study show that ASF is still present in the Lithuanian wild boar population. A joint interdisciplinary effort is needed to identify weaknesses in the control of ASF in Lithuania and to combat the disease more successfully.

Keywords: disease control; surveillance; epidemiological course; prevalence; population density

1. Introduction

African swine fever (ASF) was first identified in 1921 [1]. In the following decades, it mainly affected the African continent, but after the first introduction into Portugal in 1957, several countries in Europe also experienced outbreaks caused by ASF virus genotype I [2]. The disease was almost completely eliminated from the European continent in 1995; only Sardinia remained endemic until recently [3,4]. In 2007, ASF virus of genotype II was introduced into Georgia, presumably through contaminated waste on a ship arriving from East Africa [5]. Since then, the disease has spread widely, now affecting a large number of countries—not only in Europe, but also in Asia [6–8]. In 2014, the new ASF epidemic also hit the European Union (EU) when the disease was introduced from the east, most probably Belarus. Lithuania was the first affected EU member state [9,10]. Shortly afterwards, but still in the course of the year 2014, the first ASF cases emerged in Poland, Latvia and Estonia [11]. In the following years, further EU member states became affected, including Belgium, the Czech Republic and Germany [12–14]. Except for Romania, where outbreaks in domestic pig holdings dominate [15], the disease is mainly carried by the wild boar population in most affected countries [16,17]. The involvement of wild boar makes the disease extremely difficult to control [18–20], particularly if ASF is introduced by wild boar migrating across borders, as happened in the Baltic states, Poland and recently in Germany [12,21,22]. By contrast, disease elimination has been possible in Belgium and in the Czech Republic, where ASF was introduced only at a single point in time and space, presumably by human activities [14,23]. It is widely known that ASF can be transmitted from domestic pigs to wild boar and vice versa. It is important to note that countries where only the wild boar population is infected suffer from international trade restrictions, even if the domestic pig population if free from ASF [24]. Thus, control of ASF in wild boar and ultimately its elimination is crucial to protect the pig industry, to maintain global trade and to secure the economy of affected countries.

Since ASF reached Lithuania in 2014, great efforts have been made to combat the disease. Intensive disease surveillance and control measures were implemented and hunting management was adapted. Biosecurity in the context of hunting was improved and strengthened. Hunting was intensified and incentives were paid for targeted hunting of adult and sub-adult females, for reporting wild boar found dead and for disposing the carcasses safely by burial. Despite these control measures, the disease is still present in the wild boar population and has now spread throughout the country [9]. Only in the west of the country are small parts (less than 2000 km^2) still free from ASF. In addition, outbreaks have occurred in domestic pig holdings every year since 2014 [10,25]. Recent data analyses in Estonia and Latvia suggest that the efforts to fight ASF are rewarded, since the number of ASF virus (ASFV)-positive wild boar has decreased. In contrast, the number of hunted wild boar that tested positive for ASFV-specific antibodies, but negative for ASFV, has increased [24,26–28]. This course of disease may result from an accumulation of surviving animals and the simultaneous absence of new infections [27,28]. The circulation of an attenuated virus or the existence of ASFV carrier animals has also been discussed as possible causes for this epidemiological course of ASF [26,29–31]. In Lithuania, however, the analyses of ASF surveillance data performed until 2018 indicate an ongoing epidemic situation with regular occurrence of newly infected wild boar [9,25,26].

The epidemiology of ASF in wild boar and the drivers for persistence, transmission and spread are still not fully understood. Thus, the options for controlling the disease reliably and effectively are limited and constantly under debate. The recent introduction of ASF into the German wild boar population, its further spread and the ongoing epidemic in Poland and Lithuania emphasize the unabated need for further research and thus for a continuing effort to close knowledge gaps. In an attempt to support these efforts, we analyzed comprehensive ASF surveillance and wild boar population data from Lithuania. By including the most recent data and advanced analyses, we aimed at complementing and completing existing research results.

2. Materials and Methods

2.1. Surveillance Data

Lithuanian ASF wild boar surveillance data were obtained from the CSF/ASF wild boar surveillance database of the European Union [32]. The relevant authorities approved the use of the data. Each data record corresponds to the surveillance data for a single wild boar and contains information about place (county, municipality and the smallest administrative unit (the eldership)), where the animal was found or shot, the time (day/month/year) of sampling, age (<1 year and >1 years), sex of the wild boar and the origin of sample. The "origin" refers to how it was recorded: whether the sample was taken from an apparently healthy hunted wild boar (active surveillance) or from a wild boar found dead, shot because it was sick or killed in a road traffic accident (RTA) (passive surveillance). Furthermore, the data of the laboratory test results were recorded.

Laboratory investigations were conducted at the National Food and Veterinary Risk Assessment Institute, which is the National Reference Laboratory for ASF in Lithuania. The laboratory methods and sampling procedure were deployed as previously described [9,10].

Data from 2 January 2016 to 12 April 2021 (64 study months) at the municipality level were used. Data records before 2016 were excluded because they were often incomplete; in particular, geographical information was lacking. For each year, the number of samples examined per km^2 was calculated for each municipality.

Samples were mainly recorded as originating from hunted wild boar and wild boar found dead. Only 32 samples (recorded in 2016 and 2017) originated from wild boar shot, because they were sick. No wild boar samples were recorded that originated from wild boar killed in RTAs. Data records from wild boar shot sick were excluded in all analyses to avoid inconsistencies. In addition, all data records lacking age information were excluded ($n = 2965$). Figures illustrating the numbers of samples from hunted wild boar and wild boar found dead per year were created using the packages "ggplot2" and "cowplot" in the software R (https://www.r-project.org/, accessed on 29 June 2021). All maps were generated using the software ArcGIS ArcMap 10.8.1 (ESRI, Redlands, CA, USA).

2.2. Prevalence Estimates

ASFV prevalence estimates were calculated for hunted wild boar and for wild boar found dead separately. To this end, the number of hunted wild boar that had tested positive for ASFV genome, irrespective of the serological test result, was divided by the total number of hunted wild boar with a conclusive ASFV genome detection result. The same was done for wild boar found dead. Furthermore, seroprevalence estimates were calculated for apparently healthy hunted wild boar that had tested positive for ASFV-specific antibodies and were negative for ASFV genome by PCR. Thus, the number of wild boar with the above result were divided by the total number of wild boar that had been tested serologically and virologically and had a conclusive test result in both assays.

Prevalence estimates were determined for each study month and for each of the 54 affected municipalities. Yearly prevalence estimates were calculated for each of the two age classes. These prevalence estimates were tested for differences using the non-parametric Kruskal–Wallis test. A p-value < 0.05 was considered as statistically significant. All prevalence estimations, statistical tests and confidence intervals were calculated following Clopper and Pearson [33] with R (https://www.r-project.org/, accessed on 29 June 2021).

2.3. Model Analysis

The temporal course for prevalence estimates was calculated using a Bayesian space–time model and BayesX 2.0.1 (http://www.uni-goettingen.de/de/bayesx/550513.html, accessed on 29 June 2021). The logistic regression model allowed to examine the relationship between the probability of disease (prevalence) and potential influencing variables via the logit transformation of the prevalence p ($\log(p/(1-p))$). Calculations were done for ASFV logit prevalence and logit seroprevalence estimates for hunted wild boar on a monthly basis. Age was categorized in two age classes (<1 years and >1 years). Age was the fixed independent variable and the calculated prevalence estimates constituted the dependent variable. Time, space and season were used as random factors (see the Supplementary Materials, Figures S1 and S2). To incorporate spatial dependencies, the smallest administrative unit of Lithuania, the eldership, was used. The model was adapted from Staubach et al. [34] and is described in more detail in Schulz et al. [28].

2.4. Wild Boar Population Data

Wild boar population data were available per hunting season from the 2013/14 hunting season to the 2019/20 hunting season (1 April to 31 March of the following year). These data were estimations based on sightings and snow tracks. In addition, population density estimates based on yearly hunting bag data from 2016 to October 2020 were available. In contrast to the estimations based on sightings and snow tracks, the hunting bag data were available per calendar year (1 January to 31 December of the same year). Both data sets were available at the municipality level as the total numbers of estimated or hunted wild boar, respectively.

For population density calculations, the total number of wild boar per municipality was divided by the area (in km^2) of the respective municipality. Thus, the estimated population density per municipality is indicated as the number of wild boar per km^2. Differences in the population density based on estimations and based on the hunting bag number between the different hunting seasons or years, respectively, were calculated using the Kruskal–Wallis test. Assigning potential statistically significant differences to the respective hunting season/year (p-value < 0.05), a Mann–Whitney U test was used for pairwise comparisons. Bonferroni correction was applied to control for type I error [35]. To make the population density comparable to the surveillance data and for data mapping, only the population density estimates based on the hunting bag number per year were used in the comparison with the surveillance data.

3. Results

3.1. Descriptive Analysis of ASF Surveillance Data

For the analyses, 75,804 data records were available. Most samples of all the wild boar (hunted and found dead) were investigated in 2017 (18,191 samples), but most samples from hunted animals were retrieved in 2016 (17,284 samples). From 2017 onwards, the total number of samples clearly decreased over time. Particularly, the number of samples obtained from wild boar found dead decreased by more than half from year to year (Figure 1, Table S1). On average, most samples from hunted wild boar were taken in January (n = 2101), whereas the lowest average number of hunted animals was sampled in April (n = 457). Overall, the proportion of samples from hunted wild boar in the individual months was relatively similar over the years. By contrast, the number of samples originating from wild boar found dead showed the highest values in the months of the second half of 2017, whereas, in 2018, the majority of these samples were taken in the beginning of the year. For wild boar found dead, most samples were taken in July (n = 98), whereas the lowest number of samples was obtained in June (n = 33) (Figure 1, Table S1).

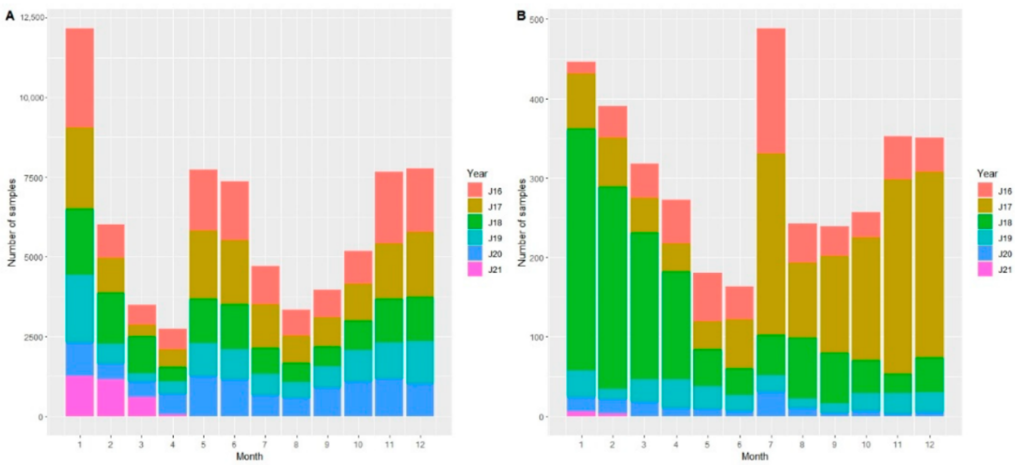

Figure 1. Numbers of samples from hunted wild boar (**A**) and from wild boar found dead (**B**) per month for the years 2016–2021. In 2021, only data from January to 12th April are included.

In all years, the majority of samples originated from wild boar older than one year for both hunted animals and those found dead (Table S1).

In 2016 and 2017, most wild boar were sampled in the eastern and central municipalities, particularly in Kedainiai (2016: 1.05 samples/km^2; 2017: 1.14 samples/km^2)

and Anyksciai (2016: 0.84 samples/km^2; 2017: 0.63 samples/km^2). Over the years, the number of samples taken in the east and the center of Lithuania decreased, while those from the western part of the country increased. However, the sample size exceeded 0.75 samples/km^2 in all of the municipalities after the year 2017 (Figure 2, Figures S3–S6).

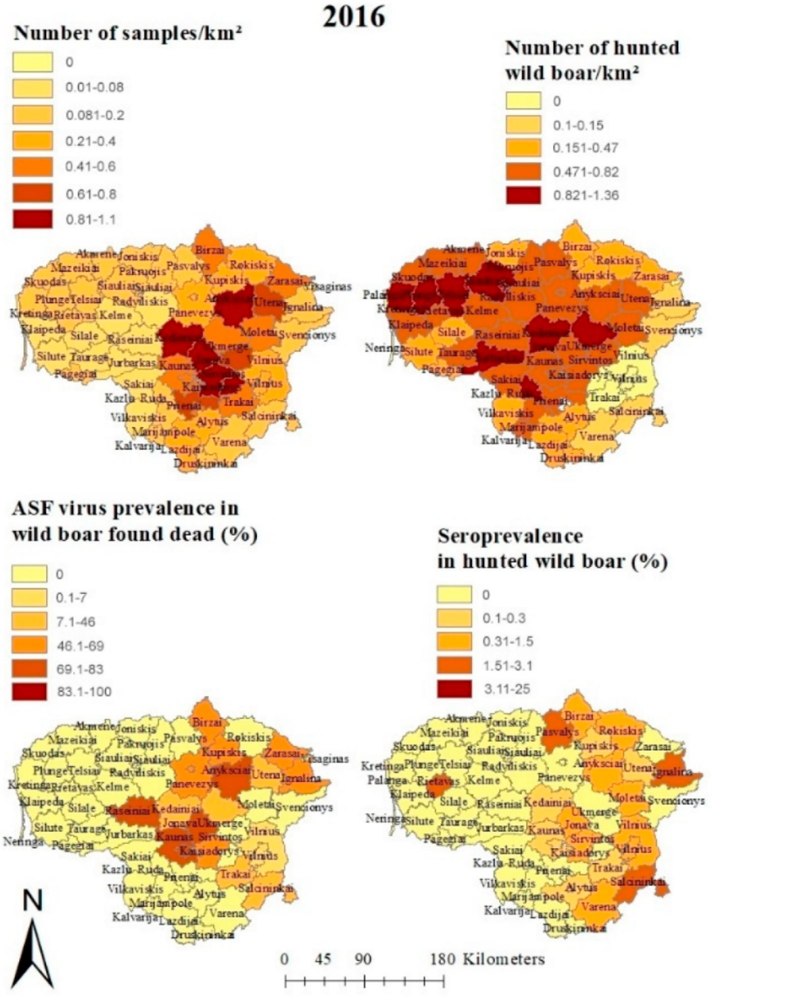

Figure 2. Numbers of investigated ASF samples and hunted wild boar, ASF virus prevalence estimates from wild boar found dead and seroprevalence estimates for hunted wild boar per municipality in Lithuania in 2016.

3.2. Prevalence Estimates

In almost all months of the study period, ASFV prevalence estimates in wild boar found dead were many times higher than in hunted wild boar. The ASFV prevalence estimates in hunted wild boar increased slightly until the end of 2017 and tended to decrease towards the end of the study period (Figure 3, Table S2). Although no major difference was found in the ASFV prevalence estimates in wild boar found dead over time, higher prevalence estimates with narrow confidence intervals were detected between August 2017 and April 2018. After that, no significant differences in the ASFV prevalence estimates over time were found until the end of the study period (Figure 4, Table S2).

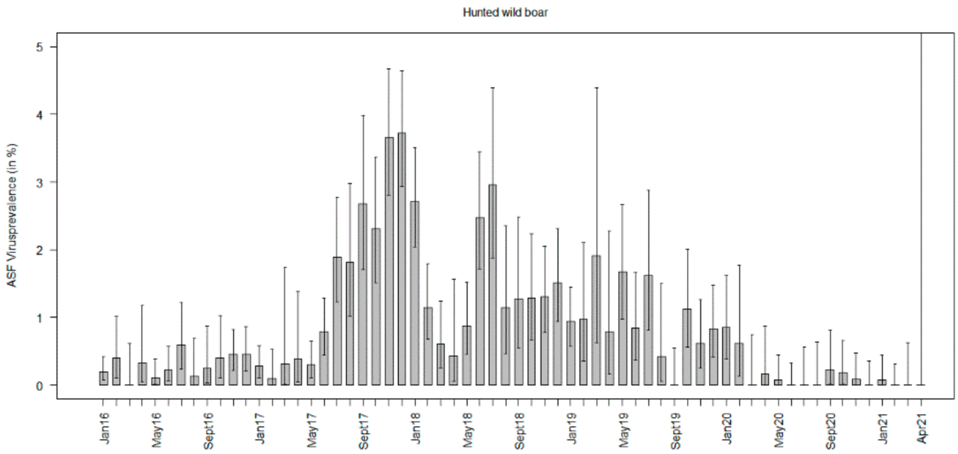

Figure 3. African swine fever virus prevalence estimates per month in hunted wild boar between January 2016 and November 2020.

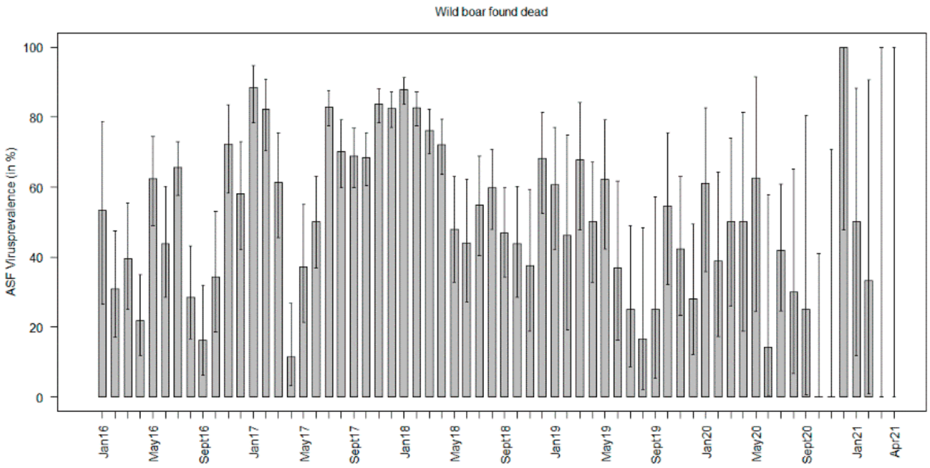

Figure 4. African swine fever virus prevalence estimates per month in wild boar found dead between January 2016 and November 2020.

In 2016, only in the municipality Ignalina, located in the eastern part of Lithuania, did the ASFV prevalence estimates in hunted wild boar exceed 1% (4.4%, 95% CI: 1.6–9.4%). In 2017 and 2018, ASFV prevalence estimates in hunted wild boar showed the highest values in municipalities located in the center of the country (Panevezys 2017: 4.1%, 95% CI: 2.8–5.7%, 2018: 14.3%, 95% CI: 7.1–24.7%; Pakruojis 2017: 3.6%, 95% CI: 1.3–7.6%, 2018: 6.8%, 95% CI: 3.7–11.3%). However, in 2018, the highest ASFV prevalence in hunted wild boar was found in Akmene (10.6%, 95% CI: 6.3–16.5%). In 2019 and 2020, ASFV prevalence estimates in hunted wild boar were generally lower. They showed the highest values in the central and western parts of Lithuania (Mazeikiai 2019: 3.1%, 95% CI: 1.6–5.5% 2020: 2.5%, 95% CI: 0.5–7.1%). Until 12 April 2021, only a single ASFV-positive hunted wild boar was found. It was detected in Plunge in western Lithuania (Table S3).

In 2016, ASFV prevalence estimates in wild boar found dead reached values of up to 80%, particularly in the center of Lithuania (Figure 2 and Table S4). As the ASFV prevalence estimates in hunted wild boar, the estimates in wild boar found dead increased in many municipalities, particularly in the center and the west of Lithuania, in 2017 and 2018 (Table S4 and Figure S3). In 2019, only four municipalities showed an ASFV prevalence above 70% in wild boar found dead. In 2020, no dead wild boar were detected in 25 municipalities. In 20 further municipalities, no ASFV-positive wild boar were detected. In Ignalina, a municipality located at the eastern border of the country, a single sample was taken and tested positive (95% CI 2.5–100%). Also, in 2021, the ASFV prevalence was 100% in this municipality with a narrower 95% CI (40–100%). In Mazeikai, located in the west of Lithuania, the ASFV prevalence in wild boar found dead was also high in 2020 (85.7%, 95% CI: 42.1–99.6%). No wild boar were found dead in 2021 in this municipality. In the majority of municipalities, no dead wild boar were detected during the first months of 2021 (Table S4).

The differences in the yearly ASFV prevalence estimates were not statistically significant in the two age classes, neither in hunted wild boar (p-value = 0.42) nor in wild boar found dead (p-value = 0.75) (Table 1, Figure 5).

Table 1. Median yearly African swine fever virus and seroprevalence estimates in Lithuanian wild boar from 2016–April 2021 for each age class.

	Median ASFV Prevalence in Hunted Wild Boar in %	Median ASFV Prevalence in Wild Boar Found Dead in %	Median Seroprevalence in Hunted Wild Boar in %
<1 year	0.80	51.75	1.02
>1 year	0.56	59.23	1.45

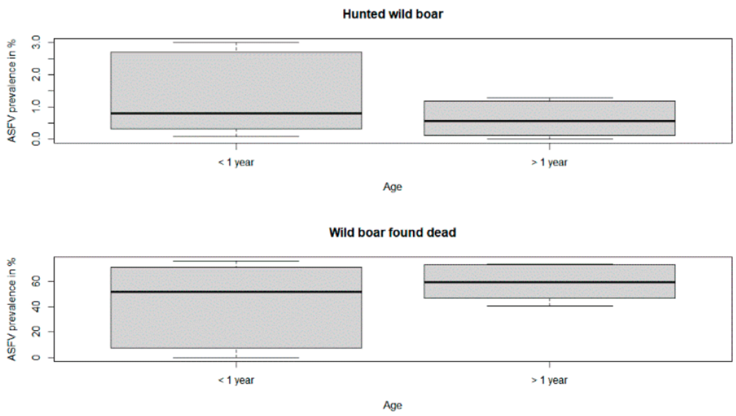

Figure 5. African swine fever virus prevalence estimates of hunted wild boar and wild boar found dead in the two age classes. The horizontal lines that form the top of the boxes illustrate the 75th percentile. The horizontal lines that form the bottom indicate the 25th percentile. The horizontal lines that intersect the box represent the median ASFV prevalence per age class. Whiskers indicate maximum and minimum values that are no more than 1.5 times the span of the interquartile range and the open circles represent outliers, which are single values greater or less than the extremes indicated by the whiskers.

Seroprevalence estimates in hunted wild boar increased over time, showing the highest values in October 2018 (3.1%, 95% CI: 2.0–4.4%) and 2019 (3.0%, 95% CI: 2.0–4.3%). By the end of 2019, they had decreased, but the overlapping confidence intervals suggest that the prevalence estimates in the following study months were not significantly lower than in the two years before (Figure 6, Table S2).

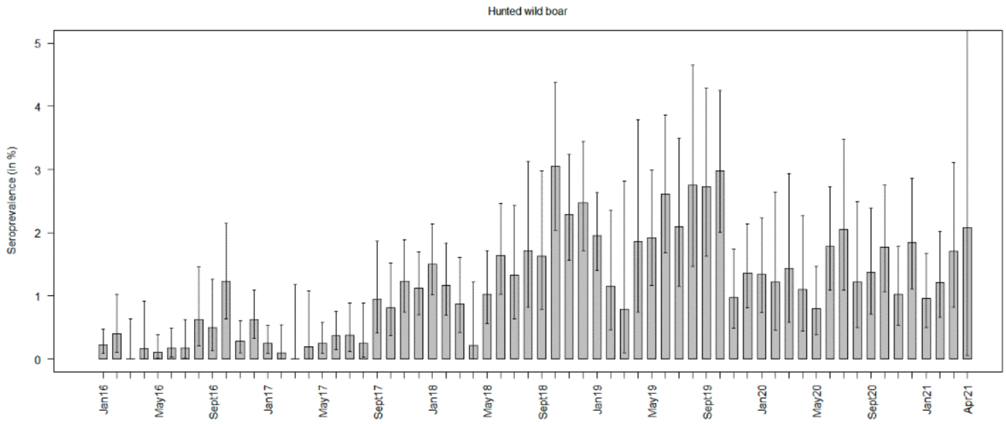

Figure 6. African swine fever virus-specific antibody prevalence estimates (seroprevalence) in hunted wild boar per month from January 2016 to April 2021.

The highest seroprevalence estimate in 2016 was found in Salcininkai (2.6%, 95% CI: 1.0–5.6%), located in the southeast of Lithuania (Table S5 and Figure 2). In 2017, the small easterly municipality of Visaginas in particular showed a high seroprevalence estimate (25%, 95% CI: 0.6–80.6%). However, the 95% confidence intervals were very wide, suggesting a small sample size. In 2017 and 2018, the highest seroprevalence estimates were found in the northern part of central Lithuania (Table S5 and Figures S3 and S4). The number of municipalities with seroprevalence estimates above 1% increased from 24 in 2018 to 31 in 2019. In addition to the centrally located municipalities, some municipalities in the west also showed an increased seroprevalence in hunted wild boar in 2019, 2020 and 2021 (e.g., Mazeikiai 2019: 3.2%, 95% CI: 1.6–5.7%; 2020: 1.7%, 95% CI 0.2–6.0%; 2021: 5.6%, 95% CI: 0.1–27.3%). In 2020, however, the seroprevalence estimates were lower than in the years before in some of the central municipalities (e.g., Birzai 2.1%, 95% CI: 0.6–5.2%) (Table S5 and Figures S5 and S6). In the four analyzed months of 2021, seropositive wild boar were only hunted in 24 municipalities. The highest seroprevalence estimates were detected in municipalities located in the northwest of Lithuania (Siauliai: 9.6%, 95% CI: 4.3–18.1% and Joniskis: 6.7%, 95% CI: 0.2–31.9%) (Table S5).

The differences in the yearly seroprevalence estimates were not statistically significant when the two age classes were compared (p-value = 0.75) (Figure 7). However, the median yearly seroprevalence estimate in animals older than one year showed a slightly higher value than that of the younger animals (Table 1).

3.3. Model Analysis

The model analyses showed an increase in the temporal trend of the logit prevalence of ASFV-positive hunted wild boar from February 2017 until October 2018. In May 2019 and in the subsequent study months, the logit prevalence decreased (Figure 8). The temporal trend of the logit seroprevalence of hunted wild boar started to increase a few months later than the logit ASFV prevalence and the rise was less pronounced. Starting in May 2019, the logit seroprevalence seemed to decrease slightly, but rose again by the end of the study period (Figure 8).

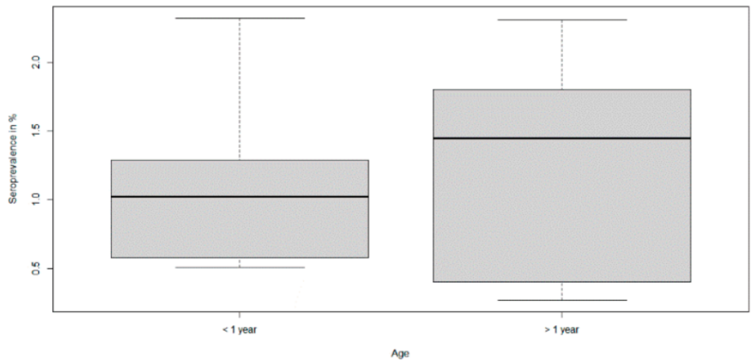

Figure 7. Seroprevalence estimates of hunted wild boar in the two different age classes. The horizontal lines that form the top of the boxes illustrate the 75th percentile. The horizontal lines that form the bottom indicate the 25th percentile. The horizontal lines that intersect the box represent the median seroprevalence per age class. Whiskers indicate maximum and minimum values that are no more than 1.5 times the span of the interquartile range and the open circles represent outliers, which are single values greater or less than the extremes indicated by the whiskers.

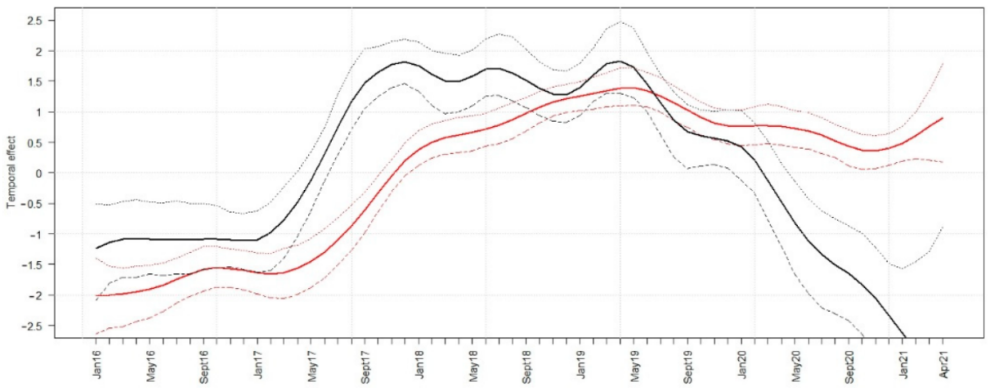

Figure 8. Median temporal effect on the logit prevalence for samples that tested positive for ASFV (black lines) and exclusively seropositive for ASFV-specific antibodies (red lines). The 95% Bayesian credible intervals (BCIs, dashed lines) are indicated for ASFV-positive wild boar (black) and animals exclusively seropositive for ASFV-specific antibodies (red).

3.4. Wild Boar Population Data

The estimated wild boar population density based on sightings and snow tracks decreased over time. The estimated population density in the 2018/19 hunting season was statistically significantly lower than in the previous hunting seasons. However, in the 2019/2020 hunting season, the population density was only significantly lower than the one estimated for 2013/14 and 2014/15 (Table S6 and Figure S7). Estimated population density data based on hunting bags were only available after 2016 and on a yearly base. The number of wild boar/km^2 calculated on the basis of the hunting bags decreased significantly over time. In 2020, it was significantly lower than in 2016, 2017 and 2018 (Table S7 and Figure 9).

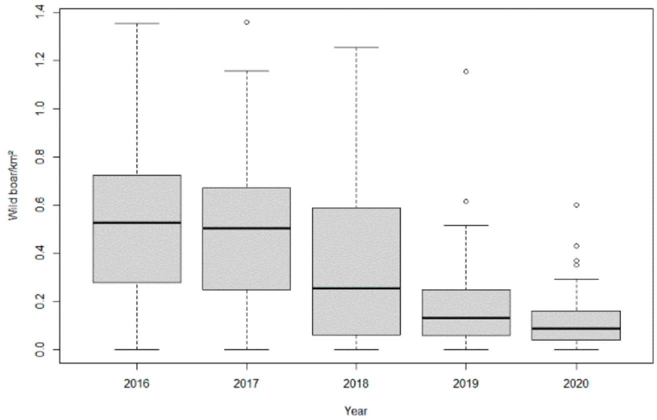

Figure 9. Estimated wild boar population density (wild boar/km^2) based on hunting bag data per year. The horizontal lines that form the top of the boxes illustrate the 75th percentile. The horizontal line that forms the bottom indicate the 25th percentile. The horizontal lines that intersect the box represent the median number of wild boar per square kilometer. Whiskers indicate maximum and minimum values that are no more than 1.5 times the span of the interquartile range and the open circles represent outliers, which are single values greater or less than the extremes indicated by the whiskers.

In all years, the estimated population density based on hunting bag data showed the highest values mainly in the western municipalities of Lithuania. However, in 2016 and 2017, a few municipalities in the center also had a population density above 0.8 wild boar/km^2. In 2019, the population density was higher than 1 wild boar/km (1.15) only in Kretinga, a municipality in the west of Lithuania with a commercial hunting ground that is considered free from ASF. The highest population density value was also reported from this municipality in 2020, although it was somewhat lower than in the year before (0.60 wild boar/km^2) (Figure 2 and Figures S3–S6).

4. Discussion

Several studies have described the epidemiological situation of ASF in Lithuania in certain periods [9,10,25]. In the present study, all available and evaluable ASF wild boar surveillance data were used to illustrate the course of the disease over time and to obtain an overview of the current epidemiological ASF situation in wild boar in Lithuania. In addition, surveillance efforts were described and the wild boar population density estimated for each year individually. Therefore, a comprehensive description of the epidemiological situation concerning ASF in wild boar in Lithuania was possible and several influencing factors could be taken into consideration.

The huge numbers of samples that were available for the analysis show the strong effort of Lithuanian authorities to combat the disease. Mačiulskis, et al. [9] showed an increase of the number of investigated samples over time starting in 2014, with a peak in 2016 and 2017. This peak was discussed as a result of paying incentives to hunters and other people who reported finding dead wild boar since 2016. This study confirms these results. The large number of samples originating from wild boar found dead in 2017 might have been due to an increased awareness, incentives for reporting and disposal and the willingness of hunters and the public to support passive surveillance. Moreover, the estimated wild boar population was still high at that time. Thus, the large number of susceptible animals combined with the high case/fatality ratio of ASF could have led to a large number of detected dead wild boar. The huge number of wild boar found dead indicates a high viral load in the environment and could thus explain the high number of ASF outbreaks in domestic pig holdings in 2018. The subsequent constant decrease in the numbers of samples during the following years was certainly due to the decrease of the susceptible wild boar population, but signs of fatigue within the hunters' community may have played a role, too. In a recent study, however, it was found that Lithuanian hunters claimed to be willing to support ASF control measures with increased hunting, indicating rather that the lower number of wild boar was the reason for the lower sample size. In contrast, passive surveillance (i.e., the detecting, reporting and sometimes also the disposal of detected wild boar carcasses by burial) was not very popular among the participating hunters [36], supporting the assumption that the decreasing number of samples from wild boar found dead could be due to the lack of hunters' motivation. Similar decreasing trends in sample sizes over time were also found in the other two Baltic states, Estonia and Latvia [24,27]. In 2021, data were only available until the 12th April, but in the first three months of 2021, the number of samples from hunted animals seemed to increase again compared to the numbers in the same months of the previous year. This may indicate a slow recovery of the wild boar population. The population density data also revealed a stabilization of the number of wild boar/km^2 in 2020 compared to 2019.

The usually larger number of samples of hunted wild boar in January and the lower numbers in April are not surprising. January is part of the main hunting season, involving driven hunts, while in April hunting activities are usually suspended due to ethical reasons (it is the main reproduction season of wild boar).

On average, most dead wild boar were detected in July, which may have been due to increased leisure activities in the forests during summer, thus resulting in a higher detection probability. Moreover, most of wild boar hide in crop fields at this time of the year, which may lead to an increased probability of direct and indirect contacts between different groups of wild boar and result in an increased probability of disease spread. The peak of detected dead wild boar in July may have been due to the fact that there are more susceptible wild boar in summer due to the main reproduction period occurring in spring and an increased case/fatality ratio of ASF in young wild boar. However, the low average number of wild boar found dead in June contradicts these explanations, as it can be assumed that the external circumstances do not differ to a great extent between June and July. Probst et al. [37] found that wild boar carcasses decompose much faster in the warm season and that mammalian scavengers visit the carcasses more frequently in summer. Dead wild boar may therefore disappear faster from the environment in this season, which might explain the low numbers of dead wild boar detected in June. In Poland, similar patterns were found and the numbers of samples from passive surveillance were high in July, while it seems that they were significantly lower in June [38].

Most samples came from animals that were estimated to be older than one year. This is not surprising, since animals between one and two years usually constitute the main target group for hunters [16,39,40]. Similar results were also obtained in other countries [27,41]. The spatial shift in the numbers of samples collected towards the west suggests that sampling was increasingly performed in areas where ASF was mainly present in the respective year. The population density decreased over time, particularly in the areas affected in the beginning of the epidemic, which could be an additional explanation for the decrease in the number of samples taken in these municipalities.

ASFV prevalence estimates were separately performed for hunted wild boar and wild boar found dead. The ASFV prevalence estimates in hunted wild boar reflect the true prevalence within the wild boar population much more accurately than the ASFV prevalence estimates in wild boar found dead. The probability that a wild boar found dead in an ASF-endemic region will be ASF-positive is much higher than for an apparently healthy wild boar that has been shot. This may lead to an overestimate of the true ASFV prevalence among wild boar found dead. Detected wild boar carcasses are often decomposed, which may impair the quality of samples and ultimately lead to an underestimation of the true prevalence. In essence, the uncertainty of the estimate is substantially increased for samples from wild boar found dead. All wild boar and wild boar carcasses are regularly tested for the presence of the ASFV genome, but samples obtained from (heavily decomposed) carcasses are not usually tested for ASFV-specific antibodies. Thus, to ensure consistency, seroprevalence estimates were only calculated for hunted wild boar.

The much higher ASFV prevalence estimates in wild boar found dead are not surprising, since several studies have demonstrated the high case/fatality ratio of ASF and the resulting higher probability of obtaining ASFV-positive samples from wild boar found dead, as already mentioned [20–22,26,42]. The findings of the present study also confirm the results of previous analyses of Lithuanian surveillance data [9]. Only data from 2016 and later were used for the analyses, since data records from previous years were often incomplete and had to be excluded. However, the epidemic had already started in Lithuania in 2014; thus, ASF had been spreading for two years in 2016 when we started this analysis. Pautienius et al. [10] and Mačiulskis et al. [9] already observed an increase in the ASFV prevalence from the beginning of the epidemic until 2017 and 2018. This temporal trend could be confirmed by our analyses, but it seems that the ASFV prevalence in hunted and wild boar found dead declined in recent years. This course of the disease may be explained by the decreasing number of susceptible wild boar and the resulting slowdown of disease spread. However, the wide confidence intervals, particularly in wild boar found dead, indicate a small sample size and may explain why the results lack statistical significance.

The findings concerning the course of the disease in wild boar were also confirmed by our model analysis. We included the prevalence estimates for neighboring administrative units and time points in a Bayesian model. Thus, the modeling results may have yielded more robust estimates, which are less prone to bias. We used the smallest administrative unit, i.e., the eldership, as the reference in our spatial analysis and calculated only the temporal course of the logit prevalence for ASFV- and seroprevalence for hunted animals. The logit transformation of the prevalence was the best way to demonstrate the variation of the prevalence. The number of samples originating from wild boar found dead per eldership was too small to yield reliable model results.

The increasing seroprevalence in hunted animals, which showed a slight delay when compared to the ASFV genome prevalence, and the subsequent slight decrease indicate that ASF took a similar course in Lithuania as observed in other countries [24,27,28]. However, in the last few months of the study period, the seroprevalence seemed to increase again. Moreover, the detection of ASFV in hunted animals even in late 2020 and early 2021 proves that the virus is still circulating, although the prevalence may be very low, resulting in a clearly decreased detection probability. The risks that attenuated virus strains may circulate or that surviving animals might shed the infectious virus ("carriers") are strongly disputed and cannot be excluded as hypothetical explanations for the observed epidemiological course of ASF in Lithuania [29–31,43,44]. It should be noted, however, that the existence of ASF "carriers" has been controversially discussed [29,30].

In contrast to previous findings, the present analyses did not show any significant differences in the prevalence estimations for the two age classes [20,21,24], suggesting that surveillance efforts should include all age classes equally according to the current knowledge. However, there is still an urgent need to continue data analyses, in other ASF-affected countries also, in order to evaluate the age distribution in various prevalence estimates further and thus to support the design of targeted surveillance measures or allow for their adaptation in an evidence-based manner.

Wild boar population density estimates are always accompanied by limitations. Also, hunting bag data does not represent the true population [45,46]. In Lithuania, every hunting club can determine the targeted number of wild boar to be hunted. There are no governmental regulations regarding the numbers of wild boar that should be hunted during the hunting season. Since the start of the epidemic in 2014, hunting rules were amended in Lithuania and wild boar could be hunted all year, irrespective of the age or sex of the animals. In our study, we compared numbers based on the same methods over the years to obtain estimates that are as reliable as possible. When calculating the numbers of wild boar in the individual municipalities, we assumed an even distribution of the population within the municipality and did not include landscape features, which may affect the distribution.

Similar to previous studies, the wild boar density decreased over time [20,24,41]. It can be assumed that this reduction of the number of wild boar was mainly due to the high case/fatality ratio of ASF [39,47]. In all analyzed years, the wild boar density was higher in the west of Lithuania, which is probably due to the start of the epidemic in the eastern part of the country and to the intensive hunting of wild boar, including females, in that part of the country. In 2016 and 2017 particularly, the numbers of samples per municipality diverged from the number of hunted wild boar per municipality and were higher in central Lithuania. This might have been due to increased surveillance efforts in ASF-affected areas and the accompanying high ASFV prevalence estimates. Over time, a shift towards the west could be observed and, in the last few years, only seroprevalence estimates showed higher values in the center of Lithuania. A similar epidemiological course of the disease was observed in Estonia and Latvia, where ASF was also introduced into the eastern part of the respective countries, presumably from Belarus or the Russian Federation, respectively, and spread towards the west over time [20,21,28].

Despite the comparable epidemiology of ASF in wild boar and its temporal and spatial course, an elimination of the disease in Lithuania is currently not within reach. Possibly, the occurrence of ASF outbreaks in Lithuanian domestic pig holdings—which mainly take place in the newly affected areas or in the areas with continuous circulation of ASFV in the wild boar population—and thus the potential indirect transmission between wild boar and domestic pigs and vice versa, might play a role in this difficult situation. Furthermore, it is still common in rural areas of Lithuania to keep a small number of pigs in backyards at a level of biosafety/biosecurity that is not sufficient to prevent the introduction of ASFV. It is obvious that pigs in these holdings are prone to become infected with ASF, thus feeding the epidemic [15]. However, in Estonia, where all backyard farms were eliminated, no outbreaks in domestic pig farms have been reported since 2017 and no ASFV-positive wild boar were detected for more than one year, new ASFV-positive wild boar cases were reported in August 2020 [24,48]. Thus, engagement, motivation and the willingness of all involved stakeholders must be continuously maintained to keep all surveillance and control efforts at a high level and the disease situation needs to be permanently monitored and analyzed to obtain the knowledge required to adapt surveillance and control as needed. Moreover, open questions like the potential role of surviving animals and of attenuated viruses, the threat of re-introduction from neighboring countries, the possibilities of disease detection at a very low prevalence and the effectiveness of different control measures need to be addressed.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/v13071276/s1, Figure S1: Median seasonal effect on the logit prevalence of samples obtained from hunted wild boar in Lithuania that tested PCR-positive, irrespective of the serological result. 95% Bayesian credible intervals (BCIs) are indicated (dotted lines). Figure S2: Median seasonal effect of samples that had tested exclusively serologically positive on the logit prevalence. 95% Bayesian credible intervals (BCIs) are indicated (dotted lines). Figure S3: Numbers of investigated ASF samples, numbers of hunted wild boar, ASF virus prevalence estimates from wild boar found dead and seroprevalence estimates for hunted wild boar per municipality in Lithuania in 2017. Figure S4: Numbers of investigated ASF samples, numbers of hunted wild boar, ASF virus prevalence estimates for wild boar found dead and seroprevalence estimates for hunted wild boar per municipality in Lithuania in 2018. Figure S5: Numbers of investigated ASF samples, numbers of hunted wild boar, ASF virus prevalence estimates for wild boar found dead and seroprevalence estimates for hunted wild boar per municipality in Lithuania in 2019. Figure S6: Numbers of investigated ASF samples, numbers of hunted wild boar, ASF virus prevalence estimates for wild boar found dead and seroprevalence estimates for hunted wild boar per municipality in Lithuania in 2020. Figure S7:

Estimated wild boar population density (wild boar/km^2) based on sightings and snow tracks per hunting season. The horizontal lines that form the top of the boxes illustrate the 75th percentile. The horizontal lines that form the bottom indicate the 25th percentile. The horizontal lines that intersect the box represent the median number of wild boar per square kilometer. Whiskers indicate maximum and minimum values that are no more than 1.5 times the span of the interquartile range and the open circles represent outliers, which are single values greater or less than the extremes indicated by the whiskers. Table S1: Numbers of samples originating from wild boar hunted or found dead in Lithuania per age class, year and month. Table S2: ASF virus prevalence and seroprevalence estimates for hunted wild boar and ASF virus prevalence estimates for wild boar found dead, including the 95% confidence intervals, on a monthly basis from January 2016 until April 2021. Blank fields indicate a lack of data in the respective month. Table S3: ASF virus prevalence of hunted wild boar, including the 95% confidence intervals, at the municipality level in the years 2016–2021 (only the first 4 months for 2021). Blank fields indicate a lack of data for the respective municipality. Table S4: ASF virus prevalence for wild boar found dead, including the 95% confidence intervals, at the municipality level in the years 2016–2021 (only the first 4 months for 2021). Blank fields indicate a lack of data for the respective municipality. Table S5: Seroprevalence in hunted wild boar, including the 95% confidence intervals, at the municipality level in the years 2016–2021 (only the first 4 months for 2021). Blank fields indicate a lack of data for the respective municipality. Table S6: Median of estimated number of wild boar/km^2 based on sightings and snow tracks per hunting year and the results of the Mann–Whitney U test. Significant differences between the hunting seasons are highlighted. Table S7: Median of the estimated number of wild boar/km^2 based on hunting bag data per year and the results of the Mann–Whitney U test. Significant differences between the hunting seasons are highlighted.

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3.2.3 ASF surveillance and control in wild boar

Not only disease patterns and pathogen-host interactions have to be understood for a successful and effective disease surveillance, but also risk factors for a higher probability to detect the disease should be considered. Thus, to deploy the available resources as effectively as possible, risk-based surveillance measures should be designed, where appropriate (Stärk et al., 2006). Surveillance activities can be active or passive. Active surveillance means the planed and structured sampling of apparently healthy animals, in case of ASF in wild boar, of a defined number of hunted wild boar. By contrast, passive surveillance means the sampling or reporting of animals, which are suspicious, thus, the number of sampled animals was not planed beforehand, is not predictable and can only be influenced to a limited extent (Schulz, 2016). In case of ASF in wild boar, passive surveillance means the reporting or sampling of animals that were found dead, shot sick or that were injured in a road traffic accident (RTA). Due to the high case/fatality ratio of ASF and the consequently low proportion of surviving wild boar, it is known that passive surveillance is most effective to detect ASF-infected wild boar (Nurmoja et al., 2017b, European Food Safety Authority, 2018, European Food Safety Authority, 2019, Schulz et al., 2019a). Montgomery (1921) had already noted that ASF-diseased pigs sought proximity to water. These findings were supported by the increased detection of dead wild boar close to or even in water bodies in the current epidemic and can help to design effective risk-based surveillance measures (European Food Safety Authority et al., 2017, Morelle et al., 2019).

Several studies showed that a high wild boar population supports the spread and maintenance of ASF (Nurmoja et al., 2017b, European Food Safety Authority, 2018, Schulz et al., 2019a). Moreover, the spread of ASF in the three Baltic States slowed down over time. Simultaneously, the population density declined significantly, probably due to the high case/ fatality ratio of ASF (Morelle et al., 2020). Despite of the knowledge that wild boar population density plays a role in the spread of ASF, the accurate estimations of these densities are difficult (European Food Safety Authority, 2018, ENETWILD-consortium et al., 2019). Currently, mainly hunting bag data are used for wild boar density estimations, usually taking the associated limitations into account.

In a narrative review, environmental risk factors for ASF in Europa were summarized. Thus, more evidence was created and the results can be used to improve ASF surveillance and control.

- Contribution **(Publication 9)**: BERGMANN, H., SCHULZ, K., CONRATHS, F. J. & SAUTER-LOUIS, C. 2021. A Review of Environmental Risk Factors for African Swine Fever in European Wild Boar. Animals, 11, 2692. https://doi.org/10.3390/ani11092692

Although it is known that passive surveillance is the most effective surveillance measure to detect the disease early, little is known about the risk of a wild boar that was killed in an RTA to be ASF-positive. Thus, the question arose whether wild boar injured or killed in an RTA should be sampled and tested for ASFV or whether the risk that they are ASF-positive is negligible and the resources should rather be saved. ASF wild boar surveillance data from Estonia and Latvia were analyzed and detection probabilities were compared.

- Contribution **(Publication 10)**: SCHULZ, K., CONRATHS, F. J., STAUBACH, C., VILTROP, A., OĻŠEVSKIS, E., NURMOJA, I., LAMBERGA, K. & SAUTER-LOUIS, C. 2020. To sample or not to sample? Detection of African swine fever in wild boar killed in road traffic accidents. Transboundary and Emerging Diseases 67, 1816-1819. https://doi.org/10.1111/tbed.13560

Surveillance efforts do not only depend on the probability of disease detection, but also on the stage of the epidemic and the respective surveillance objective (Hoinville et al., 2013). In case of early detection of a new ASF introduction, enhanced passive surveillance is crucial. However, in the case of an almost endemic situation like in the Baltic States, the number of detectable dead wild boar decreases and due to the significantly reduced wild boar density, it becomes difficult to detect any ASF-positive wild boar. Thus, surveillance efforts should be increased to determine the disease status within a country, even under these epidemiological conditions. In Estonia, no ASFV-positive wild boar were detected from February 2019 until August 2020. Before, in 2018, an increase of wild boar that tested solely positive for ASFV-specific antibodies and a decrease of ASFV-positive wild boar could already be found (Schulz et al., 2019b). The long absence of the detection of ASFV in the country raised the question about the requirements for a country to declare the freedom from ASF in wild boar. The international requirements of the World Organization for Animal Health (OIE) do not differentiate between ASFV-positive and serologically positive wild boar. Thus, even in absence of detectable ASFV, but in presence of wild boar with ASFV-specific antibodies, a country cannot be declared officially free from ASF. It is not yet known, how long ASFV-specific antibodies can be detected in wild boar that survived an ASF infection, which complicates the discussion regarding the status of freedom from the disease. Thus, a study was carried out that dealt in detail with the issues raised above.

- Contribution **(Publication 11)**: SCHULZ, K., STAUBACH, C., BLOME, S., NURMOJA, I., VILTROP, A., CONRATHS, F. J., KRISTIAN, M. & SAUTER-LOUIS, C. 2020. How to Demonstrate Freedom from African Swine Fever in Wild Boar-Estonia as an Example. Vaccines, 8, 336. https://doi.org/10.3390/vaccines8020336

Unfortunately, and despite all efforts to eliminate the disease, after the apparent absent of ASFV in Estonia for 1.5 years, the virus was detected again in a wild boar found dead in August 2020. Further cases followed swiftly. Thus, we investigated different hypotheses, which could serve as an explanation of what happened in Estonia.

- Contribution **(Publication 12)**: SCHULZ, K., SCHULZ, J., STAUBACH, C., BLOME, S., NURMOJA, I., CONRATHS, F. J., SAUTER-LOUIS, C. & VILTROP, A. 2021. African Swine Fever Re-Emerging in Estonia: The Role of Seropositive Wild Boar from an Epidemiological Perspective. Viruses, 13, 2121. https://doi.org/10.3390/v13112121

Similar to other wildlife diseases, the control of ASF in wild boar is complex. In domestic pigs, the control is much easier as the affected epidemiological unit is usually defined and directly and indirectly affected animals can be eliminated. To control ASF in wild boar populations, several control measures are applied and discussed. Measures range from a hunting ban to increased targeted hunting of female wild boar. In several countries, incentives are paid to support passive surveillance or increased hunting efforts. Fencing affected areas is often used to limit the further spread of the virus (European Food Safety Authority, 2015, Guinat et al., 2016, Šatrán, 2019). Despite the various control measures and their intensive implementation, the virus continues to spread within and between countries. Aiming to assess the effectiveness of different ASF control measures and thus to potentially improve ASF control in wild boar, control measures were evaluated based on Latvian ASF wild boar surveillance data.

- Contribution **(Publication 13)**: SCHULZ, K., OĻŠEVSKIS, E., STAUBACH, C., LAMBERGA, K., SERŽANTS, M., CVETKOVA, S., CONRATHS, F. J. & SAUTER-LOUIS, C. 2019. Epidemiological evaluation of Latvian control measures for African swine fever in wild boar on the basis of surveillance data. Scientific Reports, 9, 4189. https://doi.org/10.1038/s41598-019-40962-3

In the context of the current ASF epidemic, so far only two European countries succeeded in eliminating ASF from their wild boar populations. In Belgium and in the Czech Republic, where the disease was probably introduced through human behavior and only at one single location ('point source exposure'), the disease has only been present in wild boar. The fast implementation of intensive control measures and the lack of new virus introduction supported the containment of the spread of the disease and made an elimination possible.

In 2020, ASF emerged in German wild boar for the first time and it was obvious that the choice of control measures and their implementation was based on those used by the two countries that had successfully eliminated ASF. However, in contrast to Belgium and the Czech Republic, the disease was introduced into Germany by immigration of infected wild boar from western Poland over a line of more than 100 km. Thus, instead of having to control a single virus introduction, in Germany, the disease was introduced in different locations, which required a revision of existing and the design of new control measures, but also a prudent use of the available resources. The emergence of ASF in Germany was described and the first six months of the epidemic were compared with those in Belgium and the Czech Republic. The aim was to show that the situations differed and that the choice of control measures therefore had to be adapted.

- Contribution **(Publication 14)**: SAUTER-LOUIS, C., FORTH, J. H., PROBST, C., STAUBACH, C., HLINAK, A., RUDOVSKY, A., HOLLAND, D., SCHLIEBEN, P., GÖLDNER, M., SCHATZ, J., BOCK, S., FISCHER, M., SCHULZ, K., HOMEIER-BACHMANN, T., PLAGEMANN, R., KLAAß, U., MARQUART, R., METTENLEITER, T. C., BEER, M., CONRATHS, F. J. & BLOME, S. 2020. Joining the club: First detection of African swine fever in wild boar in Germany. Transboundary and Emerging Diseases, 68: 1744–1752. https://doi.org/10.1111/tbed.13890
- Contribution **(Publication 15)**: SAUTER-LOUIS, C., SCHULZ, K., RICHTER, M., STAUBACH, C., METTENLEITER, T. C. & CONRATHS, F. J. 2021. African swine fever: Why the situation in Germany is not comparable to that in the Czech Republic or Belgium. Transboundary and Emerging Diseases, 1-8. https://doi.org/10.1111/tbed.14231

3.2.3.1 A Review of Environmental Risk Factors for African Swine Fever in European Wild Boar

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Highlights

- Risk-based surveillance can increase the detection probability and save resources
- Active wild boar carcass search should be increased during times of peak disease occurrence
- Wild boar prefer forested areas
- Including risk factors in the design and implementation of surveillance and control is vital to increase the chances of success

animals

MDPI

Review

A Review of Environmental Risk Factors for African Swine Fever in European Wild Boar

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Simple Summary: African swine fever (ASF) is a viral haemorrhagic pig disease that continues to spread in Europe and severely damages pig production and economy, disrupts trade with pigs and porcine products and even has an impact on social welfare in affected areas. Wild boar and domestic pigs are both susceptible to infection with the ASF virus, which causes generalised haemorrhagic illness, fever and rapid death of most infected animals within a few days. ASF occurrence in wild boar dominates the spread and persistence of this disease in Europe and poses an imminent threat for spill-over infections with ASFV in domestic pig holdings. Wild boar represent an intelligent and adaptable wildlife host for ASF with an expansive distribution range in Europe and complex biology. Wild boar thus intricately link ASF with their habitat, making ASF inherently complicated and resource-hungry to control in the environment. This work reviews the currently known environmental risk factors for ASF in wild boar and specifically assesses the role that climate, land cover, human activity, wild boar and disease distribution play in the occurrence of ASF in wild boar. The reviewed risk information guides the implementation of ASF control measures in wild boar.

Abstract: A detailed understanding of environmental risk factors for African swine fever (ASF) in wild boar will be not only essential for risk assessments but also for timely and spatially informed allocation of resources in order to manage wild boar-targeted ASF control measures efficiently. Here, we review currently known environmental risk factors that can influence the occurrence of ASF virus infection in wild boar when compared to disease occurrence in wild boar of a non-exposed reference scenario. Accordingly, the exposure of wild boar to environmental risk factors related to (1) climate, (2) land cover, (3) human activity, (4) wild boar and (5) ASF were evaluated. As key environmental risk factors in this review, increased ASF occurrence in wild boar was associated with seasonal patterns, forest coverage, presence of water, human presence, farming activities, wild boar density and ASF nearness. The review highlights inconsistencies in some of these risk factor associations with disease detection in space and time and may provide valuable insights for the investigation of ASF transmission dynamics. The examined risk information was applied to consider potential improvements of the ASF control strategy in wild boar regarding disease surveillance, hunting, wild boar carcass searches and ASF barrier implementation.

Keywords: African swine fever; risk factor; wild boar; epidemiology; disease control; surveillance; environment

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1. Introduction

African swine fever (ASF) has continuously spread in Europe in recent years. The current Eurasian epidemic originated in Georgia in 2007. From there, it first spread in the Caucasus region, then north and northwest, finally reaching central Europe, but also spreading east into Asia [1]. The causative agent of this disease, ASF virus (ASFV), is a large, complex and enveloped DNA virus that is characterised by its high environmental stability [2–5]. Especially at low temperatures, infectious ASFV can be recovered from pig tissues for several months, or even years, particularly from blood, muscle and skin tissues kept at −20 °C or 4 °C [6–9]. ASFV infects only pigs; it is harmless to humans. In European wild boar and domestic pigs, infection with ASFV usually causes severe generalised illness, leading to fever within a matter of days, bleedings and rapid death of most infected animals [5]. The presence of ASF in a region severely damages pig production and economy, disrupts trade in pigs and porcine products and may even affect social welfare [10,11]. Therefore, informed, risk-based decisions are not only needed to allocate limited resources for ASF control in affected regions but also to protect disease-free regions from new ASF introductions. Whilst no sufficiently protective ASF vaccine is currently available [11], the development and inclusion of vaccination strategies into future ASF control programs should be considered on risk-based principles [1,12,13], thus maximising the effectiveness of ASF vaccines that may become available in the future [14]. Taken together, these considerations highlight the need for ASF risk factor identification and mitigation [15].

For the identification of ASF risk factors, we must consider the current epidemiological situation, the predominant disease hosts, potential pathogen vectors or 'vehicles' that could relocate ASFV and the environmental backdrop of ASF disease control in Europe. With the exception of two resolved point incursions of ASF, one into the Czech Republic [16] and one into Belgium [17], disease spread has so far been very difficult to control in the current European epidemic.

Since 2007, approximately 47,000 wild boar cases and domestic pig outbreaks of ASF have been reported overall in Europe [18]. The vast majority of these notifications, at over 86%, were made for ASF in wild boar. Less than 14% of the reported events concerned ASF in domestic pigs, indicating that wild boar currently represent the predominant host for ASF in Europe (Figure 1).

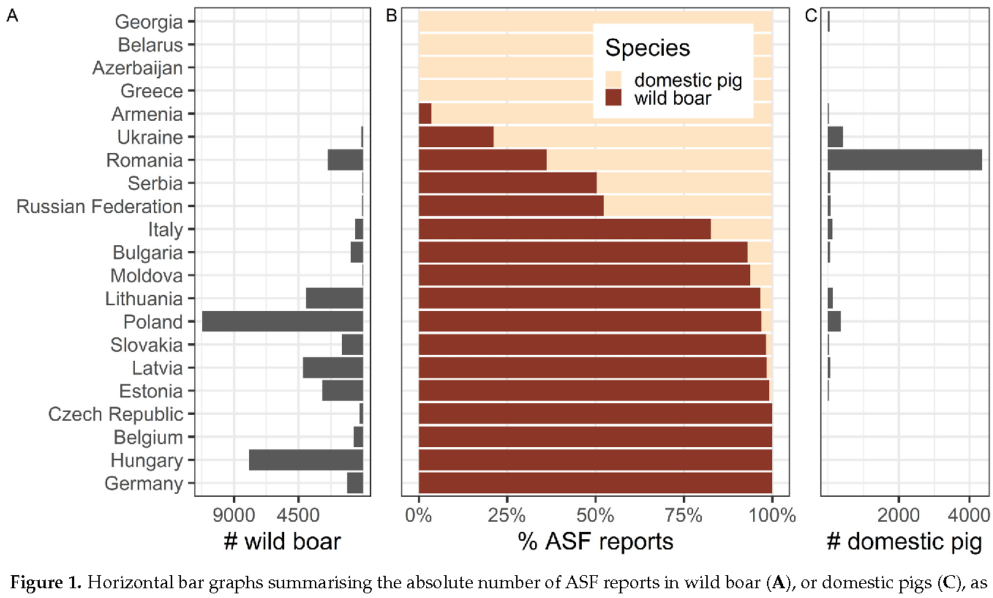

Figure 1. Horizontal bar graphs summarising the absolute number of ASF reports in wild boar (**A**), or domestic pigs (**C**), as well as the relative proportions of ASF, reports for each pig species (**B**) in the indicated countries from April 2007 to May 2021. The mean percentage of the horizontal bar graph in panel B is 62.95%. ASF—African swine fever; Source: OIE, ADIS; considered reports include the detection of ASFV genome (PCR) and humoral immune responses to ASFV (ELISA).

This conclusion also appears to hold true if the ASF-affected areas in Europe are compared on the basis of individual ASF report distribution. If a 5 km buffer is applied to each ASF report as a simplified proxy for the affected area around each notification and the resulting area is subsequently merged for all reported cases and outbreaks, ASF reports in wild boar relate to a 2.51-fold larger total area (350,193 km^2) than ASF reports in domestic pigs (139,391 km^2). A 5 km buffer was chosen as a crude estimate of the area that could be affected by ASF in the vicinity of ASF reports, potentially through animal or human-mediated movements. However, this type of comparison might be biased across all of Europe due to differences in surveillance and ASF reporting among the countries that were considered, as shown in Figure 1. Furthermore, individual ASF reports in domestic pigs generally refer to farm outbreaks with multiple pigs involved, whereas reports in wild boar usually denominate only a single animal. We, therefore, disengaged spatial ASF distribution from individual reports by mapping ASF occurrence to 20 km hexagonal grid cells and only included countries of the European Union, for which similar surveillance and reporting procedures could be assumed (Figure 2). Consistent with the report-based estimation, the grid-based approach showed that ASF in wild boar (613,839 km^2) affected an area almost twice as large as ASF in domestic pigs (308,998 km^2) within the European Union (Figure 2). Additionally, on a per-country basis, the disease in wild boar predominates in most ASF-affected countries in all of Europe (12 out of 21) and the European Union (11 out of 13) (Figure 1b).

The detection of ASF in places that were far away from previously known outbreak areas highlights the importance of human activities as a factor associated with ASF spread [19–22]. Notably, some of these long-range, likely human-mediated ASF incursions that affected the Czech Republic [16], Belgium [17] and West Poland [23,24] occurred in wild boar. Once ASF has been introduced into a previously ASF-free area, the disease can spread among wild boar through currently recognised ASF transmission pathways. These include direct pig-to-pig interaction and indirect contact, e.g., through carcasses of wild boar that died of the disease and environmental contamination [1,20,21,25]. Particularly the carcass-mediated transmission pathway seems to represent a characteristic mechanism of ASF spread in the current European context [2,3,7,25,26]. The introduction of ASF into a naïve wild boar population initially results in disease-mediated deaths and increases the environmental density of wild boar carcasses [27]. The environmental persistence of ASFV in the decomposing carcass is understood as a source of infection for wild boar [7,26]. The association of ASF with wild boar and environmental factors in the current European scenario and the resulting occurrence of ASF in wild boar habitats has led to the description of a 'wild boar habitat' ASF transmission cycle [28,29]. It thus appears fair to conclude that wild boar play a major role in ASF spread and persistence in Europe. Complex wild boar biology and environmental factors that influence the wild boar habitat should therefore be a major focus for ASF control efforts [30].

From this perspective, the relevance of wild boar biology and ASFV-persistence in wild boar carcasses highlight environmental risk factors as a useful category of risk factors to focus on [30]. ASF does not occur in a vacuum, and it is therefore helpful to consider the concept of the epidemiological triad (pathogen, host, environment) to define environmental risk factors [31,32].

The epidemiological triad highlights appropriate categories that need to be considered as elements of disease causation. Thus, for the purpose of this review, an environmental ASF risk factor for wild boar was defined as follows: any factor that occurs in the environment and exposes wild boar populations, which then become infected with ASFV, whereas a comparator wild boar population that has not been exposed to the factor as a suitable reference scenario does not become infected.

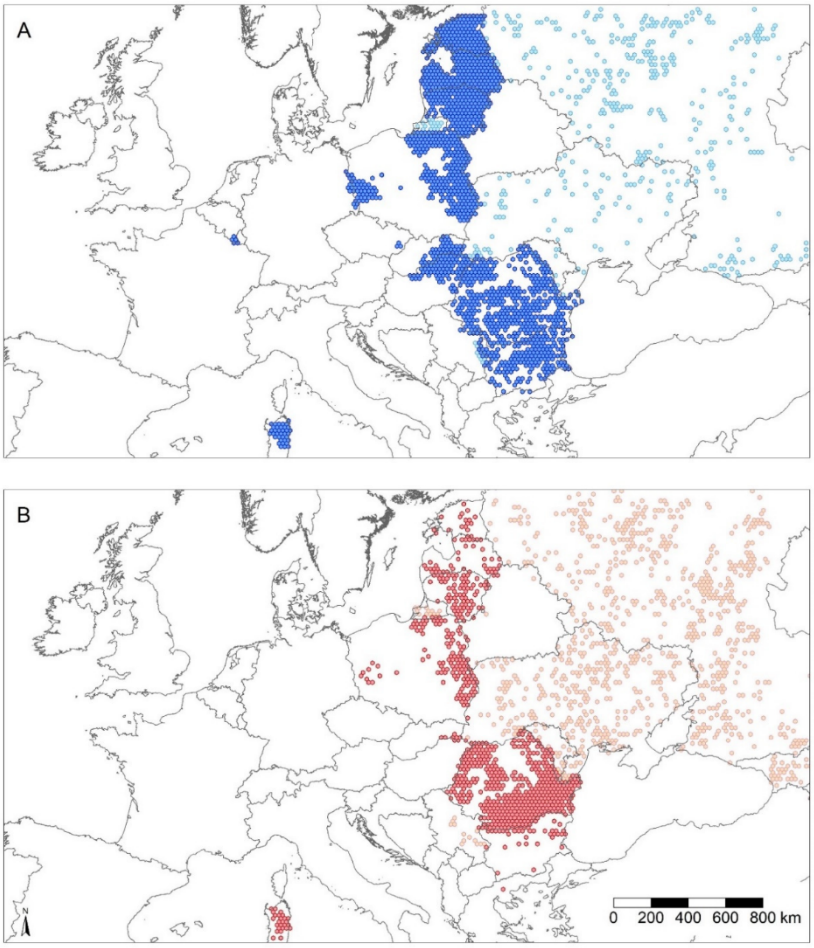

Figure 2. Area estimate maps of European countries affected by ASF in wild boar and domestic pigs. The maps show an estimate of the spatial extent of ASF in Europe, comparing ASF reports from April 2007 to May 2021 in wild boar ((**A**), blue), with reports in domestic pigs ((**B**), red) mapped to a 20 km hexagonal grid across the shown area. One 20 × 20 km grid cell covers an area of 346.41 km^2. Grid cell regions with ASF reports in countries of the EU are marked by dark blue (wild boar, (**A**)) or dark red colour (domestic pig, (**B**)), whereas grid cell regions with ASF reports outside of EU countries are indicated by light blue (**A**) or light red colour (**B**), respectively. Clear areas of the map with no grid cells shown did not report ASF. The total number of ASF affected grid cells during the mapped time segment in all EU countries (dark cells only) was 1772 cells for ASF reports in wild boar ((**A**), total area of approximately 613,839 km^2) and 892 cells for ASF reports in domestic pigs ((**B**), total area of approximately 308,998 km^2). EU—European Union; ASF—African swine fever; Source: OIE, ADIS; Considered reports include the detection of ASFV genome (PCR) and humoral immune responses to ASFV (ELISA).

It is the objective of this work to review the current literature reports about environmental risk factors associated with ASFV infection in European wild boar, but not domestic pigs, and to review factors, which are, as much as possible, in line with the provided definition. Therefore, the scope of this paper is limited to review only studies that compare ASFV infected and non-infected wild boars for their exposure to environmental risk factors. The selection of relevant publications in accordance with the objective for this narrative review was identified based on systematic literature review principles (manuscript in preparation). The environmental-type of ASF risk factors in relation to wild boar populations are considered especially relevant in the European context. A thorough understanding of environmental ASF risk factors in wild boar is expected to help explain ASF transmission dynamics, risks of ASF incursion and spread, but also to critically inform resource allocation and strategy development for ASF surveillance and control efforts in wild boar.

2. Environmental ASF Risk Factors

When considering the environment of ASFV infected wild boar in Europe, several elements of biotic and abiotic factors come to mind that influence or occur in the environment and could be investigated in detail for their association with ASF. As illustrated in Figure 3, these elements non-exhaustively include:

Climate factors, such as temperature, precipitation, humidity, wind, cloud coverage, ultraviolet light conditions, climate changes or season;
Land cover and geomorphology factors, such as vegetation-type, coverage, distribution pattern, altitude, soil type and water availability or type;
Human activity factors, such as human population density, traffic, pollution, artificial structures, housing, roads, farm density, livestock density as well as human outdoor activity types and levels;
Wild boar host-related factors, such as wild boar presence in terms of density, distribution or measurable effects as a result of their activity (e.g., crop damage);
ASF disease factors, such as disease presence, disease type (e.g., a high proportion of ASFV seropositive wild boar present), distribution, distance in space and time from susceptible animals and the viral load, infectious pressure or contamination level.

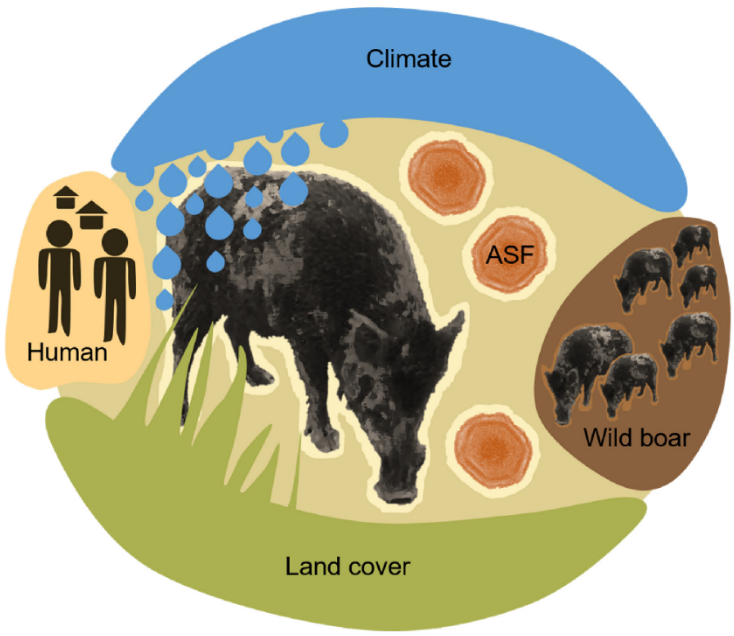

Figure 3. Environmental ASF risk factors for ASF in wild boar. Schematic drawing illustrating the reviewed environmental risk factor elements for ASF in wild boar. ASF—African swine fever.

3. Climate

Many climate parameters are readily measurable and have a pervasive impact on life. Therefore, understanding the potential association of ASFV infection in wild boar with any climate factor would not only provide a well-defined determinant of disease and guide the mechanistic investigation but also offer an easily applicable indicator of disease risk. Investigation of climate factors over longer periods could reveal the association of ASF with climate change. Climate indicators could then be used to allocate or adapt disease control efforts in space and time.

3.1. Seasonality

Whilst detailed weather or climate parameters, such as temperature or rain, might be quite variable over space and time, the broad change in climate that occurs through seasonal fluctuations each year appears to be a robust indicator that could be particularly suitable for examination of association with far-spreading trans-boundary diseases, such as ASF. Along with seasonal change, the behaviour of wild boar undergoes distinct changes. Parameters such as higher temperatures, associated insect and scavenger activity or ultra-violet radiation change and may influence the degradation rate of ASFV in the decomposing carcass, and also the activities of humans interacting with wild boar habitats may change from season to season [9,33,34]. While the interplay and relevance of these factors are unclear, it would be reasonable to expect a seasonal pattern of ASF occurrence.

To examine seasonality as an environmental risk factor, ASFV infections in wild boar exposed to a specific season would have to be measured and compared to the infections detected in wild boar that are not exposed to the season under examination. Samples collected in Poland between 2014 and 2016 from found-dead or hunted wild boar were tested for the presence of the ASFV genome. Temporal analysis consistently identified a seasonal peak of ASFV infected wild boar during summer in several studies [21,35,36]. By contrast, similar studies analysing data from 2014 to 2017 in Lithuania identified a seasonal peak of ASFV genome-positive samples from found-dead wild boar during winter, whereas viral genome-positive samples from hunted wild boar rather peaked during summer to autumn [37]. These findings were confirmed later by further data from Lithuania through to 2018, which also identified a seasonal peak of ASFV-infected wild boar among found-dead animals in winter and a peak of viral genome-positive samples among hunted wild boar in autumn [38].

An overarching analysis of similar data from several European countries by the European Food and Safety Organisation (EFSA) was based on sample collections from found-dead or hunted wild boar in the Baltic States, Poland, Czech Republic, Hungary and Belgium between 2014 and 2019 [9,19,22,39]. Early analyses, looking at data up to 2018, found seasonal peaks of ASFV genome-positive wild boar (found-dead animals) in the summer for Poland, Latvia and Estonia, but less pronounced for Lithuania, and in winter for Poland, Lithuania and Latvia, but not for Estonia. By comparison, a seasonal peak for viral genome detection among hunted wild boars was noticeable for late summer and autumn in all Baltic States but not in Poland. Statistical testing confirmed the existence of seasonal variation in the detection probability of ASFV infected wild boar; however, a specific peak season could not be assigned across all examined countries [9]. An update of the same analysis for detection of ASFV genome in found-dead wild boar included data from more European countries and expanded the study period up to August 2019 [22]. These analyses confirmed summer peaks in Latvia and Estonia and, to a lesser degree, in Lithuania and Poland, winter peaks for all Baltic States, Poland and the Czech Republic, whereas for Hungary and Belgium, peaks in spring were observed [22]. The proportion of ASFV genome detections among all hunted and tested wild boar was again higher in late summer and autumn in the Baltic States, whereas in Poland, ASF detection among hunted wild boar peaked in summer and winter. For the Czech Republic, Hungary and Belgium, seasonal variation in hunted wild boar were unclear, probably due to smaller affected areas and the shorter periods during which ASF was present in these regions [22].

Seasonal differences in the probability to detect the ASFV genome in either found-dead or hunted wild boar were again statistically confirmed for all examined countries [22]. In conclusion, it appeared that seasonal patterns for the occurrence of ASF in wild boar exist. Nevertheless, these patterns were inconsistent in space and time.

3.2. Precipitation and Temperature

Specific climate factors, such as temperature or precipitation, could potentially influence the occurrence of ASF in wild boar, and observations in association with these parameters might help to explain the seasonality of ASF occurrence. Liang et al. modelled the global ASF occurrence and examined the contribution of bioclimatic variables in confirming ASF occurrence based on the model prediction [40]. The underlying model was developed with global ASF outbreak data that did not differentiate whether the disease occurred in wild boar or domestic pigs. It was found that meteorological information was strongly correlated with the overall global ASF occurrence. Particularly, precipitation measures strongly contributed to confirming ASF occurrence, including precipitation in the driest month, precipitation in the coldest quarter and precipitation in the driest and wettest quarter [40]. Temperature-related bioclimatic variables also contributed to the modelled ASF occurrence predictions. Dominant variables included the minimal temperature of the coldest month and the mean temperature of the coldest quarter [40]. These findings indicate that precipitation, particularly during potential low rainfall seasons, might represent factors that influence ASF occurrence globally. Likewise, temperatures during cold seasons appear to be relevant indicators for global ASF occurrence. By contrast, examination of the ASF occurrence in Estonian wild boar from 2014 to 2019 and testing possible associations with temperature or precipitation measures did not reveal any significant contribution of either yearly average minimum temperatures or average yearly snow depth in a hierarchical Bayesian approach modelling the occurrence of ASF [9,22]. Investigations of found-dead wild boar on a much smaller spatial scale (hundreds of meters) in the Czech Republic suggested that increases in mean air temperature reduced the average distance of ASFV infected wild boar occurrence in relation to water sources, which would not be detectable at a regional or global level [16].

In summary, ASF occurrence in European wild boar follows inconsistent seasonal patterns and meteorological parameters seem to be able to predict the global occurrence of ASF coarsely. However, it is unclear whether the disease is directly influenced by climate factors, and if so, which factors matter. It is also possible that climate factors have rather indirect effects on ASF by modifying wild boar behaviour, human behaviour, influencing plant growth or the persistence of ASFV in the environment.

4. Land Cover

The presence and distribution pattern of various land cover types defines the direct interface of ASF on the surface of the earth. Wild boar as ASF hosts depend on minimal land cover requirements to support sustainable populations. Thus, land cover greatly influences wild boar habitat quality, distribution ranges, and ultimately, the spatial distribution of ASF occurrence [41,42]. Moreover, land cover types may also determine the persistence of wild boar carcass material and associated ASFV in the environment by providing protection in the shade and thus minimising decay of infectivity in the sun or supporting scavenger biodiversity and insect activity [33]. Finally, land cover may also influence the accessibility of areas to ASF surveillance in wild boar, so that a land cover-mediated delay in disease detection might hold up implementation of control measures and potentially facilitate the establishment of ASF in inaccessible areas. Therefore, identification of land cover types and patterns that associate with the occurrence of ASF seems valuable for managing disease control measures in ASF-affected regions, for example, through guiding fence building or wild boar carcass search activities.

4.1. Forest

From a perspective of habitat suitability, any land cover type that provides the basic requirements of wild boar habitats regarding shelter, water and food would be important to consider in the context of environmental ASF risk factors [41–43]. Some forest types, such as broad-leafed nut-bearing trees, provide food and shelter. Forest growth supporting water abundance is a prerequisite for forest coverage in the first place. It is probably sufficient to sustain wild boar populations, thus highlighting forest coverage with appropriate tree species as a key land cover type to satisfy the minimal requirements for a wild boar habitat [41]. Consistent with this view, forest coverage at a regional scale was found to associate with the occurrence of ASF in wild boar across distinct geographical regions in Europe, including the Baltic States [39] and Italy [44], indicating that the probability of detecting ASF in wild boar is greater in regions with large forest-covered areas.

At finer spatial scales, which should be more representative of wild boar home-range structures, observations similar to the regional forest studies were made in Poland. When the proportion of forest coverage surrounding a tested wild boar carcass within a 2 km radius was examined, it was found that the probability of detecting the ASFV genome in a wild boar carcass increased with increasing proportions of forest coverage surrounding the animal [36]. Small, spatially scaled studies, conducted in the Czech Republic in the area that was affected by ASF from 2017 to 2018, provided additional information about the relative influence of forest coverage on detecting ASF in wild boar [16]. Whilst a homogeneous search effort throughout the relatively small ASF-affected area can be assumed, over 70% of all carcasses in this area were found in forests. However, by comparison to other land cover types, the odds of detecting ASFV infected wild boar were actually greater in the meadow (OR 1.98) and field (OR 1.61) areas but lower in wetlands (OR 0.87), although fewer carcasses were found in these types of land cover overall [16]. In the same study, it was also found that the probability of detecting ASFV infected wild boar increased in juvenile forest stands aged 40 years or younger and at greater distances from forest edges, although the majority (over 80%) of carcasses were found within 200 m of the forest edge [16]. Notably, almost no wild boar carcasses were found more than 500 m away from forest edges, highlighting forest edges and associated land cover transition zones as key areas for ASFV-infected wild boar detection and thus for surveillance [16]. Overall, logistic regression modelling conducted to explain spatial detection patterns of ASFV-positive wild boar carcasses in the Czech Republic outbreak area revealed that (younger) forest stand age and the ratio of broad-leafed forest trees (their increased presence) were significant predictors of finding ASFV infected wild boar carcasses [16].

4.2. Water and Meadows

Whilst forest land cover potentially satisfies wild boar habitat needs, the explicit presence of surface water appears to be another key land cover factor for ASF occurrence. The occurrence of ASF in wild boar in the Baltic States and Poland from 2014 to 2016 was examined for association with surface water by using a classification tree model [39]. During the examined period, it was found that for the Baltic States, but not Poland, the probability of detecting at least one ASF-positive wild boar case was associated with the proportion of maritime wetlands, inland wetlands (inland marshes and peat bogs) and water bodies, which included watercourses, water bodies, coastal lagoons and estuaries [39]. Investigations of the detection of wild boar carcasses in relation to their distance to surface water sources in the Czech Republic revealed that 59.6% ASFV-positive and 76.2% ASFV-negative were found within 100 m of water. Almost all wild boar carcasses were found within 500 m of water sources, albeit these measures appeared to be air temperature-dependent [16]. Characteristics of meadow environments were also examined in the same study, suggesting that carcasses of ASFV-positive wild boar were more likely to be found in meadows with herb layer heights above 100 cm, whilst about 90% of all carcasses were detected in herb layers heights below 120 cm [16].

4.3. Wild Boar Habitat Quality

Individual land cover parameters might contribute to the probability of ASF occurrence in wild boar by supporting wild boar habitat requirements. Comprehensive modelling of the quality of wild boar habitats includes a range of land cover types and is a more direct way of examining the role of wild boar suitable land cover patterns in permitting ASF occurrence in wild boar [41,42]. Such an approach was taken by EFSA to analyse ASFV-genome detection data of wild boar found-dead in Estonia using a Bayesian hierarchical model. The analysis was repeated sequentially over several years [9,19,22]. It was found that average regional scores for wild boar habitat quality contributed only significantly to explaining ASF occurrence patterns in wild boar when ASF data from 2014 to 2017 were analysed. The analysis of Estonian ASF report data during this time segment suggested that the odds of detecting ASFV-infected wild boar increased by 0.74 for each unit increase in the habitat quality score [19]. Interestingly, the contribution of the wild boar habitat quality to explain ASF occurrence in wild boar in Estonia was not statistically significant when additional data from 2018 [9] and 2019 [22] were included in the analysis.

In summary, land cover types, such as forest and water, affect the occurrence of ASF in wild boar. It is unclear whether these effects are mediated through influences on wild boar habitat quality, ASFV persistence in the environment, local ASF surveillance and control efforts, a combination of these factors or potential interactions with further unknown parameters. An additional study is needed to examine the role of land cover factors in the occurrence of ASF in wild boar.

5. Human Activity

Human activities influencing the environment of ASFV infected wild boar are manifold. Human activity can directly cause the sudden spread of ASF over long distances [17,22–24], impact the environment, climate, land cover and consequently, wild boar habitats due to human presence, hunting or farming activities [9,45]. While climate and land cover factors are difficult to control and rather guide ASF management in space and time, it may be possible to regulate some types of human activity in the environment to help control ASF in wild boar. Hence, it may be useful to identify human activities in the environment that associate with the occurrence of ASF in wild boar.

5.1. Human Presence and Environmental Impacts

The simple presence of humans in numbers could be measured through population density. The influence of the human population on ASF in wild boar in a particular region was examined using classification tree models in the Baltic States from 2014 to 2016 [39]. During these years, an increasing human population density was associated with increased ASF occurrence in wild boar in Estonia and Lithuania, but not in Latvia.

While population density directly measures the presence of humans, additional factors can be considered to estimate the environmental impact of human presence, including urban coverage and the abundance of roads as indicators of transportation and residential development. Consistent with the effects found for human population density, the number of settlements was associated with the occurrence of ASF in wild boar in all Baltic States during 2014 to 2016 in the classification tree model [39]. However, these associations of population density and human settlement density/km^2 as risk factors were lost if a Bayesian hierarchical model was used instead and information on ASF-positive and -negative wild boar from Estonia for 2017 [19], 2018 [9] or 2019 were analysed [22]. Similar findings were made in Poland, where no association of built-up areas with ASF in wild boar was found [36], and the Czech Republic, where the distance to settlements had no measurable effect on finding ASFV-infected wild boar carcasses when compared to non-infected carcasses [16].

The number of roads, road length and road density was found to have a variable influence on the occurrence of ASF in wild boar. From 2014 to 2016, an increasing abundance of roads was associated with disease occurrence in Latvia and Lithuania, but not in

Estonia [39] and Poland [36]. The analysis of road abundance effects on the detection of ASF in wild boar in Estonia from 2014 to 2017 identified the regional road length as a risk factor that increased the odds of disease detection [19]. A similar analysis of Estonian data up to 2018 found the opposite effect, i.e., increasing road density reduced the odds of detecting ASF in wild boar [9], and analysis of Estonian data up to 2019 found no significant effect exerted by road density on disease occurrence [22]. By comparison, in the Czech Republic, ASFV-positive wild boar carcasses tended to be further away from roads (about 300 m) than ASFV-negative carcasses (about 100 m) [16], also suggesting a dispersing effect of roads on the probability of detecting ASFV-infected wild boar. Dead wild boar found in close proximity to roads could also be a result of road traffic accidents [46]. Experiences in Estonia and Latvia have shown that road traffic-associated wild boar deaths are not frequently examined for ASF and that the prevalence of ASF in wild boar that died by traffic accidents appears to be low [46]. These experiences suggest that road traffic accidents could contribute to increasing the occurrence of ASFV-negative wild boar carcasses in close proximity to roads slightly.

Renewable energy production and the generation of municipal waste represent interesting examples of outcomes of human activities in the environment, whereby particularly waste production is an obvious parameter in the context of ASF in wild boar. It appears plausible that increased waste production also increases ASF spill-over chances through littering by making ASFV contaminated pig products accessible to susceptible wild boar populations. From 2015 to 2018, Loi et al. examined the influence of waste production and energy production from renewable resources in Sardinian municipalities on ASF occurrence in wild boar. It was indeed found that municipalities with increased waste production observed more ASF cases in wild boar [44]. The production of energy from renewable sources had a protective effect and was associated with fewer observations of ASF infections in wild boar [44]. Whilst a relationship of waste production with ASF is conceivable, it is unclear how energy production relates to ASF occurrence in wild boar.

5.2. Hunting

Hunting and targeted wildlife management represent environmental human activities that directly affect wild boar. Hunting-related activities are thus expected to have a measurable effect on ASF in wild boar. However, repeated examination of the occurrence of ASF in wild boar and its association with various hunting-related activities in Estonia from 2014 to 2019 did not support this assumption [9,22]. Hunting and wildlife management associated parameters, including the density of hunters, the density of hunting dogs, the density of feeding or baiting places and the density of hunted wild boar, were not found to be associated with ASF detection in wild boar [9,22]. The reasons for the lack of association in this study are not known. It is possible that any wildlife management-related effects on wild boar populations and disease occurrence are so small when compared with ASF-related effects [27] that they are non-detectable in the examined data [9,22].

5.3. Farming

Farming likely influences the environment of ASFV-infected wild boar through most agricultural activities, which include crop production; effects of pig husbandry, such as manure management, pest control, feed storage, cadaver management, keeping pigs outdoor or in backyards, pig transportation; and travel and other activities of farm workers. Whilst many of these effects on ASF occurrence in wild boar may be difficult to measure, available information about the presence of farms, farmed livestock and farm types could be examined instead. In the Baltic States, the number of pig farms and the number of domestic pigs were indicators of the regional ASF occurrence in wild boar in Estonia and Latvia, but not in Lithuania, whereas the number of small pig farms increased the probability of disease occurrence in Latvia only [39]. Later in the ASF epidemic, the number of pig farms was identified as a risk factor for increasing the odds of detecting ASFV-infected carcasses when the ASF test results for wild boar carcasses from Estonia were analysed up to 2017 [19]. Similarly, data analysis from Estonia up to 2018 suggested the density of domestic pigs as a risk factor for the occurrence of ASF in wild boar [9]. Data ranging up to 2019 indicated that the density of pigs in small holdings keeping up to 10 pigs was a risk factor for ASF in wild boar [22].

In conclusion, human activities in the environment of ASFV-infected wild boar influence the epidemiology of the disease. Environmental risk factors for ASF related to the presence of humans and to farming were inconsistently identified among European countries over time, indicating that either disease dynamics or the influence of the examined risk factors may change in time and space, possibly in accordance with the local stage of the ASF epidemic. Hunting activities were not found to influence the occurrence of ASF in wild boar.

6. Wild Boar

Environmental risk factors for ASF in wild boar may consider the overall abundance or occurrence pattern of these animals in space and time, as well as any signs of their presence in the environment, such as tracks, tree markings, signs of ground rooting, crop damage or traffic accidents. The presence of wild boar defines the availability of a susceptible host for ASF and is thus a prerequisite for ASFV infection in wild boar, whereby modulation of the numbers of wild boar must be expected to influence disease occurrence. Despite the implied relevance of wild boar density for ASF, it is inherently difficult to determine the numbers of this adaptable wildlife species accurately and to monitor it continuously. It is, therefore, necessary, however, to estimate wild boar density values based on hunted wild boar counts per area [47], to use wild boar habitat models [41,42] or other techniques, such as photo-trapping or genetic capture–recapture approaches [48]. It is, therefore, important to keep in mind that wild boar density estimates are likely crude approximations of the true absolute value and likely biased by the hunting effort in accordance with how much a particular density estimate relies on underlying hunting bag data.

During the course of the current ASF epidemic in the European Union, the association of wild boar density with the occurrence of ASF in wild boar was studied in Estonia, Latvia, Lithuania and Poland, considering different time segments of the epidemic. Classification tree modelling of potential factors for ASF detection in wild boar from 2014 to 2016 in the Baltic States identified wild boar density as a risk factor for Latvia and Estonia, but not for Lithuania during this period [39]. These findings were confirmed by other studies that considered data during the same time segment for Estonia and for Lithuania from 2014 to 2017 [37,47]. Follow-up investigation of wild boar density in Estonia during 2014 to 2017 found, by using a generalised additive model and a Bayesian hierarchical approach, that an increase in the estimated wild boar density by one wild boar per square kilometre increased the odds of detecting ASF in wild boar by over two units [19]. This analysis was repeated with the Bayesian model, this time including data from an additional year on ASF in wild boar in Estonia, i.e., from 2014 to 2018, with the same result [9]. Another follow-up repeat of the same analysis now included Estonian data up to 2019 [22]. This analysis, however, did not find a significant association of wild boar density with the occurrence of ASF in wild boar [22]. This observation suggests that the effect of risk factors in this context could be time-dependent and that the underlying stage (e.g., early or late) of the examined ASF epidemic may influence whether a risk factor plays a role or not. A similar observation was made in Poland from 2014 to 2015 when a correlation between wild boar numbers and ASF cases in wild boar was detected for the affected forest regions only during the first year of the observations [35]. In the following year, this correlation was lost, as the ASF-affected area in Poland had further increased [35]. Another study examined the effect of wild boar density in the ASF-affected area in East Poland from 2014 to 2016 by using a generalised linear mixed model and found that wild boar density was a significant factor in predicting the occurrence of ASF in wild boar [36].

Taken together, high wild boar density appears to be associated with increased odds of ASF occurrence in wild boar in Europe. However, this observation might be time-dependent and influenced by the local stage of the ASF epidemic.

7. ASF Disease

Environmental factors directly related to ASF itself describe spatial and temporal patterns of the occurrence of ASF. Evidence of the occurrence of ASF in wild boar or domestic pigs in the environment of a susceptible wild boar population is likely to pose a distance-dependent risk of ASFV infection for that population. In other words, the nearness of ASF increases the probability of ASF occurrence in susceptible animals, which represents an underlying principle for animal disease control. In the European Union, the establishment of disease control zones around detected ASF outbreaks is currently implemented through Regulation (EU) 2021/605. Understanding the relevant distances that associate nearness to previous ASF cases with the increased likelihood of ASF occurrence in wild boar would help with decisions on the zoning for ASF surveillance and control measures. Knowledge about the role of spatiotemporal distance to previous ASF cases could also be used to differentiate epidemiological scenarios for possible ASFV introduction pathways into susceptible wild boar populations [9].

Temporal and spatial relationships of ASF cases in wild boar in the European Union from 2014 to 2019 were used to construct networks, in which cases represented the nodes and links between cases were assigned based on the previous occurrence in time and on minimal distance criteria [9,22]. The resulting network provided an estimation of the speed distribution of ASF spread from one case report to a neighbouring wild boar report in the network. When considering only proximity-based networks, it was found for the Baltic States and Poland during 2014 to 2018 that the median distance, with which ASF spread among wild boar during a year was 11.7 km (range of country-specific medians was 9.4–16.3) [9]. Inclusion of additional case reports up to 2019 with data from Belgium, the Czech Republic, Hungary and Romania resulted in lower ranges for the annual median spread of ASF cases in wild boar; a median ASF spread in wild boar of 5 km (the Czech Republic) to 31.4 km (Romania) per year. The 75 percentiles of the annual spread distance distributions for these countries revealed more extreme ranges of 11.7 km (the Czech Republic) to 120.3 km (Romania) [22]. Similar spread distances for ASF in wild boar were identified at about 18 km per year in Poland from 2014 to 2015 [21]. These types of analyses illustrate the distribution of distances at which susceptible wild boars may be at an increased risk of ASFV infection due to the nearness of ASF cases in their environment within a year.

The influence of the distance to previous ASF cases and the distance to the border with Belarus (as a proxy for an unknown ASF disease status) were compared for carcasses of ASFV-infected and non-infected wild boar examined during 2014 to 2016 in the outbreak area in East Poland [36]. Inclusion of the distance variables in a generalised linear mixed model revealed their significant contribution to predicting ASF occurrence in wild boar. It was found that the probability of detecting ASFV-positive wild boar carcasses increased by 19% when distances from previous cases decreased from 64 to 7 km [36]. A similar observation was made when the distance from the border with Belarus decreased from 34 to 0.07 km. Then, the probability of detecting ASF in wild boar increased by 20% [36]. Proximity to the border of another country as a substitute for ASF nearness and proximity to previously reported ASF cases were also identified as significant factors in predicting wild boar ASF occurrence in a spatial zero-inflated Poisson modelling study of ASF-positive wild boar carcass occurrence in South Korea from 2019 to 2020 [49].

The concept of ASFV transmission cycles in domestic pigs, which may involve ticks (pig–tick cycle) [50,51], or ASFV-contaminated pork products (domestic cycle), suggests that wild boar may also become infected through similar pathways in areas where ASF occurs in domestic pigs [28,29,52].

This section emphasises the nearness of ASF in the environment as an important risk factor for the occurrence of ASF in wild boar. Specifically, distances of up to about 30 km to the nearest ASF case appear to increase the probability for the occurrence of most ASF cases in wild boar during one year of exposure.

8. Discussion

The information reviewed herein summarises current knowledge on potential environmental risk factors that may influence the occurrence of ASF in European wild boar (Table 1). Exposure of wild boar to environmental risk factors related to climate, land cover, human activity, wild boar and ASF nearness were found to contribute to ASFV infection in this pig species (Figure 3).

Table 1. Summary of potential environmental risk factors for the occurrence of African swine fever in wild boar.

Risk Factor	Summary of the Possible Effect	Reference
Seasonality	Seasonal disease patterns of disease occurrence observed	Podgorski et al., 2018 [21]; Smietanka et al., 2016 [35]; Podgorski et al., 2020 [36]; Pautienius et al., 2018 [37]; Maciulskis et al., 2020 [38]; EFSA, 2018 [9]; EFSA (Abrahantes et al.), 2017 [39]; EFSA, 2020 [22]; EFSA (Depner et al.), 2017 [19]
Precipitation	Precipitation during extreme dry, wet or cold periods influences disease occurrence	Liang et al., 2020 [40]
Temperature	Temperatures, particularly during extremely cold periods, may influence disease occurrence and spatial association with water sources	Liang et al., 2020 [40]; EFSA, 2018 [9]; EFSA, 2020 [22]; Cukor et al., 2020 [16]
Forest	More forest, proximity to forest, younger tree ages of broad-leafed forest associated with disease	EFSA (Abrahantes et al.), 2017 [39]; Loi et al., 2019 [44]; Podgorski et al., 2020 [36]; Cukor et al., 2020 [16]
Water	Presence and proximity to surface water associated with disease	EFSA (Abrahantes et al.), 2017 [39]; Cukor et al., 2020 [16]
Meadows	Growth height of meadow vegetation between 1 and 1.2 m associated with disease detection	Cukor et al., 2020 [16]
Wild boar habitat quality	High wild boar habitat suitability likely associated with disease	EFSA, 2018 [9]; EFSA (Depner et al.), 2017 [19]; EFSA, 2020 [22]
Human population density	Greater human population density may be associated with disease	EFSA (Abrahantes et al.), 2017 [39]
Human settlements	Human settlements unlikely associated with disease	EFSA (Abrahantes et al.), 2017 [39]; EFSA (Depner et al.), 2017 [19]; EFSA, 2018 [9]; EFSA, 2020 [22]; Podgorski et al., 2020 [36]; Cukor et al., 2020 [16]
Roads	More roads may increase detection of disease, but roads may also have a dispersing effect on disease occurrence	EFSA (Abrahantes et al.), 2017 [39]; Podgorski et al., 2020 [36]; EFSA (Depner et al.), 2017 [19]; EFSA, 2018 [9]; EFSA, 2020 [22]; Cukor et al., 2020 [16]; Schulz et al., 2020 [46]
Renewable energy production	More energy production from renewable resources may reduce disease	Loi et al., 2019 [44]
Waste production	More waste production likely associated with disease	Loi et al., 2019 [44]
Hunting	Hunting was not found to associate with disease	EFSA, 2018 [9]; EFSA, 2020 [22]
Farming	More domestic pigs and pig farms, particularly smaller pig farms, associated with disease occurrence	EFSA (Abrahantes et al.), 2017 [39]; EFSA (Depner et al.), 2017 [19]; EFSA, 2018 [9]; EFSA, 2020 [22]
Wild boar presence	High wild boar density associated with disease	EFSA (Abrahantes et al.), 2017 [39]; Pautienius et al., 2018 [37]; Nurmoja et al., 2017 [47]; EFSA (Depner et al.), 2017 [19]; EFSA, 2018 [9]; EFSA, 2020 [22]; Smietanka et al., 2016 [35]; Podgorski et al., 2020 [36]
ASF nearness in wild boar	Proximity to ASF in wild boar associated with disease	EFSA, 2018 [9]; EFSA, 2020 [22]; Podgorski et al., 2018 [21]; Podgorski et al., 2020 [36]; Lim et al., 2021 [49]

The fact that ASF in wild boar dominates the current situation in Europe, especially since 2014, and the ongoing spread of ASF in this species, focuses much attention on controlling the disease in wild boar. Therefore, a comprehensive understanding of environmental risk factors for ASF in wild boar is urgently needed and essential for successful disease management. In this regard, the information gathered for this article is judged overall to be mostly of recent origin, sometimes scarce and partially inconsistent. Importantly, most of the available studies that reported information on environmental ASF risk factors and met the criteria to be selected for the review of evidence-based risk information associated with ASF occurrence in wild boar were reported from the Baltic States and Poland. This may at least in part be due to the emerging situation of ASF in wild boar in Europe. Conclusions drawn from the available data principally apply to similar geo-climatic regions. However, extrapolations to other areas of Europe, for which no comparable data exist, should be made with care. Naturally, environmental risk factors for ASF in wild boar are part of a greater ecosystem and are expected to be interlinked and interdependent. This context implies that unifactorial analysis of potential ASF risk factor effects would likely overlook relevant factors among confounding variables. Thus, multifactorial approaches were mostly employed in the reviewed studies to model the association of ASF occurrence in wild boar with the examined variables [9,16,19,22,36,39,44,49], thereby highlighting the relevance of identified risk factors among other co-variables.

Studies that investigate environmental ASF risks would mainly rely on detailed knowledge about ASFV infections in wild boar with accurate and precise information on time and space of disease occurrence, including the same data from suitable reference scenarios with non-infected wild boar. These type of data may be difficult to obtain, but the implementation of appropriate frameworks could support the collection of important information that would otherwise be lost. An example of such a framework is the CSF/ASF wild boar surveillance database (https://surv-wildboar.eu, accessed on 1 June 2021) of the European Union, run by the German Friedrich-Loeffler-Institut on behalf of the EU Reference Laboratories for Classical and African swine fever. It is expected that similar surveillance databases exist in Europe (likely at a national level) that record positive and negative test results, as well as relevant metadata for ASF samples from wild boar.

Much of the risk information contained in this review was obtained from expert-based EFSA reports [9,19,22,39]. This information is likely to be peer-reviewed in the relevant EFSA working groups and panels but may not necessarily be published in peer-reviewed journals. In any case, concordant conclusions were reached in situations where the findings of EFSA analyses were reproduced at least in parts by similar peer-reviewed studies using alternative methodologies [36,47]. Two pathways to access data on ASF in wild boar for the analysis of environmental risk factors were apparent. Epidemiologists either used information from their home country or collaborated with colleagues from other countries. Both pathways appear fruitful and should be supported to facilitate further studies of environmental risk factors for ASF in wild boar to improve ASF control in the future.

Inconsistencies were noticed among the reviewed risk factors. Some of these were differences in seasonal peaks of ASF occurrence in wild boar among the examined countries. These observations suggest that there are country-specific and seasonal differences in the factors that influence the frequency with which wild boar are ASFV infected in the studied outbreak areas. Since most ASF cases in the Baltic States and Poland fall inside a window of 15 degrees latitude, it appears unlikely that the observed inter-country variability of seasonal ASF peaks is directly caused by spatial variation in meteorological patterns alone, but more probably through variability in other, secondary seasonal factors. Such factors could be related to virus persistence in the environment, wild boar ecology, seasonal farming activities or other seasonal human activities [9]. Another reason for the observed seasonal inconsistencies could be a shorter duration of the epidemic, consequently a lack of data over several years and differences in disease control strategies. For instance, ASF outbreaks in the Czech Republic [16] or Belgium [17] were much smaller in areal size and duration, whilst the Baltic States and Poland have managed ASF in a relatively large area for multiple years now. Therefore, periodically high levels of ASF detection in smaller outbreak scenarios could be caused by intensive search efforts and subsequent elimination of the disease as a result rather than by underlying seasonal patterns. Similar effects may also contribute locally in larger outbreak scenarios. Whilst these possibilities or other potential causes remain to be investigated, the identified differences in seasonal peaks of ASF occurrence in wild boar among the examined countries could be very useful to identify additional underlying risk factors for ASF. At least for a subset of large ASF-affected European areas over long periods, such as for the Baltic States and Poland, the general seasonal pattern of ASF detection in found-dead wild boar appears to be similar in principle, presenting with more or less pronounced peaks in winter and summer [22]. Boklund and colleagues discuss potential reasons for these seasonal peaks [9], but further study is required to understand the underlying causes for the seasonal patterns of ASF occurrence in wild boar.

Another interesting inconsistency in the association of risk factors with the occurrence of ASF in wild boar appeared to be time-dependent. EFSA risk analyses of wild boar habitat quality- and road density-related risks for disease occurrence were conducted in a sequential manner for Estonia, starting with data from 2014 to 2016 [39], included additional data that became available each year thereafter until 2019 [9,19,22]. This approach provided insights into temporal dynamics for the ability of these factors to predict the occurrence of ASF in wild boar in Estonia. It was observed that both factors, wild boar habitat quality and road density, were initially associated with increasing the odds of disease occurrence, but this association was lost as the ASF epidemic progressed in 2018 and 2019 [9,22]. These results indicate that ASF spread continuously in wild boar in Estonia to most of the examined administrative units during 2018 and 2019, ultimately affecting almost the entire country [53]. This process appeared to be independent of the regional wild boar habitat quality or road density in the later stages of the epidemic in Estonia [9,22]. This conclusion further suggests that some environmental risk factors may only be relevant during the early phases of ASF outbreaks or at the epidemic front of larger outbreak areas by modulating the progression of the epidemic wave-front through the landscape [54,55]. The ASF induced collapse of the wild boar population, which may occur as the epidemic passes through an area, could be a key reason that disengages ASF occurrence from wild boar density and related risk factors, thus stunting this type of risk factor associated with disease occurrence as the epidemic progresses over time [27,47]. It is consistent with this interpretation that the correlation of detected ASF cases in wild boar and wild boar density estimates in Polish forest units was only noticeable during the first year of the epidemic in the study area but was lost during the second year [35]. These findings imply that environmental ASF risk factors should be examined during the early stages or at the leading edge of the prevailing epidemic in the study area. It is further implied that the examination should occur on a fine spatial scale [16,56], rather than a coarse regional level, to help identify relevant factors of ASF occurrence in naive wild boar populations. All things considered, it could be hypothesised that many of the reviewed risk factors are, in fact, modulating the occurrence and distribution of wild boar in the environment, rather than occurrence of ASF under the condition that wild boar are present in an area. This could explain why the association of some risk factors with ASF is lost as the epidemic progresses over time. These temporally dependent risk factors (such as wild boar habitat quality) may identify indirect, wild boar-related, rather than disease-related factors and may warrant future ASF risk factor analyses that consider ASF occurrence under the condition of wild boar presence.

9. Conclusions

The current understanding of environmental ASF risk factors has implications for disease management in European wild boar and provides further insights into ASF transmission dynamics. While climate, land cover and ASF-related factors would be difficult to control, they may provide guidance on how to allocate resources. Knowledge of human activity and wild boar-related factors might offer opportunities for direct control. The reviewed information about environmental ASF risk factors in wild boar implies the following considerations for managing the disease in regards to ASF surveillance, hunting, wild life management, wild boar carcass searches, implementation of barriers to reduce wild boar migration, forestry and cropping activities [57]:

9.1. Timing

Adapt the timing of disease surveillance and control efforts to the seasonal ASF occurrence pattern in wild boar. Increasing active wild boar carcass search and removal efforts during times of peak disease occurrence, such as during summer or winter, may improve the efficiency of removing carcasses of ASFV-infected wild boar from the environment. However, constraints due to extreme weather or vegetation may impede such seasonal efforts but could also be advantageous. During warm temperatures in summer, it may be more likely to find carcasses of ASFV-infected wild boar closer to water sources, thus spatially restricting the targeted search area in summer. Seasonally focussed search efforts may release resources during other times of the year, such as spring and autumn, which could then be used for other control activities, such as regulatory work. Seasonal considerations may further be adapted to known seasonal wild boar behaviour patterns and the regulation of agricultural cropping activities in the area, as cropping activities likely influence land cover, wild boar habitat suitability and potentially also occurrence patterns of ASF. Likewise, active surveillance may be improved by well-planned hunting of wild boar during late summer and autumn, particularly in areas currently not affected by ASF and which are adjacent to outbreak regions, all whilst following suitable biosecurity measures. This strategy may increase the chance of detecting the recently infected live animals at the epidemic ASF front.

9.2. Spatial Targeting

Adapt the spatial targeting of disease surveillance and control efforts to the spatial pattern of ASF occurrence in wild boar by prioritising areas to which many spatially informative environmental ASF risk factors apply. High priority areas for wild boar carcass searches may be forested areas, particularly those with younger tree stands, as well as forests with large proportions of broad-leafed trees, areas that are no more than 500 m away from the edge of the forest and from water sources, whilst potentially focussing on areas over 100 m away from roads. In open meadows, tall herb layer areas could be prioritised, again less than 500 m from water. These land cover factors may identify areas of high habitat suitability for wild boar and thus high wild boar densities. Since associations of land cover- and wild boar-related factors with the detection of ASF in wild boar were lost over time, prioritisation of these high-risk areas may be particularly important in the early phases of an outbreak or at the edges of established outbreak areas. Similar spatial considerations could be applied for surveillance purposes to target the hunting of wild boar adjacent to ASF outbreak areas, but also to position fences for ASF control or to establish wild boar culling zones (‘white zones’). By mapping high-risk areas of ASF occurrence in wild boar according to the identified criteria, it may be possible to identify landscape corridors of high and low disease risk. Bosch et al. previously proposed a similar concept to harness knowledge about areas of high wild boar habitat suitability for the management of ASF [41]. The characterisation of disease risk areas on a small spatial scale may allow locating fences and ’white zones’ along low-risk corridors to potentially enhance their barrier function. High-risk corridors, on the other hand, would represent anticipated pathways of ASF spread and can be targeted with appropriate measures to prevent ASF spread.

9.3. Dynamic Disease Control

The spatial association of known and newly detected ASF cases over time implies continuous spatial disease spread. This may therefore indicate that the size of ASF restriction zones, which define disease control activities, need to be continuously increased as well. A concept of dynamically growing ASF restriction zones over time and in relation to known disease occurrence could improve the spatial overlap of the ASF affected area and the applied control measures, thus avoiding that disease control lags spatially behind ASF occurrence. It is important to note, however, that a system of continuously expanding restriction zones needs to be effectively managed and would require the appropriate allocation of limited resources. Moreover, the expectance of ASF spread in wild boar is based on the analysis of spatially extended disease events in the Baltic States and Poland that may not apply to other ASF-affected areas in Europe. Therefore, a model of disease-anticipating, dynamic expansion of ASF restriction zones may not only be undesirable but also not justified in a specific local context.

In summary, the presence of ASF in European wild boar has a devastating impact on the European pig industry and causes significant hardship for people that work with or are dependent on pigs and pig products. The awareness of potential environmental ASF risk factors is critical in this situation, as ASF is very difficult to control in wild boar. Due to their inherent nature, the association of many environmental factors with ASF is challenging to study, and as a result, evidence is scarce. Nevertheless, at least some aspects of ASF occurrence in wild boar appear to be far from random, and environmental risk factors conducive to the observed disease pattern remain to be determined through careful epidemiological study. Notably, unpredictable, human-mediated spread events of ASF in wild boar occur, and reasonable allowances for these events have to be made for the investigation of environmental ASF risk factors.

The conclusions that can be derived from the current knowledge about environmental ASF risk factors for European wild boar should be applied to disease management. In the meantime, and particularly if ASF has been reported nearby, it would be fair to exercise extreme caution in relation to human activities that directly or indirectly interact with wild boar in the widest sense. These activities include domestic pig husbandry, waste handling, wildlife management, hunting practices, forestry work, recreational outdoor movements or any similar type activities for which adequate control and biosecurity measures should be carefully implemented.

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3.2.3.2 To sample or not to sample? Detection of African swine fever in wild boar killed in road traffic accidents

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Highlights

- In 5 years and from 108,224 data records, only 99 samples originated from wild boar killed through an RTA
- Reporting errors should be avoided to allow reliable epidemiological evaluations
- The probability to detect an ASF-positive animal among hunted wild boar is not significantly higher than among wild boar involved in a RTA
- Increasing the sample size and thus the power of surveillance, all wild boar found dead no matter if killed through an RTA or died due to any other reason should be tested for ASF virus

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RAPID COMMUNICATION

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To sample or not to sample? Detection of African swine fever in wild boar killed in road traffic accidents

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Abstract

African swine fever (ASF) in wild boar remains a threat for the global pig industry. Therefore, surveillance is of utmost importance, not only to control the disease but also to detect new introductions as early as possible. Passive surveillance is regarded as the method of choice for an effective detection of ASF in wild boar populations. However, the relevance of wild boar killed through road traffic accidents (RTA) for passive surveillance seems to be unclear. Using comprehensive ASF wild boar surveillance data from Estonia and Latvia, the prevalence of ASF-infected wild boar was calculated and the probability of infection as measured by PCR compared for animals that were hunted, found dead, shot sick or killed in a RTA. The number of samples originating from wild boar killed in a RTA was low and so was the ASF prevalence in these animals. However, the reasons for this low number of RTA animals remain unknown. Therefore, we recommend to sample wild boar killed in a RTA to a greater extent, also to explore, if this approach can increase the detection probability, and to avoid missing disease introduction.

KEYWORDS

African swine fever, detection probability, prevalence, road traffic accidents, wild boar

1 | INTRODUCTION

African swine fever (ASF) continues to threaten the pig industry globally. The current epidemic of ASF genotype II that started in Georgia in 2007 has now lasted for more than 10 years and has not been brought under control so far (Cwynar, Stojkov, & Wlazlak, 2019; Sanchez-Cordon, Montoya, Reis, & Dixon, 2018).

It is known that the probability of ASF detection in wild boar is highest through passive surveillance, that is the sampling of dead, injured or sick animals (European Food Safety Authority, 2018, 2019; Nurmoja et al., 2017; Schulz, Oļševskis, et al., 2019). Thus, estimating the prevalence of ASFV-positive, that is PCR-positive, wild boar helps evaluating the extent of the disease. Passive surveillance activities are implemented in many countries including member states of the European Union. The European Commission (EC) emphasized in a working document the focus to sample found carcasses and sick wild boar. Sampling wild boar killed in RTA in areas free from ASF and in neighbouring areas of affected zones is also recommended (European Commission, SANTE/7113/2015—Rev 11). The European Food Safety Authority (2019) also stated that it differs from country to country, if wild boar killed in RTA are included in passive surveillance activities or not. In general, wild boar are frequently involved in RTAs (Benten, Balkenhol, Vor, & Ammer, 2019; Jakubas, Rys, & Lazarus, 2018; Kruuse, Enno, & Oja, 2016; Kusta, Keken, Jezek, Hola, & Smid, 2017; Saenz-de-Santa-Maria & Telleria, 2015). It is not clear, however, whether the risk of an ASF-infected wild boar of getting involved in a RTA is increased as compared to an uninfected wild boar. This issue has been intensively discussed, and there are arguments in favour and against the hypothesis, but the number of available datasets has not been sufficient to come to a scientifically sound conclusion. These observations and the recent detection of an ASF case in a wild boar potentially involved in a RTA in a newly affected area in the province Lubusz in Western Poland initiated the present study. To use every chance for improving the efficacy of ASF surveillance and minimize the risk of missing an early ASF case, we aimed to investigate the potential role of wild boar killed through a RTA in the detection of ASF in wild boar.

2 | MATERIALS AND METHODS

To investigate and to validate the role of samples from wild boar killed in RTA in ASF surveillance and the early detection of the disease, data from ASF-affected countries had to be used. Thus, for the study, ASF wild boar surveillance data from Estonia and Latvia were used. The data originated from the CSF/ASF wild boar surveillance database of the EU Reference Laboratory (https://public.surv-wildboar.eu/Default.aspx). The available dataset consisted of 108,617 data records. Each record included information on a single animal. It comprised information about the date of sampling, the age, the ASF PCR result and the origin of the sample ('hunted', 'found dead', 'shot sick', 'killed in a RTA', these categories are referred to as 'carcass code' in the following). Data with missing information regarding the carcass (nine samples) or the PCR test result (384 samples) were excluded. Therefore, 108,224 data records were used in the analyses. The final dataset covered the period from 25th June 2014 until 30th September 2019 and included only months, in which PCR-positive wild boar were detected.

All statistical analyses were performed using the software package R (http://www.r-project.org). To test for statistically significant associations between the carcass code and the ASF PCR result on animal level, a Fisher's exact test was performed. In the case of a significant association, each carcass code was tested separately against each other using Fisher's exact test. To control the type I error for multiple testing, a Bonferroni adjusted p-value was calculated for each individual test (Dunn, 1961). A p-value of $\leq .05$ was considered statistically significant.

The ASF prevalence for each carcass code was determined on the basis of ASF PCR-positive results, and confidence intervals were calculated according to Clopper and Pearson (1935).

3 | RESULTS

Most samples that were available and tested by ASF PCR originated from hunted animals, and wild boar found dead. Only 26 samples were investigated from wild boar shot sick, and 99 samples came from animals that had been killed in a RTA (Table 1).

Out of 99 RTA samples in total, 36 were recorded in 2017, whereas in 2015, no sample were registered as originating from animals killed in a RTA. In the remaining years, between 6 and 33 samples came from wild boar involved in RTA. Except for 2014, where no samples were recorded as originating from wild boar killed in RTA in Estonia, the majority of samples of wild boar killed in a RTA came from Estonia (Figure 1).

The estimated ASFV prevalence was highest in wild boar found dead (0.735; CI 0.722–0.747), whereas the prevalence in animals killed in a RTA yielded the lowest value (0.010; CI 0.0003–0.055) (Figure 2). The one ASF-positive wild boar killed through a RTA was found in Estonia in 2017 (infected zone).

The association between the carcass codes and the ASF PCR result was statistically significant ($p < .001$). In the direct comparison of the individual carcass codes vice versa, most differences were significant ($p < .001$; Table 2). Only the difference between the probability to find an ASF-positive PCR result in hunted wild boar and wild boar killed in a RTA was not statistically significant ($p = 1$).

TABLE 1 African swine fever PCR-positive and negative wild boar samples from Estonia and Latvia and their origin

	Hunted	Found dead	Shot sick	Road traffic accident
PCR negative	100,627	1,372	19	98
PCR positive	2,301	3,799	7	1

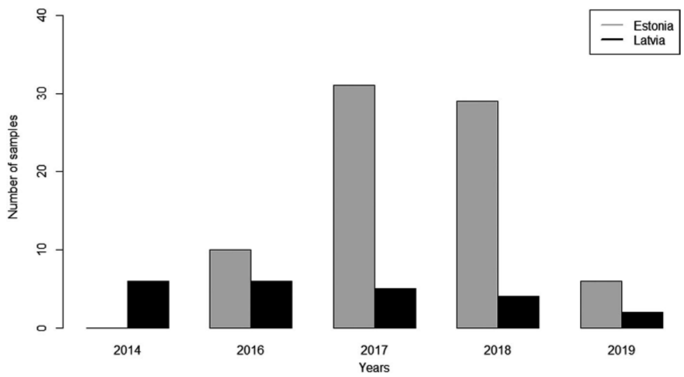

FIGURE 1 Number of samples originating from wild boar killed in road traffic accidents (RTA) by country and year. In 2015, no samples came from animals killed in a RTA

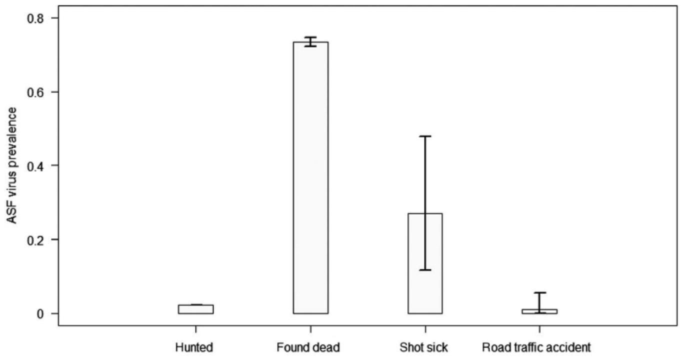

FIGURE 2 African swine fever virus prevalence in hunted, found dead, shot sick wild boar and animals killed in a road traffic accident. The whiskers indicate 95% confidence intervals

4 | DISCUSSION

Recommendations regarding passive surveillance have so far focused mostly on the sampling of wild boar found dead. However, a large number of road traffic accidents including wild life are caused by wild boar (Kruuse et al., 2016; Kusta et al., 2017; Saenz-de-Santa-Maria & Telleria, 2015). ASFV-infected wild boar may be altered in their behaviour, and their risk of becoming involved in a RTA may be increased. Therefore, the chance of detecting ASF-positive wild boar killed in a RTA may also be heightened, particularly in the case of external manipulations like driven hunts. However, there is no scientific evidence so far of an increased probability for ASF-diseased wild boar to become involved in a RTA.

The plenitude of surveillance data originating from the Baltic States allowed to investigate the ASF prevalence in wild boar killed in RTA and to compare the probabilities of detecting ASF-positive wild boar in hunted, found dead, shot sick wild boar and animals killed in a RTA. Despite of the huge number of investigated samples, only 99 samples were obtained from animals registered as killed in a RTA. This small number might be due to several reasons. Wild boar killed in a RTA has not always been recorded in the laboratory database as such, because the information was sometimes missing in the sample submission form as the main source of epidemiological information. Therefore, there may be a reporting bias. Specifically, in Estonia, a field, where pre-formed information on RTA or shot sick as the cause of death could be entered, was missing on the sample submission form until February 2017. In Estonia, hunters have been obliged to report wild boar involved in RTA to the veterinary authority since 2015. Thus, all earlier reports of RTA originate from additional remarks made by veterinary officers or hunters on the sample submission forms. In all other cases, we may suppose that the animals killed in a RTA, were reported as animals 'found dead'. Also, it is conceivable that animals, which were originally involved in RTA and not immediately killed and could retract, are not easily identifiable as such and therefore wrongly reported as 'found dead'. Therefore, an underestimation of samples coming from wild boar killed by RTA is very likely.

TABLE 2 Results of the Fisher's exact tests, testing the ASF prevalence within each carcass code separately against each other

	Found dead	Shot sick	Road traffic accident
Hunted	$p < .001$	$p < .001$	$p = 1$
Found dead		$p < .001$	$p < .001$
Shot sick			$p < .001$

Moreover, the disposal of wild boar carcasses associated with RTA is often organized through road agencies, which may dispose carcasses before sampling. Even in Estonia, where hunters are responsible for the disposal of wild boar killed in RTA, reporting of these animals has not been compulsory. It can be assumed that wild boar density, wild boar habitats, road conditions and traffic volume differ between countries, so that our study, which was conducted with data from Estonia and Latvia, may have yielded results that cannot necessarily be generalized. However, as mentioned earlier, several studies from different countries contain a large proportion of wild boar killed in RTA (Benten et al., 2019; Jakubas et al., 2018; Kusta et al., 2017). A study from Estonia reported an increasing number of wild boar and as a consequence also an increasing number of wild boar killed in a RTA during the years 2004–2013 (Kruuse et al., 2016). This study was conducted before the wild boar density in Latvia and Estonia had decreased due to ASF and measures to control the disease had been implemented (Nurmoja et al., 2017; Schulz, Staubach, et al., 2019). Therefore, the number of wild boar killed in RTA might has decreased after 2015. Due to regulations, in Estonia, no samples were recorded as originating from wild boar killed in a RTA in 2014 and 2015, making an evaluation of ASF results from animals killed in RTA before 2016 impossible. Nonetheless, the numbers of samples originating from wild boar killed in a RTA probably represent only a small proportion of wild boar that were in fact killed in a RTA. The ASFV prevalence in wild boar killed in RTA was very low; however, the low sample size and the very low number of positive cases have to be considered. Following these findings, sampling those animals might be considered as unnecessary and uneconomical. Although the detection, sampling and disposal of wild boar found dead usually requires huge personal and financial efforts, the significantly higher probability of detecting a new ASF case clearly justifies this. If wild boar killed by a RTA is sampled, however, the benefits compared to the efforts, also for the traffic authorities, which often have to be involved in many countries, are questionable.

Yet, additional efforts to sample wild boar killed in a RTA should still be considered because it is not clear, whether the risk for an ASF-infected wild boar to be killed in a RTA is increased as compared to an uninfected animal. Also, neither the true number of wild boar involved in RTA nor the true proportion of sampled animals is known. To clarify this, more samples from wild boar killed in RTA are necessary.

In our study, the probability to detect an ASF-positive animal among hunted wild boar was not significantly higher than among wild boar involved in a RTA. Therefore, our results suggest that there is no additional benefit in sampling wild boar involved in RTA compared to sampling hunted wild boar. However, the results have to be interpreted within the framework of this study and the available data. More data with more reliable reporting of the origin of the samples might support the recommendations to sample wild boar killed through RTA. Also, samples originating from wild boar involved in RTA are not dependent on hunters, hunting preferences and hunting season and might therefore be better and more reliably available, especially in countries with high wild boar density and traffic volume.

Along with the current ASF strategy of the EC (European Commission, SANTE/7113/2015 - Rev 11), we recommend, as far as feasible and economically reasonable, to sample every wild boar found dead, no matter if killed through a RTA or died due to any other reason, and test it for ASF. This applies for affected areas, in which testing and removal also wild boar that were killed in an RTA can reduce the number of ASF-positive cadavers in the environment and thus contribute to mitigating the risk of new infections. Also, in risk areas, particularly in the case of a high risk of disease introduction, any delayed detection of ASFV introduction could thus be avoided. At the same time, meticulous recording of the cause of death of the animals (hunted, found dead, shot sick or killed in a RTA) is needed to allow further analyses.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as no animal experiments were conducted.

DATA AVAILABILITY STATEMENT

The original data used for the analyses can be obtained from the author after approval by the responsible institution in Estonia and Latvia.

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3.2.3.3 How to demonstrate freedom from African swine fever in wild boar –Estonia as an example

Katja Schulz[1], Christoph Staubach[1], Sandra Blome[2], Imbi Nurmoja[3,4], Arvo Viltrop[4], Franz Josef Conraths[1], Maarja Kristian[5] and Carola Sauter-Louis[1]

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Highlights

- For an official declaration of freedom from ASF in wild boar, the country specific surveillance has to demonstrate that no ASFV and no wild boar with ASFV-specific antibodies are present
- The low population density developing in the course of an ASF epidemic stresses out the urgent need to maintain strong active and passive surveillance measures
- Due to the lack of scientific evidence regarding the role of seropositive animals in the transmission of ASF, a way of dealing with such animals must be considered within the framework of the demonstration of freedom from ASF
- ASF should be added on the disease list of the OIE for which an official procedure for recognition of disease status already exists

vaccines

MDPI

Article

How to Demonstrate Freedom from African Swine Fever in Wild Boar—Estonia as an Example

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Abstract: Estonia has been combatting African swine fever (ASF) for six years now. Since October 2017, the disease has only been detected in the wild boar population, but trade restrictions had to remain in place due to international regulations. Yet, the epidemiological course of the disease has changed within the last few years. The prevalence of ASF virus (ASFV)-positive wild boar decreased steadily towards 0%. In February 2019, the last ASFV-positive wild boar was detected. Since then, positive wild boar samples have exclusively been positive for ASFV-specific antibodies, suggesting the possible absence of circulating ASFV in the Estonian wild boar population. However, as the role of seropositive animals is controversially discussed and the presence of antibody-carriers is regarded as an indication of virus circulation at EU and OIE level, Estonia remains under trade restrictions. To make the disease status of a country reliable for trading partners and to facilitate the process of declaration of disease freedom, we suggest to monitor the prevalence of seropositive wild boar in absence of ASFV-positive animals. The possibility to include ASF in the list of diseases, for which an official pathway for recognition of disease status is defined by the OIE should be evaluated.

Keywords: African swine fever; disease freedom; wild boar; serology; surveillance

1. Introduction

African swine fever (ASF) emerged in Estonia in September 2014 for the first time. First cases were reported in wild boar and domestic pig farms were first affected by ASF outbreaks in 2015. The last ASF outbreaks in domestic pig holdings were notified in 2017 [1]. However, Estonia is still subject to trade restrictions and suffers economic losses as the country does not fulfil the criteria for disease freedom, although no ASFV-positive wild boar were detected since February 2019. Only the fact that there are still seropositive wild boar is taken as an indication for possible virus circulation in the population. With the exception of the island of Hiiumaa, where ASF has never been detected, the whole territory of Estonia has been designated as an infected zone and restrictions according to the Commission Implementing Decision of 9 October 2014 concerning animal health control measures relating to African swine fever in certain Member States (MS) and repealing Implementing Decision 2014/178/EU (2014/709/EU) apply (area with ASF occurrence in wild boar: Part II). Correspondingly, the dispatch of live pigs and pig by-products is restricted. By way of derogation, pigs can be dispatched to other areas and MS listed in part II or part III. However, the dispatch is accompanied by increased personal and financial resources (2014/709/EU Article 3). The exportation of live pigs in third countries is completely prohibited [2]. Also, the exportation of animal by-products is subject to strict regulations [2]. Within the EU, all listed regulations can be lifted as soon as a country shows the required evidence of disease freedom. However, the World Trade Organization (WTO) as well as third countries conduct their own risk assessments. Thus, third countries could impede the import of pigs and animal by-products based on their own risk assessment, even if the trade restrictions within the EU were lifted. A possibility to prevent arbitrary restrictions through third countries could be an official declaration by the OIE-Assembly. However, the procedure to gain official recognition of disease status by the OIE is currently reserved to seven defined diseases, which do not include ASF (https://www.oie.int/animal-health-in-the-world/official-disease-status/official-recognition-policy-and-procedures/, accessed 3 April 2020).

Following the Commission Decision of 26 May 2003 approving an African swine fever diagnostic manual (2003/422/EC) and the current OIE Terrestrial Animal Health Code chapter 15.1.1, infection with ASF is present, when ASFV has been isolated from a suid sample or ASFV antigen identified in a sample or if ASFV-specific antibodies have been found in samples coming from domestic pigs or wild boar. The latter two definitions apply to samples, which originate from suids that showed clinical or pathological signs, were epidemiologically linked to ASF cases or were suspected to be associated with ASFV [2]. The current OIE definitions differ from the ones in the previous edition of the Terrestrial Animal Health Code, where the detection of ASFV-specific antibodies during the past 12 months was only considered as relevant in wild boar aged between 6 and 12 months [3].

Accordingly, a country can be considered free from ASF when no infection with ASF is observed and further criteria specified by the EU or the OIE are met. These include appropriate laboratory investigations, awareness programs, presence of competent veterinary authorities, sufficient surveillance activities, and the implementation of appropriate biosecurity measures [2]. No evidence has so far been found for the potential involvement of Ornithodoros ticks in the transmission and spread of ASF in Estonia [4]. Therefore, sufficient surveillance activities have to be shown for 12 months and the importation of pigs has to follow the regulations of the EU and the OIE before the country might be eligible to be declared free from ASF [2]. With regard to sufficient surveillance measures, the EU working document "Strategic approach to the management of African Swine Fever for the EU" (SANTE/7113/2015–Rev 11) recommends differentiating the surveillance activities between the aims of eradicating or controlling the disease. As Estonia aims to eliminate the disease, the focus is on both, enhanced passive surveillance, i.e., testing of all wild boar found dead for ASFV by PCR; and on active surveillance, i.e., testing all hunted wild boar for ASFV by PCR and for ASFV specific antibodies.

For areas or countries that are in the process of disease elimination, the current EU (2003/422/EC) and OIE recommendations prescribe the implementation of real-time PCR for virus detection and ELISAs for the detection of antibodies against ASFV as the standard procedure. As serological confirmation tests, IPT (indirect immunoperoxidase test), IBT (immune blotting test), and IFAT (indirect fluorescent antibody test) are recommended [5]. The respective standard operating procedures are provided by the European Union Reference Laboratory for ASF (https://asf-referencelab.info/asf/en/procedures-diagnosis/sops, accessed 14 May 2020) and their implementation in Estonia is described in detail elsewhere [6].

The case fatality ratio of ASF is known to be very high [7]. However, animals usually develop antibodies against ASFV within 7–10 days post infection, irrespective of the final disease outcome. They can be tested positive for ASFV and for antibodies simultaneously for at least 100 days after infection [8,9]. Samples from wild boar surviving this period are usually positive for ASFV-specific antibodies, but negative for ASFV and its genome [8–10]. Since a vaccine against ASF is not available [11,12], wild boar showing such test results more than 100 days post infection apparently survived the disease.

It is not yet known for how long ASFV-specific antibodies persist and may be detected in wild boar; however, Penrith et al. [13] and Pujols Romeu et al. [14] found them several years after infection.

Penrith et al. [13] also found ASFV-specific maternal antibodies in piglets younger than six months of age. Although these findings refer to domestic pigs, one can assume that the situation in wild boar is similar. While diaplacental virus transmission to the fetus can occur after infection with Classical Swine Fever (CSF) virus [15,16], for ASF, scientific evidence for intrauterine infection is scarce and inconclusive [8,17–21].

The role of surviving animals in the spread or in the maintenance of ASF is still controversially discussed. In a recent study, it was hypothesized that survivors with virus persisting in lymphatic tissues could start shedding virus when stressed or immunocompromised [22]. However, there is no scientific evidence that survivors really serve as carriers and could thus pose a risk for maintaining the disease in the population [9,10,23,24].

Recent analyses of Estonian wild boar surveillance data showed a clear decline of wild boar samples that were ASFV-positive by PCR. Even in areas where the first cases were not detected before 2016, the prevalence of PCR-positive wild boar started to decrease by the end of 2017. At the same time, an increase of seropositive, but PCR-negative test results was observed. Furthermore, in areas, where the epidemic had started in 2014 and 2015 (Southern and Northeastern part of the country), the initially increasing seroprevalence already started to decrease in July 2018 [10]. Since March 2019, all positive ASF samples have been exclusively serologically positive, i.e., showing ASFV-specific antibodies, although all surveillance activities in Estonia are still performed in accordance with the regulations for an infected country and intensive surveillance is implemented.

In a previous study, data for the period December 2014–July 2018 was analyzed to investigate the course of ASF in the East and the West of Estonia [10]. Based on this first investigation, the presented study broadens the scope by including recent surveillance data and assesses the situation in the whole of Estonia. Based on our findings, we propose adapting the current EU and OIE regulations, taking the latest scientific results into account [9,10,23–25]. Due to the presumably long half-life of ASFV-specific antibodies [13,14], seropositive wild boar may be present for several years without constituting a risk of disease transmission. In contrast to CSF, which could be controlled by oral vaccination in wild boar [26], it is of utmost importance to explore alternatives to re-gain ASF-free status without vaccination. To protect countries like Estonia from individual trade restrictions through third countries, we propose not only defining the current EU and OIE requirements more precisely, but to add ASF to the list of diseases, for which official recognition of disease status can be granted by the OIE. These changes may support elimination efforts in other countries, where the epidemic of ASF seems to run a similar course [25] and could acknowledge the global importance, ASF has gained in recent years.

2. Materials and Methods

2.1. Data

Wild boar surveillance data from the whole of Estonia that covered a period from January 2015 until February 2020 were used (62 study months, 6 study years). The data were entered by the Estonian veterinary authorities into the CSF/ASF wild boar surveillance database of the European Union (https://surv-wildboar.eu), from which they were obtained with the approval of the Estonian authorities for the analyses described here. The structure of the data set, the sampling process and the laboratory investigations are described in detail elsewhere [6]. Samples originated either from active (hunted wild boar) or passive surveillance (wild boar that were found dead, shot due to sickness, or killed in a road traffic accident). Each data record contained information about the virological (PCR) and the serological (ELISA and for confirmation IPT) test result. Blood samples were taken from hunted wild boar that were tested for ASFV genome and for ASFV-specific antibodies. From animals found dead, tissue or bone marrow samples were collected and investigated for ASFV genome [6]. All samples were processed following the Diagnostic Manual valid in the EU (2003/422/EC).

All data records lacking a conclusive virological test result (i.e., neither positive nor negative) (n = 135) or information on the origin of sample (active or passive surveillance) (n = 2) were excluded.

Estonia is divided into 15 counties, which represented the basis of our analyses. Due to an administrative reform in 2017, all data records from 2015–2017 were assigned to the new administrative counties.

Population data from the Estonian Environment Agency (Nature department) were used to investigate the trend of the wild boar population density in Estonia in the context of ASF. The analysis continued those performed by Schulz et al. [10] by adding data from the hunting seasons of 2018/19 and 2019/20 to those from the hunting seasons 2012/13–2017/18. Data was provided on county level and the estimated number of wild boar per km^2 was calculated for the whole of Estonia. The detailed description of the data has previously been provided [6].

2.2. Prevalence Analyses

Prevalence estimates were calculated for PCR-positive wild boar (irrespectively of their serological test result), for wild boar that were PCR and serologically positive and for wild boar with a PCR-negative, but a positive serological test result, in the same way as described by Schulz et al. [10] and Olševskis et al. [25]. The prevalence estimates were calculated for the whole of Estonia, for each age class (younger than one year, between one and two years, and older than two years) and for each of the 62 study months. Prevalences were estimated using the software package R (http://www.r-project) [27] and 95% confidence intervals were computed according to Clopper and Pearson [28].

2.3. Modeling Temporal Effect

Model analysis was applied to data from wild boar samples that had yielded a conclusive serological test result (negative or positive) and were PCR-negative. The temporal course of the raw seroprevalence estimates was calculated for each study month using a Bayesian space–time model and BayesX 2.0.1 (http://www.uni-goettingen.de/de/bayesx/550513.html). A detailed description of the model and the variables is provided elsewhere [10,29]. In brief, age was defined as a fixed independent variable. In contrast to the prevalence estimations, age was categorized into two classes (animals younger than two years and animals older than two year) for the model analysis. The seroprevalence was defined as a dependent variable and time, space, and season were included as random factors. From the original data set, all data records with a positive PCR result were removed (n = 2936). Also, samples from passive surveillance (n = 672), samples without information for age (n = 547) and samples lacking a conclusive serological test result (n = 82) were excluded for the model analysis. All figures were generated using the software package R (http://www.r-project) [27].

2.4. Population Density

The number of wild boar per km^2 in all 15 counties of Estonia were summarized and analyzed for the whole country. Differences between the population densities in the hunting seasons were analyzed as described previously [10]. A p-value of <0.05 was considered as statistically significant.

3. Results

3.1. Data

After excluding all data records with invalid information, 46,093 data records were available. In the 62 study months, the number of samples originating from hunted wild boar was clearly higher than the number of those coming from passive surveillance. The fluctuations in the sample size followed a clear seasonal pattern with the highest number of samples taken during winter. This applies to samples from both active and passive surveillance, likewise (Figure S1 Supplemental Material). The largest numbers of samples (14,947 samples from active surveillance and 978 samples from passive surveillance) were obtained in 2016. In 2018 and 2019, the numbers of samples were clearly lower in both categories. However, in 2020, where information was so far only available for the first two months, already 1346 samples from active surveillance were investigated and 10 samples originated from passive surveillance (Table 1).

Table 1. Numbers of investigated wild boar samples for each year of the study period from active (i.e., from hunted animals) and from passive surveillance (i.e., from animals found dead, shot due to sickness or killed in road traffic accidents)

Number of Samples \ Year	2015	2016	2017	2018	2019	2020 *
Active surveillance	8580	14,947	9150	4884	4715	1346
Passive surveillance	941	978	401	79	62	10
Total	9521	15,925	9551	4963	4777	1356

* For 2020, only data from the first two months were included.

3.2. Prevalence Analyses

Of all three prevalence groups (PCR-positive, only seropositive or positive for ASFV and ASFV-specific antibodies), PCR-positive wild boar showed the highest prevalence estimates, whereby the prevalence estimates resulted in the highest values in animals younger than one year (Table 2). The ASFV prevalence estimates in wild boar younger than one year were higher in spring and summer (Figure 1). The PCR-prevalence decreased over time and reached 0%, where it remained since March 2019 in all three age classes (Figure 1).

Table 2. Median, minimum, and maximum prevalence estimates for wild boar of three different age classes for the period January 2015–February 2020. The animals had tested positive by PCR, were seropositive or positive for ASFV, and had ASFV-specific antibodies.

Age Classes	Prevalence ASFV (PCR-Positive)			Prevalence Seropositive			Prevalence ASFV- (PCR-) and Seropositive		
	Median	Min	Max	Median	Min	Max	Median	Min	Max
<1 year	0.057	0.000	0.602	0.011	0.000	0.250	0.007	0.000	0.119
1–2 years	0.051	0.000	0.209	0.019	0.000	0.130	0.003	0.000	0.025
>2 years	0.027	0.000	0.419	0.022	0.000	0.160	0.000	0.000	0.035

The seroprevalence was clearly lower in all three age classes than the ASFV prevalence (Table 2). Especially in animals older than two years, an increase in the seroprevalence over time was observed. During the last six months of the study period, the seroprevalence decreased in all three age classes, but particularly in wild boar younger than one year. In this age class, the last two seropositive wild boar were detected in July and August 2019 (Figure 2).

The prevalence of wild boar that tested positive for both, ASFV-specific antibodies and ASFV by PCR were even lower than the seroprevalence estimates (Table 2). The lowest values were observed in wild boar older than two years. From May 2018 onwards, the prevalence estimates were extremely low in all three age classes, with a 0% prevalence in the last 12 (younger than one year), the last 13 (between 1 and 2 years), or the last 15 months (older than 2 years) of the study period, respectively (Figure 3).

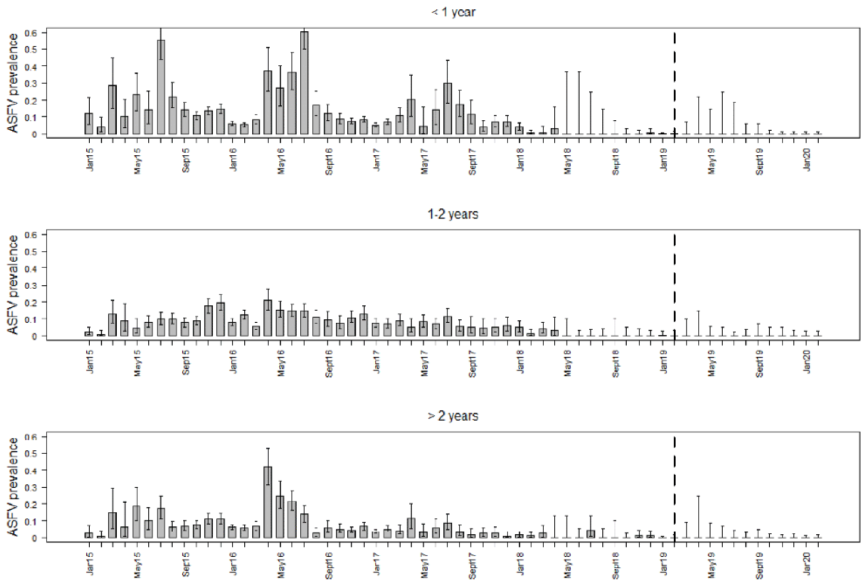

Figure 1. Prevalence estimates for ASFV PCR-positive wild boar, irrespective of their serological status for each study month and three different age classes. The whiskers indicate 95% confidence intervals. The broken vertical line highlights February 2019, when ASFV-positive wild boar were last detected by PCR.

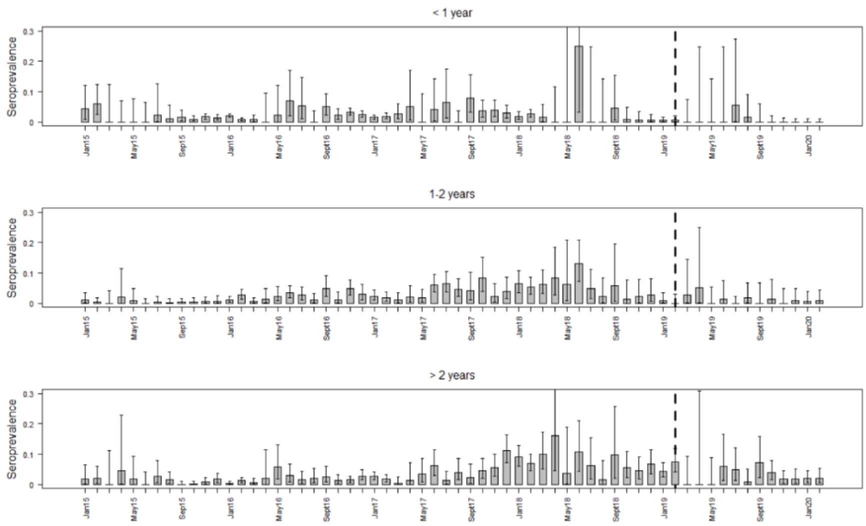

Figure 2. Prevalence estimates for seropositive wild boar that were ASFV PCR-negative for each study month and three different age classes. The whiskers indicate 95% confidence intervals. The broken vertical line highlights February 2019, when ASFV-positive wild boar were last detected by PCR.

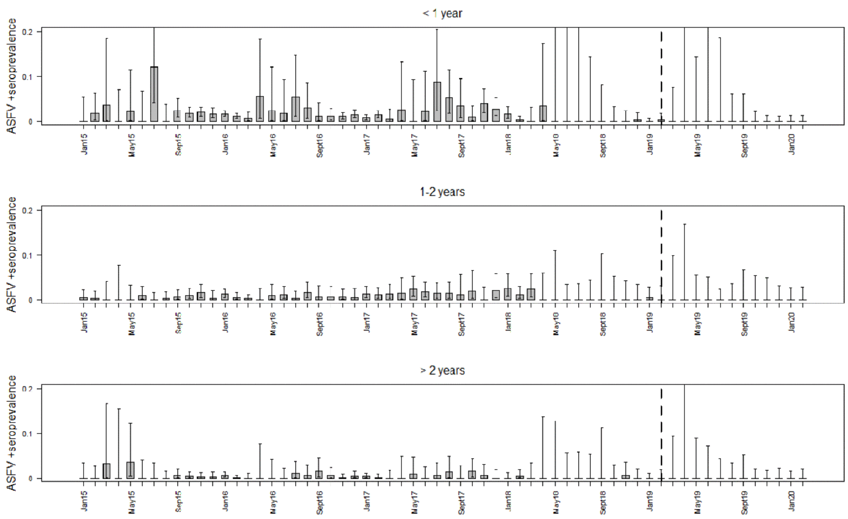

Figure 3. Prevalence estimates for ASFV PCR-positive and seropositive wild boar test results for each study month and three age classes. The whiskers indicate 95% confidence intervals. The broken vertical line highlights February 2019, the last month, in which ASFV-positive wild boar were detected.

3.3. Modeling Temporal Effect

For the model analysis of the temporal effect on the prevalence, 41,856 data records were used. Adjusted for seasonal effects (Figure S2 Supplemental Material), the temporal course of seroprevalence estimates was investigated. Until April 2018, an increase in the temporal trend of the logit prevalence of seropositive wild boar was observed. Subsequently, the logit prevalence decreased steadily until the end of the study period (Figure 4).

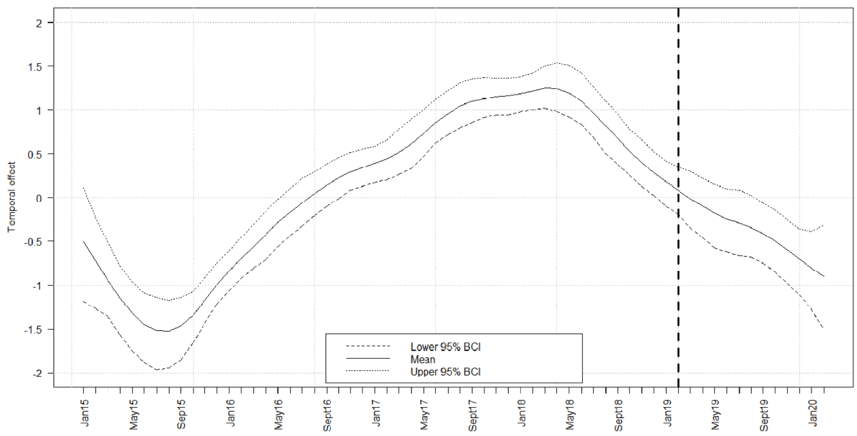

Figure 4. Median temporal effect on the logit prevalence for all samples that tested exclusively serologically positive, 95% Bayesian credible intervals (BCI) are indicated. The broken vertical line highlights February 2019, when the last ASFV-positive wild boar were detected by PCR.

3.4. Population Density

The wild boar population in Estonia was stable from 2012/13 until 2015/16 with no significant difference between the single hunting seasons. However, the population density dropped statistically significantly (p-value = 0.015) from hunting season 2015/16 to hunting season 2016/17. Also from 2016/17 to 2017/18, the population density decreased, but the difference was not statistically significant (Figure S3 Supplemental Material). From hunting season 2016/17 onwards, the reported number of wild boar and the calculated number of wild boar per km^2 was highest on the island of Hiiumaa, where no ASF cases have occurred so far (Table S1 Supplemental Material).

4. Discussion

In Estonia, the last ASFV PCR-positive wild boar was detected in February 2019. In December 2018 and January 2019, only nine ASFV-positive wild boar were detected, from which eight were found close to the eastern border of the country. Most likely, these cases were epidemiologically linked to the ASF situation in the western part of the Russian Federation, where ASFV circulation was reported between September and November 2018 (http://www.fsvps.ru/fsvps/asf, accessed 7 March 2019).

Since then, all cases reported as ASF-positive were ASFV-negative by PCR and only seropositive. According to EU regulation 2003/422/EC and the current Terrestrial Animal Health Code of the OIE [2], it makes no difference for the status of the country, if animals are ASFV-positive, i.e., virus positive; or seropositive (if an epidemiological link to ASFV cases exists), i.e., the country remained under trade restrictions [2]. With regard to the seropositive test results, the judgement concerning the presence of an epidemiological link to ASFV-positive cases is left to the individual countries, but it will be difficult for a country like Estonia to argue that the seropositive cases found there are not epidemiologically linked to previous ASFV infections in wild boar. Yet, the regulation leaves already some room for interpretation, while it was stricter in the former OIE Code text, where seropositive test results were only considered as relevant, if they originated from wild boar aged between six and 12 months [3].

In the current ASF epidemic, which started in Georgia in 2007 [30], the Czech Republic provided the documents to the OIE in support of a self-declaration of re-gaining an ASF-free status. This declaration must not be confused with the official recognition of disease status by the OIE (https://www.oie.int/animal-health-in-the-world/official-disease-status/official-recognition-policy-and-procedures/, accessed 3 April 2020).

In accordance with the requirements of the OIE [2], the Czech Republic declared the freedom from ASF in April 2019, after one year of the last confirmed ASFV-positive cases in wild boar (https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Self-declarations/2019_05_CzechRep_ASF_ANG.pdf, accessed 3 April 2020). In this self-declaration of the Czech Republic, data from the time of the epidemic (26 June 2017 till 28 February 2019) has been presented (https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Self-declarations/2019_05_CzechRep_ASF_ANG.pdf, accessed 3 April 2020). No information is provided about the kind of investigations performed (virologically or serologically) in this document. In an official presentation, more detailed data have been shown, indicating that serologically positive hunted wild boar were sampled in July and October 2018 (http://web.oie.int/RREurope/eng/Regprog/docs/docs/SGE%20ASF12/BTSF/02_Sampling_and_laboratory_testing_Pavel_Bartak.pdf, accessed 22 April 2020). This indicates the presence of seropositive wild boar only few months before the country submitted the self-declaration of freedom from ASF to the OIE. However, the seropositive animals may not have originated from wild boar younger than one year and thus, the Czech Republic may not have considered them as directly linked to a suspected or confirmed case of ASF. Therefore, these findings were not regarded as contradicting the requirements of the OIE for a self-declaration of freedom from ASF [2]. Moreover, no new cases of ASF were so far detected in the Czech Republic, suggesting the absence of ASFV circulation in the country, despite the potential presence of seropositive wild boar.

In the majority of countries that are affected by the current ASF epidemic, infected wild boar populations play a major role. This experience contrasts with previous African swine fever epidemics in Europe, which mainly involved domestic pigs and were often under control after a few outbreaks had occurred [31]. After the successful implementation of control measures, the disease status was resolved. On the Iberian Peninsula, however, the situation was more complicated and the disease became endemic for decades. In 1995, when Spain was officially declared as free from ASF in domestic pigs, wild boar and their ASF status were not included in the statement [31,32]. Perez et al. [33], who detected a seroprevalence of almost 10% in wild boar in some areas of Spain, recommended repeating serological investigations after the declaration of freedom, but wild boar were not considered as a relevant reservoir for ASFV at that time and trade restrictions regarding Spain were ultimately lifted [34]. Although Mur et al. [31] found many years later no ASFV or ASFV-specific antibodies, the seroprevalence in Spanish wild boar was unknown in 1995.

This example from the past, the ambiguity of the self-declaration of disease freedom, the temporal trend of ASF test results in Estonia, the continuous huge surveillance effort in countries like Estonia and the lack of scientific evidence for the existence of carrier animals and their obvious negligible epidemiological role, if they existed [9,23], were the motivation to initiate the present study. The aim was to analyze ASF surveillance data with regard to the temporal trend of the ASFV prevalence and seroprevalence and to evaluate surveillance activities. The results lead us to propose a revision of the current EU and OIE definitions regarding the freedom from ASF status or even adding ASF to the diseases that can gain an official recognition of disease status by the OIE.

The decrease in the sample size over time correlates most probably with the decreasing population density. Particularly regarding the reduced numbers of samples originating from passive surveillance, decreased chances to detect a dead wild boar can be assumed due to the lower population density and the reduced circulation of ASF. Following the working document of the EU "Strategic approach to the management of African Swine Fever for the EU" (SANTE/7113/2015–Rev 11), the number of wild boar carcasses detectable declines in the phase of a decreasing epidemic. This supports the hypothesis that the ASF epidemic in Estonia is subsiding. However, the effort to find dead wild boar and to sample every detected wild boar, thus performing surveillance according to the EU (SANTE/7113/2015–Rev 11) and to the Article 15.1.30 to 15.1.32 of the OIE Terrestrial Code [2] is of course still high and must remain so for the foreseeable future to detect any re-incursion or re-emergence as soon as possible.

The study results also show a clear seasonality in the number of ASF samples. In the months of hunting season (October–February), the numbers of samples are higher, particularly the ones taken from hunted wild boar. Also the number of samples originating from passive surveillance are higher in the winter months, which may be due to the less dense vegetation and the increased presence of hunters in the forests. However, to increase the certainty in the assessment of freedom from ASFV, more efforts should be made to increase the sample size also in times of usually low hunting activities. This is particularly important as the results of this study suggest a high ASFV prevalence in the summer months. Although high ASFV prevalence estimates were mainly found in wild boar younger than one year, which is likely to be due to the generally high case fatality ratio in young animals, these results are in accord with observations from other affected countries [24], even further emphasizing the need to raise sampling efforts in the summer months (risk-based sampling).

The seroprevalence, which increased until May 2018 in Estonia [10], has since decreased steadily, whereas the ASFV prevalence as measured by PCR has remained at a point estimate of 0% for more than 12 months. In Latvia, a similar course of disease can currently be observed [25]. In Sardinia, where the disease has been endemic since 1978, a recent survey yielded a very high mean seroprevalence above 50% and a low mean ASFV prevalence (2.6%) in free-ranging pigs [35]. Between 2015 and 2018, intensive control measures were applied in Sardinia, including the elimination of free ranging pigs, which are known to play an important role in the spread of ASF on the island [36]. Thus, this positive course might be the beginning of disease elimination. However, a continued endemic situation of the disease in Sardinia cannot be excluded at this stage. In Estonia, however, only seropositive wild boar have been found since more than 12 months, which may suggest that ASFV is no longer circulating and the epidemic might fade out, if there is no new introduction or re-emerging infection from an yet undetected focus. The latter seems unlikely in view of the intensity and spatial representativeness of the surveillance activities in Estonia.

The last seropositive wild boar younger than one year was detected in August 2019. Unfortunately, age is only classified as younger than one year, between one and two years and as older than two years in the records of the surveillance data. It is therefore not possible to tell if the seropositive wild boar that was hunted in August 2019 was between one and six months of age or between 6 and 12 months [37,38]. This increases the uncertainty regarding the determination of the time interval, when the last ASFV infections occurred in Estonia.

According to the definitions of the previous OIE Code, Estonia does therefore not fulfil the requirements for a self-declaration of freedom from ASF before September 2020. The slight changes in the previous and the current definitions probably resulted from the debate regarding the possible existence of carrier animals and their potential role in ASFV spread [23]. In addition, the differentiation between these two age classifications in the previous OIE Code was probably due to the assumption that maternal antibodies may be present in wild boar younger than six months [13,19,37,39]. As long as ASFV is not detected in such animals, indicating that no new infection has occurred, it can be assumed that seropositive young animals do not pose a risk for the spread the disease. By contrast, seropositive wild boar between 6 and 12 months could have been infected several months prior to sampling, suggesting ASFV circulation within the previous months. However, EU regulations and the current edition of the OIE Code do not include the above-mentioned age distinction anymore. It rather considers seropositive wild boar of all ages as relevant as long as they show clinical or pathological signs of an ASFV infection or are epidemiologically linked to ASF cases [2]. However, due to the lack of the differentiation between animals younger or older than six months, we propose to re-evaluate this definition. If no ASFV-positive wild boar have been detected for at least one year, seropositive animals between one and two years might still suggest circulating ASFV, so that a disease-free status is not warranted. By contrast, isolated seropositive animals younger than one year may be present due to maternal antibodies. These animals would not increase the risk of disease spread. At the same time, wild boar older than two years are old enough to have become infected at the time, when ASFV was still circulating. They have obviously cleared the infection as they are PCR-negative for ASFV and do therefore not pose a risk of spreading the disease.

Previously, the evolution of attenuated ASFV strains that could complicate detection and control has been suggested [8,40,41]. Indeed, a virus strain with reduced virulence was detected in Estonia. However, this could only be demonstrated regionally and over a short period of time [8]. Despite intensive searching for the attenuated virus with a tailored PCR, it could not be found in any of the samples originating from the affected region in the following years. However, even the potential circulation of such a strain would not change the role of seropositive animals, i.e., animals, which survived infection. Experimental data do not suggest a higher risk for carriers or chronically infected animals [40]. To include the risk of the potential circulation of attenuated virus strains in surveillance activities, the course of the seroprevalence has to be evaluated carefully. A sudden increase of seropositive animals without concurrent increase of ASFV-positive animals may indicate the presence of an attenuated ASFV strain and thus require more intensive testing. So far, this has not been the case. Although an accumulation of seropositive animals was observed over time, there was no significant peak after May 2018.

The potentially long half-life time of ASFV-specific antibodies [13,14] and the resulting accumulation of seropositive wild boar support the hypothesis that the ASFV circulation has come to an end in Estonia. The presumed accumulation of seropositive animals has probably led to the presented study results, which show a higher seroprevalence in older animals, particularly at the end of the study period. This has also been observed in Latvia [25]. When this knowledge about the potential long half-life and accumulation of seropositive wild boar is taken into account in the evaluation of surveillance data, a long time period can be expected, during which seropositive wild boar might be detected [37]. Therefore, a more precise definition regarding the role of seropositive, but ASFV-negative wild boar during the late phase of an ASF epidemic is needed, as the current regulations may impede the self-declaration of freedom from ASF status in a country in a way that is not warranted by scientific evidence. One way to define the disease status of a country more clearly might be to attribute wild boar only to two age categories, namely animals younger than two years and those older than two years. The vague transition period between young and sub-adult wild boar [38,42,43] could thus be avoided and more accurate epidemiological conclusions could be drawn based on the laboratory test results and the age of the animals. Finally, the possibility to provide a self-declaration of disease freedom, including the inconclusive definition of the existence of an epidemiological link, does not support a clear and reliable statement about the disease status of a country. The consideration of a more precise definition could clearly help to increase the certainty for potential trading partners.

5. Conclusions

In Estonia, no ASFV has been detected for more than one year now. At the same time, strong biosecurity measures, surveillance activities, and ongoing awareness campaigns are in place. The wild boar population density decreased significantly, probably due to a combination of ASFV circulation and intensified hunting. This low population density supports the control of ASF and should therefore be maintained. In addition, surveillance activities have to be retained, possibly focusing on risk-based sampling. The last few ASFV-positive cases emphasize in particular the increased need to sample in risk areas close to the external borders of Estonia. Moreover, sampling should be increased during summer months and younger wild boar should be investigated for ASFV and ASFV-specific antibodies, while the testing of older animals for ASFV-specific antibodies should be paramount. If Estonia upholds its strong efforts to detect any new introduction as early as possible and to monitor the age and the current course of seropositive wild boar continuously, the country should be able to submit a self-declaration regarding the recovery of freedom from ASF in due course. In the case of attained disease freedom, surveillance activities must be maintained so that early detection of any re-introduction or re-emergence is warranted. Freedom from disease must be repeatedly demonstrated. To circumvent the uncertainties regarding the status of freedom from ASF status, we propose to add ASF to the list of diseases for which an official procedure for recognition of disease status by the OIE already exists. This may decrease potential risks in the international trade and help to prevent trade restrictions that are not based on scientific evidence.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-393X/8/2/336/s1, Figure S1: Number of all investigated wild boar samples for each study month starting in January 2015 and ending in February 2020. The numbers refer to samples originating from active surveillance (i.e., from hunted animals; white bars) and from passive surveillance (i.e., from animals found dead, shot due to sickness or killed in road traffic accidents; black bars); Figure S2: Median seasonal effect of all samples that tested exclusively serologically positive on the logit prevalence. 95% Bayesian credible intervals (BCI) are indicated. The broken vertical line highlights February 2019, the last month, in which ASFV-positive wild boar were detected; Figure S3: Wild boar population density (number of wild boar/km^2) in Estonia during the study period (hunting seasons 2012/2013; 12/13, to 2019/2020; 19/20) The horizontal line that forms the top of the box marks the 75th percentile, the line that forms the bottom the 25th percentile. The horizontal line that intersects the box indicates the median number of wild boar per square kilometer. Whiskers represent the maximum and minimum values that were within the 1.5 times span of the interquartile range. Open circles represent outliers, i.e., single values greater or smaller than the extremes indicated by the whiskers, Table S1: Number of wild boar per km^2 in the counties of Estonia in the hunting seasons 2012/2013–2019/2020.

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3.2.3.4 African Swine Fever Re-Emerging in Estonia: The Role of Seropositive Wild Boar from an Epidemiological Perspective

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Highlights

- ASFV circulation at a low prevalence without detection for 1.5 years is very unlikely
- Disease spread by seropositive, but ASFV-negative wild boar seems unlikely but cannot be completely ruled out
- New introduction of ASFV into the Estonian wild boar population seems to be the most likely explanation for the re-emergence of ASFV-positive animals after 1.5 years
- Long-term collaborative and interdisciplinary efforts are needed to successfully control ASF in wild boar

viruses

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African Swine Fever Re-Emerging in Estonia: The Role of Seropositive Wild Boar from an Epidemiological Perspective

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Abstract: African swine fever (ASF) emerged in Estonia in 2014. From February 2019 to August 2020, no pigs or wild boar tested positive for ASF virus (ASFV), only ASFV-specific antibodies could be detected in shot wild boar. However, ASF recently re-emerged in wild boar. We tested three hypotheses that might explain the current situation: (i) ASFV may have been present throughout, but at a prevalence below the detection limit; (ii) seropositive wild boar may have remained infectious (i.e., virus-carriers) and kept the epidemic going; or (iii) ASF was gone for 1.5 years, but was recently re-introduced. Using Estonian surveillance data, the sensitivity of the surveillance system and the confidence in freedom from ASF were estimated. Furthermore, the detection probability was determined and cluster analyses were performed to investigate the role of serological positive wild boar. The results suggest that the surveillance system was not able to detect virus circulation at a design prevalence below 1%. With respect to the confidence in freedom from ASF, the results indicate that circulating virus should have been detected over time, if the prevalence was ≥2%. However, the decreasing wild boar population density and ongoing surveillance activities made ASFV circulation at a low prevalence unlikely. Cluster analyses provided no evidence for a significant accumulation of serologically positive wild boar in temporal connection to the re-emergence of ASFV. Further targeted research, such as long-term experimental studies and molecular epidemiology, is necessary to improve our knowledge on the epidemiology of ASF and to control the disease more effectively.

Keywords: African swine fever; Estonia; ASFV-carrier; confidence in freedom; detection probability; sensitivity of surveillance; wild boar; cluster analysis

1. Introduction

African swine fever (ASF) was first described by Montgomery [1] in 1921. In the following decades, ASF virus (ASFV) mainly circulated in Africa. However, it was introduced into Europe on various occasions since 1957, but had almost completely been eliminated from the European continent by 1995 [2,3]. It was only in Sardinia that ASF became endemic; it has been constantly present on the island since 1978 [4]. In 2007, ASFV of genotype II was introduced into Georgia, where it spread over various European countries, both inside and outside of the European Union (EU); it reached China and several other Asian countries in recent years [5]. After ASF appeared in Lithuania, Poland, and Latvia in the first half of 2014, Estonia has been affected since September 2014 [6–9].

In contrast to previous outbreak situations in Europe, where mostly domestic pigs were affected with some spillover infections into wild boar, ASF mainly circulates among wild boar in the current epidemic in Europe, described as the "Wild Boar–Habitat Epidemiologic Cycle" [10]. Controlling ASF in wild boar is incomparably more difficult than in domestic pigs, which usually constitute a defined population in a distinct area, normally the premises of a farm. Yet, controlling ASF is much more challenging in wild boar, and the surveillance of the disease is more complex in these animals. The case/fatality ratio of ASF is extremely high, which leads to a significantly higher detection probability in wild boar found dead [7,11,12]. Thus, surveillance activities focus on passive surveillance, i.e., the detection of wild boar carcasses and testing them for ASF. However, the probability of detecting wild boar carcasses depends on a variety of factors, including land-use, coverage of the area with crops, grassland, shrubs, bushes, or forest, and the decomposition speed of the carcasses as a function of temperature, the presence of scavengers, insects, etc. Although the number of dead wild boar is expected to increase during an ASF epidemic, the animals often hide when they feel sick, and die; thus, making discovery more difficult. Moreover, carcasses may be hidden or eaten by scavengers, also reducing the potential for discovery [13,14]. This makes successful passive surveillance labor-intensive. Furthermore, in countries such as Estonia, where ASF has been present in wild boar for several years throughout most of the country, and since around 50% of the country is forest and wetland, maintaining surveillance at a high level is a major challenge. By combining reduced intensive surveillance efforts with a decreased population density, as a result of the high lethality due to ASFV infection [15], it is obvious that the probability of detecting wild boar infected with ASF decreases in such a situation. Yet, passive surveillance is usually supplemented with active surveillance, i.e., the sampling and testing of wild boar that were apparently healthy when shot. In an advanced stage of an ASF epidemic, it was found that the number of hunted wild boar that tested positive for ASFV-specific antibodies (i.e., seropositive wild boar) increased, and were, at the same time, negative for ASFV. This was probably due to an accumulation of the number of animals that had survived the infection [16,17]. Thus, active surveillance is an essential addition to passive surveillance, especially in a later phase of an ASF epidemic.

In Estonia, the last ASF outbreaks in domestic pig holdings for several years occurred in September 2017 [18]. However, in July 2021, one domestic pig farm had to report an ASF outbreak and, thus, the long period without ASF in domestic pigs ended. By contrast, wild boar that tested ASFV-positive, seropositive, or both, were regularly detected until February 2019. Thereafter, only seropositive, but not ASFV-positive wild boar, were found for more than 1 year, suggesting a lack of new ASFV infections and, thus, a potentially subsiding epidemic [17]. In August 2020, however, an ASFV-positive wild boar was found in Rapla County in the central part of Estonia, followed by several detections of ASFV-positive wild boar in this region. Shortly afterwards, new ASFV cases were detected in Lääne-Viru County in the northeast of the country, around 120 km from the cases in Rapla County. The re-emergence of ASFV-positive wild boar after a time, when there was reason to hope that the elimination of ASF from Estonia might be possible, fueled controversial discussions regarding the detection probability of circulating ASFV, and the role of seropositive wild boar in the ASF epidemic. Before discussing the latter, agreement must be reached on the definition of so-called ASFV-carriers. According to Putt, et al. [19], a true virus-carrier is an infected individual that sheds the pathogen, but neither sickens nor shows any clinical signs. There is general consent in the scientific community—that after infection with ASFV, infected individuals shed the pathogen from 24–48 h post infection, until at least 35 days. During that time, laboratory-testing yields ASFV-positive test results. In some laboratory experiments, ASFV was (intermittent) detectable up to 100 days post-infection [20,21]. After about 7 to 10 days, antibodies are formed and, thus, there is a time period, in which ASFV- and serologically-positive laboratory test results are observed simultaneously [5,22]. In case of survival of the infected wild boar, it will clear the infection and laboratory testing usually yields seropositive, but ASFV-negative test results. Petrov et al. [20] and Nurmoja, et al. [23] showed that, in their experiments with genotype I and II strains, ASF survivors did not transmit the virus to sentinels commingled later than 50 days post-infection. After 100 days of survival, no virus was found in the respective animals, but genome copies could be detected. Some scientists would define wild boar that shed ASFV up to 100 days post-infection as an ASFV-carrier. However, another group of experts discussed whether ASFV could be reactivated in a serological positive, but ASFV-negative wild boar, at a later point in time, e.g., in case of immunosuppression, stress, or death. Thus, there is a debate whether serologically-positive wild boar should be considered as potential ASFV-carriers that might play a role in maintaining the epidemic. Blome et al. [5] doubt that genome copies have a significant impact on disease spread and transmission dynamics. These findings and the resulting different definitions of a (potential) ASFV-carrier emphasize once again the importance of a similar understanding of terminology. Despite the controversial discussion, there is currently no evidence that wild boar that have survived an ASF infection play a major long-term role in the maintenance of ASF [24]. The same is probably true for domestic pigs under field conditions. Very recently, Oh et al. [25] conducted a long-term follow-up of convalescent pigs and their offspring in Vietnam, and did not observe any transmission events.

This scientific discussion and the course of the epidemic in Estonia, including the long absence of ASFV-positive wild boar, motivated us to conduct the present study. Besides discussing the terminology of (potential) carriers and the impact of such animals on ASF epidemiology, we aimed to understand the cause of the ASFV re-emergence in Estonia. We therefore investigated the probability of ASFV detection, potential ASFV circulation at a low prevalence, and the role of seropositive wild boar in the course of ASF in Estonia. Accordingly, we investigated the three hypotheses:

1. Persistence: ASFV was present in wild boar or the environment throughout the period with no ASFV detections, but at a low prevalence ($\leq$1% or 2%, respectively).
2. Virus carrier-hypothesis: seropositive wild boar could remain infectious and, thus, kept the epidemic going.
3. Reintroduction: the virus was absent for 1.5 years and newly introduced.

2. Materials and Methods

2.1. Study Areas and Data

Estonia consists of 15 counties divided into 79 municipalities (Figure 1). The first study area, Rapla County, is located in central Estonia, and consists of four rural municipalities. The second study area, Lääne-Viru County, is located in northeast Estonia, and consist of eight municipalities. Information on samples taken from hunted wild boar and from passive surveillance (found dead, shot sick, or died in a road traffic accident) was extracted from the CSF/ASF wild boar surveillance database of the European Union (https://surv-wildboar.eu, accessed on 8 December 2021) for all of Estonia, for the period January 2015 to April 2021 (n = 56,622). For each data record, information about the origin of the sample (wild boar hunted or found dead), the sampling location, and the results of serological and virological laboratory tests was available. For the detection of ASFV-specific antibodies in hunted wild boar, a commercially available blocking ELISA (Ingezim PPA COMPAC, Ingenasa, Madrid, Spain) was used. If the ELISA result was positive or inconclusive, the sample was retested by an indirect immunoperoxidase technique (IPT) for confirmation using the protocols provided by the European Union Reference Laboratory (EURL) for ASF (CISA-INIA, Valdeolmos, Spain). Real-time PCR was applied to detect the ASFV genome in wild boar hunted or found dead according to the protocols reported by Tignon et al. [26]. Whenever possible, positive PCR results were confirmed by an alternative real-time PCR assay, the Universal Probe Library (UPL) real-time PCR [27]. The samples that finally yielded an inconclusive test result in serological or virological laboratory tests were excluded from the analyses (n = 121).

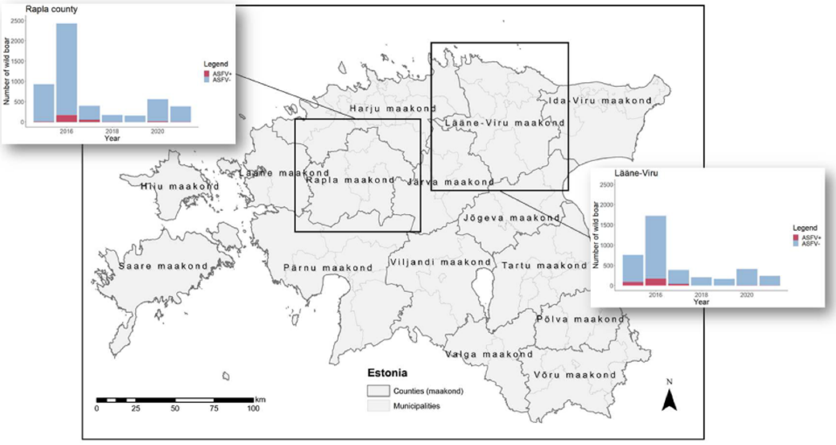

Figure 1. Map of Estonia. The two study areas Rapla County and Lääne-Viru County are highlighted by black rectangles. For each county, the figure shows the numbers of virological investigations from 1 January 2015 through to 30 April 2021. Red bars represent ASF virus positive wild boar by a PCR test, blue bars indicate negative PCR test results.

Wild boar population estimates for each county were calculated based on the number of hunted wild boar (N_{hunt}). We assumed that a proportion of 45% of the total wild boar population will be hunted or die during one year ($p_{dead} = 0.45$) [28]. In addition, we assumed that for 90% of wild boar, the cause of death is hunting ($p_{hunt} = 0.9$), whereas 10% of the animals die due to other reasons (age, disease, or predation) [29]. Thus, the wild boar population N_{pop} was calculated as

$$N_{pop} = \frac{1}{p_{dead}} \cdot \frac{1}{p_{hunt}} \cdot N_{hunt} = 2.469 \cdot N_{hunt}. \quad (1)$$

Finally, N_{pop} was rounded up to the next larger whole number.

2.2. Persistence

To evaluate, if ASFV might have been present, undetected at a low prevalence between the last ASFV-positive cases in February 2019 and the new emerging cases in August 2020, we calculated the sensitivity of the surveillance system and the confidence in freedom.

2.2.1. Sensitivity of the Surveillance System

The sensitivity of the surveillance system on a county level was calculated using Epitools for simple risk-based surveillance, in which a high-risk population is targeted [30,31]. To this end, the population was divided into two groups according to their risk of ASF occurrence: (1) The high-risk group included all dead wild boar (carcasses) and (2) the low-risk group contained all living wild boar. Based on the surveillance data, the proportion of wild boar found dead, shot sick due to sickness, or killed in road traffic accident from all wild boar considered for ASF surveillance decreased from 1.7% in 2018 to 0.6% in 2021 (only data until April 2021 were considered). To account for a potential underestimation of the number of dead wild boar (represented by 'found dead' included in the surveillance data), we assumed a proportion of dead wild boar (carcasses) of 3% in this study.

To calculate the surveillance sensitivity, the following information was needed as input:

1. Relative risk. This measure describes the relative risk of ASF-infected wild boar in the high-risk group, relative to the risk of ASF-infected wild boar in the low-risk group. Based on the surveillance data, the risk that wild boar in the high-risk group are

ASFV-infected was estimated to be 60 times higher compared to the risk that hunted wild boar were ASFV-positive (low-risk group).

2. Test characteristics. The sensitivity of the test performed on individual wild boar (i.e., of the virological laboratory test) was assumed as 0.999. The specificity of the surveillance system was assumed equal to 1, expecting that all positive wild boar were followed up by a confirmatory test to ensure that they were not false positive.
3. Number of animals tested in the high- and low-risk group. The surveillance sensitivity was calculated for 2018, 2019, and 2020. However, in each year, only those months during which no ASFV-positive wild boar was detected in the two study areas, were included in the calculations. The number of animals tested in the high- and low-risk group was, thus, calculated for the considered time periods, based on the surveillance data.

As an output, the surveillance sensitivity was recorded as the probability that the surveillance system will detect at least one infected wild boar, if ASF is present at design prevalence values of 1% or 2%, respectively. The design prevalence of 1% or 2% was chosen, because these values correspond to an effective probability of infection (EPI) in the high-risk group (i.e., in wild boar carcasses) of 21.7% or 43.3%, respectively, and an EPI in the low-risk group (i.e., in hunted wild boar) of 0.4% or 0.7%. A surveillance sensitivity above 95% was considered sufficient to detect ASFV in the wild boar population.

2.2.2. Confidence in Freedom from ASF

In addition, the confidence in freedom from infection with ASFV for multiple periods was estimated using Epitools [30,32]. Using the estimated surveillance sensitivity for the three considered periods, we evaluated the ability of the surveillance system (confidence in freedom) to detect ASFV at the two design prevalence values of 1% and 2%. The initial prior confidence in freedom was set to 0.5, since no information was available.

No information was available regarding the probability of ASFV introduction into the wild boar population. Assuming that a new introduction of ASFV into two non-neighboring counties could be considered as independent, we estimated the probability of introduction for Estonia as a whole PI_{EE} as:

$$PI_{EE} \geq 1 - \left(1 - PI_{county}\right)^{n}, \tag{2}$$

with PI_{county} as the assumed probability of introduction per county and n as number of counties in Estonia (n = 15). Conservative values for $PI_{EE} \geq 79\%$ and $PI_{county} = 10\%$ were used and complemented by a sensitivity analysis to assess the uncertainty when choosing the above values.

A confidence in freedom from ASFV above 95% was considered sufficient.

2.3. Virus Carrier Hypothesis

To evaluate the potential role of ASF-seropositive wild boar in maintaining the epidemic, we assumed that ASF-seropositive wild boar might still be intermittent infectious. Thus, the hypothesis is that the number of ASFV-positive wild boar increases during the subsequent period, once a seropositive wild boar has been detected (positive index case). We therefore examined if the number of investigated wild boar was sufficient to detect ASFV-positive wild boar, once an index case had occurred, by calculating the detection probability. Furthermore, a cluster analysis was performed to identify potential spatial and spatiotemporal clusters of seropositive wild boar.

2.3.1. Detection Probability

We estimated the probability of detecting at least one ASFV-positive wild boar after a seropositive wild boar had been hunted (positive index case) and compared the detection probability to the estimates obtained after hunting a seronegative wild boar (negative index case). Therefore, for every hunted wild boar that was investigated serologically during the study period (index wild boar), the number of virological investigations was recorded for periods of 90 and 120 days after the date of the serological investigation of the index wild boar. The result was used to estimate the detection probability for each index wild boar, i.e., the sensitivity of detecting ASFV in the two considered periods. Again, we used design prevalence values of 1% or 2%, respectively. The calculation was based on a previously presented formula for surveys to substantiate freedom from disease considering imperfect test characteristics and a finite population size [33]. The formula requires the wild boar population (estimated as detailed above), the number of virological investigations in the considered time period (calculated based on the surveillance data), the expected number of ASFV-positive wild boar in the population (equals the design prevalence multiplied with the population size N_{pop}) and the sensitivity of the performed virological laboratory test (sensitivity = 0.999) as input parameters. Results were presented as histograms of the resulting detection probabilities. Values above 95% were considered as sufficient to detect ASFV-positive wild boar at the chosen design prevalence value.

2.3.2. Cluster Analysis

To investigate if more seropositive wild boar were found in the two Estonian study areas compared to other Estonian counties, a cluster analysis was performed. To test for global clustering, we used the Tango test [34] and Oden's I pop, which account for varying wild boar population densities in Estonia [35]. SaTScan analysis was used to identify spatial, temporal, and spatiotemporal clusters in serological positive wild boar [36].

2.4. Software

If no specific software was mentioned in the previous sections, statistical analysis was performed using the software R version 4.0.3 (http://www.r-project.org, accessed on 9 January 2021), which was also used to generate figures. The following R packages were used: lubridate [37], RSurveillance [38], ggplot2 [39], gridExtra [40], ggpur [41], spdep [42], and DCluster [43]. Figures 1–3 were generated using ArcGIS ArcMap 10.8.1 (ESRI, Redlands, CA, USA, http://www.esri.com/, accessed on 9 January 2021).

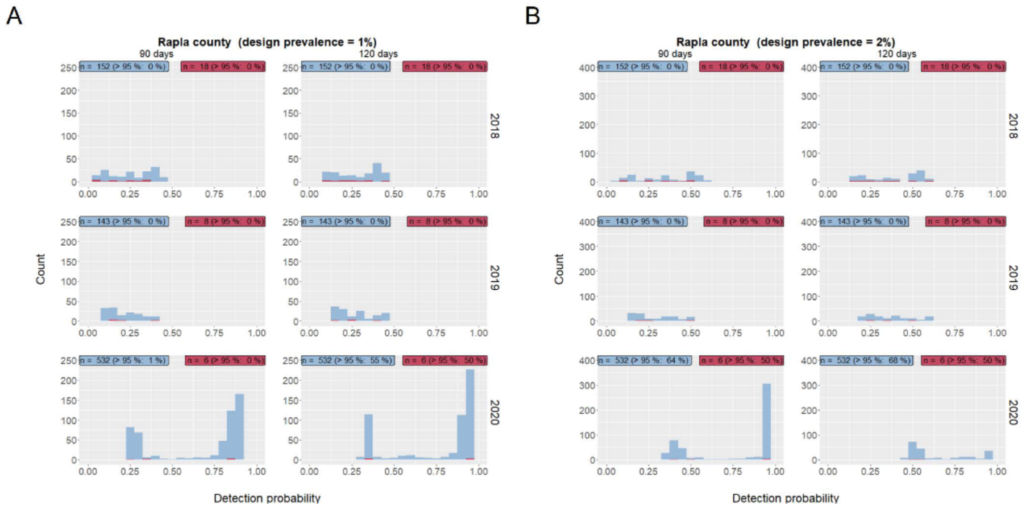

Figure 2. *Cont.*

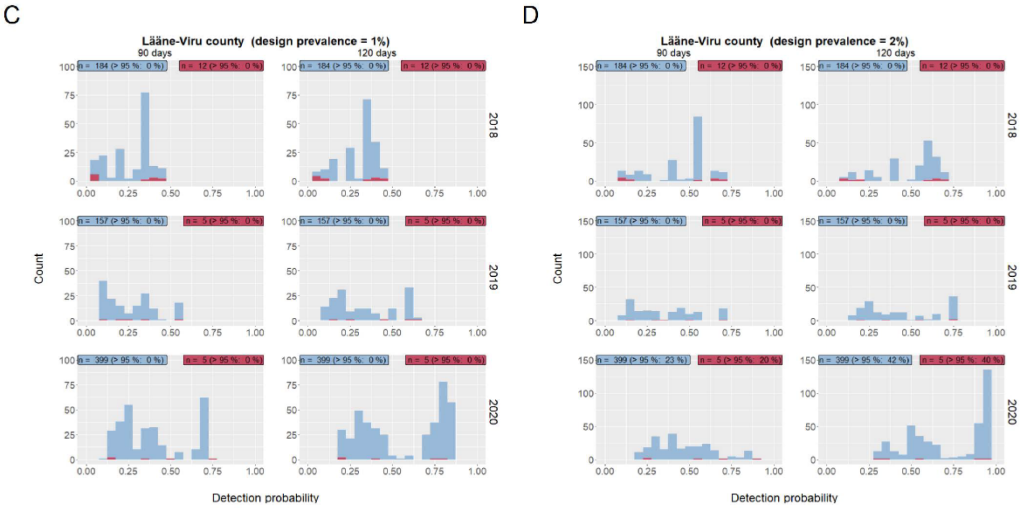

Figure 2. Map of Estonia. The two study areas—Rapla County ((**A**): design prevalence 1% and (**B**): design prevalence 2%) and Lääne-Viru County ((**C**): design prevalence 1% and (**D**): design prevalence 2%)—are highlighted by black rectangles. For each county, the figure shows the numbers of virological investigations from 1 January 2015 through to 30 April 2021. Red bars represent ASF virus positive wild boar by a PCR test, blue bars indicate negative PCR test results.

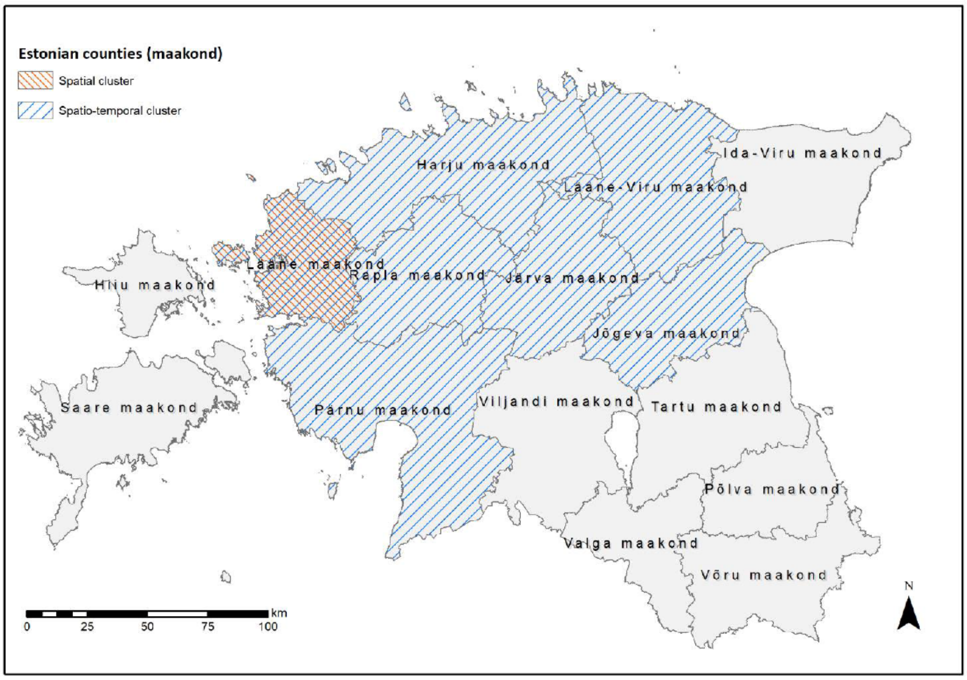

Figure 3. Significant spatial (red) and spatiotemporal (blue) clusters of seropositive wild boar detected by SaTScan analysis in Estonia.

3. Results

The sensitivity of the surveillance system and the confidence in freedom from ASF were investigated to assess, if ASFV might have gone undetected at a low prevalence between the last ASFV-positive cases in February 2019 and the cases that re-emerged since August 2020.

3.1. Persistence

3.1.1. Sensitivity of the Surveillance System

In both study regions, the number of wild boar carcasses investigated for ASFV in each considered period varied between zero and four (Table 1). The surveillance sensitivity for an assumed design prevalence of 1% was below 95% in both study areas for all three considered periods (Table 1). Assuming a design prevalence of 2%, in Lääne-Viru County, the estimate of the surveillance sensitivity remained above 95% in two periods, and was thus considered sufficient to detect ASFV circulation.

Table 1. Number of investigated wild boar and sensitivity of the surveillance system (risk-based) assuming design prevalence (dp) values of 1% and 2% in Rapla County and Lääne-Viru County for the considered periods. Values above 95% are marked in bold.

Study Area	Time Period	Number of Wild Boar Investigated for ASFV		Surveillance Sensitivity (in %)	
		Hunted	Found Dead	dp = 1%	dp = 2%
Rapla County	March 2018–December 2018	81	1	41.5	68.4
	2019	150	3	72.0	93.8
	January 2020–July 2020	171	0	46.1	71.0
Lääne-Viru County	2018	196	4	81.4	**97.5**
	2019	163	1	56.5	82.6
	January 2020–November 2020	313	4	87.8	**98.9**

3.1.2. Confidence in Freedom from ASF

For an assumed design prevalence of 1%, the estimated confidence in freedom considering the three periods individually was below 90% in both study areas (Table 2). Increasing the design prevalence to 2% led to sufficient levels of confidence (i.e., above 95%) in Lääne-Viru for two considered time periods (2018 and January–November 2020, Table 2).

Considering multiple consecutive time periods, we achieved a cumulative confidence in freedom after three periods for both design prevalence values in Lääne-Viru County (Table 2). In contrast, in Rapla County, a cumulative confidence in freedom above 95% was only observed for 2% design prevalence (Table 2).

As no information on the probability of introduction was available, a sensitivity analysis was performed. Choosing lower values for the probability of introduction (e.g., PI_{county} =5% (PI_{EE} $\geq$ 54%)) led to increased values for the confidence in freedom. Increasing the introduction probability (e.g., PI_{county} = 15% ($PI_{EE} \geq$ 91%)) led to decreasing values for the confidence in freedom. However, compared to the probability of introduction of PI_{county} = 10%, the result regarding confidence in freedom altered only marginally.

Table 2. Cumulative confidence in freedom assuming a probability of introduction of 10% and design prevalence (dp) values of 1% and 2% in Rapla County and Lääne-Viru County for the considered periods. Values above 95% are marked in bold.

Study Area	Individual Time Periods	Confidence in Freedom for Individual Time Periods (in %)		Consecutive Time Period	Cumulative Confidence in Freedom Over Consecutive Periods (in %)	
		dp = 1%	dp = 2%		dp = 1%	dp = 2%
Rapla County	March–December 2018	63.1	76.0	March–December 2018	58.3	72.1
	2019	78.1	94.2	March 2018–December 2019	79.8	**96.8**
	January–July 2020	65.0	77.5	March 2018–July 2020	82.5	**95.9**
Lääne-Viru County	2018	84.3	**97.6**	January–December 2018	81.5	**97.0**
	2019	69.7	85.2	January 2018–December 2019	86.3	**97.5**
	January–November 2020	89.2	**98.9**	January 2018–November 2020	**96.6**	**99.9**

3.2. *Virus-Carrier-Hypothesis*

3.2.1. Detection Probability

In Rapla County, the number of serologically tested wild boar varied between 151 in 2019 and 538 in 2020 (all of the tested wild boar tested negative for ASFV) (Figure 2A,B). Between 6 and 18 animals were found seropositive. These animals were considered as potential ASFV-carriers. In Rapla County, the estimated detection probability was below 95% in 2018 and in 2019, regardless of the chosen design prevalence value of 1% or 2%. In 2020, a detection probability above 95% was reached for 3 of 6 index wild boar (50%), assuming a design prevalence of 1% and considering a period of 120 days after the detection of the index case (Figure 2A). Assuming a design prevalence of 2% led to detection probabilities above 95% also for a period of 90 days after the detection of the index case in 2020 (Figure 2B).

In Lääne-Viru County, between 162 and 404 wild boar were tested, and 5 and 12 were serologically positive, but ASFV-negative (Figure 2C,D). In none of the scenarios described above for Rapla County, a detection probability above 95% was reached in Lääne-Viru County, assuming a design prevalence of 1% (Figure 2,C). Increasing the design prevalence to 2% led to a proportion of index wild boar with detection probabilities above zero in 2020 (Figure 2,D). In both study areas, we did not observe a difference in the proportion of index wild boar leading to detection probabilities above 95%, following a seropositive or a seronegative index wild boar (Figure 2).

In general, the median proportion of cases on Estonian county level, in which the detection probability was above 95%, was zero, except for one situation (Table 3): in 2020, the proportions of cases, in which the detection probability was above 95%, ranged between 0 and 83% on the county level in Estonia, assuming a design prevalence of 2%. A median of 42% was calculated regardless of the virological status of the index wild boar.

3.2.2. Cluster Analysis

Both global cluster tests, the Tango test and Oden's I pop showed p-values below 0.05 (Tango test $p = 0.010$, Oden's I pop $p < 0.001$). Thus, both tests indicated that statistically significant clusters of seropositive wild boar existed in Estonia. A spatial cluster covering Lääne County (not to be confused with Lääne-Viru County) with a statistically significantly higher number of seropositive wild boar was identified by SaTScan analysis (Figure 3). Lääne County is located in direct neighborhood to the study area Rapla County (Figure 1). The temporal cluster analysis found the whole of Estonia as a cluster in March 2019. Combining spatial and temporal analysis led to a cluster for February–March 2019 covering seven counties (Figure 3). Thus, no link could be identified to the periods, when the new ASFV cases had occurred.

Table 3. Median (minimum–maximum) number of serological investigations in Estonian counties that were used to identify index wild boar cases to estimate the probability of detecting ASFV-positive wild boar 90 and 120 days after detection of the index wild boar case. Sero+ indicates estimates after seropositive, but ASFV-negative index wild boar. Sero +/− indicates estimates for the median [minimum-maximum] number of index wild boar cases regardless of the serological test result for ASFV-negative wild boar. For the two periods of 90 and 120 days after detection of the respective index case, the median (minimum–maximum) proportion of the detection probability above 95% is presented for assumed design prevalence (dp) values of 1% and 2%.

	Time Period	Number of Index Wild Boar (Median (Minimum-Maximum))	Days after Serological Investigation of Index Wild Boar	Proportion of Detection Probabilities above 95%	
				dp = 1%	dp = 2%
Sero+	2018	10 (0–71)	90	0 (0–21)	0 (0–45)
			120	0 (0–27)	0 (0–54)
	2019	4 (0–12)	90	0 (0–0)	0 (0–60)
			120	0 (0–50)	0 (0–60)
	2020	4 (0–17)	90	0 (0–35)	0 (0–88)
			120	0 (0–88)	0 (0–88)
Sero+/−	2018	256 (101–1031)	90	0 (0–230)	0 (0–54)
			120	0 (0–27)	0 (0–65)
	2019	244 (83–955)	90	0 (0–0)	0 (0–53)
			120	0 (0–28)	0 (0–64)
	2020	352 (137–1445)	90	0 (0–39)	0 (0–75)
			120	0 (0–67)	42 (0–83)

4. Discussion

After five years of ASFV circulation and a decreasing ASFV prevalence in Estonia, no more ASFV-positive wild boar were detected from March 2019 until August 2020. During that time, seropositive wild boar were still hunted, although at a low prevalence [17]. Newly detected wild boar carcasses that had tested positive for ASFV were found in two counties, in which the virus had not been detected for 28 months in Rapla County and 37 months in Lääne-Viru county. Thus, the hope that freedom from ASF in wild boar might be a realistic task was destroyed. The question was raised—how did ASFV re-emerge in Estonia? We set up the following hypotheses: (1) Persistence: ASFV was present all the time in wild boar or in the environment (e.g., carcass), but at a low prevalence. (2)Virus carrier-hypothesis: seropositive wild boar may be infectious and can maintain the epidemic. (3) Reintroduction: ASFV was not found in the entire country for 1.5 years and then newly introduced.

To evaluate the probability of ASFV detection, the sensitivity of the surveillance system and the confidence in freedom from ASF were calculated. Several assumptions were made in these calculations. The chance to detect ASFV in dead wild boar was estimated to be 60 times higher than in apparently healthy animals. This was estimated based on the Estonian surveillance data. Similarly, a significant higher probability to detect ASFV in wild boar carcasses than in animals shot apparently healthy was found in several other studies [7,12,44,45]. Moreover, the assumed proportion of wild boar carcasses (3%) relative to all sampled wild boar was defined based on available surveillance data, and is similar to the data composition in other countries [16,46,47]. In a recent analysis by the European Food Safety Authority [48], this proportion was estimated to be only 1%. The higher proportion in our study may at least in part be explained by the fact that our estimates include the assumed proportion of undetected wild boar carcasses. Furthermore, following the World Organisation for Animal Health [49], almost perfect test sensitivity and specificity were assumed for calculating the sensitivity of the surveillance system. The chosen values were supported by findings by Schoder et al. [50]. To calculate the confidence in freedom, the estimated probability of introduction was incorporated. Since substantiated data are lacking, we chose a rather conservative value of 10% per county. To account for the uncertainty of the chosen values, the influence of this choice on the results was tested by performing a sensitivity analysis. This analysis investigated introduction probabilities for the whole of Estonia between 54% and 91%. Larger values were regarded as unrealistic. By using the lower value of 54%, we attempted to reflect the current ASF situation in neighboring countries and in Estonia as a whole. If the probability of introduction was even lower than anticipated, the confidence in freedom would increase and thus lead to the same conclusion.

Only in Lääne-Viru County, but not even there in all three periods, the sensitivity of the surveillance system was high enough to detect the disease at a design prevalence of 2%. It must therefore be assumed that the surveillance system was not sufficient to detect virus circulation at this low prevalence. When we took previous periods into account when calculating the confidence in freedom from ASFV, the results indicated that the circulating virus should have been detected over time, if a prevalence of 2% was assumed. The result for a prevalence of 1%, in which ASFV circulation is likely to be missed by the surveillance system, corroborates the analyses of the European Food Safety Authority [48]. In several studies, it was found that the wild boar population density significantly decreases during an ASF epidemic [15,22,44,46]. The low ASFV prevalence combined with a low population density may suggest that the epidemic is fading. However, considering the calculations for confidence in freedom, ASFV may be re-discovered after some time, if the prevalence starts to rise and exceeds the detection threshold [48]. Thus, it seems possible that the virus circulation at some stage exceeded the detection limit in the two counties under study again, so that the virus was detected. However, it could be expected that the number of detected carcasses would further increase in case of exceeding the detection threshold.

Moreover, it seems unlikely that ASFV circulated throughout Estonia for more than 1 year at such a low level and remained undetected, particularly when considering the low wild boar population density and assuming the wild boar habitat infection cycle [10].

The role of seropositive wild boar and its potential role as a "carrier" in the course of ASF has been controversially discussed for several years [24]. Blome et al. [5] provided clear definitions, calling a "true" survivor an animal, in which ASFV-specific antibodies, but no ASFV is detected. The probability that such animals shed an infectious virus and play a role as virus-carriers in disease spread is virtually zero. Eble et al. [21] argued, however, that animals that survive an ASFV infection (i.e., showing ASFV-specific antibodies), could shed ASFV and, thus, transmit the disease. Therefore, they called these animals ASFV-carriers. The pigs in their study had tested positive for ASFV during the whole study period, which was only 55 days. It therefore remains open for how long virus shedding might have continued. Thus, the findings of Eble et al. [21] do not contradict the statements by Blome et al. [5], who argued that ASFV or viral genome can be detected for approximately 60- and 100-days post-infection, respectively, and that an infected wild boar could play a role as virus-carrier during that period. Consequently, if a virus-carrier in the context of ASF is an animal that survived an ASFV-infection, but still carries and sheds detectable amounts of ASFV for a certain period, then there is no doubt that virus-carrier animals exist. If, however, all wild boar that have survived ASF (as shown by the presence of ASFV-specific antibodies in apparently healthy animals) are regarded as potential carriers, even in absence of detectable virus, more evidence is needed. Gallardo et al. [51] hypothesized that ASFV might be reactivated under immunosuppression, stress, or death and, thus, these potential carriers might play a role in ASF persistence in swine-populations. So far, evidence for ASFV transmission has neither been obtained in animals surviving more than 100 days, nor has excretion of an infectious virus been detected from such animals [20,23]. It cannot be excluded, however, that a very small number of wild boar might transmit ASFV even after 100 days. Moreover, sample matrix and quality may have influenced the probability of detecting low amounts of viral genomes or viral genomes confined to one organ or organ system. In this respect, screening of various tissues could aid detection of potential virus-carriers.

In this study, we tested the role of potential ASFV-carriers in Estonia (i.e., wild boar with ASFV-specific antibodies, but not ASFV-positive) by estimating the detection probability of ASFV-positive wild boar emerging after the occurrence of a seropositive wild boar. Since there is no doubt that surviving animals can transmit ASFV for some time, we only evaluated the hypothesis that seropositive, but ASFV genome negative wild boar may spread ASF and, therefore, represent apparently healthy ASFV-carriers. We assumed that ASFV-positive animals would be detected in a period of 90 or 120 days after detection of the seropositive wild boar, if these animals were infectious. These time spans were chosen because ASFV has been detected 60–70 days post-infection and viral genome up to approximately 100 days post-infection [20,23]. These calculations were conducted only for the time periods in which no ASFV-positive wild boar were detected in Estonia (between February 2019 and July 2020). The significantly decreased population density was considered. The probability to detect ASFV-positive wild boar after a seropositive animal had been found was too low to exclude the possibility that seropositive animals could spread ASF.

To further investigate possible causes for the re-emergence of ASFV in the two affected counties with regard to seropositive animals and their potential to spread ASFV, cluster analyses were performed. In the spatial analysis, a statistically significant cluster was identified in a county bordering Rapla County. In this county (Lääne County), the last ASFV-positive wild boar was detected in February 2019, representing the last ASFV-positive animal in Estonia for more than 1 year. An increasing seroprevalence subsequent to a decreasing ASFV prevalence was described in several countries [16,22,46]. These findings may explain the identified cluster in Lääne County, which could simply be a result of the temporal course of the epidemic on a county level, without any epidemiological link to the new occurrence of ASFV in Rapla County. The distance between the last ASFV-positive wild boar in February 2019 and the first one detected in August 2020 was approximately 75 km. The European Food Safety Authority [48] found a median speed of spread of 3–12 km/year in the affected Member States. In Poland, ASF has also been described as spreading rather slowly [52,53]. These findings, the generally limited home range of wild boar [54,55], and their tendency to avoid contact between individual packs [56], support the hypothesis that the re-emergence of ASFV in Rapla County may be independent from the cases in Lääne County in February 2019. The only significant cluster obtained from the spatio-temporal cluster analysis was identified at the beginning of the study period, in which Estonia was potentially free from ASFV and included almost the entire country. Similar to the spatial and temporal cluster analysis, but on country level, this result is likely to be due to the temporal course of ASF, i.e., the decrease of the ASFV prevalence and the subsequent increase in the proportion of seropositive animals [16,22]. Thus, the spatiotemporal expansion is not surprising. The lack of further spatiotemporal clusters at a later stage suggests the absence of ASFV circulation and makes it unlikely that seropositive animals played a role in virus transmission. In addition, clusters could also be the result of different ASFV strains with varying survival rates. Thus, a less virulent strain might lead to a higher number of seropositive wild boar and consequently results of cluster analysis should be interpreted with care.

Our results suggest that it was unlikely that ASFV circulated at a low prevalence without detection for 1.5 years (Persistence). Unfortunately, the study did not yield unambiguous results regarding the potential role of seropositive wild boar in transmitting ASFV (Virus carrier-hypothesis). Although it seems unlikely that seropositive, but ASFV negative wild boar shed ASFV and spread the disease, this could not be completely ruled out. It still needs clarification if (1) a considerable proportion of infected wild boar can survive ASF, still harboring and shedding the virus for longer periods than currently discovered, and thus playing a significant role in the further spread of ASF. Furthermore, the question if (2) ASFV could be reactivated in seropositive wild boar under immunosuppression, stress or in case of death [57] has to be pursued further. To find scientific evidence regarding these questions, it will be inevitable to conduct long-term experimental studies.

The third hypothesis (Reintroduction of ASFV) seems the most likely explanation for the re-emergence of ASF in Estonia. The virus could have been newly released locally e.g., by dropping infected wild boar meat from illegally hunted wild boar. Moreover, the virus could have been introduced from neighboring countries, where ASF is still present in various regions [58]. The outbreak in Lääne-Viru County was detected several months after the Rapla County outbreak and could be the result of human mediated transmission from there. Preliminary results of molecular sequencing of ASFV strains emerging in Rapla and Lääne-Viru County in 2020 indicate a 100% match to the Georgia 2007/1 strain [59]. This strain currently circulates in Russia, Belarus, Poland, and several other countries. Thus, reintroduction of this virus seems possible. Yet, the genome of ASFV has proven to be very stable, so that available sequencing results do not allow rejecting the other hypotheses.

Despite the huge effort to test various hypotheses that might explain the re-emergence of ASF in Estonia, we must confess—with Socrates—that we (only) know that we know nothing. Yet, appropriate consequences have to be drawn to improve ASF control in Estonia. Collaborative and interdisciplinary efforts are needed more than ever to develop and investigate further hypotheses to increase the chances of combating ASF successfully. With increasing availability of ASFV full-length sequences [60], further molecular epidemiology can help to solve open questions about potential virus origins. Nevertheless, the epidemiological course of the disease in affected countries must be analyzed and jointly discussed with scientists to increase the knowledge and improve our understanding of ASF and its epidemiology.

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3.2.3.5 Epidemiological evaluation of Latvian control measures for African swine fever in wild boar on the basis of surveillance data

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Highlights

- Also in Latvia, a significant reduction of the population density could be observed over time
- Paying incentives to all persons who report dead wild boar to the veterinary authorities and permission to use silencers and night vision devices for wild boar hunting indicated a potential slight effect on the estimated ASF prevalence
- Incentives should help to motivate hunters also to hunt outside the hunting season
- None of the control measures applied in Latvia during the present ASF epidemic showed a statistically significant effect on the prevalence estimates

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A wild boar population infected with African Swine Fever (ASF) constitutes a constant threat to commercial pig farms and therefore to the economy of the affected country. Currently, ASF is still spreading in several countries and the implementation of intensive measures such as reducing wild boar population densities seems not to be able to stop the further spread of the disease. In addition, there are still substantial knowledge gaps regarding the epidemiology of the disease. To identify risk factors for a higher probability of a wild boar sample being virological or serological positive, comprehensive statistical analyses were performed based on Latvian surveillance data. Using a multivariable Bayesian regression model, the effects of implemented control measures on the proportion of hunted or found dead wild boar or on the estimated virus prevalence were evaluated. None of the control measures applied in Latvia showed a significant effect on the relevant target figure. Also, the estimated periodic prevalence of wild boar that had tested ASF positive by PCR appeared to remain unaffected over time. Therefore, there is an urgent need to reconsider the implemented control measures. The results of this study and the course of ASF in other affected countries, raise the question, whether an endemic situation of ASF in wild boar is reversible.

Due to its high case-fatality ratio, African Swine Fever (ASF) is one of the most dreaded viral diseases in swine, especially in countries with a considerable pig industry[1]. As yet, there is no effective treatment or vaccination available[2,3].

ASF was introduced into South-Eastern Europe through Georgia. Shortly afterwards, the virus was detected in several other countries in the region[4–7]. It took seven years, until the epidemic had reached countries in the East of the European Union. Initially, Lithuania and Poland were affected, followed by Latvia and Estonia[6,8]. However, in the course of time, the disease also reached the Czech Republic, Romania, Hungary, Belgium and Bulgaria. As additional non EU countries, Moldova and since August 2018 also China are affected (OIE WAHID interface, visited online 13 August 2018). ASF has first been detected in the Latvian wild boar population in June 2014 and has been present there since then.

Outbreaks of ASF in domestic pigs occurred in several countries, but were usually controlled using conventional measures of animal disease control such as culling of affected pig holdings, safe disposal of the carcasses, cleaning and disinfection, movement restrictions, monitoring and surveillance. In regions, however, where ASF had the chance to spread in the wild boar population, controlling the disease in wild boar, not to mention eradicating it, was largely unsuccessful[4,9,10]. This is of particular importance as wild boar have been shown to play a significant role in disease transmission and maintenance[7,11]. Several strategies have been proposed to control ASF in wild boar population, including the ban of large-scale drive hunts, the implementation of massive targeted hunting and the removal and safe disposal of wild boar carcasses from the environment[12,13]. The effect of these measures is controversially discussed and many are assumed to have a low efficacy, e.g. use or ban of supplementary

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Measure	Control measure	Time period of measure implementation
A	Incentives to all persons who report dead wild boar to the veterinary authorities	July 2014–March 2015
B	Incentives to hunters (200 Euros per hunted wild boar older than 1 year, 50 Euros for wild boar of less than 1 year)	July 2014–Sept 2014
C	Collection and safe disposal of dead wild boar carcasses (done by the Food and Veterinary Service)	26 June 2014–March 2015
D	Notification, collection and safe disposal of dead wild boar carcasses (done by hunters)	April 2015–Jan2016
E	Collection and safe disposal of dead wild boar carcasses (Responsibility of local municipalities - mostly done by hunters)	Feb 2016–Dec 2017
F	Winter feeding ban	since 10 Dec 2014
G	Baiting of wild boar only allowed for hunting purposes	since 10 Dec 2014
H	Restrictions on driven hunts	Oct 2014–Feb 2015
		Oct 2015–Feb 2016
I	Incentives for hunting adult and sub-adult female wild boar	Nov 2015–March 2016
		Oct 2016–Dec 2017
J	Permission to use sound moderators (silencers) and night vision devices for wild boar hunting	since April 2015

Table 1. Measures implemented to control African swine fever in wild boar in Latvia.

feeding of wild boar[8,14]. However, the restricting or prohibiting of drive hunts and paying incentives for carcass removal are considered to be reasonably effective[8,12].

Nurmoja, *et al.*[9] showed a positive association between wild boar population density and an increased incidence of ASF cases in wild boar. Accordingly, it is generally accepted that a drastic reduction of the wild boar population, at least in the surroundings of foci with the occurrence of ASF in wild boar may help to control ASF in this species[8,15]. Although this approach appears promising in the model, its practicability is doubtful. Lange[15] showed by mathematical modelling that conventional strategies of wild boar population management (e.g. targeted hunting) must be conducted over several years to lead to a clear decrease of the wild boar population size. Furthermore, a recent study suggests that the role of the population density in the spread of ASF within wild boar might be less important than previously assumed[16].

In Latvia, various control measures were implemented for different periods of time during the current epidemic (Table 1).

However, the continuous spread of ASF in the wild boar population in Latvia suggests that these measures were not sufficient to contain the epidemic.

Following these observations, the present study aimed to evaluate the effects of the Latvian control measures on the respective target figures on the basis of available surveillance data, i.e. the effect on the proportion of samples originating from animals hunted or found dead or on the estimated ASFV genome prevalence.

Due to the existing knowledge gaps regarding the epidemiology of ASF, risk factors for a higher probability to detect ASF positive samples were additionally identified.

The results of the study may be used to improve assessments of the success of control measures for ASF in wild boar. They also allow adjusting the applied measures as appropriate.

Material and Methods

Study area. The study area consisted of three different regions in Latvia, from where a sufficiently large sample size was available, i.e. Latgale region, which is bordering Belarus, Vidzeme region, bordering Estonia, and Madona County, which is located between the two regions (Fig. 1). In total, the study area included 20 counties and comprised of approximately 15,146 km^2. The towns of Daugavpils and Valmiera were excluded from the analyses.

Data. Surveillance data were extracted from the CSF/ASF wild boar surveillance database of the European Union (https://surv-wildboar.eu). The study period ranged from 25.06.2014 (first detection of ASF in wild boar in Latvia) to 31.10.2017 (41 months). There was one individual data set for each wild boar, which contained information about date and location where the sampled animal had been hunted or found dead, the estimated age of the animal and the laboratory test result of the sample (detection of virus genome by PCR and serology). Regarding age, animals were categorized in two classes, younger than one year and one year or older. Furthermore, information was recorded for the origin of each sample (in the following text termed carcass type), i. e. shot apparently healthy (active surveillance) or shot sick, found dead or involved in a road traffic accident (passive surveillance). Data with an inconclusive result in any of the variables were excluded.

Laboratory testing of samples for ASFV genome and ASF-specific antibodies was performed in the Latvian Institute of Food Safety, Animal Health and Environment (BIOR) by the Animal Disease Diagnostic Laboratory. For antibody detection and ASFV genome, blood samples were taken by the hunters from hunted wild boar. Organ (kidney, spleen, lymph node) or bone marrow samples were collected from animals found dead and analyzed for virus genome.

In 2014 and 2015, detection of ASFV genome was performed by real time PCR[17], but from 2016 onwards, the UPL protocol established by Reference Laboratory for ASF of the European Union[18] was used. Antibody testing was done by using a commercial blocking enzyme-linked immunosorbent assay (ELISA) (INGENASA,

Figure 1. The study area comprised of three regions, i.e. Latgale region in the South, Vidzeme region in the North and Madona County in between. Map was generated by using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA).

INGEZIM PPA COMPAC, Spain) and positive results confirmed with the indirect immunoperoxidase test (IPT), validated by the Reference Laboratory for ASF of the European Union.

The State Forest Service provided wild boar population data that were used to assess the wild boar population density. Data were available on game management unit for the hunting seasons (April of a year until March of the following year) during the ASF epidemic (2014/15, 2015/16, 2016/17). The definition of the game management units is based on habitat and human related landscape characteristics (e.g. human population density, land use, major roads and rivers). They are used to manage all species of game. The number of wild boar in each game management unit was estimated on the basis of the hunting bag, population structure data (number of hunted animals divided by their age and gender), current occurrence evidences (e.g. visual observations, foot prints etc. observed by hunters or forest rangers) and information on wild boar damage to crop production claimed by local farmers. Estimations were annually done with a deadline on the 1st of April of each year for the previous year, for each game management unit by a local official of the State Forest Service, who collected and amalgamated the information.

For analysis, the data was aggregated at municipality level. The software ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA (http://www.esri.com/) was used to calculate the wild boar density per km^2 based on the estimated number of wild boar per game management unit. Areas of game management units that overlapped with the territories of at least two municipalities were proportionally attributed to the territory of each municipality. On the basis of the wild boar density per km^2 and the adapted game management units, the total number of wild boar per municipality was calculated. The average values of the wild boar densities of the three hunting seasons were determined and assigned to each corresponding municipality. An additional dataset on wild boar population density was available from the time before ASF had emerged in Latvia (hunting season 2011/12). In contrast to the hunting data of the years during the ASF epidemic, these data were only available on regional subunits of the State forest service (geographically larger than municipalities). These data were assigned to the administrative district level. To ensure comparability, the data of the hunting seasons during the ASF epidemic had to be adapted accordingly. To this end, the centroid of the municipalities was used to assign the wild boar densities of the municipalities to the respective administrative district. The number of wild boar in the individual municipalities, which belonged to one particular administrative level, were summarized and the wild boar densities for each administrative district calculated.

Maps and figures. Maps were generated using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/). Figures were generated using the software package R (http://www.r-project.org)[19].

Statistical analyses. Statistical analyses were performed using the software package R (http://www.r-project.org) if not stated otherwise. Confidence intervals were calculated according to Clopper and Pearson[20]. A p-value of ≤ 0.05 was considered statistically significant.

The statistical analyses of population data and the association between potential risk factors for a higher probability to detect ASF-positive samples and the ASF laboratory test result were performed essentially as described by Nurmoja, *et al.*[9]. Correspondingly, potential associations between age and test result as well as carcass type and test result were examined using Fisher's exact test. Potential associations between carcass type and age were analyzed in the same manner. The estimated periodic prevalence of ASFV genome-positive wild boar and the seroprevalence of ASF-specific antibodies with the respective 95% confidence intervals were calculated and the temporal course of the prevalence data was analyzed.

Potential differences in population densities between different hunting seasons were investigated using a non-parametric Kruskal-Wallis test. If a statistically significant difference was detected, pairwise Mann–Whitney U tests were performed. To control the type I error for multiple testing, the Bonferroni correction[21] was applied in these comparisons.

To study the effect of control measures on the wild boar population, or the estimated periodic ASFV genome prevalence, an appropriate control period was defined for each period, during which the specific measure was in place (Table 2, Figs 2 and 3). When determining the control period, we aimed to choose the same number of month and the same season as in the period, during which the measure was applied. Ideally, no other measure with a potential impact on related parameters (e.g. increase of hunted wild boar/increase of animals found dead) was in place during the control period. Accordingly, for some measures the control periods had to be chosen before, and for others after the periods, when the measures were applied.

For some measures, no control period could be established. These measures were excluded from the analysis (measures F and G in Table 1). Moreover, only measures with a direct effect on the composition of the population, the carcass type (animals hunted or found dead) or the estimated ASFV genome prevalence were considered. Accordingly, measures C, D and E (Table 1) were excluded from further analysis.

To test the Null hypotheses and thus the effect of the measure on the estimated ASFV genome prevalence or the proportion of hunted/found dead wild boar a multivariable Bayesian regression model was used[9,22]. A proportion of mean/Std.Dev. >1.96 was regarded as statistically significant.

Each measure was defined as the independent variable. In each model, age was included as fixed effect. Accounting for the epidemiological situation over time, in the case of carcass type (proportion of hunted/found dead wild boar) as dependent variable, the estimated ASFV genome prevalence in each month of the respective measure/control period was included as fixed effect. The origin of sample (hunted/found dead) was included as fixed effect in the models where the estimated ASFV genome prevalence constituted the dependent variable. Time (in months) was treated as a random effect. To estimate the parameter values, a Markov Chain Monte Carlo algorithm (MCMC) was used. 50,000 iterations were performed and at every 50th iteration a sample was selected. For the burn-in 1,000 iterations were chosen. The model was implemented using BayesX 2.0.1 (http://www.uni-goettingen.de/de/bayesx/550513.html) and convergence of the MCMC chain was assessed using standard diagnostic plots and tests. A detailed model description can be found in the supplementary information.

Stepwise model building using forward selection were used to find the best model for each measure. The models were evaluated on the basis of their Deviance Information Criterion (DIC) and the Effective number of parameters (pD). For each measure the model with the smallest DIC were used for the final analyses. In the case of comparable DICs, the model with the smallest pD was chosen (Supplementary information Tables S2, S8).

Results

Data. In total, 12,978 data records were available for analysis. Of these, 5,224 records originated from Latgale region, 5,379 from Vidzeme region and 2,375 from Madona County.

Statistical analysis. A significant association between age and positive laboratory test results (both, ASFV genome and serology) was found. When analyzing all samples, the probability to find a positive test result was higher in young animals (Table 3). The probability that a sample originating from an animal found dead came from a younger animal was significantly higher as compared to older wild boar. The probability to detect a positive test results in wild boar found dead was significantly higher than in hunted animals (Table 3).

Temporal changes in population density were also detected: Compared to the hunting season of 2011/12, the wild boar density decreased significantly starting with 2014 until the hunting season of 2016/17 ($p < 0.001$) (Fig. 4).

The estimated ASF prevalence, regardless of the laboratory testing method, i.e. ASFV genome detection or serology, hardly showed any statistically significant changes during the study period of 41 months (Fig. 5).

For the model analyses, models with the lowest DIC were used (Table 4, Supplementary information Tables S2, S8). None of the measures resulted in a significant effect on the variable of interest (carcass type or estimated ASFV genome prevalence).

Paying incentives to all persons who reported dead wild boar to the veterinary authorities (control measure M1, Table 2) yielded a greater effect on the estimated ASFV genome prevalence (1.20) than most of the other measures. However, the difference between the estimated prevalences in the two time periods was nearly zero (Supplementary information Table S1). Also, permission to use silencers and night vision devices for wild boar hunting (control measure M5) seemed to have a stronger effect, albeit not significant, on the proportion of hunted wild boar and on the estimated ASFV genome prevalence (Table 4). In addition, the number of hunted wild boar was larger in the period when control measure M5 had been implemented (Supplementary information Table S1).

Paying incentives to hunters for hunted wild boar (control measure M2) had no statistically significant effect on the proportion of hunted animals (Table 4) but also a lower number of hunted animals in the time when the measure was applied as compared to the time, when it was not applied (Supplementary information Table S1).

	Measure	Measure/Control period
		Null hypothesis
M1	Incentives to all persons who report dead wild boar to the veterinary authorities (corresponds to A in Table 1)	Measure period (M1 = C5): July 2014 – March 2015 Control period (C1 = M5): July 2015 – March 2016
		$H_0$1: No significant effect of M1on the proportion of animals found dead $H_0$2: No significant effect of M1on the estimated ASFV genome prevalence
M2	Incentives to hunters for hunted wild boar (corresponds to B in Table 1)	M2: July 2014 – Sept 2014 C2: July 2015 – Sept 2015
		H_0: No significant effect of M2 on the proportion of hunted animals
M3	Restrictions on driven hunts (corresponds to H in Table 1)	M3a: October 2014 – February 2015 M3b: October 2015 – February 2016 C3a + b: October 2016 – February 2017
		H_0: No significant effect of M3 on the estimated ASFV genome prevalence
M4	Incentives for hunting adult and sub-adult female wild boar (corresponds to I in Table 1)	M4a: November 2015 – March 2016 M4b: October 2016 – October 2017 C4a: November 2014 – March 2015 C4b: October 2014 – October 2015
		H_0: No significant effect of M4 on the proportion of hunted animals
M5	Permission to use sound moderators (silencers) and night vision devices for wild boar hunting (corresponds to J in Table 1)	M5 = C1: July 2015 – March 2016 C5 = M1: July 2014 – March 2015
		$H_0$1: No significant effect of M5 on the proportion of hunted animals $H_0$2: No significant effect of M5 on the estimated ASFV genome

Table 2. Control measures for African swine fever in wild boar in Latvia analyzed for their effect on the estimated ASFV genome prevalence, the proportion of hunted animals or found dead.

The estimated ASFV genome prevalence was higher in the period when driven hunts were restricted (control measures M3a and M3b) as compared to the time, when there were no limitations of driven hunts (Supplementary information Table S1). These finding was also supported by the model analyses. However, the effect of the measure on the estimated ASFV genome prevalence was not significant (Table 4). Paying incentives for hunting adult and sub-adult female wild boar (control measure M4a and M4b) also failed to show a statistically significant effect on the proportion of hunted wild boar in any of the periods, during which this control measure was applied (Table 4).

Discussion

The aim of the study was to evaluate the effect of measures that had been applied in Latvia to control ASF in wild boar. In view of the fact that there are still substantial gaps in our knowledge about the epidemiology of ASF, particularly in wild boar[23,24], we also tried to address some of these gaps. Therefore, risk factors for a higher probability to detect ASF-positive wild boar were investigated.

In close cooperation with the Latvian veterinary authorities, three regions were determined as the study area. From these regions, which were typical for other parts of the country, also with regard to the occurrence of ASF in wild boar, a wide range of surveillance and wild boar population data originating from different epidemiological situations were available.

According to EU legislation (Council Directive 2002/60/EC), it is mandatory to sample all hunted and dead wild boar in ASF-affected areas. It can thus be assumed that the obtained sample size in areas affected by ASF corresponds to the entire hunting bag, i.e. usually at least 60–80% of the population. However, due to hunting habits such as the desire for trophies, some older and very young wild boar are usually not shot. Therefore, the composition of the hunting bag does not completely mirror the structure of the wild boar population[25–28]. Nonetheless, the sample size available from ASF- affected areas is still large as compared to other wildlife studies[29–32]. Consequently, although respecting the uncertainty regarding the true population size and the true epidemiological situation of ASF in Latvian wild boar, we assumed that the sample size was sufficient for our analyses to estimate the periodic ASFV genome prevalences and seroprevalences with a high confidence.

The results regarding the association of risk factors and laboratory test results were in line with results obtained in a study recently conducted in Estonia[9]. The majority of samples resulted from adult wild boar and was obtained by active surveillance. However, the probability to find an ASF-positive animal was significantly higher in wild boar found dead, i.e. passive surveillance. This result is in accord with previous findings and emphasizes the urgent need to enhance the number of samples resulting from passive surveillance[7,9,33–35]. The fact that similar results were obtained in several countries affected by ASF in wild boar suggests that the risk factors for a higher probability to detect an ASF-positive sample are also similar, regardless of the environment, the region or the population density. Therefore, one can assume that the course of ASF within wild boar populations is comparable between affected countries or regions. The increased probability that a wild boar that was found dead was younger than one year suggests that young animals are at a higher risk to die. The cause of death may be ASF in the study area, but other reasons such as infections with agents other than ASFV or predation cannot be excluded[28,36].

Although estimated population densities in different hunting seasons may not be independent, due to the lack of a suitable alternative, independence was assumed and the conservative non-parametric Kruskal-Wallis test used for statistical comparison.

Over the years, a significant reduction of the estimated wild boar population density was observed. This is probably due to the continuous spread of ASF and its high case-fatality ratio. To rule out completely that the

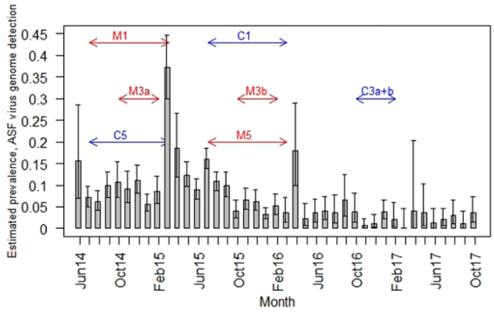

Figure 2. Estimated prevalence of African Swine Fever virus genome-positive wild boar (boxes) and 95% confidence intervals (whiskers) for each month of the study period. Red arrows illustrate the time span for the different measures, which aimed to influence the prevalence. Blue arrows illustrate the corresponding control periods. The numbering of measures and controls follows Table 2. Figure was generated by using the software package R (http://www.r-project.org).

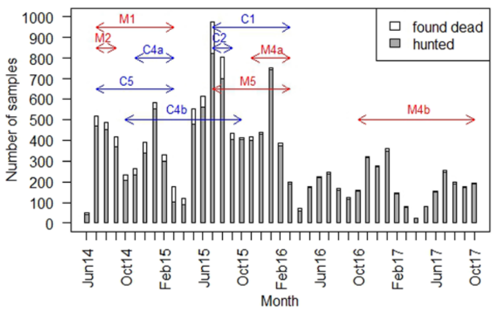

Figure 3. Number of samples originating from hunted wild boar or wild boar found dead. Red arrows illustrate the periods, when various measures that aimed at influencing the proportion of hunted/found dead wild boar were applied. Blue arrows illustrate the corresponding control periods. The numbering of measures and controls was performed according to Table 2. Figure was generated by using the software package R (http://www.r-project.org).

decrease was not just an effect of intensified hunting in these areas, further data, from the western part of Latvia should be analyzed and compared. In these regions, ASF cases occurred after 2015 but hunting strategies were the same as in affected regions. However, such analyses were beyond the scope of the present study as only data of the defined study area were used. Moreover, only data of one hunting season (2011/12) before the emergence of ASF were available. Therefore, it remains unknown, if the population density in the hunting years 2012/13 and 2013/14 was higher than in the years after ASF had been introduced into the wild boar population. Furthermore, when interpreting the temporal trend of the wild boar population density, it has to be kept in mind that data were available on different administrative levels, which may have caused some bias. Since population density was estimated with some degree of uncertainty, the effect of the bias caused by aggregating data originally obtained on different administrative levels is likely to be negligible. As wild boar population densities usually represent estimates, these data have always to be regarded as benchmarks rather than precise figures. However, currently these rough estimates of population densities based on hunting bag etc. are the only data available for analyzing wild boar population density data.

Due to the inclusion of all available samples in the analyses, the estimated periodic prevalences could be determined. The analyses of estimated ASFV genome prevalences during the study period showed hardly any change during the study period. This finding is in accord with other studies where the course of ASF in wild boar was analyzed and no significant change in the ASF prevalence in wild boar could be found over time[9,35]. The small variation in the prevalences did not indicate any seasonality of the ASF infection in wild boar. This is in contrast to studies conducted in the Russian Federation, Poland and Sardinia, where seasonality in the occurrence of ASF was found[35,37,38].

Only some of the control measures that had been implemented by the Latvian authorities during the study period could be analyzed with regard to their effect on the course of ASF. For measures that started shortly after

Age	ASFV genome detection	p	<0.001
		OR (95% CI)	0.55 (0.47–0.64)
	Serology	p	<0.001
		OR (95% CI)	0.72 (0.60–0.87)
	Carcass type (hunted/found dead)	p	<0.001
		OR (95% CI)	0.70 (0.60–0.82)
Carcass type (hunted/found dead)	ASFV genome detection	p	<0.001
		OR (95% CI)	192.51 (157.37–235.49)
	Serology	p	<0.001
		OR (95% CI)	9.30 (6.83–12.67)

Table 3. Associations between potential risk factors (age and carcass type) and a positive laboratory test result (ASFV genome detection and serology) and association between age and carcass type.

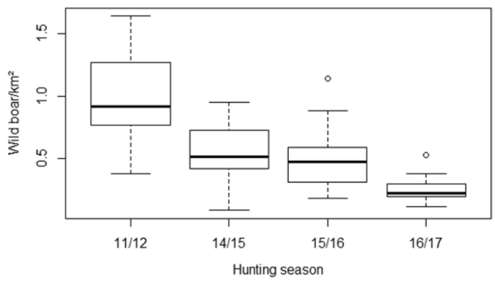

Figure 4. Temporal course of wild boar population density. Figure was generated by using the software package R (http://www.r-project.org).

the first ASF-positive wild boar had been detected in Latvia and were in place during the entire study period, e.g. the winter feeding ban (Table 1), an appropriate control time period could not be defined. Due to lack of transferability to other countries or areas, measures, in which only the allocation of responsibilities (e.g. veterinary authorities were responsible for collection of dead wild boar vs. hunters were responsible; C, D and E in Table 1) changed, were excluded from the analyses.

To analyze the effects of different control measures on the carcass type (hunted wild boar, animals found dead) or the estimated ASFV genome prevalence, a multivariable Bayesian regression model was used. Age and carcass type were statistically significantly associated with the estimated ASFV genome prevalence (Table 3). Therefore, accounting for the effect of age and either carcass type or estimated ASFV genome prevalence on the dependent variable, these variables were included in the model as fixed effects. Due to the low number of available geographical units, no spatial effect was included.

In contrast to most control measures, paying incentives to all persons who report dead wild boar to the veterinary authorities (control measure M1) and permission to use silencers and night vision devices for wild boar hunting (control measure M5) indicated a potential slight effect on the estimated ASF prevalence. Moreover, the number of hunted wild boar increased in the period, when control measure M5 was applied. The analyses of the effect of control measure M1 (incentives for notification of found dead wild boar) could not be separated from the analyses of control measure M5 (hunters could use silencers and night-vision device for wild boar hunting), because the control period of M1 corresponded to the period, when control measure M5 was applied. Therefore, the potential effect of M1 may be a result of M5 or vice versa. In addition, the combination of these measures may have also led to the observed results. The available data suggest that the wild boar population density decreased over time, presumably due to the increased hunting pressure which might have been supported by control measure M5 (usage of supporting tools). These results demonstrate the need to adapt hunting regulations in the case of the emergence of ASF or even before as a preventive measure in an area at risk. The permission to use these tools, however, does not necessarily indicate the extent of their use by hunters.

Paying incentives for hunted wild boar (M2) in the summer months of 2014 was mainly done to facilitate hunting. The season for driven hunts starts in October and the number of hunted animals therefore usually increases from this month onwards without any additional measures. Measure M2 showed no effect in the present study However, motivating hunters financially to reduce the population density might still support the control of ASF, as the wild boar population density probably plays an important role in the spread of ASF[8,9,15]. This applies especially to the summer months, during which the hunting activity is usually decreased.

As driven hunts end in February, restrictions regarding this form of hunting were only applied until February 2015 (M3a) and in a second period until February 2016 (M3b). The restrictions of driven hunts had no significant effect on the estimated ASFV genome prevalence. In both time periods when control measure M3 was

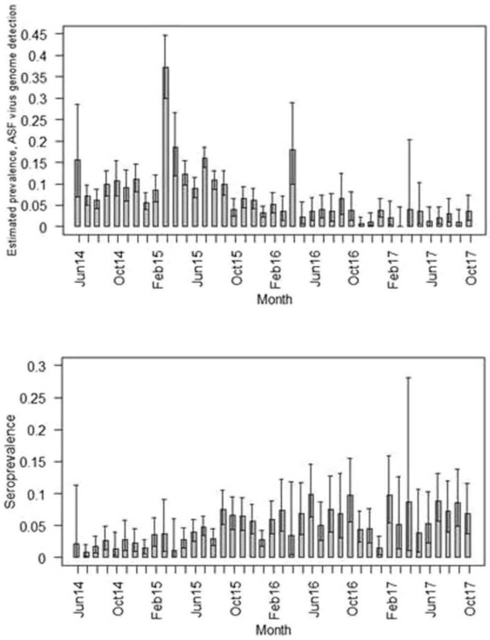

Figure 5. Estimated ASF prevalence (upper panel ASF virus genome detection by PCR; lower panel, detection of ASF-specific antibodies). The boxes represent point estimates and the whiskers 95% confidence intervals. Figure was generated by using the software package R (http://www.r-project.org).

Measure	Null hypotheses	Mean/ St.Dev.*
Incentives to all persons who report dead wild boar to the veterinary authorities (M1)	$H_0$1 No effect on the proportion of animals found dead	0.72
	$H_0$2 No effect on the estimated virus prevalence	1.20
Incentives to hunters for hunted wild boar (M2)	H_0 No effect on the proportion of hunted animals	0.65
Restrictions on driven hunts (M3a)	H_0 No effect on the estimated virus prevalence	0.61
Restrictions on driven hunts (M3b)	H_0 No effect on the estimated virus prevalence	0.58
Incentives for hunting adult and sub-adult female wild boar (M4a)	H_0 No effect on the proportion of hunted animals	0.09
Incentives for hunting adult and sub-adult female wild boar (M4b)	H_0 No effect on the proportion of hunted animals	0.78
Permission to use sound moderators (silencers) and night vision devices for wild boar hunting (M5)	$H_0$1 No effect on the proportion of hunted animals	1.71
	$H_0$2 No effect on the estimated virus prevalence	1.30

Table 4. Results of the multivariable analyses regarding the effects of the control measures on the estimated ASFV genome prevalence, the proportion of hunted animals or found dead. *Mean/Std.Dev. >1.96, indicating statistical significance.

implemented, the estimated ASFV genome prevalence was even higher than in the respective control periods. These findings contradict the hypothesis that driven hunts increase the risk of spreading ASF, as infected wild boar might be disturbed, driven apart and stimulated to move in a wider radius as usual.

Control measures 4a and 4b consisted of paying incentives for hunted female wild boar. Due to ethical considerations (hunting restrictions during the time of wild boar reproduction), no incentives were paid from March 2016 to September 2016. The implementation of control measure M4a led to an increase of the number of hunted wild boar, but the effect of the measure was not statistically significant, when age and prevalence were taken into account. When control measure 4b was in place, the incentives were lower than during the period, when control measure 4a was applied. This was simply to reduce expenses as limited funds were available. Although control measure 2, which was implemented in summer 2014, already included paying incentives for hunted wild boar, the payments only started again in November 2015. At that time, it had become obvious that ASF continued to spread. Accordingly, the European Commission decided to provide Latvia financial support to reduce the wild boar population more intensively. Statistical analysis showed that similar to 2014, paying incentives had a direct positive effect on the proportion of hunted wild boar.

None of the control measures applied in Latvia during the present ASF epidemic showed a statistically significant effect on the relevant target variable (increase of the number of hunted wild boar or wild boar found dead). Also, no significant effect on the estimated ASF prevalence could be observed. However, when interpreting the results, the partly low sample size has to be considered. Especially the samples showing a positive test result for ASFV were often very small (Supplementary information Table S1) and a decrease of the effect size due to a small sample size could therefore not be excluded.

Over the study period of 41 months, the prevalence failed to decrease significantly, irrespectively of the implemented measures. Therefore, analyzing surveillance data, this study demonstrates what many experts had already feared and what also became evident in other affected countries: Once ASF has emerged in the wild boar population in a region, it seems hard or even impossible to eradicate the disease, at least if the epidemic is not stopped very early on. However, any potential long-term effects of the control measures remain unknown. A minor or moderate decrease of the ASF incidence in the wild boar population over several years due to the implemented control measures is still possible. Also Lange[15] stated that an effect on the course of ASF and the population will probably take many years. However, due to available data, in the present study the effects were only investigated for the measure and the defined control period.

Recent analyses of unpublished surveillance data suggest that the proportion of wild boar samples that tested positive for antibodies to ASFV but negative for ASFV genome by PCR increases in Latvia. This may indicate a decrease in the incidence of ASF in wild boar[39,40].

Our findings also demonstrate that more detailed knowledge on the transmission and excretion of ASFV, the tenacity in wild boar carcasses and on the role of potential carriers is necessary[40]. Once the epidemiology of the disease in the wild boar population becomes clearer, it might be possible to improve control measures and to utilize them in a more targeted way. Also, alternative measures such as fencing, which were used in controlling an epidemic of ASF among wild boar in the Czech Republic, need to be considered. Further studies are required to verify the success of such alternative control measures but also to investigate the possible long-term effects of measures that were already applied.

Although the effect of the wild boar population density on the course of an ASF epidemic remains disputed[8,9,16], additional strategies to reduce the wild boar population density should be taken into account (e.g. intensive targeted hunting on adult females or trapping). However, finally the question remains, whether affected countries will have to accept the presence of ASF within their wild boar population at a certain level and how they and their trade partners can learn to live with this situation. It may therefore be inevitable to focus on the biosecurity of pig farms. Unaffected countries should carefully evaluate their surveillance with the aim of preventing ASF entry and early detection in the case of ASF introduction[41].

Data Availability

In accordance with the responsible persons from Latvia, the original data used for the analyses can be obtained from the author.

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Author Contributions
K.S. designed the study, performed the data analyses and drafted the manuscript. E.O. designed the study, provided surveillance data and information about control measures and reviewed the manuscript. C.S. designed the study, supported the statistical analyses and reviewed the manuscript. K.L. provided input to the ASF situation in Latvia and reviewed the manuscript. M.S. provided hunting and wild boar population data and reviewed the manuscript. S.C. prepared a description of laboratory methods used for ASF diagnostics and reviewed the manuscript. F.J.C. supported the study with epidemiological considerations and carefully reviewed the manuscript. C.S.-L. designed and supervised the study and reviewed the manuscript.

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3.2.3.6 Joining the club: First detection of African swine fever in wild boar in Germany

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Highlights

- The first ASF-positive wild boar carcass in Germany was detected in September 2020
- All first cases were detected close to the Polish border
- The virus was probably introduced into Germany in early July 2020 or even earlier
- The ASFV Germany 2020/1 sequence showed 99.9% nucleotide sequence identity to the virus, which originated from an outbreak south of Warsaw in 2017

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Joining the club: First detection of African swine fever in wild boar in Germany

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Abstract

African swine fever (ASF) has spread across many countries in Europe since the introduction into Georgia in 2007. We report here on the first cases of ASF in wild boar detected in Germany close to the border with Poland. In addition to the constant risk of ASF virus (ASFV) spread through human activities, movements of infected wild boar also represent a route of introduction. Since ASF emerged in Western Poland in November 2019, surveillance efforts, in particular examination of wild boar found dead, were intensified in the regions of Germany bordering with Poland. The first case of ASF in wild boar in Germany was therefore detected by passive surveillance and confirmed on 10 September 2020. By 24 September 2020, 32 cases were recorded. Testing of samples from tissues of carcasses in different stages of decomposition yielded cycle threshold values from 18 to 36 in the OIE-recommended PCR, which were comparable between the regional and national reference laboratory. Blood swabs yielded reliable results, indicating that the method is suitable also under outbreak conditions. Phylogenetic analysis of the ASFV whole-genome sequence generated from material of the first carcass detected in Germany, revealed that it groups with ASFV genotype II including all sequences from Eastern Europe, Asia and Belgium. However, some genetic markers including a 14 bp tandem repeat duplication in the O174L gene were confirmed that have so far been detected only in sequences from Poland (including Western Poland). Epidemiological investigations that include estimated postmortem intervals of wild boar carcasses of infected animals suggest that ASFV had been introduced into Germany in the first half of July 2020 or even earlier.

Sauter-Louis and Forth contributed equally to this work.

KEYWORDS
African swine fever, epidemiology, first case, Germany, virus sequence

1 | INTRODUCTION

Over the past decade, African swine fever (ASF) has developed into a panzootic threat to domestic pigs and wild boar worldwide (Blome et al., 2020). Since 2014, ASF virus (ASFV) has been spreading in the European Union (Chenais et al., 2019). The virus was introduced into wild boar in Belgium in 2018 (Linden et al., 2019) and into the western part of Poland in 2019 (Mazur-Panasiuk et al., 2020) close to the respective German borders. While the situation in Belgium appears to be under control, the epidemic in Poland worsened. The first reported case of ASF in wild boar in Western Poland was found about 79 km from the German border. Between 1 November 2019 and 9 September 2020, a total of 1,037 cases in wild boar and eight outbreaks in domestic pigs were reported from this region (ADNS, as of 10 September 2020) with restriction zones in Poland extending to the German border. The nearest case of ASF in wild boar in Poland was located 10.4 km from the German border (confirmed on 26 March 2020). Here, we report on the first cases of ASF in wild boar in Germany, detected close to the German-Polish border in the Federal State of Brandenburg, including the results of epidemiological investigations, diagnostics and genetic characterization of the German ASFV isolate using whole-genome sequencing.

2 | MATERIAL AND METHODS

2.1 | Epidemiological investigations

Information on ASF cases in wild boar obtained by the local veterinary offices was entered into the German electronic animal disease information system (TSN, 'Tierseuchennachrichtensystem', German National Animal Disease Database) (Kroschewski et al., 2006). These data sets contain the date of suspicion and disease confirmation, the location (geographical coordinates), where the animal had been hunted or found dead, as well as the age and gender of each individual wild boar.

In addition, for most carcasses, photographs and a 'checklist for wild boar carcasses found in the field' (https://www.fli.de/fileadmin/FLI/IfE/AG_Forensik_Wildschwein/Erhebungsbogen_Wildschwein.pdf) were made available for epidemiological investigations or retrieved by epidemiologists of the Friedrich-Loeffler-Institut (FLI). These data were used to estimate the minimum postmortem interval (PMI) as previously described (Probst, Gethmann, Amendt, et al., 2020; Probst, Gethmann, Hohmann, et al., 2020). The minimum PMI, that is the latest possible time point of death, was estimated based on morphological characteristics of the carcass, that is the decomposition stage of different body parts and accumulated decomposition (fresh, bloated, active decay, advanced decay, dry and remains), mean temperature (at 2 m height) and rainfall in the previous weeks (retrieved from the nearest weather station in Coschen, Brandenburg; German Weather Service, Offenbach, Germany), type of microhabitat (dry/moist, direct sunlight/shade, etc.), entomological activity, characteristics of the decomposition island and evidence for vertebrate scavenging. Euclidian distances between the cases in Germany and those in Western Poland were calculated in km using the software package sp (Pebesma & Bivand, 2005) within the open-source software R (http://www.r-project).

Data on ASF surveillance prior to the emergence of ASF in Germany were extracted from the CSF/ASF wild boar surveillance database of the European Union (https://surv-wildboar.eu) for the period from 1 November 2019 to 9 September 2020. This covered the whole period since cases of ASF had been reported from Western Poland. There is one individual data set for each wild boar, which contained the date and location, where the animal had been hunted or found dead. Furthermore, information was recorded on the circumstances of death for each animal, that is shot apparently healthy (active surveillance) or shot sick, found dead or involved in a traffic accident (passive surveillance).

2.2 | Laboratory diagnosis

For qPCR at the regional laboratory (Landeslabor Berlin-Brandenburg, Frankfurt/Oder, Germany, LLBB), the commercial RealPCR ASFV DNA Test (IDEXX) was used following the manufacturer's instructions. For confirmatory purposes, the OIE-listed protocol by King et al. (2003) was used with slight modifications. At the German national reference laboratory (NRL at the FLI), qPCRs were performed according to the protocols published by King et al. (2003) and Tignon et al. (2011). Both assays were used with adapted internal control systems. In addition, a commercial qPCR kit was employed (virotype ASFV; Indical Bioscience) according to the manufacturer´s instructions. To isolate the causative ASFV in serum and tissue samples, a haemadsorption test (HAT) was performed using PBMC-derived macrophages according to slightly modified standard procedures (Carrascosa et al., 2011).

2.3 | Next-generation sequencing

For whole-genome sequencing, DNA was extracted from 200 µl of bone marrow homogenate using the NucleoMagVet kit (Macherey-Nagel) according to the manufacturers' protocol. For rapid generation of sequence information, DNA from the same sample was sequenced on the Illumina MiSeq platform (San Diego) as described elsewhere (Wylezich et al., 2018).

The resulting read data were mapped against a custom database including all ASFV sequences available from International Nucleotide Sequence Database Collaboration (INSDC) databases as of 15 September 2020 using Bowtie2 v.2.3.5.1 (Langmead &

Salzberg, 2012) with the highest sensitivity settings followed by de novo assembly of the mapped reads using SPAdes v.3.13 (Antipov et al., 2016) with default parameters. The resulting whole-genome sequence was aligned with all other available ASFV whole-genome sequences from INSDC databases (as of 21 September 2020) using MAFFT v7.388 (Katoh & Standley, 2013) in Geneious Prime v.2019.2.3 (Biomatters), annotated on the basis of ASFV Georgia 2007/1 (FR682468.2) using Geneious, and a phylogenetic tree was constructed with IQTREE v 1.6.5 (Hoang et al., 2018; Kalyaanamoorthy et al., 2017; Nguyen et al., 2015). Whole-genome sequences from Poland were kindly provided by N. Mazur and G. Woźniakowski ahead of publication in INSDC databases and compared to the ASFV Germany 2020/1 whole-genome sequence using MAFFT v.7.388 with default parameters in Geneious prime.

3 | RESULTS AND DISCUSSION

3.1 | Case description and epidemiological situation

The carcass of an adult female wild boar was found in a ditch between two harvested maize fields at a dry location exposed to direct sunlight, about 200 m off a country road in a community of the Spree-Neiße district, Brandenburg, Germany *approx.* 6 km from the border with Poland (Figure 1) by a person walking the dog. Foreleg bones of the largely decomposed carcass were submitted by the responsible hunter for ASFV testing, and the carcass was buried on site on 7 September 2020. At the time of detection, the hunter noticed large numbers of maggots on and around the carcass. The mean temperature was 15°C (1–7 September 2020) or 19°C (6 August–7 September 2020), respectively. From 1 August–9 September 2020, the average rainfall was 6 mm. The carcass was found in an advanced stage of decomposition, and the minimum PMI was estimated at 2 weeks. At that time, the adjacent region in Poland was classified as a part I-region according to the implementing decision EU 2014/709, that is with a certain risk regarding ASF due to proximity of the infection in the feral population (Figure 1).

Since the occurrence of ASF in wild boar in Western Poland, the risk of introduction of ASF into Germany by migrating infected wild boar had substantially increased. Preventive measures such as building a mobile fence along the Polish border and increased surveillance in wild boar and domestic pigs were introduced. The direct distance from the first detected case in Brandenburg to the nearest ASF case in Poland, which was confirmed within the preceding 4 weeks (13 August–9 September 2020), was 33.9 km. The ASF case in Poland nearest to Germany was detected at a distance of 10.4 km from the German border, but south of the now affected area. Due to lack of information on the surveillance intensity between the national border and the ASF focus in Western Poland, direct evidence for a westward expansion of the infected area in Western Poland is missing. Yet, it seems likely that the disease has been introduced by migrating wild boar. (A new case, confirmed on 30 September 2020, located approx. 60 km north of the focus of the first introduction into Germany, was also detected less than 2 km away from the border with Poland.) However, it cannot be excluded that human activity (e.g. unsafe disposal of contaminated material) may have caused the initial infection. ASFV remains infectious for a long time in pig products, especially if blood is contained (Fischer et al., 2020; Kolbasov et al., 2011; Mebus et al., 1997).

Wild boar and domestic pigs have been intensively monitored for ASF in Germany in recent years. Especially in areas close to the

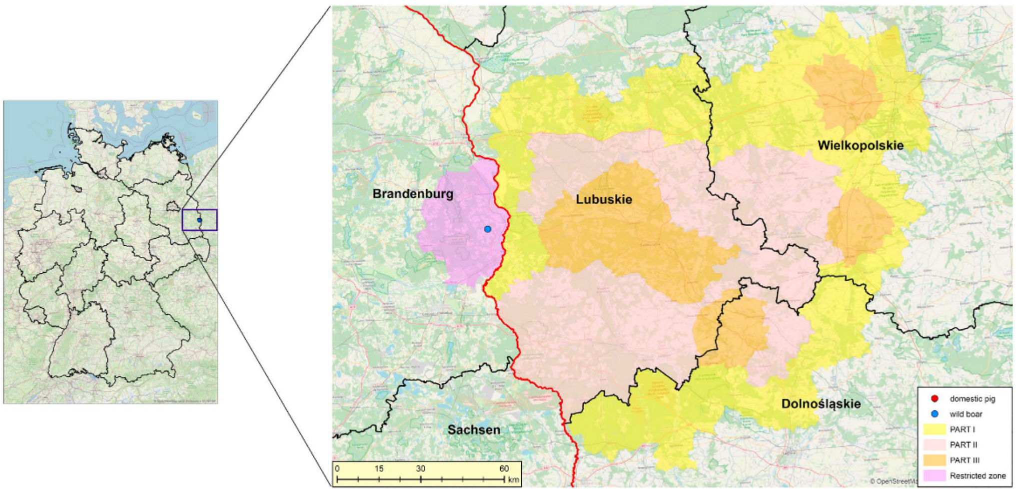

FIGURE 1 Location of the first wild boar found dead in Germany in relation to the ASF restriction zones in Western Poland (as of 10 September, 2020). Red line: German-Polish border, black lines: borders between federal states in Germany and Voivodeships in Poland

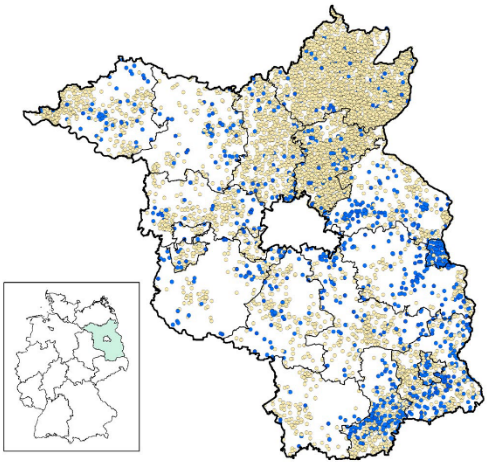

FIGURE 2 Surveillance effort since 1 November 2019 until 9 September 2020 in the federal state Brandenburg. (Blue dots: passive surveillance: shot sick, found dead, involved in road traffic accidents; yellow dots: active surveillance: shot apparently healthy.)

German-Polish-border, the efforts were intensified after the emergence of the disease in Western Poland. Figure 2 shows the results of surveillance within the affected region close to the German-Polish border for the period from 1 November 2019 to 9 September 2020. A total of 7.125 samples of wild boar (6.081 apparently healthy shot wild boar, 312 wild boar found dead, 74 animals shot sick and 658 animals killed in road traffic accidents) tested negative for ASF. Most wild boar are examined during winter, when hunting is at its maximum. Nevertheless, in the 4 months before the detection of the first case of ASF in Germany, 50 wild boar found dead, 12 animals shot sick and 165 animals killed in road traffic accidents also tested negative for ASFV.

During the following days of intensified carcass search, four more carcasses and a hunted wild boar with clinical signs were found 6 km away from the first site. These carcasses were fresh with an estimated minimum PMI of only a few days. Until 24 September 2020, a total of 32 cases of ASF were confirmed, of which nine were found close to the first site and 23 in a small area further north (Figure 3). In both locations, fresh and old carcasses of young and old wild boar were found (14 piglets, six subadults, six adult animals; the age of six animals could not be assessed) (Table 1). At the place, where most carcasses were found, the carcasses with the longest minimum PMI of 80 days were detected (Table 1).

FIGURE 3 Locations of ASF cases in wild boar in Germany (as of 24 September 2020). Yellow star: first detected case with estimated death date in August 2020; red dots: cases with estimated death date in July 2020; yellow dots: cases with estimated death date in August 2020; green dots: cases with estimated death date in September 2020; black dots: cases where the death date could not be estimated

TABLE 1 Wild boar samples examined for ASF in Brandenburg, Germany (from 10th September to 24th September 2020)

ID	County	Matrix	Age class	Gender	Sample origin[a]	Estimated minimum PMI (days)[b]	Estimated latest date of death[c]	LLBB IDEXX ASFV	NRL ASF King PCR	NRL ASF virotype ASFV	NRL ASF Tignon PCR
1	LK Spree-Neiße	Bone marrow	Adult	Female	✝	14	24.08.2020	24	23	21	25
2	LK Oder-Spree	Swab suspension	Subadult	Female	💧	0	11.09.2020	30	29	27	29
3	LK Oder-Spree	Blood clot suspension	Piglet	n.d.	✝	1	11.09.2020	20	21	21	21
4	LK Oder-Spree	Swab suspension	Subadult	Female	✝	Few days	10.09.2020	30	25	29	25
5	LK Oder-Spree	Swab suspension	Adult	Female	✝	Few days	11.09.2020	25	25	24	25
6	LK Oder-Spree	Swab suspension	Piglet	n.d.	✝	Few days	08.09.2020	25	25	24	32
7	LK Oder-Spree	Swab suspension	Subadult	Female	✝	3	10.09.2020	24	26	23	n.d.
8	LK Oder-Spree	Swab suspension	Piglet	n.d.	✝	5	10.09.2020	28	28	25	29
9	LK Oder-Spree	Swab suspension	Piglet	n.d.	💧	0	15.09.2020	26	25	24	27
10	LK Spree-Neiße	Bone marrow	Subadult	Male	✝	n.d.	n.d.	27	30	27	31
11	LK Spree-Neiße	Bone marrow	Piglet	n.d.	✝	30	17.08.2020	33	35	29	37
12	LK Spree-Neiße	Bone marrow	Subadult	Male	✝	n.d.	n.d.	29	*no cq*	33	38
13	LK Spree-Neiße	Bone marrow	Piglet	n.d.	✝	7	09.09.2020	31	36	30	37
14	LK Oder-Spree	Swab suspension	Subadult	Female	✝	1	16.09.2020	23	21	n.d.	n.d.
15	LK Oder-Spree	Bone marrow	Adult	Female	✝	60	20.07.2020	30	33	31	34
16	LK Oder-Spree	Swab suspension	Piglet	n.d.	✝	4	14.09.2020	26	25	23	25
17	LK Spree-Neiße	Bone marrow	Piglet	n.d.	✝	n.d.	n.d.	37	35	*no cq*	34
18	LK Spree-Neiße	Bone marrow	Piglet	n.d.	✝	n.d.	n.d.	19	20	17	20
19	LK Spree-Neiße	Bone marrow	Piglet	n.d.	✝	2	17.09.2020	21	18	17	19
20	LK Spree-Neiße	Bone marrow	Adult	Female	✝	14	05.09.2020	23	23	22	24
21	LK Oder-Spree	Swab suspension	Piglet	n.d.	✝	7	12.09.2020	22	21	18	22
22	LK Oder-Spree	Swab suspension	Piglet	n.d.	✝	1	18.09.2020	26	25	24	27
23	LK Oder-Spree	Bone marrow	n.d.	Female	✝	80	01.07.2020	35	36	29	35
24	LK Oder-Spree	Bone marrow	n.d., only bones left	Female (?)	✝	80	01.07.2020	32	31	29	32
25	LK Oder-Spree	Bone marrow	Piglet	n.d.	✝	7	12.09.2020	20	19	18	21
26	LK Oder-Spree	Bone marrow	n.d., only bones left	Male (?)	✝	10	09.09.2020	26	27	23	28
27	LK Oder-Spree	Bone marrow	n.d., decomposed	n.d.	✝	10	10.09.2020	28	27	26	29
28	LK Oder-Spree	Bone marrow	Adult	Female	✝	6	13.09.2020	26	25	24	27
29	LK Oder-Spree	Bone marrow	n.d., decomposed	n.d.	✝	10	09.09.2020	25	22	21	24

(Continues)

TABLE 1 (Continued)

ID	County	Matrix	Age class	Gender	Sample origin[a]	Estimated minimum PMI (days)[b]	Estimated latest date of death[c]	LLBB IDEXX ASFV	NRL ASF King PCR	NRL ASF virotype ASFV	NRL ASF Tignon PCR
30	LK Oder-Spree	Bone marrow	n.d.	Female	✚	30	22.08.2020	20	24	21	n.d.
31	LK Oder-Spree	Bone marrow	Piglet	n.d.	✚	14	07.09.2020	19	19	18	n.d.
32	LK Oder-Spree	Swab suspension	Adult	Male	✚	2	19.09.2020	25	27	23	n.d.

Abbreviation: n.d., not determined.

[a]Sample origin: ✚: carcass; ●: shot sick.

[b]Estimated minimum Postmortem Interval (PMI).

[c]Estimated latest date of death: calculated as: finding date minus minimum postmortem interval.

PMI estimates were based on the macroscopically visible stage of decay, the presence of insects and the microclimatic conditions at the sites. However, when decomposition had reached an advanced stage and only single skeletal remains were found, dating the PMI became difficult, in particular when carcasses had obviously been used by large vertebrate scavengers (red foxes and wolves are present in the area).

Based on the available data, the virus was probably introduced into Germany in early July 2020 or even earlier. Since most of the detected ASFV-infected wild boar died in September 2020 (Table 1), the situation is still evolving.

From all these animals, dry blood swabs or bones (carcasses) or serum (shot wild boar) were sent for laboratory diagnosis to the LLBB and for confirmation to the NRL at the FLI.

3.2 | Routine diagnosis and confirmation

The standard procedure in Germany for the diagnosis of ASF includes an initial testing in an accredited state laboratory in the affected federal state. These laboratories regularly participate in an inter-laboratory comparison test performed by the NRL (see also tasks of the NRL according to Council Directive 2002/60/EC). Samples with a positive or inconclusive result are sent to the NRL for confirmation. Only the diagnosis of the NRL confirms an outbreak or case. Suitable diagnostic tests are listed in the official method collection published by the FLI, taking into account the EU Diagnostic Manual (Commission Decision 2003/422/EC).

Despite the advanced state of decomposition of the first carcass and the resulting low sample quality, the LLBB detected moderate amounts of genome sequences of ASF virus. At the FLI, these results were confirmed with very similar cq-values, despite the use of different cyclers and conditions. By 24 September 2020, 32 cases were detected at the LLBB and confirmed by FLI. Sample matrices were 19 bone marrow specimens from carcasses of varying stages of decomposition, 12 blood swab suspensions, and one blood clot suspension and the corresponding serum. Cycle threshold values ranged from 18 to 36 in the OIE-recommended PCR by King et al. (2003) and were comparable between the regional and national laboratory. As expected, PCRs that amplified short genome fragments performed better on poor quality samples. A combination of different test systems, also for internal control, is therefore advisable and has proven to be effective in the present case. Details are shown in Table 1. Blood swab suspensions, which had been included into the official method collection following extensive validation studies under experimental and limited field conditions at the NRL (Carlson et al., 2018; Petrov et al., 2014), yielded reliable results also in this outbreak situation. The hemadsorbing virus causing this outbreak was isolated from the serum sample mentioned above.

Following the confirmation of the first case, the local veterinary authority had the remains of the carcass exhumed for further pathological examination at the LLBB. All samples taken from the carcass even muscle and fatty tissues were positive in qPCR (see Table S1). This fact underlines that any sample should be sent if the

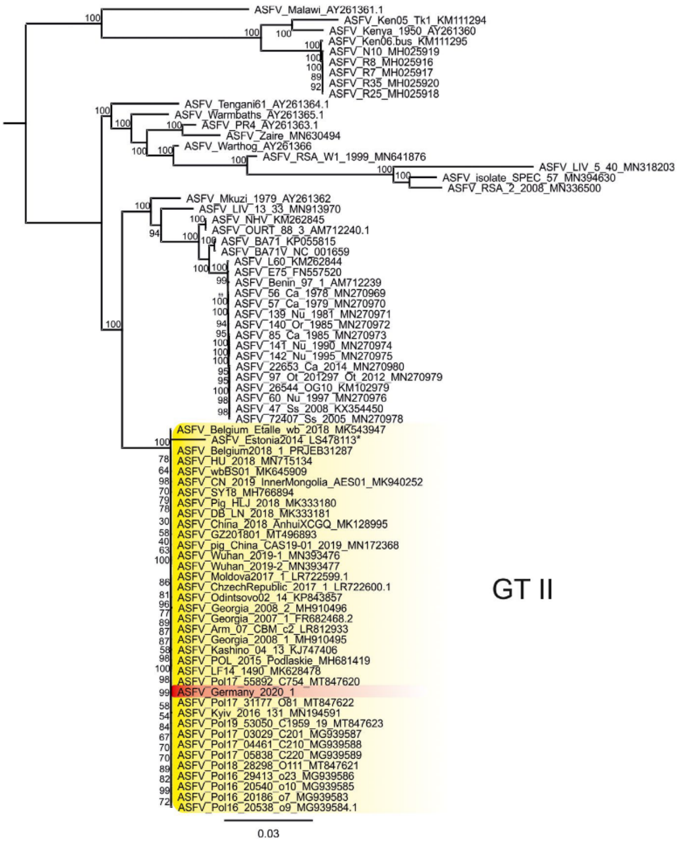

FIGURE 4 Phylogenetic reconstruction of ASFV whole-genome sequences. The phylogenetic tree was constructed from MAFFT v7.388 aligned whole-genome sequences kindly provided by N. Mazur-Panasiuk and G. Woźniakowski and downloaded from INSDC databases on 21 September 2020 using IQTREE v 1.6.5 with automatically selected models GTR + F+ R3 (general time reversible model with unequal rates and unequal base frequency + empirical base frequencies + FreeRate model with 3 categories). Statistical support of 10,000 ultrafast bootstraps using the ultrafast bootstrap approximation (UFBoot) (percentage values) is indicated at the nodes. Taxon names include, where available, ASFV designation and INSDC accession number. The scale bar represents number of substitutions per site. The ASFV P72 genotype II sequences (yellow) and the ASFV Germany 2020/1 sequence (red) are highlighted

recommended samples are not available. In this case, communication should be established with the receiving laboratory.

Positive samples were also obtained when diluting the samples 1:5. The latter would mimic the pooling options currently provided in the official method collection.

3.3 | Next-generation sequencing and phylogenetic analyses

Sequencing resulted in 18.865.783 single-end and 46.020.040 paired-end 300 bp reads, from which 1.228.953 reads (1.89%) mapped to the ASFV reference database and were assembled with a coverage of 2082. The ASFV Germany 2020/1 whole-genome sequence encompasses 190,592 bp with a G/C content of 38.4%. Phylogenetically, it groups with sequences from ASFV genotype II including all sequences from Eastern Europe, Asia and Belgium (Figure 4). The ASFV Germany 2020/1 sequence shows 99.9% nucleotide sequence identity to the whole-genome sequence of ASFV_Pol17_55892_ C754 (MT847620), which originated from an outbreak south of Warsaw (Piaseczno province, Mazowieckie voivodeship) in 2017 (Mazur-Panasiuk et al., 2020). Furthermore, some characteristic markers, for example a 14 bp insertion in the O174L gene, a single nucleotide variation in the K145R gene, the MGF 505-5R gene and the K205R gene as well as a one tandem repeat integration in the intergenic region between the genes I73R and I329L described in Poland (Mazur-Panasiuk et al., 2019, 2020) were also present in ASFV Germany 2020/1. These are in accordance with characteristics found in ASFV sequences from Southern Warsaw and Western Poland (Mazur-Panasiuk et al., 2019, 2020) and clearly distinguish ASFV Germany 2020/1 from all other ASFV GTII whole-genome sequences including those from the Czech Republic (LR722600.1), Belgium (LR536725.1), Moldova (LR722599.1) and Georgia (FR682468.2).

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ETHICAL APPROVAL

This work does not include any animal experiments. Only dead wild boar were sampled. Sampling of these animals is prescribed according to the German Swine Fever Ordinance (Schweinepest-Verordnung) in accordance with the German Animal Health Law (Tiergesundheitsgesetz). Ethical approval for sampling the carcasses was therefore not required.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. The sequence data generated in this study are available from INSDC databases under study accession number PRJEB40536.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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3.2.3.7 African swine fever: Why the situation in Germany is not comparable to that in the Czech Republic or Belgium

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Highlights

- All cases in the Czech Republic and in Belgium clustered in one single defined area, suggesting point-source introductions, whereas in Germany four distinct spatial clusters were observed
- Germany experienced several independent ASFV introductions
- The course of the disease was similar in all clusters but the overall situation differed between Germany and the other two countries
- Control measures cannot be copied from Belgium and the Czech Republic but have to be adapted according to the constant infection pressure being present in Germany

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African swine fever: Why the situation in Germany is not comparable to that in the Czech Republic or Belgium

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Abstract

After the first occurrence of African swine fever (ASF) in Germany in September 2020, control measures were implemented that resembled those taken in the Czech Republic and Belgium, the only two countries that succeeded in eliminating ASF from their territory so far in the current epidemic. In the present study, the epidemiological course of ASF in the first 6 months since introduction in these three countries is compared. Within 6 months, Germany experienced more cases than the Czech Republic and Belgium. The affected area in Germany, measured using minimal convex polygons, is much larger than the respective areas in the Czech Republic and in Belgium. All cases in the Czech Republic and in Belgium clustered in one single defined area, suggesting point-source introductions, whereas in Germany four distinct spatial clusters were observed, which indicates that multiple incursions had occurred along the border with Poland. While the overall course of the disease was comparable, when individual clusters were considered, the summarized data showed clear differences between the situation in Germany compared to that in the Czech Republic and Belgium. Germany experienced several independent introductions, caused by continuous infection pressure along the border to Poland, while the infection was only introduced on a single occasion each into the Czech Republic and Belgium. These differences may require appropriate adaptation of control measures, in particular concerning fencing along the border.

KEYWORDS
african swine fever, emerging, epidemiology, surveillance, wild boar

1 | INTRODUCTION

African swine fever (ASF) is a devastating disease of domestic pigs and wild boar with a tremendous socio-economic impact (Penrith et al., 2004). The occurrence of ASF in domestic pigs or wild boar in a country usually leads to substantial trade restrictions regarding pigs and porcine products.

The first case of ASF in a wild boar in Germany was confirmed on 10th September 2020 at a distance of approximately 6 km to the Polish border. Only 3 weeks later, an ASF-positive wild boar was found 60 km north of the first case, also close to the Polish-German border at a distance of less than 2 km. One month later (31 October 2020), an infected wild boar was detected approximately 60 km south of the first case less than 200 m away from the Polish-German border (Sauter-Louis et al., 2020). According to the estimated minimum post-mortem intervals (Probst et al., 2020), which were estimated based on the decomposition status of the wild boar carcasses at the time, when they were found, ASF virus (ASFV) was most likely introduced into Germany in the beginning of July 2020 at the latest. With the detection of the first cases, various control measures were applied with the aim of preventing further spread of the disease. Based on the successful elimination of ASF in the Czech Republic and in Belgium, the measures applied in these countries served as guideline for the German authorities. Thus, the first control measures focused on defining affected areas, intensifying the search for carcasses and fencing the areas at risks (Food & Agriculture Organization of the United Nations et al., 2019; Marcon et al., 2020).

In the Czech Republic, the first ASF cases in wild boar were detected in June 2017 and in Belgium in September 2018, respectively (Linden et al., 2019; Šatrán, 2019). Only wild boar were affected in both countries and no outbreaks in domestic pigs occurred. This has so far also been the case in Germany[1]. In addition, the first cases identified in the two countries emerged several hundred kilometres away from the closest reported ASF case, suggesting a human-mediated disease introduction over a long distance (Food & Agriculture Organization of the United Nations et al., 2019; Saegerman, 2018; Šatrán, 2019). Consequently, the control measures applied in these two countries were tailored to control and eliminate a focal introduction of ASF (point-source exposure). They were based on the establishment of various zones, which were defined around the respective index cases and regularly adapted, when new cases were detected outside the previously defined zones. The main aim was to minimize the risk of disease spread (Dellicour et al., 2020; Food & Agriculture Organization of the United Nations et al., 2019). Within 1 to 2 years, both countries had successfully eliminated ASF (World Organisation for Animal Health, 2020).

In Poland, where ASF has been circulating since 2014, only the eastern part of the country and the Warsaw region were affected until 2019. It has been hypothesized that the infection was originally introduced by infected wild boar that had crossed the border between Poland and Belarus (Frant et al., 2020; Pejsak et al., 2014, 2018; Smietanka et al., 2016). In November 2019, however, new ASF cases in wild boar were reported from Western Poland, about 270 km distant from the nearest ASF case in the country, suggesting a human-mediated virus spread (Mazur-Panasiuk et al., 2020). Since the detection of the first ASF-positive wild boar in Western Poland, surveillance measures resulted in the detection of 1683 ASF-positive wild boar in that area until mid-2020 (Mazur-Panasiuk et al., 2020). One of the cases was found only 10.4 km away from the Polish-German border (Sauter-Louis et al., 2020). According to Pejsak et al. (2018), the region bordering to Germany is the area with the highest wild boar density in Poland. It can be assumed that wild boar regularly cross the border between the two countries. It was, therefore, not surprising that the first German ASF case was detected only 6 km away from the German-Polish border (Sauter-Louis et al., 2020).

[1] First outbreaks of ASF in domestic pigs were detected in Brandenburg, Germany, on 15 July, 2021.

The successful ASF elimination strategies implemented in the Czech Republic and in Belgium were used as a template for designing control measures in Germany. However, in contrast to the Czech Republic and Belgium, where ASF was locally introduced in a single event in each country, Germany faces a constant infection pressure along the border with the affected region in Poland. Thus, the epidemiological situation in the three countries are different, resulting in the requirement to adapt measures to the actual situation in Germany and Western Poland to prevent further spread of ASF in the wild boar population.

To address these questions, we compared the course of the ASF epidemic in the first 6 months between the three countries.

2 | MATERIALS AND METHODS

ASF wild boar surveillance data from the Czech Republic, Belgium, and Western Poland were obtained from the European Animal Disease Information System (ADIS) or the former Animal Disease Notifications System. German ASF surveillance data were taken from the National Animal Disease Notification System (Tierseuchennachrichtensystem, TSN). These data are also used to inform ADIS. The extracted records contained information on the date and the location (coordinates) of ASF-infected wild boar (regardless of the diagnostic method) in each of the countries for the first 180 days after introduction, except for Western Poland, where data were extracted from the first occurrence in November 2019 to September 2020 (300 days).

The distances between the first occurrence in Germany to the cases of ASF in wild boar in Western Poland were calculated using the R (R Core Team, 2017) package sp (Pebesma et al., 2005) as previously described (Sauter-Louis et al., 2020). A moving average distance was calculated using a window size of 14 days, that is, a series of all cases of ASF occurring in Western Poland in the previous 14 days was created. The mean and the standard deviation of this average distance were calculated from day 50 to 200.

The cumulative numbers of cases detected in the first 6 months of the epidemics in the Czech Republic, Belgium, and Germany were separately calculated for each country. For data display, the date of the first occurrence was set as day 0 in each country.

Clusters in the geographical distribution of the cases were derived using a hierarchical clustering approach that defines clusters that occurred within a specified distance. The method of agglomerative clustering (agglomerative nesting, AGNES) has been used, employing complete linkage clustering, whereby pairwise distances between the elements in one cluster and elements in the other clusters are computed (Kassambara, 2017).

The size of the affected area was estimated using the minimum convex polygon (MCP) of cases of ASF. The MCP technique, often used in home-range analyses (Boyle et al., 2009; Kumbhojkar et al., 2020), is a nonparametric method using the locations of all observed positions by creating the smallest convex polygon (Anderson, 1982). The MCPs were calculated per day, including all cases that had occurred until that day. For the cases in each of the identified clusters, the MCPs were calculated and the sums of these MCPs calculated for each country. To

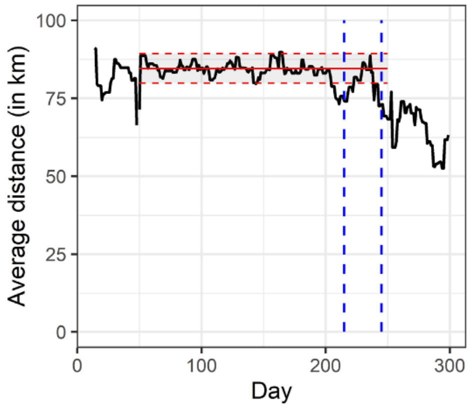

FIGURE 1 Average distance between the locations of the first ASF case in Germany and ASF cases in wild boar in Western Poland (using a moving window of 14 days prior to the day calculated). The red line represents the mean of the average distance between day 50 and 200 with the 95% confidence interval containing two standard deviations of this average distance (dashed red lines). The blue dashed lines represent the time of the presumed first introduction of ASF into Germany (mid-June to mid-July 2020)

compare the numbers of ASF-positive wild boar per kilometre square affected area, the cumulative sum of the ASF-positive wild boar cases was divided by the cumulative area of the MCPs. Data were stored and managed in Excel (Microsoft, version 2019). Calculations were performed and figures prepared using R Version 4.0.3 (R Core Team, 2017). Clusters were identified using the function hclust in R, after displaying the locations using the packages sp and rgdal (Keitt et al., 2010; Pebesma et al., 2005). Convex polygons were calculated using the R package geosphere (Hijmans et al., 2019).

The R package ggplot2 was used to prepare figures (Wickham, 2016) and a map produced with ArcMap (version 10.5.1., ESRI).

3 | RESULTS

The ASF case closest to the first ASF-infected wild boar detected in the Czech Republic in June 2017 was observed in a wild boar in the Ukraine at a distance of more than 300 km. The equivalent distance between the first ASF case in Belgium in September 2018 was more than 800 km from the nearest previous ASF cases in the Czech Republic. In Germany, the first ASF case in a wild boar was detected only 30 km away from the closest known case in Western Poland (detected in February 2020). The average distance of all cases reported from Western Poland before September 2020 ranged between 75 and 90 km from the location of the first ASF case in Germany (Figure 1). Especially from early-June 2020 onwards, the average distance decreased and reached 52 km shortly before the detection of the first case in Germany. During the presumed time of introduction, the average distance was partially below the lower 95 % confidence limit of the distance observed between day 0 and 200.

All cases in the Czech Republic and in Belgium clustered in a single defined area within the country. In Germany, four distinct spatial clusters of ASF cases were observed (Figure 2). The first case in cluster 1 was confirmed on 10th September 2020. By the end of September 2020, another area, approximately 60 km north of the first case, but also close to the border with Poland, was affected. In early March 2021, a case in the region between cluster 1 and 2 was detected, which was associated with cluster 2 in the hierarchical cluster analysis. This case was also located close to the border with Poland. In cluster 3, the first ASF-positive carcass was detected by the end of October 2020. At the same time, a first case was identified in cluster 4 in Saxony, approximately 60 km south of cluster 1 (Figure 2).

In the beginning, i. e. until day 50, the number of cumulative cases in cluster 1 increased in a similar way as the numbers in the Czech Republic and Belgium (Figure 3A). In contrast, the other German cluster areas showed slower increases. The numbers of the Czech cluster showed a flattening curve from day 50 onwards, while this is not the case for Belgium during the first 180 days. The cumulative number of cases in cluster area 4 (Saxony) remained low until day 90, when it started to rise.

The Czech Republic had the smallest affected area of only 46 kmš in the MCP at the end of the study period (Figure 3B). The sizes of the MCPs of the four individual cluster areas in Germany varied between the areas found in the Czech Republic and Belgium with similar increases over time. Cluster areas 1 and 2 showed a flattening in the curve from day 50 to day 110. For Belgium, such a flattening of the curve could not be observed during the study period.

When calculating the numbers of ASF-positive wild boar per affected area (Figure 3C), a wide range of values was obtained between the different areas. While the Czech Republic had the highest numbers with 4 to 6 ASF-positive wild boar per kilometre square affected area, the four cluster areas in Germany varied between 0.5 and 5 ASF-positive wild boar per kilometre square affected area. Both, cluster areas 2 and 4, showed a marked decrease in their curves, which were due to the increase in area, indicating that new cases had occurred outside the previously observed areas.

When the ASF case numbers in all four German cluster areas were accumulated and compared to those in the Czech Republic and Belgium, the numbers of the cumulative cases in the Czech Republic and Germany were very similar until day 45, while Belgium had a lower increase in the beginning (Figure 4A). However, from day 45 onwards, the numbers in Germany rose more rapidly than the numbers in the Czech Republic and Belgium.

When the sizes of the affected areas in each country were compared using the sums of the four individual cluster areas for Germany, the Czech Republic had the smallest area (46 kmš) affected at the end of the study period, while it was 463 kmš in Belgium and 628 kmš in Germany. Until day 60, the affected area in Belgium increased faster than that in Czech Republic and the summarized cluster areas in Germany (Figure 4B). Between days 60 and 133, the affected areas in Germany and Belgium were similar. On day 133, a new introduction

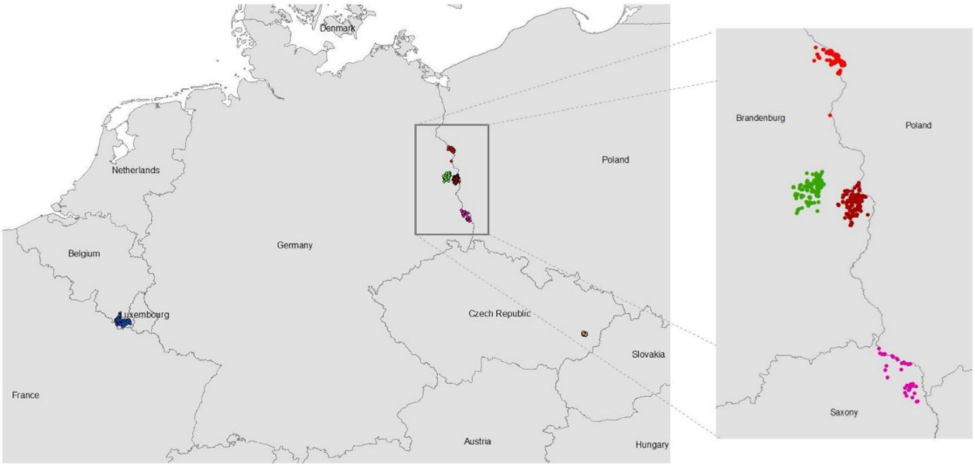

FIGURE 2 Spatial clusters of ASF cases in wild boar observed in the Czech Republic, Belgium and Germany for the first 6 months of the ASF epidemic in each country. Inset: distinct clusters in Germany (dark red: Cluster 1; red: Cluster 2; green: Cluster 3, all in Brandenburg; purple: Cluster 4 in Saxony)

into Germany was observed (cluster 4), and new cases occurred approximately 20 km westwards of the location of the first introduction (cluster 3), leading to an increase in the size of the affected cluster area. In Belgium, a similar increase was seen on day 156 (Figure 4B).

Differences in the numbers of ASF-positive wild boar per affected area between the three countries were also noted (Figure 4C). The respective number for Germany was in between the ones observed for the Czech Republic (4-6 ASF-positive wild boar per kmš) and Belgium (approx. 1.5 ASF positive animals per kmš) (Figure 4C).

4 | DISCUSSION

The aim of the present study was to compare the first 6 months of the ASF epidemic in wild boar in Germany with the equivalent period of ASF occurrence in the Czech Republic and Belgium. Due to the absence of any outbreaks in domestic pig holdings in all three countries, the applied control measures focused on minimizing spread of the infection between wild boar, and preventing disease transmission into domestic pig holdings. The rigorous measures implemented in the Czech Republic and in Belgium, such as enhanced passive surveillance, erecting fences to limit the movements of wild boar and drastic reduction of the wild boar population in the affected area, led to a successful elimination of the infection in both countries (Food & Agriculture Organization of the United Nations et al., 2019; World Organisation for Animal Health, 2020). This success, which in the current ASF epidemic that started in Georgia in 2007 (Rowlands et al., 2008), has so far been limited to these two countries. Thus, it seems reasonable that the effective control strategy applied in the two countries could serve as a blueprint for other ASF-affected countries including Germany. A direct comparison of the course of ASF between the three countries may help to evaluate the situation and the control measures.

Similarities in the ASF epidemics between the three studied countries were indeed observed. In Germany and Belgium, the first cases were detected in September (Linden et al., 2019; Sauter-Louis et al., 2020). This may have been by coincidence, but is seems also possible that the detection of the cases was facilitated by the harvest of agricultural fields, which usually occurs just before September in these regions. The harvested fields make it easier to detect wild boar carcasses, which may otherwise remain undetectable. This hypothesis is supported by the decomposition stage of the first carcasses found in both countries, which suggested that the disease had been introduced a few months ago (Federal Agency for the Safety of the Food Chain Belgium, 2018; Sauter-Louis et al., 2020). In contrast, in the Czech Republic, the first case was detected very shortly after the ASF-infected wild boar had died and there was no indication that the disease had been present in the area for months (Šatrán, 2019). Interestingly, the presumed introduction into Germany was around the same time of the year (June/July) (Sauter-Louis et al., 2020), when the first case was observed in the Czech Republic.

Evaluation and comparison of the areas affected may be performed by comparing the restriction zones defined by the European Commission (Commission Implementing Regulation 2021/605, formerly Commission Implementing Decision 2014/709/EU). However, several factors, such as the size, the territorial and geographical continuity with adjacent territories, the typology of biotope, the administrative divisions and the surveillance in place must be taken into consideration, when restriction zones are established. Thus, the sizes of the restriction zones do not allow an objective comparison between countries. We, therefore, used the MCP method to estimate the affected area. An

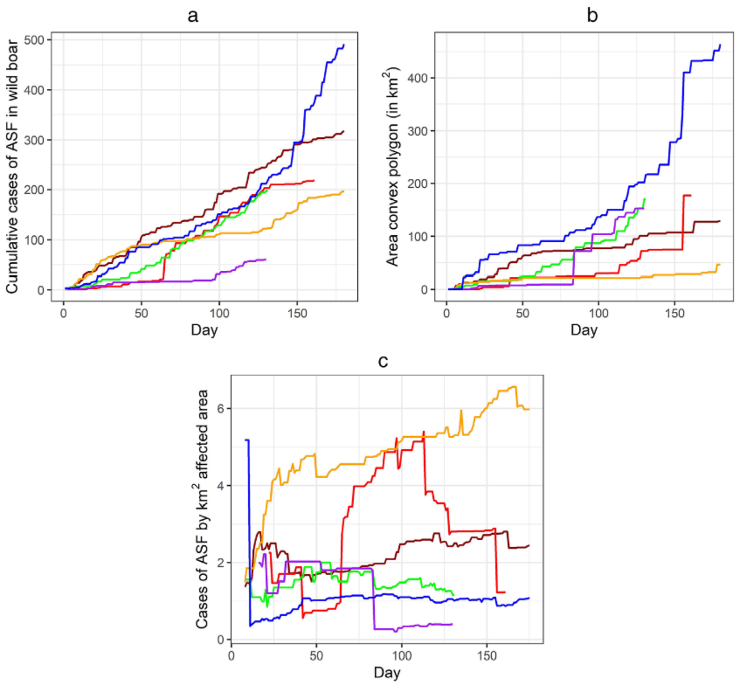

FIGURE 3 (a) Cumulative ASF cases in wild boar in the four cluster areas in Germany and in the Czech Republic and Belgium over the first 6 months after the first detection of ASF. (b) Cumulative area (minimum convex polygon) of ASF cases in wild boar for the four cluster areas in Germany and in the Czech Republic and Belgium over the first 6 months after the first detection of ASF. (c) Numbers of cases of ASF in wild boar by square kilometre affected area, as calculated in a minimum convex polygon for the four cluster areas in Germany and in the Czech Republic and Belgium over the first 6 months after the first detection of ASF (Orange: Czech Republic; blue: Belgium; dark red: cluster area 1, red: cluster area 2, green: cluster area 3, purple: cluster area 4 in Germany)

MCP describes only the area that encloses the detected cases. However, the area calculated in this way may not completely cover the truly affected area, as undetected ASF-positive wild boar might have been present outside the defined area.

In Germany, four different clusters were observed. Three weeks after the first introduction, further ASF cases emerged approximately 60 km north of the first cluster, which were also located close to the Polish border. Usually, the home range of wild boar does not exceed more than 20 km (Food & Agriculture Organization of the United Nations et al., 2019; Podgorski et al., 2013). It therefore seems unlikely that these new cases were directly related to the first cluster. This hypothesis is supported by the findings of the intensive carcass searches around the first case, which led to the detection of further ASF cases within a 6 km radius. No ASF-positive wild boar was found outside a radius of 12 km around the first case. In addition, the area between the two clusters has been monitored closely, and only in March 2021, that is, 6 months after the first detection of ASF, the first ASFV-positive carcasses were detected there. Likewise, cluster 4 emerged 60 km south of the first cluster. Despite intensive regular carcass search, no cases were found between the two clusters before March 2021. Consequently, a separate virus introduction may also be assumed in this case. Thus, there have been at least three separate introductions into Germany along the border with Poland, probably caused through wild boar migrating from Poland across the border. Despite intensive but negative carcass search immediately west of cluster 1, the emergence of cluster 3 might have been caused by migrating wild boar from cluster 1.

When comparing the cumulative numbers of infected animals, the cumulative affected area and the numbers of ASF-positive animals per kilometre square affected area for the four clusters in Germany with those in the Czech Republic and Belgium, similarities are noticeable. Our results suggest that the course of ASF in each individual German cluster area resembles those in the Czech Republic and in Belgium. This is not surprising, as the epidemics were caused by the same virus, affected the same species, that is, wild boar, and occurred under similar environmental conditions. However, when summarizing the data of the different cluster areas and comparing the cumulated area to those of the Czech Republic and Belgium, the results diverge, suggesting

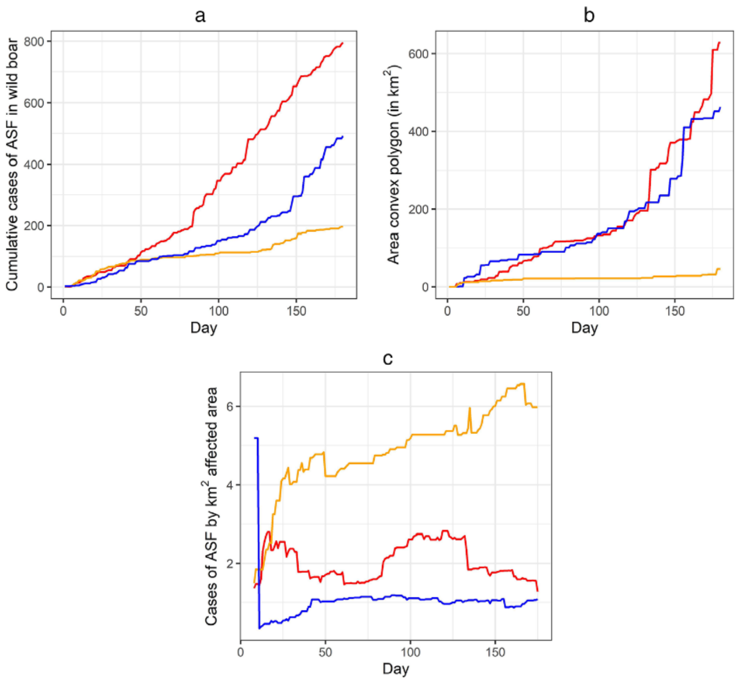

FIGURE 4 (a) Cumulative ASF cases in wild boar in Germany (red), the Czech Republic (orange) and Belgium (blue) over the first 6 months after the first detection of ASF. (b) Cumulative area (minimum convex polygon) of ASF cases in wild boar in Germany (red), the Czech Republic (orange) and Belgium (blue) over the first 6 months after the first detection of ASF. (c) Numbers of cases of ASF in wild boar by square kilometre affected area, as calculated in a minimum convex polygon, in Germany (red), the Czech Republic (orange) and Belgium (blue) over the first 6 months after the first detection of ASF.

differences. Despite all influencing factors, such as population density, search effort, landscape and other environmental factors like settlement structure and road network, the main and probably most influencing difference is the way of ASFV introduction. In the Czech Republic and Belgium, the virus had 'jumped' over several hundred kilometres. These introductions were most likely caused by human activities leading to a point-source exposure of wild boar at the site of introduction (Food & Agriculture Organization of the United Nations et al., 2019; Saegerman, 2018; Šatrán, 2019). Thus, the disease was only introduced at one single point in time and space, so that disease control measures could be implemented that focused on one defined area. The comparable course of the disease in each of the individual clusters demonstrates that a successful diseases elimination implementing the current control measures could also be possible, if the ASFV had been introduced only once at one point. In Germany, however, this was unfortunately not the case. Germany borders Poland over almost 400 km and a substantial proportion of this borderline extends over a region, where ASF occurs in Poland. In the western part of Poland, ASF has been present in the wild boar population since almost 2 years (Mazur-Panasiuk et al., 2020) and it does not seem to subside. Accordingly, the disease has not only been introduced at one point, but in several locations along the border with Germany and on several occasions.

The constant infection pressure, Germany is exposed to, is comparable to the one that resulted in the introduction of ASF into the Baltic States and Poland in 2014 and in the following years. These states border to ASF-affected countries, mostly in the East (European Food Safety Authority, 2015; Nurmoja et al., 2017; Oļševskis et al., 2016; Pejsak et al., 2014; Smietanka et al., 2016). However, at the time, when ASF reached these countries, knowledge about ASF in wild boar and possible control measures was scarce. Control measures, therefore, focused on hunting and the removal of carcasses, but did not include fencing measures to limit the movement of wild boar. The infection pressure along the southern part of the border between Poland and Germany is likely to persist for the foreseeable future. In addition to migrating wild boar, also travelling tourists or workers, who cross the German-Polish border regularly, might carry infectious material with them, for example, contaminated food. Consequently, control measures have to focus on the border area, while prevention and keeping disease awareness at a high level must be maintained in the entire country. Already in the beginning of 2020, only 2 months after the first cases had occurred in Western Poland, mobile fences were erected along the border on German territory. These fences had to be maintained, replaced by permanent fences and extended after the first ASF cases had emerged in Germany. It is obvious that controlling a disease in several locations needs approaches that differ, at least in part, from those required for controlling the disease in a single area. In the Czech Republic, the detected ASF cases were so far away from the border to Slovakia, that all control measures and zonings could be exclusively performed in the own country according to EU and national legislation. In Belgium, several cases were close to the border with France and Luxembourg. France established protection zones and thereby supported the control of ASF in Belgium. Regarding the situation in the border area between Poland and Germany, a joint effort of both countries is necessary to control and eventually eliminate the ASF epidemic in wild boar. If the infection pressure along the border continues, new introductions are likely and there is an increased risk of ASF spread in western direction.

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ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as no animal experiments were conducted.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

AUTHOR CONTRIBUTIONS

C.S.L. designed the study, performed the analysis and drafted the manuscript. K.S. supported the design of the study and drafted the manuscript. M.R. designed the study, provided data and reviewed the manuscript. C.S. supported the analysis and reviewed the manuscript. T.C.M. supported the design and carefully reviewed the manuscript. F.J.C. designed the study, provided epidemiological advice and carefully reviewed the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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4 Participatory epidemiology

4.1 Scientific background

The term participatory epidemiology (PE) was first used in 1993 (Catley, 2020). However, participatory approaches had already before been integrated into applied veterinary epidemiology, for example within the framework of rinderpest eradication (Mariner et al., 2012). The methods originate from social sciences, where the knowledge about the importance of communication and mutual understanding is deeply rooted. To understand PE and its concept, the term participation has to be explained. Arnstein (1969) developed a ladder of citizen participation, which illustrates that participation has different levels ranking from non-participation to citizen control. She emphasised the importance of considering these differences in the gradations when using the term (Arnstein, 1969). Similarly, Wright (2021) described the different levels of participation, whereby also only three levels are regarded as true participation. Considering the described model, co-determination, partial decision-making power and decision-making power can be understood as true participation. Due to legal regulations and practical feasibility, participation of affected communities and people in the field of animal disease control is often only possible to a limited extent. Catley et al. (2012) defined PE in animal health systems as “the systematic use of participatory approaches and methods to improve understanding of diseases and options for animal disease control.”

The use of participatory approaches in veterinary medicine mainly evolved from the need in developing countries to overcome cultural barriers and logistic limitations (Jost et al., 2007, Alders et al., 2020). It became evident that to monitor and control livestock diseases successfully and to evaluate the effectiveness of the adopted measures, local communities, their perceptions and particularly their priorities had to be included in the design and the implementation of any measures. Particularly in multicultural settings, it became clear that scientists needed to engage more closely with the local culture to develop an understanding and to formulate research questions relevant to the potentially affected communities (Catley et al., 2012). The awareness grew that not only the locals can learn from the scientists, but the scientist themselves can learn from the locals (Jost et al., 2007). Thus, in contrast to conventional “top-down” approaches, where the experts tell people, how to implement a promising strategy, more and more “bottom-up” and demand-driven approaches are being employed (Alders et al., 2020).

The application of participatory methods usually includes the use of different tools, whereby communication plays a crucial role. In conventional interviews, close questions usually prevail and the room for the respondent to express his or her own opinion or to formulate wishes and concerns are limited. In contrast, semi-structured interviews and focus group discussions (FGD) are only moderated by the researchers and commonly, they only follow a rough checklist to keep the thread going, while avoiding to influence the direction of the discussion too much

(Mariner and Roeder, 2003). Thus, the implementation of these methods give participants the opportunity to express themselves freely and to share thoughts that may bring to light important discussion points that were not considered beforehand by the researcher. Further tools can be used to visualize, rank or score certain aspects (Jost et al., 2007, Catley et al., 2012). Methods like mapping local conditions or illustrating relationships or information flows with the help of diagrams are easy to understand and provide very good insights into the views of those affected. The use of ranking and scoring tools has the advantage that different aspects, such as disease symptoms, control measures, etc., can be weighted against each other with regard to the factor of interest, e. g. importance or acceptance (Bedelian et al., 2007, Molla and Delil, 2015). Furthermore, the use of these methods usually yields quantitative data in addition to qualitative ones, thus allowing comparisons (Jost et al., 2007, Shiferaw et al., 2010).

In their comprehensive review, Allepuz et al. (2017) confirmed that until 2015, the great majority of PE studies had been applied in Africa and Asia. The main topics, which were approached by PE, were needs assessments, prioritization and animal disease surveillance and control. For a long time, the scientific community took a rather critical position towards qualitative research and qualitative results. Their quality, reliability and generalizability were doubted (Mays and Pope, 2000), which may at least in part explain the main use of PE in developing countries. In the meantime, however, scientists increasingly realize that the use of participatory methods can also be beneficial in industrialized countries (Wright, 2021). The increasing complexity of animal disease surveillance and control due to globalization, the growing awareness of potential zoonotic epidemics and the raising health threat due to antimicrobial resistance illustrates the urgent need of the establishment of approaches, which include stakeholders and the affected communities.

Probably the most recent and prominent example of the urgent need to involve affected groups of people and to improve transdisciplinary communication is the current COVID pandemic. Particularly with regard to the acceptability of the vaccination, communication with the different groups of people is inevitable and it is quite possible that the involvement of the community including PE can increase the acceptance of vaccination (Burgess et al., 2021). Roche et al. (2020) showed that the inclusion of PE in disease management on dairy farms can be economically worthwhile. In a European participatory study with the aim to identify options to improve dairy herd health, Sjostrom et al. (2019) experienced a very positive feedback by the participating farmers and veterinarians. They appreciated to be perceived as an equal part of the discussion in contrast to previous experiences of top-down communication (Sjostrom et al., 2019). Similarly, Schulz et al. (2016) reported a high degree of acceptability of participatory methods among hunters regarding the surveillance of CSF in wild boar. This positive feedback of participants and involved key figures demonstrates the high potential of PE to engage affected stakeholders and thus to increase the long-term effectivity of animal disease surveillance and control. The mere fact that people realize that scientists and decision makers listen to them and take them serious has the potential to increase the willingness of communities and individuals to cooperate and support disease control measures.

4.2 Contributions

4.2.1 General considerations

Participatory epidemiology includes a wide range of different methods. When facing complex and difficult situation such as the current ASF epidemic in wild boar, an interdisciplinary approach is inevitable. The participatory "World Café" method can be used to gather expert opinions or perceptions of affected people. However, currently, it is mainly used in public health settings (Stockigt et al., 2013, MacFarlane et al., 2017, Coetzee et al., 2020). Collecting expert opinions regarding ASF control measures, a participatory workshop was successfully conducted and the "World Café" method was applied.

- Contribution **(Publication 16)**: JORI, F., CHENAIS, E., BOINAS, F., BUSAUSKAS, P., DHOLLLANDER, S., FLEISCHMANN, L., OLSEVSKIS, E., RIJKS, J. M., SCHULZ, K., THULKE, H. H., VILTROP, A. & STAHL, K. 2020. Application of the World Café method to discuss the efficiency of African swine fever control strategies in European wild boar (Sus scrofa) populations. Preventive Veterinary Medicine, 105178. https://doi.org/10.1016/j.prevetmed.2020.105178

The increasing appreciation of the potential of PE in veterinary epidemiology is promising. However, this increased use of the methodology also carries the risk of professionally and scientifically inadequate implementations. In addition to the general ability of the moderator to communicate in a comprehensible and attentive manner, which is unfortunately all too often taken for granted (Westberg et al., 2010, Berglund et al., 2013), other aspects must be taken into account in the planning and successful implementation of PE studies.

- Contribution **(Publication 17)**: FISCHER, K., SCHULZ, K. & CHENAIS, E. 2020. "Can we agree on that"? Plurality, power and language in participatory research. Preventive Veterinary Medicine, 180, 104991. https://doi.org/10.1016/j.prevetmed.2020.104991

4.2.1.1 Using the World Café model to evaluate methods for controlling African swine fever in European wild boar (*Sus scrofa*) populations.

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Highlights

- Using the participatory World Café method successfully supported the interactive identification of different expert opinions regarding the control of ASF
- Regular awareness campaigns and motivation incentives are necessary to maintain the involvement of stakeholders
- The implementation of fences is controversially disputed
- The success of passive surveillance highly depends on the willingness of involved stakeholders to support this measure

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Application of the World Café method to discuss the efficiency of African swine fever control strategies in European wild boar (*Sus scrofa*) populations

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ABSTRACT

In the current epidemic of African swine fever (ASF) in Europe, the maintenance and spread of the disease among wild boar populations remains the most important epidemiological challenge. Affected and at-risk countries have addressed this situation using a diversity of wild boar management methods with varying levels of success. The methods applied range from conventional animal disease intervention measures (zoning, stakeholder awareness campaigns, increased surveillance and biosecurity measures) to measures aimed at reducing wild boar population movements (fencing and baiting/feeding) or population numbers (intensive hunting). To assess the perceived efficiency and acceptance of such measures in the context of a focal introduction of ASF, the authors organised a participatory workshop inviting experts from the fields of wildlife management, wild boar ecology, sociology, epidemiology and animal disease management to discuss the advantages and disadvantages of various control approaches. The discussions between professionals from different countries took place using the World Café method. This paper documents the World Café method as a tool for increasing the level of participation in multi-stakeholder group discussions, and describes the outputs of the workshop pertaining to the control measures. In summary, the World Café method was perceived as an efficient tool for quickly grasping comprehensive perspectives from the professionals involved in managing ASF and wild boar populations, while promoting engagement in multi-disciplinary discussions. The exercise achieved a good overview of the perceived efficiency and applicability of the different control methods and generated useful recommendations for ASF control in wild boar populations in Europe.

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1. Introduction

African swine fever (ASF) of genotype II has been present in the European Union (EU) since 2014[2]. During these six years collective knowledge about ASF epidemiology and control in the European context has increased and many publications and reports have been produced (Chenais et al., 2019; Schulz et al., 2019; Dixon et al., 2020). The haemorrhagic viral disease affects domestic pigs and European wild boar (*Sus scrofa*), with clinical presentations varying from peracute to chronic disease and a case fatality rate of up to 100 % (Costard et al., 2009). If protected by organic material, the virus is very resistant and can remain infective for several months (Mebus et al., 1997). Upon direct contact with blood from infected pigs, the infectivity is fairly high (Gulenkin et al., 2011), but between farms, or independent social groups formed by wild boar (sounders), the rate of transmission is quite low (Oļševskis et al., 2016; Chenais et al., 2019; Schulz et al., 2019). These epidemiological characteristics are important when considering control of the disease in wild boar populations. In the current epidemic the virus is maintained within wild boar populations independently of domestic pigs (Chenais et al., 2018; EFSA Panel on Animal Health and Welfare (AHAW) et al., 2018a). This infectious cycle involving wild boar populations, wild boar carcasses and the habitat is known as the wild boar-habitat epidemiological cycle (Chenais et al., 2018). Infected wild boar carcasses, anthropogenic spread and hunting waste left in forests are recognised as the main drivers of transmission within and between wild boar populations (Morelle et al., 2019). Largely based on experience from the ASF-incursion in the Czech Republic, a recent scientific report recommends that following a focal introduction of ASF in wild boar, different areas should be created in which specific management measures aiming at control or eradication of ASF in wild boar populations can be applied EFSA Panel on Animal Health and Welfare (AHAW) et al., 2018b). See Box 1 for a detailed description of these areas.

Current experience has shown that management of ASF in wild boar populations requires cooperation between specialists from a variety of disciplines. Indeed, the competences of veterinarians, epidemiologists, modellers, ecologists, hunters, wildlife specialists, as well as social and communication scientists are needed to improve ASF management, assess efficiency and acceptance of available control measures, and to generate innovative solutions. Discussion tools explicitly designed to foster interdisciplinarity and inclusive participation required to achieve such cooperation are currently used in various contexts. The World Café method is one such generic tool, methodologically included among participatory action research (Aldred, 2011; Steier et al., 2016) and specifically designed to facilitate multidisciplinary dialogue between participants with different scientific background, professional experience and geographical or cultural origins (MacFarlane et al., 2017).

With this in mind, the objectives of this study were: i) to document the use of the World Café method as a tool for increasing participation in multi-stakeholder discussions in the context of an animal health crisis, and ii) to gather knowledge concerning the control of ASF in wild boar populations and assess the efficiency and applicability of different control methods.

2. Material and methods

2.1. The World Café method

The World Café method is a flexible approach for facilitating group discussions that can be used to engage stakeholders and encourage participation while discussing a specific topic (Biondo et al., 2019). Initially conceived as a process for learning, planning and leading in the intersection between policy and practice, it has by now been used for many other applications, including research prioritisation (MacFarlane et al., 2017), activity planning and elicitation of community group perspectives (Biondo et al., 2019), as well as in plant and public health programme evaluation and planning (MacLeod et al., 2016; MacFarlane et al., 2017; Silva and Guenther, 2018). It is considered a powerful conversational process that helps communities and other groups of people engage in constructive dialogues, build personal relationships and foster collaborative learning (Tan and Brown, 2005). A World Café discussion covers several topics (each with an assigned host or facilitator), that are discussed by small groups of participants rotating between the topics. Progress is strived for at each rotation, supported by the facilitator giving a short introduction at the start of each rotation to inform the new group about the input of the previous groups (Carson, 2011). In the original World Café format, special effort is made to encourage informal discussions, making participants feel at ease, and create a relaxed discussion environment resembling a café (Carson, 2011). In the present study, we applied the World Café method in the context of a workshop about the assessment of ASF control strategies in wild boar populations within EU countries. To our knowledge, this is the first publication on the use of the World Café method in the context of an international animal health crisis. In this manuscript we describe the application of an adopted version of the World Café method, summarise and asses the quality of the emerging results, and place the method in the framework of participatory action research from which the veterinary version of participatory epidemiology stem.

2.2. Study design and participant selection

The study was conducted in March 2019 at a two-day workshop of the ASF-STOP COST action (www.asf-stop.com), addressing control of ASF in wild boar populations in infected and at-risk countries. The first day of the workshop comprised scientific presentations to provide some background on different management methods applicable for the control of ASF in wild boar populations. The second day of the workshop consisted of the participatory exercise described here. Participants attended both days of the workshop, and were purposively selected by the study steering group (first, second and last authors) in order to obtain maximum diversity in terms of the disciplines and geographic origins represented. All participant costs related to the workshop were covered by the project. The group of participants encompassed 36 experts, including veterinarians, wild boar managers, hunters, epidemiologists, mathematical modellers and social scientists representing governments, national and international organisations, the hunting lobby and private industry. The proportion of the different disciplines is represented in Fig. 1.

On the second day of the workshop, the World Café method was used to discuss six methods to improve control of ASF in wild boar populations: a) stakeholder engagement and public awareness, b) fencing, c) passive surveillance, d) manipulation of the carrying capacity of wild boar habitats, e) hunting management, and f) other population control methods (e.g. poisoning, fertility control). These methods were identified by the study steering group based on previous discussions and meetings within the framework of the ASF-STOP COST action, EFSA reports and scientific opinions, as well as field experience from the current epidemic. Some aspects of these topics were explored during the presentations given on the previous day. In addition, the terms and concepts needed for the discussions were defined at the start of each World Café rotation (see Table 1). The participants were asked to assess the feasibility, efficacy and constraints of each method in the context of a focal introduction. For each discussion topic (related to a specific ASF control method), a facilitator was selected by the steering group. The selection was based on area of expertise, interest in the World Café method or participatory approaches, and perceived facilitation skills. The request to act as facilitator was done a few days prior to the workshop and none of the facilitators were involved in the selection of participants. The steering group remained as overall observers in order

[2] https://ec.europa.eu/food/animals/animal-diseases/not-system_en.

Box 1
Areas to be established following a focal introduction of ASF in wild boar, according to the EFSA Panel on Animal Health and Welfare (2018b).

The core area refers to the smallest circle around all detected ASF-positive carcasses. In this area, public access and hunting activities should be restricted to keep the wild boar populations undisturbed. The buffer area surrounds the core area and should be several times larger than the size of wild boar home range (which depends on the local landscape and habitat conditions). As with the core area, wild boar populations in this area should initially be kept undisturbed. After a certain waiting time, the core and buffer areas are to be depopulated. In an intensive hunting area surrounding the buffer area, the aim is immediate preventive reduction of population numbers in order to provide unfavourable conditions for ASF spread in the event that infected wild boar leave the core or buffer areas. In the intensive hunting area, hunting activities need to be carried out at a high biosecurity level and hunting procedures that minimise the disturbance of wild boar should be used. In all areas, passive surveillance activities should be carried out, including the active search and removal of carcasses.

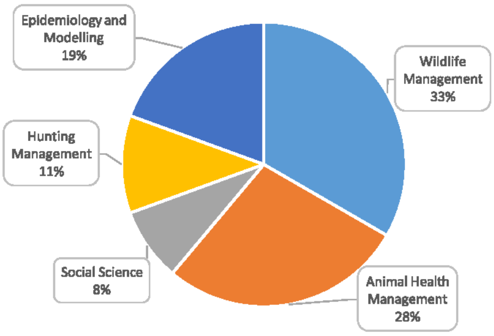

Fig. 1. Proportions of the different disciplines represented among the World Café participants (n 36).

Table 1
Definitions of terms used during the World Café discussions.

Term	Definition
Stakeholder communication	Communication between stakeholders directly involved in control measures, such as authorities and hunters, but also transparent communication of information to the general public
Passive surveillance	Observer-initiated provision of animal health-related data (e.g. voluntary notification of suspected disease) or use of existing data for surveillance[1]
Carrying capacity	Carrying capacity, the average population density or population size of a species below which its numbers tend to increase and above which its numbers tend to decrease because of shortages of particular food, shelter, and social requirements[2].
Poisoning	Oral administration (through baits) of a toxic substance that causes quick death without suffering

[1] RISK SUR https://www.fp7-risksur.eu/terminology/glossary#group-P.
[2] Encyclopaedia Britannica https://www.britannica.com/science/carrying-capacity.

to document and supervise the exercise and did not facilitate nor contribute to the group discussions. Participants were divided into six groups of five to six persons each, making sure that groups were as diverse as possible regarding the professional background of the participants. The topics were discussed around tables, with one table dedicated to each topic. The facilitator stayed at the table, while the groups rotated. Each table was equipped with flipcharts, pens and other materials for taking notes. Each group spent 20–30 min at each table before rotating. The facilitators collected the information and gave a summary of the previous discussions to each new group arriving, making sure that the discussions progressed with each group. When all groups had visited all tables, the facilitators were given 30 min to summarise and organise the notes before presenting the results to all the participants.

After the workshop, the facilitators summarised the discussions in a written report (available on the project's website) and evaluated the discussions concerning their respective topic for dissensus, consensus and saturation. The reports from the facilitators were further shortened and adopted to fit the publication format. What is presented in the result section regarding "Methods for improving control of ASF in wild boar populations" is thus the authors' summary of the facilitators' summaries of the discussions. Dissensus was considered to be present if the topic seemed to provoke debate and heated discussions, or if strong disagreements or opposing positions were detected within or between groups. Consensus was considered to be present when there was a general feeling of agreement and similarity in opinions within and between groups. Saturation was assessed based on whether new concepts or ideas were still emerging during the last rotation. The World Café method was evaluated as a tool for stimulating participation in multi-stakeholder discussions based on i) direct observation of the process, ii) informal interviews with participants and facilitators, and iii) the quality of the results emerging from the discussions. The evaluation of the quality of the emerging results was based on the assumption that increased participation and interdisciplinary communication will create outputs of higher quality, and grounded in the authors' previous expert knowledge of the topics as well as experience from other forms of group discussions (e.g. in the framework of the ASF-STOP COST action).

3. Results

The results regarding the qualitative assessment of the perceived efficiency and applicability of the control methods are summarised here per discussion topic, followed by the results pertaining to the evaluation of the World Café method.

3.1. *Methods for improving control of ASF in wild boar populations*

3.1.1. *Stakeholder engagement and public awareness*

There was consensus between all groups on the need to engage with a large panel of stakeholders. The diversity of the stakeholders was considered important per se and a non-exhaustive list of potential stakeholders was identified (see Table 2). All groups agreed that it is essential to engage in discussions with different stakeholder groups at an early stage and that stakeholders should be identified during the contingency planning process. To engage more efficiently with stakeholders, the participants considered the importance of understanding stakeholder motivations (for instance financial incentives, social acceptance, or political visibility) and identify their leverages. During an outbreak, the information flow within the veterinary sector managing notifiable diseases is top down from central decision-making level to local level. Since stakeholders on the local level need to implement the actions decided at the top level, a dialogue regarding the practical implementation of measures needs to be developed. In the context of

Table 2
List of stakeholders with whom to engage in the event of focal introduction of ASF among wild boar populations (non-exhaustive list), according to World Café discussions concerning "Stakeholder engagement and public awareness".

Sector	Stakeholders
Pig sector	Commercial pig farmers
	Backyard pig farmers
	Pig farmer associations
	Pig feed producers
	Pig transport enterprises
	Pig waste transformers
	Rendering plants
Animal health services	Animal health authorities (official veterinarians)
	Private veterinarians
	Diagnostic laboratories
Forest, fish, wildlife and nature exploitation	Foresters, wood industry
	Volunteer rangers
	Fisheries
	Hunters and their associations
	Mushroom/berry pickers
	Conservationists
	Bird watchers
	Hikers
Forestry services	Forestry/nature ministries
	Forestry rangers
	Wildlife managers/rangers
Civil service	Government/politicians
	Provinces
	Municipalities
	Army
	Police
	Border inspection posts
Other	Linear infrastructure managers (road maintenance)
	Lorry drivers
	Local farmers
	General public, e.g., dog walkers, local inhabitants
	Animal welfare associations
	Journalists and media (TV, radio for society)
	Scientists
	Citizen scientists
	Tourism agencies
	City people

ASF, risk communication further needs to be directed to the general public. The latter was considered instrumental as an uninformed public can adopt practices facilitating disease spread and is more likely to contest control measures or complicate their implementation. Communication can be implemented by different means such as publications, leaflets, posters or through the media. In the absence of clear communication from the relevant authorities, different lobby groups can fill information gaps by introducing messages that benefit their own interests. Therefore, it was considered important to communicate a simple and clear message that includes the non-zoonotic nature of ASF, its global dimension, its epidemiology and ecology, the economic impact and the absence of treatment and vaccine. There was agreement on the need to involve communication experts in designing the messages, communication tools and strategies so that messages for stakeholders become as simple and practical as possible, and tailored for long-term application.

The risk of unintentional disease spread was considered high for people having intensive contacts with wild boar and domestic pigs (e.g. hunters and farmers). Therefore, an urgent need for a proactive approach was identified, including risk-oriented awareness campaigns. The groups also discussed and expressed consensus regarding the management of reports of clinical suspicions of ASF in wild boar arising from the field by a specific structure (e.g. a central emergency number, mobile phone application) that is continually available. There was consensus about emphasising the importance of establishing a task force with defined responsibilities before ASF occurs in a country. This could include crisis management teams from domains other than the veterinary sector to achieve quick mobilisation of a workforce. It was stated that it would be beneficial to involve the experience and the knowledge of countries affected by ASF in the preparedness process.

Finally, there was a discussion around the habituation to the endemic disease situation which is likely to occur if the disease becomes endemic in a territory. In such cases, regular awareness campaigns and motivation incentives were considered necessary to maintain the involvement of stakeholders. In summary, there was a high level of consensus in this topic, especially concerning the urgent need to identify and involve all categories of stakeholders in the disease control activities and to develop proactive and risk-oriented awareness campaigns involving different disciplines, before and at all stages of the epidemic.

3.1.2. Fencing

In the discussions around this topic the participants found it necessary to distinguish between different kinds of physical barriers such as permanent or mobile fences and electric fences. This specification was considered relevant because of the specific technical characteristics and purposes of each particular type of fence. Electric fences were mentioned as being more efficient in deterring wild boar movements but requiring more maintenance and possibly having less social acceptance due to a perceived risk of electric shocks to humans and animals. In some instances, the use of fences was mentioned as being complementary to other population control methods (hunting or trapping). The efficiency of fences was considered to be variable, depending on the goal and the moment of their evaluation: if the aim is immediate restriction of wild boar movement to mitigate disease spread and give governments and administration time to react in connection to a focal introduction, then appropriate fencing might be effective. In such cases, responsiveness needs to be high and fences deployed quickly. However, if the aim is to stop spread of ASF in the long term, fencing is likely less efficient.

Participants further highlighted that the general public's acceptability of fences could vary depending on several aspects. The decision to fence a territory has high political impact and is often controversial because it can conflict with property laws and international biodiversity conservation treaties (e.g. reducing ecological connectivity and the functionality of wildlife corridors). Restricting pedestrians from entering fenced areas for recreational use increases the likelihood of protest and rejection of the implemented measures. Moreover, fences are expensive to build and maintain, while their efficiency for enhancing control of ASF in wild boar population is not guaranteed. Therefore, the media and the public can easily contest a fencing policy. In some cases, fences can even generate or increase diplomatic tensions between neighbouring countries or municipalities. Conversely, high media visibility can be used to increase public awareness of disease control and control area boundaries. Long-term measures to monitor ecological impact, plan maintenance and assess efficiency should be considered and requested from decision-makers in the case of long-term fencing policies.

In general, this topic generated highly contrasting opinions within and between groups. However, there was clear consensus that long-term fencing along national borders as well as large-scale fencing can be inefficient at preventing wild boar movements, and that these measures often are implemented for merely symbolic or political reasons. Finally, the groups discussed that high consideration should be given to maintenance costs and efforts when planning the construction of a fence, the later costs being proportional to the length and durability of the fence.

3.1.3. Passive surveillance

In all groups, the discussion started with finding a common definition of passive surveillance for the purpose of the discussion. There was agreement on the need to distinguish between "*routine* passive surveillance", i.e. the reporting of wild boar found dead in the absence of a perceived risk of ASF, and "*enhanced* passive surveillance", which is implemented when ASF is already present in an area or in the direct neighbourhood. It was noted that in some countries the term passive surveillance could also include sampling or even removal of wild boar carcasses, in addition to reporting. There was controversy in the discussion about whether wild boar involved in road traffic accidents should be included in passive surveillance or only wild boar found dead without an obvious cause of death. Low natural wild boar mortality and the tendency of diseased animals to hide can considerably reduce the success of finding wild boar carcasses, and influence both the efficiency of passive surveillance and the motivation of the persons involved in the search. The groups discussed different options for increasing the acceptability, feasibility and efficiency of passive surveillance, such as voluntary hunters (paid or unpaid) searching for carcasses even during periods with low hunting activities. Other methods suggested were the provision/availability of easy-to-use sampling material and infrastructures for carcass disposal. Furthermore, good communication with stakeholders, the authorities and hunters, as well as transparent information directed at the public, was considered as being the most important and powerful tool for improving passive surveillance. Several aspects, which could decrease the willingness for, or feasibility of, effective passive surveillance, were also discussed. In many countries, hunters were asked to volunteer to search for carcasses. However, if this community did not see any advantage in reporting or taking samples from dead wild boar, passive surveillance would be difficult to implement successfully. In several countries, good communication and feedback to hunters was reported as being more motivational than other incentives in this regard. Motivating stakeholders with financial compensation was also discussed as having potentially adverse consequences on disease control because dead wild boar could be moved to infected areas deliberately. This would distort any surveillance data and imply a risk of spreading ASF. The benefit of financial incentives was also questioned due to low sustainability if the outbreak is not quickly resolved, and the disease becomes endemic. Indeed, most national administrations would not be able to maintain financial compensation over extended periods of time. Another identified potential constraint for the reporting of dead wild boar was the fear of subsequent consequences, such as declaring a hunting ground as an infected area and losing the benefits of paid hunts. Such a situation, might restrict the access of authorities to private land, thus reducing the effectiveness of passive surveillance.

Moreover, research gaps needing further studies were identified. These included the development of modelling approaches to identify environmental characteristics favouring the detectability of wild boar carcasses to help target surveillance efforts towards areas with a higher probability of carcass detection. Other measures with the same goal were also mentioned, such as the use of hunting dogs to improve carcass detection and smart phone technology to speed up reporting and storage of geographic information of carcass location data. Moreover, the need for evaluation of the effectiveness and sustainability of intensive passive surveillance efforts was highlighted. In summary, there was consensus on the importance of implementing wild boar passive surveillance activities, particularly for early detection of introductions and the subsequent potential deployment of contingency plans. However, the methods and ideas for improving the probability of wild boar carcass and disease detection differed between countries.

3.1.4. Manipulation of the carrying capacity of wild boar habitats

The discussion on carrying capacity (CC) was centred around two graphs created during the World Café, one of them displayed as Fig. 2. This graph represented the theoretical population dynamics over time at and around the CC of a habitat, and the potential drop in surviving wild boar after negative CC manipulation. The second graph (not shown) represented a spatial area affected by an outbreak. The total size of this area varied between the groups. The discussions around these graphs included how different habitat manipulations in the core area, buffer area or surrounding intensive hunting area might impact on population

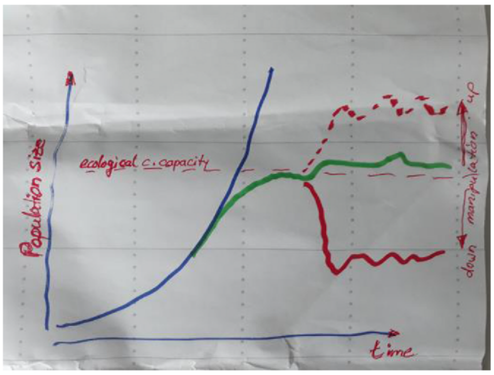

Fig. 2. Representation of theoretical population dynamics over time at and around the carrying capacity of a hypothetical habitat created during the discussions: The x-axis refers to symbolic time since the introduction of a wild boar population in a habitat area. The y-axis refers to the number of wild boar living in the habitat area. The blue line symbolises exponential growth, eventually leading to more animals than could sustain their needs in the habitat; the green line shows the population fluctuating by size around the number related to the carrying capacity; the red lines symbolise the alternative targets of manipulating carrying capacity e.g. by adding or removing feed sources.

density. The conceptualisation between participants was not univocal. During the discussion, the potential purposes of CC manipulations in wild boar habitats, the available tools to achieve CC manipulations, and some concerns related to the general idea of CC manipulation were addressed. The potential purposes of CC manipulation discussed were to reduce the survival time of the virus in a population, reduce the number of hosts, and fracture the populations and favour small-scale movements rather than long-distance migrations. Different tools to achieve CC manipulations were discussed and sorted into spatial manipulation of resource/needs (fencing off water and nutrition, manipulating agricultural harvests), reducing comfort (clearing away bushes, trees and shelter areas, stripping wetlands, burning habitats), altering mortality/survival (poisoning, fertility control, predators, hunting), and spatially structured nutrition (artificial feeding). In general, it was suggested that the effectiveness of CC manipulation was determined by the size of the habitat to be managed and the time horizon available to implement the habitat manipulations. The participants regarded 'incompatible timescales' as the most recognised problem for the use of the ecological CC as a control option for ASF in wild boar, because most measures would require multiple generation times to become effective. Feed scarcity, for example, would be an achievable outcome of CC manipulations only in very limited parts of Europe. Consequently, it was agreed that CC manipulation might be difficult to implement as a reactive, spatially limited, emergency measure. The purposefulness of the measures was further discussed in relation to the waste of effort involved in performing disproportionate treatments (e.g. clearing bushes impacts the area for longer than the epidemic lasts). It was agreed that CC manipulation tools were unlikely to have an impact in the context of small-scale spatio-temporal outbreaks, as wild boar is a resistant species that is difficult to bring to the limit of resource needs. In large areas with a long-term horizon of manipulations, the sustainability of the approaches is most important due to imposed social and ecological conflicts. Measures proposed to manipulate the wild boar CC in larger habitat areas were always considered controversial due to anticipated negative interference with ecosystem services. This might relate to ecological complications (e.g. affecting other species, birds, trees, population genetics) and difficulties in adjusting manipulations to fluctuating natural resources (e.g. mast years). There was consensus about the need for coordination among stakeholders who represent heterogeneous interests in an area, e.g. stakeholders with different agricultural traditions and practices. Specific management of CC to manipulate wild boar populations in small ASF-affected areas within a short timeframe was also discussed. Measures mentioned in that regard were keeping feed available by leaving agricultural crop fields unharvested, providing extra feed, and removing forage from the buffer area. There was a discussion that application of these measures requires a strong legal enforcement by the authorities because farmers will not be allowed to harvest their fields. In the Czech Republic, this method was considered to have had a positive effect on the reduction of movement of wild boar out of their home range areas, thus reducing the risk of further spread of the infection (Charvátová et al., 2019; Office National des Epizooties, 2019). The effect on population abundance in that country was considered slow and proportional to the mortality caused by the disease. It was concluded that habitat manipulation was considered acceptable by the general public and professionals, but encountered some resistance from local farmers directly affected by the restrictions.

In summary, it was agreed that reducing the CC might be difficult to implement as a reactive, short-term emergency measure, as in most areas there is no scarcity of feed for wild boar. Conversely, it was agreed that increasing the CC in a very small affected area (e.g. the core-area after a focal introduction) could decrease the risk of further spread of the infection from the core area as it may lead to reduced wild boar movements

3.1.5. Hunting management

All groups agreed on a temporal scenario described as "stop-search-think-do", separating the measures to be implemented into different phases. In this context, there was a consensus on the fact that hunting to eradicate wild boar in the buffer and core areas (see Box 1) must be carried out according to a plan, agreed on with the stakeholders involved, and implemented in a way that prevents the outward movement of wild boar. Ideally, wild boar should be eliminated from the outside edge of the buffer towards the centre of the core area, whilst trying to achieve a good equilibrium between the greatest possible efficiency and the least possible disturbance of wild boar. Different hunting methods are possible such as individual hunting, drive hunts, hunting with dogs, and the use of night-vision devices. The choice can be influenced by local hunting traditions, the local habitat and the hunting objective (population reduction or elimination). The groups also concluded that the feasibility and efficacy of different hunting modalities and their effect on wild boar disturbance in different hunting grounds must be assessed in peacetime. Alternative methods for eradicating wild boar in the buffer and core areas were also discussed under this topic, including poisoning, trapping or fencing. For instance, an alternative fencing approach to direct wild boar movement towards a specific area was proposed based on the experience in Australia. In this approach, some one-way gates are left open in an otherwise fenced area, enabling wild boar to be driven into an area where they are easier to eliminate.

It was concluded that different hunting modalities can be used to achieve a substantial reduction in the local wild boar population. Some countries have reported greater efficiency by using drive hunts with dogs, while other countries have reported better efficiency with individual hunting. The efficiency will depend on the duration of the hunting activities (e.g. individual nightly hunting or three times per month drive hunts may have the same effect). Involving trained shooters (police and military snipers, specifically trained hunters) allowed to use special equipment such as night vision, thermal vision and silencers in affected areas was also discussed. According to the experience of the Czech Republic, these methods were considered effective and feasible, and with the advantage of having a limited effect on animal movement compared to regular hunting. The effectiveness can be increased further if performed at baiting sites or using attractants. The method was assumed to be well accepted by both the general public and professionals, but its application in some countries might face legal constraints. The groups also discussed the use of camera trap networks to monitor populations and evaluate the efficiency of the implemented activities. Furthermore, the use of helicopters, drones or thermal cameras was proposed in open areas to localise remaining wild boar populations and target culling efforts.

In summary, despite divergent points of view due to different hunting traditions and experiences in a diversity of habitats, there was consensus in the fact that after a focal introduction of ASF, wild boar should be driven from the outside edge towards the centre of the affected area, where they are to be eliminated with the method providing the highest efficiency and the least possible disturbance.

3.1.6. Other population control methods

The methods discussed included the use of poison, fertility control, trapping and trained shooters. The results of the discussions on this topic are summarised in Table 3. As a starting point, the group suggested that the methods discussed should not be referred to as alternative but rather as complementary, given that they are not meant to substitute hunting as a population control method but to provide additional options. Moreover, it was concluded that several of the methods discussed are technically available, but are not yet currently applicable in the European context because they are controversial and politically sensitive (use of toxicants) or because they are not technically deployable at large scale or commercially available (use of contraceptives). Poisoning, i.e. culling by oral administration (through baits) of a toxic substance that causes quick death without suffering, is used in areas where wild boar or feral swine have been introduced and are considered an invasive species, such as in Australia or the USA. Technically, the method was considered feasible for application in restricted areas (possibly fenced) in emergency situations and for a short time period. Some countries might have the power to implement this measure under exceptional emergency situations, but culling with toxic substances was not considered an option at present for the control of wild boar populations (see Table 3). Considering the assumed negative perception of the application of this method among the general public as well as among professionals in Europe, the groups agreed that there is a need for additional risk assessment to evaluate the benefits and potential threats to the

Table 3
Summary of the conversation on "Other population control methods" during the World Café discussion.

Methods	Positive attribute	Negative attributes	Stakeholder acceptability
Trapping	Effectiveness varies between regions and seasons	Resource and labour intensive	General public: good Hunters: variable Other professionals: good
Poisoning	Effective, quick response	Technically feasible but currently not legal in the EU. Requires further scientific validation	General public: bad Hunters: bad Other professionals: variable
Fertility control	No adverse effects on animal welfare. Applicable in urban and inaccessible areas	Oral contraceptive not available for wild boar Potentially slow effect Impact on other species Food safety not evaluated	General public: good Hunters: variable Other professionals: variable
Trained shooters	Effective compared to regular hunting Limited effect on wild boar movement	Not applicable in urban areas	General public: good Hunters: good Other professionals: good

environment and to animal and human health. In particular, there is a lack of data on the potential threat to non-target animal species and possible environmental contamination. To increase acceptability, it was suggested that alternative expressions should be used in communication, such as "euthanasia" or "humane preventive destruction (culling) of the population", and to improve communication regarding the purpose of the culling (to prevent animals with the disease from suffering) and the potential advantages (quick and humane action, no residues). Fertility control, i.e. decreasing the fecundity of female wild boar by administration of contraceptive medicines as a complementary method of population control, was assumed to be more ethically acceptable than culling by the use of toxic substances with no legal or political constraints to its application. However, such a product is not yet commercially available. Moreover, the consensus of the group was that the impact of the method would be too slow to reduce the population in an emergency situation within affected areas. Potentially, however, it could be used in buffer zones around affected areas, for preventive population reduction in free areas, and in areas where other measures are difficult to implement, for example in urban environments. However, the groups concluded that some concerns regarding the impact on non-target species and public health (unintentional exposure of humans) still need to be assessed.

The use of traps for catching wild boar at baiting sites followed by culling was generally considered a feasible method that has been applied with variable success in several countries and under different conditions. The advantages of using large traps designed for many animals at every catch were emphasised. The groups concluded that trapping of wild boar as an alternative population control method would allow high biosecurity levels as well as a higher level of animal welfare compared to the other methods discussed, and was thus assumed to be a method that would be accepted by the general public and professionals. Nevertheless, some hunters could oppose the concept of culling (i.e. shooting) trapped animals. The drawbacks mentioned were related to costs required in terms of time, effort and human resources, since traps need to be regularly monitored and maintained. It was also mentioned that the effectiveness may vary between regions and seasons.

Furthermore, the groups discussed using trained shooters and managing forage availability in affected areas as an alternative/complementary method. However, the aspects included in those discussions are covered in the hunting strategy and carrying capacity management sections and are therefore not repeated here. To summarize, despite being resource intensive and contested by some hunters, trapping of wild boar was considered a good complementary population control method compatible with biosecurity and animal welfare requirements. Despite proven efficiency in experimental or other contexts, population control methods such as poisoning and fertility control remain controversial, politically sensitive, or require additional research before they can be deployed at a large scale in the European context.

3.2. *The World Café method*

In summary, the World Café method was perceived as suitable for reaching the objectives of this study. Key qualities of the method put forward by previous practitioners such as stimulating collective intelligence (MacFarlane et al., 2017), allowing creativity and the emergence of new ideas (Steier et al., 2016) as well as reducing felt presence of hierarchical structures (Tan and Brown, 2005) were indeed observed, and its ability to increase the level of participation confirmed. Further, the rotating format and the small discussion groups that were kept fixed throughout the exercise seemed to allow participants to express themselves with transparency and trust, and to enable the participants to evolve together as a group. This adaption of the World Café method has been used before (MacFarlane et al., 2017), whereas others promote a more informal set up with new groups forming spontaneously during the exercise (Fouché and Light, 2011). In our experience, keeping the same groups during all rotations reinforced a collaborative spirit that encouraged active participation and had a positive effect on the working atmosphere. This constructive atmosphere was observed in the creation of operational solutions, for example illustrated in the graphs (Fig. 2) to conceptualize the problem and its potential solutions. Conversations were generally fluent and intensive, with a high level of exchange between countries and disciplines despite some of the topics proving controversial. Some participants from affected countries said that they felt the World Café method was a useful process for being confronted with different or new approaches to address similar challenges. Meanwhile, some participants from unaffected countries highlighted the exercise as particularly beneficial, exposing the diversity and variety of wild boar management measures that need to be implemented to manage ASF. Country and discipline diversity allowed a rich level of exchange. The World Café method is often described to promote learning from each other and to create collective intelligence (Brown and Issacs, 2010). In that respect, participants involved in disease control appreciated the professional exchange with wildlife managers and conversely wildlife professionals appreciated learning more on the epidemiological aspects of the disease. Similarly, contrasting field management experiences with different wild *Suidae* populations such as wild boar in Europe and feral pigs in Australia was considered an added value of the discussions.

In general, there were varying viewpoints within and between groups, indicating that many of the methods discussed triggered contrasting opinions and discussions. Using the World Café method, this diversity of opinion could be captured without striving for consensus to be reached. Striving for consensus or even forcing it, have been an element of critique put forward against the way group interviews or focus group discussions have been employed in participatory epidemiology (Chenais and Fischer, 2018). Even in groups that are constructed to be homogenous (which was not the case in this study) a certain level of diversity will always be present (Ebata et al., 2020; Fischer et al., 2020). If consensus is sought for, this diversity in opinion and experiences will be lost, and especially weaker voices might not be heard.

The three dimensions of evaluation of the different topics are summarised in Table 4. Some of the control methods and topics addressed were particularly controversial, especially those including ethical judgements related to wild boar population control methods or to the building and impact of fences (see Table 4). Conversely, a high level of consensus was expressed about passive surveillance and stakeholder communication. Some conflict between practical experience and theoretical knowledge was present in all topics. The level of dissensus, consensus and the saturation level varied between the topics (see Table 4).

4. Discussion

As the related Appreciative Inquiry method (Egan and Lancaster, 2005), the World Café method traditionally focuses on promoting positive examples of solutions and producing constructive change, whereas other methods within the common field of Participatory Action Research

Table 4
Appreciation of the different topics based on the level of dissensus, consensus and saturation during the World Café discussion (Null 0, Low +, Medium ++, High +++).

Topic	Dissensus	Consensus	Saturation
Stakeholder engagement and public awareness	0	++	+
Fencing	+++	++/**	+
Passive surveillance	+	+	++
Manipulation of carrying capacity	++	+	++
Hunting management	*	*	*
Other population control methods	++	+	++

*Data were not recorded for this topic.
** This refers to the inefficiency of long fences/political impact).

(participatory epidemiology included) focuses more on finding solutions to problems (Aldred, 2011). In this study, however, the World Café method efficiently framed problems within the different topics, and especially served to make the complexity of these problems tangible. Further, and as mentioned by Aldred (2011), the method served to create understanding rather than confronting different opinions or counterparts. These are some of the key qualities of the World Café method, previously mentioned in the literature and observed in this study. In this regard, it might be relevant to mention that in our study many of the participants of different disciplines and backgrounds did not know each other before the meeting. Thus, the opportunity for interest groups or individuals to impose a specific agenda or point of view was limited. This aspect has been reported as a problem from discussions at for example open community meetings (Carson, 2011). The fact that the meeting was organised by a networking project linked to ASF control could further have contributed to the perceived sense of neutrality, transparency and trust that facilitated open communication. In that sense, the mixed groups facilitated the flow of information and exchange of views between disciplines in a cumulative, non-hierarchical process. Keeping the same groups throughout the exercise contributed to the desired informal discussion climate. This adoption of the original World Café method promoted inclusive participation and stimulated bridging of communication barriers that can otherwise be observed in multi-stakeholder and transdisciplinary discussions (Bagnol et al., 2016; Norris et al., 2016). In this study, the observed high level of participation were crucial for the quality of the end results, with the integration of views from varied stakeholders contributing to the scientific quality of the outcome concerning general priorities, knowledge gaps and recommendations in the field of wild boar management and ASF control. This ability of the method to harvest multiple opinions, without prioritising or striving for consensus has indeed been put forward as one of its strengths (Fouché and Light, 2011), compared to other participatory methods that often strive for consensus, thus failing to account for all voices (Campbell, 2002). Nevertheless, critiques have been raised arguing that the World Café (and Appreciative Inquiry) methods might actually conceal disagreements, especially if discussions are undertaken in contexts that have power inequalities (Aldred, 2011). Contrary to such critique, in this study different solutions to the same problems were frequently voiced, and it was noted that the varied stakeholders involved in wild boar management or ASF control had different professional objectives. This application of the World Café method thus efficiently dealt with the challenge of finding a trade-off between different visions and needs. An interesting outcome of this dynamic was the production of a harmonised panel of definitions for terms that do not always have the same meaning in different countries or expert fields.

Biases might be introduced at every step in the research process, from acquiring funding to study design and participants selection, data collection, analysis and publication of the results (Galdas, 2017). In our study, one of the potential biases was the facilitators and how they were selected (Bedelian et al., 2007; Fischer et al., 2020). The original World Café format[3] does not include pre-selected facilitators but let the groups themselves distribute the roles among the participants. We, however, hypothesized that it would be relevant especially for the second objective of our study ("to gather knowledge concerning the control of ASF in wild boar populations and assess the efficiency and applicability of different control methods") that the facilitators had good knowledge of the topic they were facilitating, and that their expertise would be useful for guiding the discussions and ensuring progress. These aspects, together with facilitation skills and interest in the method, thus guided the selection of facilitators. The facilitators' previous knowledge could however have influenced the power relations between the facilitators and the participants (Galdas, 2017), how discussions were engaged, and what aspects that were put forward in each topic. The extent of this potential bias cannot be assessed. Further supporting the use of pre-selected facilitators, however, MacFarlane et al. (2017) point out that skilled facilitators are able to pick up and react to group dynamics in ways that layman café or table hosts might not be.

The issue of communication among and between stakeholders seems to be both difficult and essential in the context of disease crisis management (Sell, 2017). This aspect was repeatedly mentioned as a challenge for all topics, and the importance of delivering adapted key messages to targeted stakeholders was consensual. Another aspect that transpired from the conversations was the challenge of keeping stakeholder motivation high over time if the disease becomes endemic. An additional constraint concerns the participation of hunters in activities aimed at controlling ASF: it seems obvious to involve hunters in the management of their resource, but if the management activity consists of drastically reducing the wild boar population size or requesting other services such as carcass disposal and sampling, it might become difficult to maintain motivation and engagement. Further efforts are needed to improve communication with the hunting community and increase understanding of their views to enhance their integration in disease management activities. Fencing has been used successfully in the Czech Republic to control and eradicate a focal introduction of ASF in a wild boar population (Charvátová et al., 2019). Since then, several countries have promoted fencing to prevent the introduction of ASF in their territory from neighbouring countries or to contain the spread (Mysterud and Rolandsen, 2019; Dellicour et al., 2020). One of the World Café outputs was tangible scepticism about the current efforts of many EU countries to fence large perimeters of their borders to prevent transboundary ASF spread in light of their ecological impact and maintenance costs (Woodroffe et al., 2014; Mysterud and Rolandsen, 2019).

Considering the progress of ASF and the high densities of wild boar populations in some areas of the EU (Melis et al., 2006), it seems a matter of urgency to test different options for reducing population densities. In the light of the conclusions from this study, intensive hunting methods are considered effective and feasible, but have a more limited effect on animal movement compared to regular hunting. Furthermore, to improve efficiency they should, when possible, be combined with other possible control methods, such as fencing and trapping. The potential use of alternative methods to reduce wild boar populations in localised emergency situations, such as chemical culling (Snow et al., 2016, 2017) or fertility control (Massei et al., 2011), should be investigated from a legal, environmental, animal welfare and oral administration perspectives (Ferretti et al., 2018).

5. Conclusions

Despite intensive efforts to control ASF in wild boar populations, the disease continues to spread to new territories. The complex epidemiology of ASF in wild boar necessitates the involvement of diverse stakeholders and different scientific disciplines to achieve sustainable control. The studied application of the World Café method showed comparative discussion advantages such as the production of concrete outputs and the creation of an inclusive discussion climate promoting active participation that allowed open-minded appraisal of the control methods. The importance of coordination and communication within and between different stakeholders during the preparation as well as implementation phases of disease control was highlighted. Therefore, based on the results from this study, we recommend the use of the World Café method for discussions in multinational and/or multidisciplinary fora in the context of ASF and other animal health crises.

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[3] http://www.theworldcafe.com/.

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4.2.1.2 "Can we agree on that"? Plurality, power and language in participatory research

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Highlights

- Language and translation have hardly been considered in participatory epidemiology
- Facilitators and interpreters are crucial for the research
- Striving for consensus and analysing group averages prevents weaker voices from being heard
- Power relations and plurality in focus groups need to be better integrated

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"Can we agree on that"? Plurality, power and language in participatory research

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ABSTRACT

Participatory epidemiology (PE) is a method that gathers data from groups through focus group interviews and participatory visual and scoring exercises. The method is often used in poor communities in low-income countries where it is hard to obtain conventional epidemiological data. This paper draws on research on the public sphere and democratic deliberation, along with research on language and interpretation, to suggest how PE research could be better equipped to account for diversity in local knowledge, include minority views and acknowledge power dynamics. These aspects are discussed under the three themes of 'plurality', 'power' and 'language'. A review of highly-cited PE literature suggests that PE research engages with plurality and power to a very limited extent, and only marginally more so with language and translation. Examples are taken from the authors' own PE research on African swine fever in —Uganda, classical swine fever in Germany, peste des petits ruminants (PPR) in Eastern Europe, and Ugandan pastoralists' understanding of cattle disease to provide more detail as to why conventional PE studies might fail to record issues of plurality, power and language, and also to suggest how this can be addressed. With reference to the literature on the public sphere and democratic deliberation, and on language and interpretation, this paper concludes with some suggestions as to how to take plurality, power and language into greater consideration in PE studies in future, thus improving the validity and reliability of PE data.

1. Introduction

Participatory epidemiology (PE) is based on the assumption that people are knowledgeable about issues that are important to them, such as diseases affecting their animals (Mariner and Paskin, 2000; Dunkle et al., 2013). Thus, by collecting data through engaging with local communities, results can be obtained that are both more valid (in the sense of being closer to the local reality) and of greater local relevance than by using structured questionnaires for example (Chambers, 1994; Fischer et al., 2016). The methods that have developed as the core of PE can be grouped into semi-structured interviews, visualisation tools, and ranking and scoring tools (Etter et al., 2006; Jost et al., 2007; Dunkle et al., 2013). Central to PE is triangulation, drawing on multiple methods and sources, allowing people to confirm or refute what is being said in group discussions (Mariner and Paskin, 2000; Dunkle et al., 2013). Many of the tools employed are designed to achieve one consensual answer from the group as a whole (Dunkle et al., 2013).

Despite the centrality of groups in PE and in other participatory research, there has been relatively little discussion in the literature as to how to compose groups or deal with group dynamics. Mention is sometimes made that it is important to have a 'skilled' or 'trained' facilitator to ensure everyone in a group speaks and to construct gender-homogenous groups for this purpose (Bedelian et al., 2007; Catley et al., 2012). Beyond that, little detail is generally provided on how to deal with the fact that people within a community are different (referred to in short in this paper as 'plurality'), which means that attention also needs to be paid to different forms and expressions of power (within communities, and between researchers and the studied communities) (Chenais and Fischer, 2018). Likewise, mention is frequently made that names for diseases or syndromes should be collected using local terminology (Rufael et al., 2008; Catley et al., 2012), but a more in-depth theoretical or methodological interrogation of how to deal with language when performing research in cross-cultural and multi-linguistic situations is largely absent in key PE literature (Mariner and Roeder, 2003; Bedelian et al., 2007; Barasa et al., 2008; Rufael et al., 2008; Catley et al., 2012).

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This paper aims to suggest ways of deepening the engagement in PE with the three interlinked aspects of plurality, power and language by drawing on insights from the field of deliberative democracy and the public sphere (Fraser, 1990; Habermas, 1991; Arendt, 1998; Hagendijk and Irwin, 2006) and on literature that critically reflects on the role of languages and translation in fieldwork (Borchgrevink, 2003). The twenty most cited texts on PE in the Web of Science were reviewed and the authors' own fieldwork experiences drawn on to discuss how plurality, power and language are given consideration in PE. A discussion then follows about how PE studies might engage methodologically with these aspects to a greater extent in future studies.

1.1. *Theoretical framework – the ideal PE situation*

This section starts with a description of the key tenets of participatory methodology in the tradition developed by Chambers et al. in the 1990s,commonly referred to as participatory rural appraisal (PRA) (Chambers, 1992; 1994; 1997), and pinpoints some of the basic assumptions and practical developments in PRA and PE. There is then an introduction of the authors' thinking on what theorising on the public sphere, public deliberation and language can do to assist in the development of a version of PE that more effectively represents the local diverse reality being studied.

1.1.1. *A critique of key tenets of participatory research*

Groups are central to participatory methodology (Chambers, 1994; Mariner and Paskin, 2000; Dunkle et al., 2013). The advantages put forward for organising data collection in the form of group activity are that it is quicker than carrying out numerous individual interviews or surveys, and that the group dynamic allows on-site triangulation where people in the group can confirm or refute each others' statements (Chambers, 1992; Mariner and Paskin, 2000). For such triangulation to work, the group must be sufficiently homogenous so that everyone feels comfortable expressing their views and that there is one response that is agreeable for the whole group. While it is acknowledged as good practice to conduct separate focus group discussions (FGDs) e.g. based on gender to capture women's perspectives and ensure they also feel they are free to speak (Catley et al., 2012), other dimensions of social stratification are addressed more rarely. Researchers in both PRA and PE studies often spend limited time in the community, restricting full understanding of the dimensions of social structure. Thus, groups that appear relatively homogenous on the surface might not actually be that homogenous (Chambers, 1992; Mariner and Paskin, 2000), while group activities that focus on reaching one consensual answer might actually marginalise weaker and non-dominant perspectives (Campbell, 2002; Chenais and Fischer, 2018). Many of the tools used in PE are designed to result in one (often semi-quantitative or quantitative) answer per question per group (Catley et al., 2012). Historically, it has been argued that this is one of the strengths of the veterinary adoption of PE because it enables statistical analysis (Mariner et al., 2003; Bett et al., 2009). Handling semi-quantitative or quantitative data at group level, however, does not allow diverging opinions within the groups to be taken into account. If the intention is to understand how certain animal diseases or prevention and control measures might contribute to poverty reduction, for example, it is important to ensure that the voices of the poorest or most marginalised are also heard (Chenais and Fischer, 2018). This means that attention needs to be paid to social stratification beyond gender, allowing it to influence how groups are composed and ensuring that different people within the groups are heard. The above is referred to in this paper as aspects of plurality. In this regard it is important to point out that the establishment of majority perspectives is not always negative. Depending on the research question and objective of the study, understanding how most people, or the dominant group in a society, perceive or act in relation to a certain issue might be adequate. An excellent example of this is how drawing on pastoralist intelligence on animal disease through PE tools turned out to be key to eradicating rinderpest (Mariner et al., 2012).

Early critiques of participatory research concerned the assumption that this would be a quick way of gathering data (Richards, 1995). Spending short periods in the field has been criticised for leading to insufficient understanding of the local social and cultural system, i.e. how people comprehend their world and relate to each other (Richards, 1995). A lack of understanding of the social context might lead to a failure to acknowledge local dimensions of power that affect the information given, as discussed above, and to misunderstandings and misinterpretations of the data. Time constraints have nevertheless remained a key motivation for many PRA and PE studies in practice (Bett et al., 2009; Grace et al., 2009; Goutard et al., 2015; Kimaro et al., 2017). Central to participatory methodology is also striving to 'hand over the stick' (Chambers, 1994, pp. 1254). This metaphor refers to the importance of listening to local people on their terms, facilitated through the use of tools with which local people feel comfortable, for example tools that do not require competence in reading and writing and are not pre-designed into categories assumed by external and powerful scientists and development workers (Chambers, 1992). The idea of 'handing over the stick' can partly be seen as a strategy to overcome the shortage of time. If the scientist or development worker truly takes a step back and lets local people lead data collection and organise findings in ways that make sense to them, this might improve access to local ways of knowing and acting. However, despite the idea of 'handing over the stick', in practice both PRA (Richards, 1995) and perhaps even more so PE (Chenais and Fischer, 2018) have become standardised into the use of particular techniques and tools that are sometimes even settled on beforehand, and box participants' answers into categories that make sense for science and are easy to publish (Chenais et al., 2017). Such PRA and PE work is likely to miss out on important local perspectives. It has been shown elsewhere how for PE this might lead to scientists failing to discover new diseases, for example, or to veterinary advice being given in such a way that it is completely incomprehensible to local animal owners or impossible for them to implement (Chenais and Fischer, 2018).

Related to this, the majority of PRA and PE work is performed in contexts that require an interpreter. While the role of interpreters remains practically invisible in key texts on PRA (e.g. Chambers, 1992; Pretty et al., 1995), in PE it is often emphasised that it is important in collecting information on diseases and syndromes in local terminology (Rufael et al., 2008; Catley et al., 2012). However, more in-depth discussion about the purpose of this, how to embrace other ways of understanding the world than those of the researcher or research team, or the relationship between language and power etc., is rare (Mariner and Roeder, 2003; Bedelian et al., 2007; Barasa et al., 2008; Rufael et al., 2008; Catley et al., 2012). As this paper will show, conducting PE and PRA studies with the help of an interpreter requires thorough theoretical and methodological interrogation to ensure good-quality data collection.

Considering the above key critiques of participatory research relating to plurality, power and language, these aspects might be better understood and their wider importance acknowledged by looking at academic debates about power and representation within the field of the public sphere and public deliberation. Curiously, as with the lack of discussion on language in the development and critique of PRA and PE, this debate has also been absent from the field of public deliberation (Triadafilopoulos, 1999). Given suggestions that thinking about language is essential for the development of better PE studies, there is an exploration of the limited published research that critically and constructively reflects on the use of interpreters in the field and the ensuing challenges.

1.1.2. *A focus group as the public sphere*

The 'public sphere' can be understood as the physical place and social situation in which people are able to speak freely on public matters (Fraser, 1990; Habermas, 1991; Dikeç, 2013). Adopting this definition, a well-designed group activity in PRA or PE should function like a 'public sphere'. Jürgen Habermas is one of the key thinkers on the public sphere. For him, it is characterised as an unrestricted rational discussion in which everyone is free to take part and where inequalities and social status should be temporarily bracketed to give everyone's voice equal weight (Fraser, 1990; Habermas, 1991). Thus, in the public sphere, discussion should be free of any power differentials. Habermas' idea of a public sphere is also based on the notion that the free deliberation of public matters will enable a resulting consensus about what the correct action on a certain issue would be (Canovan, 1983). The possibility of reaching consensus, or even the appropriateness of striving for it, has been questioned by scholars such as Fraser (1990) and Mouffe (2011), who point out that the consensus imagined by Habermas was only possible because the situation he studied was not in fact a situation of broad societal inclusion. Habermas' development of the theory of a public sphere was based on the specific historical context of coffee houses in the Ottoman empire, which did not admit women or the working class, for example. In effect this was consensus through exclusion.

1.1.3. Consensus versus conflict and plurality

Fraser (1990) has suggested that Habermas' idea that differences can be left behind to achieve an inclusive discussion actually serves to hide inequality. In the same vein, Arendt (1998) emphasises that plurality, i.e. that we are all different, is an essential part of what it is to be human. Therefore the starting point for any theory on public deliberation must be to embrace these differences (Arendt, 1998). If the starting point is that we are different and have unequal possibilities, some guiding principles are required to enable discussions in which everyone is heard and where consensus through exclusion can be avoided. Drawing on theories on democracy and public deliberation, these guiding principles might be to have humility and accept that we are not always right, to listen to one other, to make ourselves understood, and to let everyone speak (Arendt, 1998; Roberts-Miller, 2005). As such, the guiding principles for a functional political dialogue in the public sphere are essentially the same as those advisable for a good FGD.

Other scholars, who like Arendt criticise the idea of consensus as the final outcome of successful deliberation, are less hopeful of a fruitful discussion being possible in a plural society (Fraser, 1990; Mouffe, 2011). Mouffe emphasises that democracy is about conflict. She advocates for a "*conflictual consensus*" (Mouffe, 2011, pp. 121) where participants in a democracy need not agree on the issues debated, but must agree on the format in which this is done. This is similar to the general guiding principles discussed above. Fraser (1990), who like Mouffe sees conflict as inherent in society, suggests that a solution to a more inclusive society, which has also been repeatedly observed empirically, is the development of what she refers to as 'counter publics'. By this she means sub-groups within society that develop their own arenas in which they can deliberate together about "*their needs, objectives, and strategies*" and in doing so become better equipped at "*articulat[ing] and defend[ing] their interests in the comprehensive public sphere*" (Fraser, 1990, pp. 66). To facilitate this, Fraser (1990, pp. 64) says that it is important to "unbracket inequalities in the sense of explicitly thematising them". If this suggestion does have a bearing on PRA and PE work, it emphasises the importance of trying to identify how the local communities studied are stratified (beyond gender) and then organising separate FGDs based on these stratifications.

1.1.4. Language and different ways of knowing the world

Much of what has been touched on in the above two sections relates to how people speak to one other, which warrants an engagement with language. Language shapes, and is shaped by, the cultural and ecological context, and different languages "*carve up reality in different ways*" (Borchgrevink, 2003, pp. 106). Language is also a factor that affects power dynamics in a group (Fraser, 1990). Despite this, social sciences have largely shied away from talking about the role of language and the need for translation in cross-cultural and multi-linguistic research situations (Skjelsbæk, 2016; Gibb and Danero Iglesias, 2017). This is even the case in anthropology, where fieldwork in far-off countries and cultures is a key part of the discipline (Borchgrevink, 2003). A few studies are explored that have discussed the lack of reflection on language and interpretation in these disciplines and attempted to provide practical guidance based on experience (Borchgrevink, 2003; Bujra, 2006; Berreman, 2012; Gibb and Danero Iglesias, 2017).

Language can be used (intentionally or otherwise) to exclude particular groups of people from the public sphere if the language spoken is not accessible to everyone or if particular ways of phrasing and categorising issues are seen as more profound and simply 'better' than others (Fraser, 1990). In FGDs, this exclusion works in at least two ways. First, it can affect the dynamic within an insufficiently homogenous group, where those who are more articulate or have access to higher-status language might be heard in preference to others, possibly forging false consensus. The higher status that comes with the use of scientific language, for example, is shown to frequently obstruct full inclusion by non-scientists in public deliberation (e.g. Cook et al., 2004; Hagendijk and Irwin, 2006). Second, it is likely that, without reflection, research teams favour the more articulate or better-educated group members simply because the way they talk is more similar to that of the research team. An important way of countering this tendency is through the selection of facilitators and interpreters. It has been emphasised that the choice of an external expert as an interpreter or facilitator can lead to the reinforcement of the power relationship between the researcher and the interviewee, and more limited possibilities for the creation of a trusting and open environment in which local people will speak freely (Borchgrevink, 2003; Bujra, 2006). Even when selecting local interpreters, an interpreter's caste, ethnicity, gender or social status can have important effects on how willing different people in a community are to speak to the researcher and what information is being accessed (Borchgrevink, 2003). Skjelsbæk (2016), interviewing rape victims, describes how paying attention to gender, ethnicity and personal characteristics was central in her selection of interpreters to ensure that they would be able to build trust, sensitivity and compassion. She also explains that at times the interpreters found the victims' stories so emotionally upsetting that they (consciously or not) used other words, for example, such as 'the event' or 'it' when the interviewee had said 'rape' (which the researcher noted due to being familiar with some key words in the local language of relevance to her research topic). This example thus also points out the value of the researcher understanding key terms in local languages, even when relying on an interpreter. Berreman (2012) describes how the high-caste interpreter with whom he initially worked in India concealed practices that were deemed to show his culture in a less favourable light. This was only revealed when teaming up later with a low-caste interpreter, which gave him a more comprehensive understanding of the social organisation and conflicts than when working with the first interpreter alone (Berreman, 2012).

As indicated by the above, language is intertwined with culture and society, and therefore translation in cross-cultural contexts is much more than merely converting words from one language into another. Frequently there is not a 1:1 relationship between words in different languages. For example, Philipps (1960; in Borchgrevink, 2003, pp. 111) describes how over time he understood that there were six different Thai words to describe different ways of being angry, but that initially his interpreter only interpreted all these different words to him as 'angry', for want of other words. There is a balance (and to some extent a trade-off) between providing a literal translation and one that best conveys the meaning of what is said. As indicated by the above, in cross-cultural contexts it is often more useful to have a less literal and more meaningful translation (Borchgrevink, 2003; Bujra, 2006). This can be done by complementing the translation with explanations, e.g. by showing practically or using metaphors. Indeed, this is also what 'handing over the stick' and communicating with people on their own terms, e.g. through the visual aids, charts etc. often used in participatory research and PE, is all about. Several researchers have suggested that in order to achieve this aim, it is better to choose an interpreter "whose background and social positioning vis-à-vis the given field setting will be compatible with that of the interviewee" (Skjelsbæk, 2016, pp. 514) rather than an external expert (which might be better language-wise). In conclusion, choosing interpreters with an understanding of the local context, and to whom people are willing to open up, and aiming to understand the local context and meaning of what is said rather than literal translations are key to good-quality research in cross-cultural and linguistic contexts.

2. Materials and methods

To obtain an overview of how plurality, power and language are addressed in PE literature, a search of the abstract, title and key words in publications in English was undertaken for the term "participatory epidemiology" in the Web of Science database on 21 February 2020. The search produced 182 hits. Of these, 24 references were manually excluded due to meeting least one of the following criteria: duplicate reference (one reference); not written in English language (one reference); did clearly not concern veterinary epidemiology or animal health based on reading abstract (22 references). Appendix A lists all 182 references indicating excluded references. Of the 158 remaining hits, the top 20 cited publications were analysed as an indicator of the norm for how PE studies should be performed (Mariner and Roeder, 2003; Catley et al., 2004; Mariner et al., 2005; Catley, 2006; Etter et al., 2006; Bedelian et al., 2007; Jost et al., 2007; Barasa et al., 2008; Rufael et al., 2008; Bett et al., 2009; Leo et al., 2011; Mariner et al., 2011; Rich and Perry, 2011; Catley et al., 2012; Mariner et al., 2012; Marcotty et al., 2013; Roeder et al., 2013; Coffin et al., 2015; Brugere et al., 2017; Chenais et al., 2017). These twenty publications were published between 2003 and 2017, and each was cited between 42 and 93 times[1]. In total there were 106 different authors to these 20 papers, of which most only occur once. Eight authors occur more than once in the author lists and three authors stand out: Jeff Mariner with seven authorships, Andrew Catley with six authorships and Peter L. Roeder authoring five of the papers.The twenty publications were read in detail and all the parts of the text that addressed themes relating to plurality, power and language were noted.

The scientific publishing format rarely allows space to elaborate on the problems encountered during the research process, and negative results are usually difficult to publish. Therefore, after reviewing the twenty highly cited PE publications, some examples from the authors' own research experiences in terms of failures to take plurality, power and language sufficiently into account are given, with reflections on why that was the case. This section aims to enrich understanding of why aspects of plurality, power and language are still addressed to such a limited extent in PE research.

3. Results

3.1. How do key texts in PE address plurality, power and language?

3.1.1. Plurality

Starting with the aspect of plurality (i.e. the fact that all people are different, with different preferences and opportunities), only five of twenty publications mentioned some aspect of this (Bedelian et al., 2007; Bett et al., 2009; Rich and Perry, 2011; Catley et al., 2012; Coffin et al., 2015). Rich and Perry (2011, pp. 135) state that "*the response of different stakeholders to the disease will be contextualized in their unique circumstance and constraints*", but they do not elaborate on what this might mean more specifically or how to address it methodologically. Three of the publications (Bett et al., 2009; Catley et al., 2012; Coffin et al., 2015) mention the importance of, and/or state that they in part or fully, performed gender-homogenous groups but do not elaborate further on the reasons for this, or other aspects of plurality. The study by Bedelian et al. (2007) stands out among the reviewed papers for its engagement with plurality. Here, FGDs were complemented with household interviews for the explicit purpose of capturing the voices of those community members who did not attend meetings. Despite this, the results were subsequently only presented as group means.

3.1.2. Power

With regard to power (here referring to the possibility of being heard, for example, or being able to steer outcomes to one's own benefit (rather than someone else's)) – of the twenty most cited PE papers, only Bedelian et al. (2007) and Catley et al. (2012) address this in any respect. The review by Catley et al. (2012) does so (only) by mentioning the importance of gender-homogenous groups to access women's voices. Bedelian et al. (2007) present findings from a PE study of Kenyan Maasai people's perception of malignant catarrhal fever (MCF). In that study, the authors acknowledge the risk of dominant individuals steering group conversations and therefore state that it is important to have skilled facilitators. However, they do not elaborate on what it means to have a skilled facilitator or what kind of strategies could be helpful for ensuring that everyone is heard.

3.1.3. Language

The issue of language is addressed to a greater extent than power and plurality. Nine of the twenty reviewed publications talk about language (Mariner and Roeder, 2003; Catley et al., 2004; Catley, 2006; Bedelian et al., 2007; Jost et al., 2007; Barasa et al., 2008; Rufael et al., 2008; Bett et al., 2009; Catley et al., 2012). All of these have collected, or emphasise the importance of collecting, names for diseases and syndromes in local languages. However, only Jost et al. (2007) engage more significantly with languages and different kinds of knowledge. These authors point out that studies must be designed to be flexible, adapting data collection for the sake of comprehending local priorities and ways of conceptualising and categorising the world. To achieve this, the importance of facilitators being good listeners is emphasised. At the same time the authors provide examples of when this approach to local communities has sometimes been difficult, due to a "*tendency on the part of national governments to favour survey methods rather than embrace the investigatory approach that is at the heart of PDS [participatory disease surveillance]*" (Jost et al., 2007, pp. 540). Jost et al. (2007) also highlight the importance of acknowledging that understanding and classification of diseases and vectors differ between communities: "*A primary objective of PE is to gain an overview of the range of local disease terms and how farmers process and perceive information. For example, the Somalis have a very detailed grasp of disease vectors and have local names for most species important in disease transmission. Like the Somalis, the Karamojong of Uganda are pastoralists, but do not associate insects or arthropods with specific diseases. Understanding these factors is essential to carrying out disease investigations and designing control programmes that work*" (Jost et al., 2007, 539).

3.1.4. Summary of the review

Overall, the literature review indicated that it is not the norm to address aspects of power and plurality in PE literature. Using local terminology is common practice, and emphasised as being important, but beyond that, language is also engaged with to a limited extent. For example, none of the studies reviewed here describes the process of translation, the potential lack of clear correspondence between local and English disease names or veterinary textbook descriptions, or potential variations in knowledge and terms used within local communities. Clearly there is limited space in an article to develop all these aspects, but the almost non-existent engagement with plurality and

[1] Of the remaining publications, hits 21 – 31 were cited between 15 and 10 times and hits 31 – 52 between 10 and 5 times. Hits 92 – 158 were never cited.

power, the limited engagement with language, and the associated common practice of reporting on means or majority (or "consensus") perspectives still indicate that there is room for increased engagement with these issues within PE.

3.1.5. The authors' stories from the field

3.1.5.1. Plurality. Katja conducted a participatory field study with German hunters about the acceptability to them of different surveillance strategies for classical swine fever (CSF) in wild boar (Schulz et al., 2016, 2017). Some of the hunters lived in western Germany, others in eastern Germany. Prior to Germany's reunification, all hunting activities and disease control in eastern Germany were regulated by the state. For instance, ownership of a weapon and allocation of hunting grounds were controlled by the government. While this has now changed, historical differences were reflected in the manner of participation. Katja sensed that the hunters from eastern Germany felt more obliged to show their commitment and willingness to collaborate with the research institute where Katja worked than hunters in western Germany. At the same time, the hunters from eastern Germany expressed their feelings in general and any dissatisfaction during the participatory exercises to a lesser degree than the hunters from western Germany. This example indicates the importance of understanding historical and contextual differences between groups who seemingly share the same culture and language.

In a PE study on Basongora pastoralists' local priorities, perceptions and practices regarding cattle disease in Uganda, Klara and Erika aimed to take the diversity of voices and possibilities within communities into account (Chenais and Fischer, 2018). Effort was made to ensure sufficiently homogenous groups, for example by separating out women as well as herders (i.e. those working for others taking care of their animals). It was much harder to gather herders together than the other groups. Klara and Erika were continually being told that 'the herders are out in the pastures' even when they tried to start early or end late to make sure that the herders would be in the village. One FGD with herders was achieved and additional individual interviews were performed, meeting the herders in the grazing lands. In general, the herders were less eager to talk and it was harder to obtain their views than those of the animal owners. Clearly longer term field work would have facilitated the building of trust and a better understanding of the local context so as to be able to fully pick up this group's perspectives.

One strategy for ensuring that a diversity of perspectives was reached, not only with regard to gender but also taking account of locational and class aspects, was the undertaking of a participatory mapping and wealth ranking exercise (Chenais and Fischer, 2018). The exercise indicated that there was no notable difference in the distribution of the wealth ranks of households participating or not participating in the FGDs. However, one geographical area, where many widowed or divorced women lived, emerged as being under-represented. According to the key informants, these women did not feel comfortable with coming to a meeting and had therefore been left out. If the participatory mapping had not been done, this omission would not have been noticed during the short stay in the village.

In 2019, Erika led a PE study of peste de petits ruminants (PPR) in an eastern European country. The aim was to investigate temporal and geographical distribution, herd prevalence and case fatality rates in affected herds. The study also aimed to contribute knowledge about local sheep and goat management, drawing some conclusions on how management factors might affect the local epidemiology. The research partner at central administration level prepared all the logistics and did the practical planning of the fieldwork. The selection of participants had been delegated to district veterinary officers. Erika was told that it would be difficult to recruit women-. This was justified by comments such as "*the women don't want to/aren't able to come to public meetings*", "*these are traditional communities and those women stay at home*", and "*it's the men who are involved in the small ruminant business*". The people mobilising participants were all men. This probably aggravated the reluctance to involve the women and the women's reluctance to attend meetings. In the end, many of the FGDs were mixed male/female, often consisting of family members, with an obvious patriarchal hierarchy in place. In these cases, the facilitator had to be constantly reminded not to reinforce these structures, to make sure that the seating was arranged to engage the women, that speaking times were equally allocated, and that the women were specifically asked to lead the discussion using the PE tools. Another observation was that after insisting on it throughout the fieldwork, towards the end more female participants were included, even though the majority were still male. However, if Erika and her colleague had accepted the excuses for why the initial representation of women was so low despite the instructions given for recruitment, very few women would have been included.

3.1.5.2. Power. In Katja's study with German hunters, the fact that she was a young woman of no perceived authority or threat to the hunters, and that she expressed a genuine interest in these hunters' perspectives, helped build trust and overcome their historical resistance to government authorities, indicating the importance of choosing a facilitator with whom interviewees are comfortable and willing to speak freely. Katja drew on some PE tools designed to collect one group response, thus requiring the group to achieve consensus. A hierarchical structure could be observed in each group. There was always one person who was the most dominant and insisted on his opinion being heard. Katja, facilitating the exercise, could ensure in such instances that those who had less of a chance to express their views were also heard. These discussions leading up to the final consensus statement were noted down, as were the disagreements, and were considered in the descriptive analysis.

In Klara's and Erika's study with Basongora pastoralists in Uganda, the local research team consisted of a facilitator and a note-taker who were both male veterinarians, and a male translator who was not a veterinarian but who had previously worked with other researchers in the region. Klara and Erika made conscious efforts to ensure free discussion and that everyone was heard during FGDs, but they had to continuously remind the facilitator to allow marginal voices to be heard and not to forge consensus. Despite such reminders, the facilitator habitually paid more attention to those more articulate in the group. One possible reason for this is that the high status and education of the veterinarian-facilitator made him unused to embrace what to him were seemingly uneducated responses.

In the Basongora study, exactly the same number of all-female and all-male FGDs were achieved, and an attempt made at least to tackle power relations such as wealth, but there was still a failure to fully account for other power relations. For example, it turned out that all the participants in one all-female FGD were related, consisting of a mother/mother-in-law and her daughters/daughters-in-law. In this group the gender balance was what was aspired to, but power relations such as age, family status, experience and family ties still hindered an open discussion.

Erika and her colleagues conducted a study on the knowledge, attitudes and practices (KAP) concerning ASF among smallholder pig-owners in Uganda (Chenais et al., 2015). Data were collected in FGDs using PE tools such as seasonal calendars, proportional piling, listing and rankings, facilitated by a local veterinarian trained in PE. Answers were collected at group level. Using the participatory tools, one person would start proportional piling, for example, and the group would discuss and often change the position of the beans used as markers until the facilitator perceived that the group had reached consensus. In some cases, this consensus was clearly forged, with the stronger voices being the ones heard and noted as the group's final answer. In other cases, the facilitator managed to navigate situations when one or a few persons dominated and ensure that everyone was heard and included in the discussion. Other tools, such as listing, allowed all the individual answers to be noted down, but more dominant participants still influenced the voices of the less dominant. In an analysis of the results, only the quantitative and semi-quantitative/qualitative results were used and the group was the reporting unit for the statistical testing.

3.1.5.3. Language. Uganda has more than forty recognised languages, with English and Swahili given status as national languages. All schooling is in English. In Erika's study on ASF in Uganda, the FGDs were conducted in the local language (Luo), with notes written down in English by a note-taker. The facilitator and the note-taker were native Luo speakers, but had been educated in English from nursery to university. Luo is rarely used in writing or for talking about more technical and scientific matters, making some words difficult to translate directly from English into Luo. During the research Erika also noted that there were some false friends[2] between the British English she spoke and the Ugandan English spoken by the facilitator and note-taker. Former British colonies that have English as their official language have continued to develop their own version of English since independence (Isingoma, 2014). For example, with regard to family relations, the terms Erika used for brothers, sister and cousins were not the ones used in Ugandan English. Another false friend is "hotel", which in Ugandan English means what in British English is referred to as "restaurant". This is important because restaurant swill is a potential transmission route of ASF.

In their PE study with Basongora pastoralists in Uganda, the fact that the country has so many co-existing languages again came up as a relevant methodological issue. In this study, the pastoralists spoke Rusongora, whereas the local research team (the translator, facilitator and note-taker) spoke the related language Rutoro, the language of one of the dominant ethnic groups in the region. That the facilitator and translator did not speak the same language as the pastoralists was only made known to Klara and Erika at the beginning of field work, as this was not seen as relevant information to communicate by the local partners. Rutoro and Rusongora share many words and people speaking Rutoro and Rusongora live next to each other and are used to speaking together. Rutoro is more commonly spoken by local government authorities, including by the local veterinarian. As a result, the pastoralists were used to hearing animal disease names in Rutoro. Nevertheless, the fact that the local research team did not speak Rusongora, the native tongue of the pastoralists, would have limited the nuances that could be grasped in the research. This was addressed by including two of the Basongora key informants as translators and facilitators during some of the later FGDs and by discussing disease names with them.

The local research team had a strong urge to find and use the "correct" names of the diseases and force participants' descriptions of syndromes into scientifically accepted disease nomenclature. For example, participants described three different syndromes of "*fever*": fever, tick-borne fever and East Coast Fever (ECF). There was insufficient thoroughness when discussing how to translate the diseases with the local research team, and it was for example not discussed beforehand how to allow in translation for an openness about there possibly not being a one-to-one relationship between local and formal, as well as between Rusongora and English disease names and meanings. As ECF in Rusongora is translated as high fever, it is likely that this term groups together several reasons for a high fever, and thus that some of what was reported as ECF might in fact have been other forms of high fever in cattle. There was also a syndrome translated as "*ECF in calves*", which might equally have been translated as "*high fever in calves*" and might actually be the same disease as another syndrome translated as "*diarrhoea (and fever) in calves*". Awareness of this discrepancy between English and Rusongora terms for ECF came in the fieldwork, and then a more thorough discussion was held of what the different disease names in Rusongora meant and the signs with which they were correlated, without connecting them initially to an English term.

In the 2019 PPR study, Erika found out at the first meeting with the research team that the team members trained in PE could not join the field work due to other engagements, and that a replacement facilitator, who had never worked with PE before and was not fully proficient in English, had been selected. She subsequently found out that not all the participants spoke the national language because some were originally from a neighbouring country and spoke the language of that country. None of the research team (including the translator) spoke the neighbouring country's language. One FGD consisted only of women originating from the neighbouring country who did not speak the national language. The research team had to deal with this spontaneously on site and used one member of the community to act as a translator. It proved to be impossible to have the translator translate without answering himself when he knew the answer or adding to or deducing from the answers. In one village, the team wanted to reach more women as they were underrepresented, but none of these women spoke the national language and the spontaneously recruited translator was a man, which seemed to hinder free and open communication. It became obvious that in order to acquire some useful data from these women, a different translator would have been needed to allow for freer communication, and a longer stay in the community would have been useful to gain trust and enable direct observations for triangulating the data.

Katja's study with German hunters stands out amongst the examples presented here. No translation was needed as both the research team and all the hunters spoke German. This meant misunderstandings due to language and translation biases could be avoided. It also enabled Katja to pick up the different emotions and read between the lines. A few chats during the meetings and shared jokes certainly helped break the ice and create a pleasant environment for the participants.

4. Discussion

The engagement with research on democratic deliberation and the public sphere (Fraser, 1990; Hagendijk and Irwin, 2006) in this article has helped provide a theoretical grounding and deeper understanding of why it is important to give consideration to the organisation of participatory research. The work of Fraser (1990) on counter publics can offer an understanding of the relevance of dividing groups so that they are less heterogeneous with regard to different aspects of social stratification (beyond gender alone). One way of identifying such counter publics can be to perform participatory wealth rankings, where all households in a community are ranked according to their relative poverty or wealth, based on local definitions as identified in FGDs (Jacobson, 2013). This works well in some communities, but not in others, depending on the varying sensitivity of discussing this issue across cultures. Another strategy can be to actively target individuals and households that do not show up or are particularly quiet at group meetings, and arrange separate, individual or group interviews with them (Bedelian et al., 2007; Chenais and Fischer, 2018).

However, even when organising more homogenous groups, groups will never represent one voice alone. For example, it can be expected that a group might agree on a list of all the problems related to cattle rearing in the community, but there is more individual variation regarding the relative importance of the diseases. We would suggest that it might be relevant to think beyond consensus in FGDs and to note and analyse minority views as well. This is possible when using traditionally consensus-oriented PE tools. As shown by Katja's example with German hunters, there is nothing to prevent the researcher noting down minority perspectives, for example while performing a proportional piling exercise, and then including this data in the analysis. However, this requires the researcher to be able to catch nuances in group discussions. This was clearly easier for Katja as she spoke the same language and

[2] The term "false friends", when used in linguistics, refers to pairs of words from different languages or dialects that sound or look the same but that have different meanings. For example, Norwegian and Swedish languages are so similar that people speaking either language can often understand each other well. However, the term "rolig" means calm in Norwegian but funny in Swedish, which frequently causes confusion.

came from the same culture as the participating German hunters.

While Katja's case with the German hunters shows the importance of being able to read between the lines for the facilitator to allow minority voices to be heard, this is difficult when the researcher does not speak the same language as the study participants and places high demands on the facilitator (Bedelian et al., 2007). Theories on democracy and public deliberation (Arendt, 1998; Roberts-Miller, 2005), as well as the authors' own experiences presented here, indicate that some guiding principles for facilitators might be the ability to listen, create an open discussion climate, invite participants to share their knowledge, show interest and not lecture, as well as be sensitive and reactive to group dynamics and balance the discussion between participants. The authors' own experiences, and those of others (Berreman, 2012), indicate that facilitators sharing a culture with participants is no guarantee that these guiding principles are better adhered to; neither does it guarantee the collection of more valid and relevant data. Well-educated facilitators, for example, might judge local knowledge as irrelevant since it comes from people perceived to be uneducated or who for other reasons are judged not to contribute relevant information on the topic. In Erika and Klara's study with Basongora pastoralists, for example, the local research partner had only included animal owners, and not herders who spend a considerable time with the animals, in the initial FGDs. during FGDs the facilitator clearly prioritised formal textbook knowledge over statements considered as uneducated. Likewise, local gender relations can result in preconceived ideas about women's engagement in and knowledge of animal husbandry (Petitt, 2016). Thus, Erika's PPR study indicates for example that the local research partners did not see any reason to include women in the study. If researchers in such cases follow local recommendations to exclude women, their research might serve to cement existing cultural practices of devaluing women's work with animals and prioritise the animals traditionally seen as "men's", while ignoring species more commonly associated with women. Local people might also be uncomfortable with being completely honest with local facilitators about practices that they know are advised against by veterinarians. Likewise facilitators might want to exclude more problematic or embarrassing local practices from information given to the research team (Borchgrevink, 2003). Therefore, while it is important to choose facilitators and translators who can act as brokers with the local culture, it is essential to be observant of any negative effects on data collection from the dynamics between the facilitator and participants. It is wise to work with more than one facilitator in order to be able to triangulate the data gathered through different facilitators, and to select facilitators with whom each group of participants is comfortable talking to, e.g. based on local class and gender dynamics (Borchgrevink, 2003; Bujra, 2006; Skjelsbæk, 2016). The literature review indicates that the basis for selection of facilitators is rarely discussed. The authors' own experiences indicate that veterinary PE studies frequently prioritise linguistic and veterinary competence over communication and cultural skills for both facilitators and translators. From her KAP study on ASF in Uganda, Erika described how the facilitator was a local veterinarian and thus a local figure of authority. He spoke the same language as the local participants (Luo), but viewed from a different perspective he spoke the formal disease language of veterinarians and not the situated disease language of the participants. Since Erika was only able to observe the discussions without being able to speak the local language herself, she had limited opportunities to intervene. Despite this experience teaching her to be aware of the difficulty of obtaining good data on local peoples' views when using people in authority as facilitators, it still proved difficult to recruit facilitators who were familiar with and sensitive to the perspectives of the local community in subsequent studies. A key reason for this was that local partners saw expert competence as essential. Similar experiences are highlighted by Jost et al. (2007). In the authors' experience, local partners who mobilise participants for FGDs are often men and veterinarians, which frequently seems to encourage prioritisation of people known by the mobiliser (i.e. those who have the means to call a veterinarian when needed, for example) and the exclusion of people who the mobiliser does not think have the relevant competence to discuss the disease studied. People of perceived lower status or education, such as women and herders, are frequently excluded. In Erika's case on PPR, these groups were indeed initially excluded by the mobiliser, despite Erika specifically asking to meet them. This could be referred to as 'mobiliser bias' in PE, similar to how Chambers (1997, pp. 13) has talked about 'roadside bias', both having to do with only accessing those who are easiest to reach.

While writing this paper, it was surprising to find that language is discussed to such a limited extent in the reviewed social science and traditional PRA literature, despite reporting on research where a translator has obviously been needed. PE is a child of older participatory research, which in turn is inspired by anthropology. As shown here, anthropology has hardly engaged at all theoretically or methodologically with the multilingual empirical situations in which it is frequently performed (Borchgrevink, 2003). Despite this, the review indicated that PE takes language into account to a greater extent than traditional PRA does. However, this frequently stopped at pointing out the importance of collecting names of diseases and syndromes in local languages. In line with Borchgrevink (2003); Skjelsbæk (2016); Gibb and Danero Iglesias (2017) and others, the authors' view is that this needs to change – not only in PE but, it seems, in the vast majority of research performed in multilingual contexts. With regard to PE in particular, it is important that translators are sensitive to local ways of linguistically constructing the world, and that they are able to convey this to the researcher to ensure that local disease or symptom descriptions are not falsely forged into formal nomenclature (Queenan et al., 2017). Many of the aspects raised in this paper are easier to consider if more time is spent in the field, thus providing greater insight into the local culture, however it is expected that most PE studies will continue to face time constraints. It is therefore crucial to endeavour to include minorities and local perspectives also when working within limited time frames. Some strategies for doing so have been suggested in this paper.

5. Conclusions

In conclusion, the methodological development of PRA and PE would benefit from engaging with work undertaken on the public sphere, public deliberation of science and language studies. In summarising what these bodies of literature suggest, it is clear that striving for consensus frequently leads to weaker voices being marginalised in favour of a 'consensus of the privileged'. In this regard there needs to be awareness of the trade-off between forging consensus and understanding the perspectives of the most marginalised. One strategy to address this is to have separate FGDs based not only on gender, but on other forms of social stratification as well, noting nevertheless that the groups constructed cannot be expected to be completely free of power and plurality. Moreover, attention needs to be paid to the role of researchers here and how they perceive their own knowledge in relation to that of others. Facilitators and interpreters need to be carefully selected and there is a requirement for ongoing dialogue with them about the research situation and translation, and about the importance of trying to see the world from the study subjects' perspective.

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Appendix A. Supplementary data

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4.2.2 Acceptance studies

Participatory methods are particularly suited to evaluate the acceptability of defined measures by affected persons (Calba et al., 2015b, Calba et al., 2016). Following Meynard et al. (2008), acceptability is the "willingness of users to be involved in the operation of the system". The Centers for Disease Control and Prevention (CDC) mentioned acceptability as one of the main qualitative characteristics within disease surveillance (German et al., 2001). Within the framework of animal diseases, it is often the farmer, the farm veterinarian or, in case of wildlife diseases, the hunter, whose cooperation is essential for successful disease surveillance and control. Several studies have hypothesised that weaknesses in animal disease surveillance systems or ineffective disease control measures may be due to insufficient involvement of the key stakeholders concerned. These studies showed that the application of participatory methods can help to identify hindering factors decreasing the acceptability or motivational options, which have the potential to increase the willingness of key figures to participant in the system and support the effectivity of proposed measures (Pfeiffer, 2013, Bronner et al., 2014, Calba et al., 2016, Schulz et al., 2016, Ciaravino et al., 2017).

Schulz et al. (2016) could show that hunters oppose certain measures within the surveillance of CSF in wild boar. They listed several reasons, why hunters were not willing to support passive surveillance, i.e. the detection, reporting and sampling of wild boar carcasses. However, particularly with regard to ASF in wild boar, passive surveillance is one of the most important tools to advance disease detection and control. The willingness to report noticeable events is crucial, particularly in the surveillance and control of emerging infectious diseases (National Research Council Committee on Achieving Sustainable Global Capacity for and Response to Emerging Diseases of Zoonotic, 2009). Thus, in case of ASF, the willingness of hunters to support passive surveillance is crucial to control the spread of ASF. In Estonia and in Latvia, a participatory study was conducted to investigate the acceptability of hunters for certain ASF control measures and for motivational options to support passive surveillance.

- Contribution **(Publication 18)**: URNER, N., MÕTUS, K., NURMOJA, I., SCHULZ, J., SAUTER-LOUIS, C., STAUBACH, C., CONRATHS, F. J. & SCHULZ, K. 2020. Hunters' Acceptance of Measures against African Swine Fever in Wild Boar in Estonia. Preventive Veterinary Medicine, 182, 105121. https://doi.org/10.1016/j.prevetmed.2020.105121.
- Contribution **(Publication 19)**: URNER, N., SERŽANTS, M., UŽULE, M., SAUTER-LOUIS, C., STAUBACH, C., LAMBERGA, K., OĻŠEVSKIS, E., CONRATHS, F. J. & SCHULZ, K. 2021. Hunters' view on the control of African swine fever in wild boar. A participatory study in Latvia. Preventive Veterinary Medicine, 186, 105229. https://doi.org/10.1016/j.prevetmed.2020.105229

Identifying differences and similarities between these two studies and thus emphasizing the motives of hunters to prefer or refuse certain control measures opens up the possibility to address and improve disease control accordingly. Furthermore, by comparing the studies, external factors influencing the quality of the studies were investigated and discussed.

- Contribution **(Publication 20)**: URNER, N., SAUTER-LOUIS, C., STAUBACH, C., CONRATHS, F. J. & SCHULZ, K. 2021. A comparison of perceptions of Estonian and Latvian hunters with regard to the control of African Swine Fever. Frontiers in Veterinary Science, 8. https://doi.org/10.3389/fvets.2021.642126

4.2.2.1 Hunters' Acceptance of Measures against African Swine Fever in Wild Boar in Estonia

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URNER, N., MÕTUS, K., NURMOJA, I., SCHULZ, J., SAUTER-LOUIS, C., STAUBACH, C., CONRATHS, F. J. & **SCHULZ, K.** 2020. Hunters' Acceptance of Measures against African Swine Fever in Wild Boar in Estonia. Preventive Veterinary Medicine, 182, 105121. https://doi.org/10.1016/j.prevetmed.2020.105121

Highlights

- Estonian hunters perceived the contact to higher ministries as one-way contact just receiving orders
- Hunters trusted themselves the most in the implementation of ASF control measures
- Hunters supported control measures, which involve increased hunting activities and reject measures including hunting restrictions
- Incentives and the reduction of the infection pressure were perceived as motivating to support passive surveillance

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Hunters' Acceptance of Measures against African Swine Fever in Wild Boar in Estonia

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ABSTRACT

African swine fever (ASF) was first identified in Estonia in 2014, initially detected in wild boar and spreading to affect almost the whole country from late 2016 onwards. Passive surveillance and the control measures applied in Estonia are the main actions in the attempt to control the wild boar population and therefore limit the spread of ASF. Implementation and success of both activities depend mainly on the involvement and commitment of the executing force: the Estonian hunters. Thus, their acceptance of the measures is of utmost importance and with the help of participatory methods, their acceptability can be assessed.

Participatory epidemiology allows the involvement of key stakeholders in planning control measures and surveillance strategies and gathering information otherwise inaccessible. By conducting focus group discussions and utilizing participatory tools, this study aimed to assess the acceptance of ASF control measures by hunters in Estonia. Furthermore, the study aimed to detect means to improve the motivation of hunters to support passive surveillance.

Among hunters, the results ranked the trust in lower authorities (e.g. local official veterinarians) towards implementing control measures as high (in contrast to higher officials e.g. 'Ministry of Rural Affairs'), while perceiving themselves as the most trustworthy group among those implementing ASF control measures. Hunting and every measure supporting increased hunting, for example selective hunting, bait feeding and incentives for hunting wild boar, were deemed favourable for hunters. These measures also received the highest trust for controlling ASF. All measures hindering hunting and the movement of wildlife, for example fencing or involvement of the army in ASF control, were described as unpleasant or even unethical and trust in these measures to control the disease successfully was lacking. When assessing the perceived consequences for hunters of finding a dead wild boar, arising financial costs, additional workload and time consumption were highlighted. In line with these results, the two tools with the strongest motivational effect for taking part in passive surveillance were: (1) higher monetary incentives as compensation for the hunters' work, and (2) the reduction of the negative consequences by limiting the hunters' duties to solely reporting found dead wild boar.

In conclusion, participatory methods can be used as a highly suitable tool for the evaluation of acceptance of measures and surveillance systems. Potentially, the results can help to improve control and passive surveillance in Estonia, as well as functioning as an example for other countries battling or awaiting ASF.

1. Introduction

The epidemic wave of African swine fever virus (ASFV) genotype II starting in Georgia in 2007 appears to be unrelenting (EFSA, 2010). Huge geographical leaps amid new introductions across borders of the Caucasus region, the Russian Federation, Poland, Estonia, Latvia,

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Lithuania, Czech Republic, Ukraine, Romania, Bulgaria, Hungary and Belgium (2007-2018) indicate the impactful role of humans as a distributor for African swine fever (ASF) (EFSA, 2010, 2015, 2018b). Once introduced into a wild boar population their movements can in addition cause further spread on a local level (Podgorski, 2017). After the introduction of ASF into Estonia, presumably in autumn 2014, the disease progressed from East to West. By the end of 2016, the entire country with the exception of the island of Hiiumaa was considered as an ASF infected area (Schulz et al., 2019b). In several studies, potential associations between ASF outbreaks in domestic pigs and infected wild boar in the close vicinity were suggested (Olsevskis et al., 2016; Nurmoja et al., 2017a; Nurmoja et al., 2017b; EFSA, 2018b; Nurmoja et al., 2018).

The important role of wild boar in the spread of ASF and its establishment in a country is evident from the course of the disease in Estonia and other affected countries (Olsevskis et al., 2016; Nurmoja et al., 2017b; Pejsak et al., 2018). Consequently, Estonia focused its control measures on decreasing the wild boar population with the aim of limiting the local spread of ASF. Control measures have been decreed by the government after detection of an ASF-positive wild boar with the goal of controlling the disease. Thus, hunting more sows and increasing the number of driven hunts are flanked by extended biosecurity rules in handling wild boar carcasses, baiting wild boar for more efficient hunts and paying incentives for hunted wild boar. In Estonia, like in many other countries, implementation of the control measures regarding ASF in wild boar relies mainly on hunters.

In any case, the role of hunters in disease surveillance is of utmost importance.

Since the probability of detecting ASF is considerably higher in wild boar found dead, passive surveillance is crucial to control the disease. In countries free of ASF, it is extremely important to detect disease introduction as early as possible (Nurmoja et al., 2017b; Schulz et al., 2019a). Passive surveillance describes the sampling of animals found dead, shot due to sickness or killed in a road traffic accident. Due to their regular presence in the hunting grounds, hunters are more likely to find dead or diseased wild boar. Without their willingness to implement control measures and to support the surveillance activities stipulated by the authorities, disease control will become extremely difficult or even impossible. Although hunters play a key role in wild life management, to this date only few participatory studies have been conducted with this occupational group (Calba et al., 2015a; Schulz et al., 2016). Involving important stakeholders in planning control measures and surveillance strategies can be achieved by using methods of participatory epidemiology (PE), a scientific area able to give a voice to communities while increasing the understanding of their situation. Thus, experts have highlighted the importance of integrating critical stakeholders (Allepuz et al., 2017), which, in the case of combatting ASF, translates into the integration of e.g. local hunters (EFSA, 2018a).

Moreover, including participatory methods in research can help to gather information that can otherwise potentially stay inaccessible (Catley et al., 2012; Bronner et al., 2014; Calba et al., 2015b; McKerr et al., 2015; Schulz et al., 2016; Bach et al., 2019). The ability of these approaches to dissect complex issues from different viewpoints leads to the avoidance of impractical measures based on the lack of knowledge of key stakeholders' perspectives (Catley et al., 2012; Allepuz et al., 2017; EFSA, 2018a).

While the interaction of social sciences and public health has led to new methods and tools well implemented in developing countries, they are far less prevalent in developed countries, especially in the area of veterinary epidemiology (Chambers, 1994; Roeder and Rich, 2013; Calba et al., 2015a; Reix et al., 2015; Schulz et al., 2016; Chenais et al., 2019). The use of focus group discussions in combination with visualization, ranking and scoring methods results in numerical data which can then be analysed using conventional statistical tests (Catley et al., 2012).

Estonia was chosen as an ASF-affected country for this study to make use of the already accumulated knowledge and the experience of hunters regarding the implementation of ASF control measures and passive surveillance during the ongoing epidemic.

In the present study, by utilizing participatory epidemiological methods with Estonian hunters as the key stakeholder, we aim to assess the acceptance of ASF control measures and the possibilities to increase their motivation to support passive surveillance.

2. Material & Methods

2.1. Recruitment of participants

Hunters from multiple different regions of Estonia were recruited, leading to the inclusion of in total six (Tartu, Ida-Viru, Harju, Järva, Pärnu, Saare) out of 14 ASF-affected Estonian counties in the study (Fig. 1). The selection of these areas was based on the intention to gather information based on a variety of experiences throughout the epidemic waves of ASF. Areas of all regions of Estonia and areas with various density of ASF positive wild boars were chosen. From April to June 2019, hunters were contacted in co-operation with the Estonian Hunters' Society (EHS). With approximately 11,000 members (90% of all active and licenced hunters), the EHS is the largest hunting organisation of Estonia. The EHS offered a list of potential contact persons on a regional/local level, mostly leaders of regional/local hunting organisations. They were informed, both in writing and verbally, about the aims of the study, logistic principles of the meetings including confidentiality and recording. Based on this framework, the group leaders invited hunters to participate in a meeting. The criteria for the acquisition of hunters was their willingness to participate and experience with implementing ASF control measures.

2.2. Meetings

For each meeting, the aim was to recruit three to six hunters as a focus group for discussions. All meetings were moderated by the same, previously trained scientist. The entire communication was audio-recorded with a Jabra Speak 510 microphone. Prior to the meetings, all participants had given permission for the recording of the discussions and for anonymous publication of the results. The discussions were held in Estonian language and later translated into English by the Language Centre of the Estonian University of Life Sciences in Tartu.

2.3. Methods of focus group discussions

Participatory methods were adapted from Schulz et al. (2016) and Calba et al. (2015a). All analyses were performed semi-quantitatively and the results of the discussions were included descriptively.

2.3.1. Acceptability of control measures

By decree of the Environmental Board, 50% of all hunted wild boar must be female and 50% sub-adults (6-12 months). Furthermore, hunters are obligated to notify the Veterinary and Food Board (VFB) regional office by email concerning all wild boar hunted or found dead. For hunted wild boar, the information must contain the name of the hunter, phone number, date of hunting, the hunting licence number, sex of the hunted wild boar, age estimation and the place of hunting. Hunters must also take blood samples, mark and pack the tubes, fill in the laboratory submission form and bring the samples and the forms to the VFB local office. The biosecurity rules stipulate that all hunting equipment (vehicle, boots, clothing etc.) has to be cleansed, washed and disinfected after hunting. Vehicles used to transport hunted wild boar and animal by-products must be leak-proof. The storage rooms for hunted wild boar and equipment in hunting lodges must be cleansed and disinfected. Hunted wild boar must be kept in a cold storage room until the ASF test results have arrived (ASF-positive carcass must be placed into a special, separate container) and removal of the carcass from the hunting ground is forbidden until the results are available. If the test is

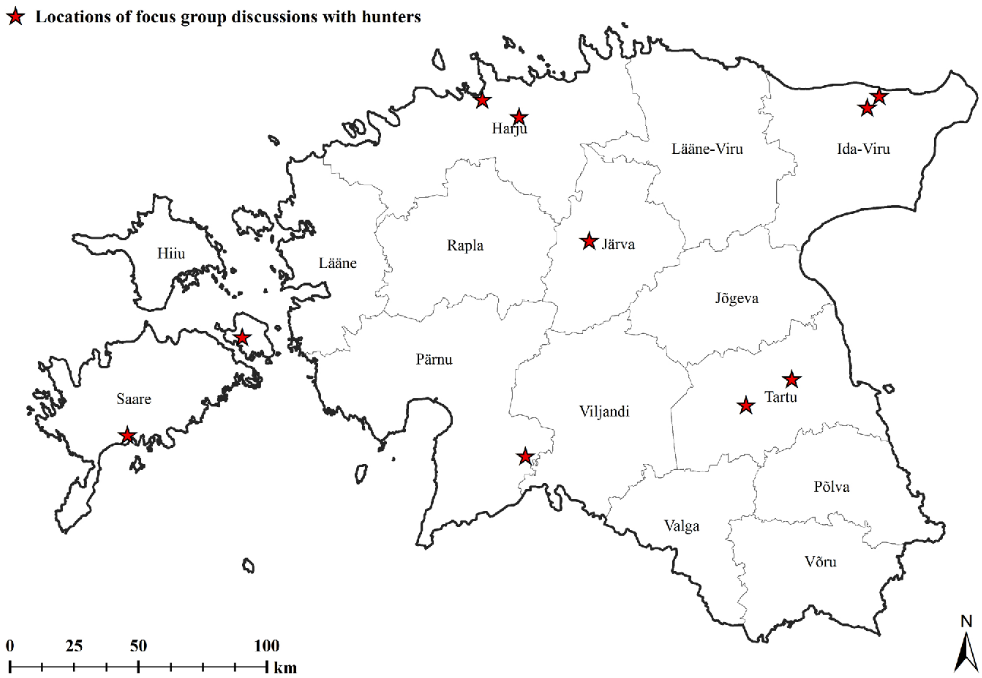

Fig. 1. Locations of the focus group discussion meetings with hunters in the counties of Estonia.

positive, the storage room, all equipment and transportation means must be disinfected under the supervision of veterinary inspector/official veterinarian. Offal of all wild boar is collected in containers and must not be left in the field. To evaluate the acceptability of control measures against ASF in wild boar, two different investigations were carried out (Supplemental material Table 1).

2.3.1.1. a. Relation diagram. Initially, the hunters were asked to list all stakeholders they believed to be involved in the design and implementation of measures to control ASF in wild boar (Supplemental material table 2). The facilitator had listed the hunters as a definite stakeholder beforehand. Simultaneously, the hunters were asked to describe the presumed role of any stakeholder they had named within the framework of control measures for ASF in wild boar. When all stakeholders who were believed relevant had been named, the hunters were asked to describe the relationships between each stakeholder and the hunters in a quantitative fashion. To this end, a group consensus on the quantity and direction of each relationship was reached by discussion between the participants, with the result visualised by an arrow (Fig. 2). If all participants agreed that there was no contact between themselves and the respective stakeholder, no arrow was drawn.

After final consensus was reached, all relationships were described qualitatively. Each hunter received three different smileys. In comparison to the first steps, each hunter was asked to individually visualise his/her satisfaction with the liaison by rating each relationship and its quantity separately by using the appropriate smiley (Fig. 2).

Finally, an evaluation step of the hunters' trust towards the performance of the listed stakeholders in respect to the control measures was executed. To visualize trust, 100 glass beans were provided to the discussion group. The participants were asked to discuss and, after finding consensus, to place the glass beans proportionally to their trust in the listed stakeholders.

2.3.1.2. b. List of control measures. Six different control measures for ASF in wild boar were presented to the hunters:

- Fencing
- Ban of hunting
- Including professionals for intensive hunting (police/army)
- Increased hunting of female wild boar
- Incentives for hunting
- Increased carcass search and removal

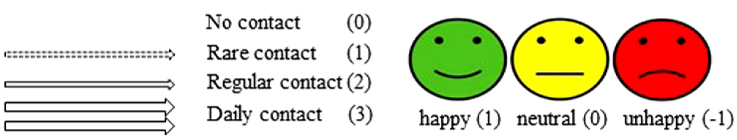

Fig. 2. Visualisation tools used in the participatory study with hunters in Estonia. The three different arrows illustrate the quantity of contacts in the relation diagram (assigned to ranks ranging from 0 to 3). Smileys helped to illustrate the hunters' feelings (assigned to ranks ranging from -1 to 1).

The six control measures were chosen, because they had already been in place in Estonia for some time (Supplemental material Table 1) or are widely discussed in the ASF community. After ensuring a shared understanding of the listed measures, the hunters had the chance to add further control measures (Supplemental material table 2). Using the smileys, the hunters should demonstrate their personal evaluation for each of the listed control measures (Fig. 2). To describe their trust in the effectivity of these measures, proportional piling was used as described above. Proportionally to the trust of the hunters, 100 glass beans were attributed to the listed control measures after reaching a group consensus.

2.3.2. Acceptability of passive surveillance and different motivation options

If a wild boar is found dead, an official from the VFB regional centre or another official veterinarian must take samples (tubular bone or organs like kidney, spleen, lymph nodes) and give instructions regarding the burial of the cadaver or the disposal in a specific container for wild boar carcasses. Burial is carried out by hunters. The burial hole must be at least 0.5 m deep and the burial place must be disinfected. If burial is not feasible, the container collection system for positive wild boar must be used. Containers are placed in the areas tested positive for ASF in wild boar, often close to the primary processing establishments (approx. 100 containers in Estonia). The rendering plant replaces containers (locked and solely used for wild boar carcasses and wild boar offal) with empty containers at least once a week, based on the information received from the responsible hunting club. Containers are collected by a separate truck, cleaned and disinfected every time. Full containers are taken to the rendering plant for incineration. Hunters are provided with disinfectants for their vehicles. They are responsible for transporting carcasses from the location of detection to the container. They are compensated with 70 € incl. VAT for burying a dead wild boar and 42 € incl. VAT for transporting carcasses to a container. The evaluation of the hunters' acceptability to support passive surveillance and different motivation tools to increase passive surveillance was also divided into two parts.

2.3.2.1. a. Impact diagram. To assess the perception towards the detection of dead wild boar and the knowledge about potential consequences, an impact diagram was utilized. The hunters should state all positive and negative consequences of detecting a dead wild boar that came to mind (Supplemental material table 2). Subsequently, the group was asked to discuss until a consensus had been reached and then distribute 100 glass beans proportionally among the listed consequences to visualize the impact on the hunters. Consequences were divided into positive and negative.

2.3.2.2. b. List of motivation options to increase passive surveillance. The following four options deemed to increase the motivation to support passive surveillance were presented to the hunters:

- Increase of currently paid incentives
- Reduction of infection pressure in the wild boar population
- Only reporting dead wild boar without any further work for the hunter
- Detailed feedback from the relevant authority to the hunter

Again, the hunters could add further items. After completing the list, proportional piling was used to assess the hunters' trust in the efficacy of different motivation tools (Supplemental material table 2). Correspondingly, the hunters were asked to visualize which motivational tools were most likely to be successful in their opinion by distributing 100 glass beans proportionally.

Any additional motivation options named by the hunters were in each case only mentioned in one group and listed in the supplementary information (Supplemental material Table 1).

2.4. Quantitative analyses of focus group discussions

2.4.1. a. Arrows

Each arrow was assigned to the appropriate rank (Fig. 2) and including the results of all groups, for each relationship, the mean of the quantity of the contact was calculated.

2.4.2. b. Smileys

The smileys, illustrating the quality of the relationship between hunters and the appropriate stakeholders were assigned to the corresponding ranks (Fig. 2). The mean of the ranks assigned by each hunter was calculated to obtain one value for each relationship for each focus group. Subsequently, the means of all groups were calculated for each relationship.

2.4.3. c. Proportional Piling

The analysis of all proportional piling tools is shown by the example of the relation diagram regarding the acceptability of control measures.

The average trust T_{SH_i} for all stakeholders SH_i ($i \in \{1, \ldots, 27\}$) listed in all groups was calculated using the results of the proportional piling exercise. To account for the varying number of stakeholders listed by the ten groups, the number of glass beans GB_{ij} received for stakeholder SH_i from group G_j with j $\in\{1,\ldots,10\}$ was weighted with the proportion P_j

$$P_j = \frac{C_j^{SH}}{\sum_{j=1}^{10} C_j^{SH}},$$

with C_j^{SH} the number of stakeholders SH noted by group G_j. Thus, the average trust T_{SH_i} was calculated by

$$T_{SH_i} = \frac{1}{N_{SH_i}} \cdot \sum_{j=1}^{10} P_j \cdot GB_{ij},$$

with N_{SH_i} the number of groups G calling stakeholder SH_i.

The analysis of the proportional piling of the list of control measures (1b), the impact diagram of consequences (2a) and the list of motivation options to increase passive surveillance (2b) was done as described by replacing the stakeholders and the number of stakeholders noted by the group with the control measures, consequences and motivation options.

3. Results

3.1. Recruitment of participants

For the study, ten meetings in different regions of the country were organised with the following composition: Ten hunters from Harju county, twelve from Tartu county, ten from Saare county, seven from Ida-Viru county, four from Pärnu county and three from Järva county (Table 1; Fig. 1).

All but one of the participants were men, with the woman joining in the group meeting in the Saare county. The estimated age ranged between 25 (minimum) and 65 years (maximum). The majority of participants were more than 50 years old. Two of the hunters had retired from active hunting while the remaining participants described themselves as regular hunters, hunting several times every week.

3.2. Meetings

In total, ten focus group discussions were conducted. The length of the meetings varied between 1 h 05 min and 2 h 15 min.

Table 1
Locations of the focus group meetings and the numbers of participating hunters in the respective study in Estonia.

County	City	No. of participating hunters
Harju	Aruküla	4
Harju	Tallinn	6
Saare	Kuressaare	6
Saare	Muhu	4
Tartu	Tartu	6
Tartu	Tartu	6
Ida-Viru	Toila	5
Ida-Viru	Jõhvi	2
Pärnu	Kärsu	4
Järva	Paide	3

3.3. Analyses

3.3.1. Acceptability of control measures

3.3.1.1. a. Relation diagram. As shown in supplemental material table 2, 27 different stakeholders were named in the relation diagram (including the hunters themselves). Even if not all mentioned stakeholders have a specific role in controlling ASF, all were included in the calculation of the weighted average. The VFB and the Environmental Board were listed most frequently by eight groups. The Veterinary and Food Laboratory and the Estonian Hunters' Society were named seven times, closely followed by the Estonian University of Life Sciences mentioned six times. All listed stakeholders are shown in supplemental material table 2.

The perceived highest contact rate existed between the hunters and varying veterinary services, for example the Veterinary and Food Laboratory, the VFB (county veterinary centre) and the respective local veterinarian, followed by hunting organisations, e.g. the hunting council of the county and the Estonian Hunters' Society. Contact with higher institutions, for instance the Environmental Board as well as the Environmental Inspectorate or agencies, different listed ministries and the Estonian University of Life Sciences, was described as rare or lacking (Fig. 3).

The quality of these contacts was not in agreement with the mentioned frequency. The satisfaction of contact with hunting organisations was generally high, as was the contact to the laboratory. Nevertheless, the amount of paperwork and the accessibility of sample submission to the laboratory was described as challenging. Especially in the summer months, the opening hours of the laboratory only until Friday afternoon was perceived as a problem due to the therefore arising necessity of the hunters to store the carcasses over the weekend. The quality of contact to local veterinarians and the county veterinary service was mostly described as neutral to slightly positive. However, the participants reported a lack of knowledge of these stakeholders regarding the paperwork in conjunction with sample submission, the procedure of handling dead wild boar and the tasks of hunters, leading to, in their opinion, loading of work onto the hunters. In accordance, the participants wished for clear instructions from veterinarians and veterinary officials. Missing or rare contact to the Estonian University of Life Sciences was perceived as neutral, but the desire for more communication, continuous workshops and training was present. Rare to non-existent contact to state level authorities, such as the Environmental Board and the Ministry of Rural Affairs, the Ministry of the Environment and the Ministry of Defence, was observed to be neutral. The existing contact was seen as a strict one-way relationship in the direction of the hunters, as only orders were given, but no information was carried back to the state level authorities.

The highest trust of the hunters towards the implementation of control measures was towards themselves and other hunters (Fig. 4). The current situation of low numbers of new ASF cases was believed to have been achieved only due to the hunters' work. Nevertheless, the participants did not see any clear advantage for themselves as a result of the work completed. The Veterinary and Food Laboratory along with hunting authorities, for example local hunting clubs, the hunting council of the county and the Estonian Hunters' Society, were perceived to have the second highest trust score, followed by the local veterinarian and Veterinary and Food Board. Official public institutions including Environmental Board and Environmental Agency, various ministries and the EU were categorized as the least-trusted group of stakeholders, possibly due to the missing connection of the stakeholders in the daily working routine. As hunters, hunting organisations and the Veterinary and Food Laboratory were involved in the work in the field, knowledge of practical implementation of the measures was presumed. Directives enforced on state level without information and understanding of the hunters' daily work were perceived as untrustworthy by the participants.

3.3.1.2. b. List of control measures. The most accepted and trusted control measures to eradicate ASF were proven to be the measures supporting hunting (Fig. 5). A general increase of hunting to reduce the wild boar population was the most favoured measure, as it was believed to have the highest impact on eradicating the disease. This was followed by hunting with bait feeding to attract wild boar to the preferred hunting ground and keep them in the area, thus boosting the efficiency of hunting, reducing wild boar roaming and ultimately limiting the spread

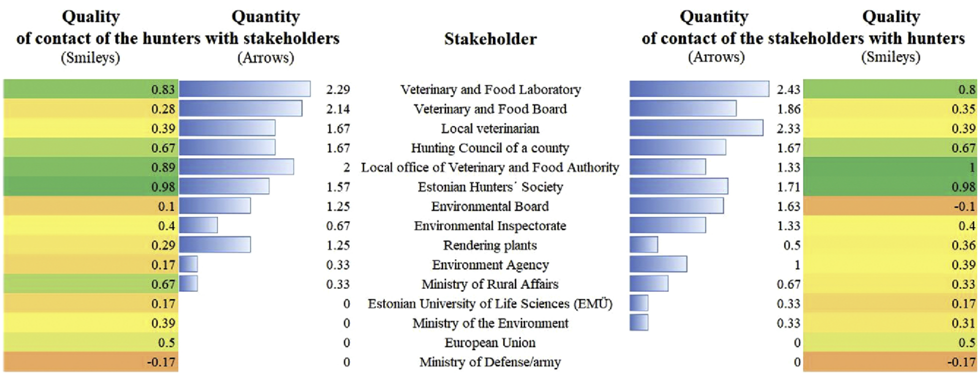

Fig. 3. Stakeholders ranked by the sum of quantity of contact with hunters supported by the perceived quality of contact by the hunters. The colours of the quality of contact to hunters illustrate the colours of the corresponding smileys.

Rank	Stakeholder	Average Trust (Piling)
1	Hunter	3.03
2	Veterinary and Food Laboratory	1.78
3	Hunting Council of a county	1.64
4	Estonian Hunters´ Society	1.61
5	Estonian University of Life Sciences (EMÜ)	1.3
6	Local veterinarian	1.15
7	Veterinary and Food Board	1.06
8	Environmental Board	0.99
9	Local office of Veterinary and Food Authority	0.77
10	Environment Agency	0.52
11	European Union	0.36
12	Ministry of Rural Affairs	0.19
13	Ministry of the Environment	0.16
14	Environmental Inspectorate	0.15
15	Rendering plants	0.07
16	Ministry of Defense (including the army)	0

Fig. 4. Mentioned stakeholders ranked by the trust of the hunters to implement control measures.

Trust (Piling)	Control Measures	Satisfaction (Smileys)
-	Vaccination	1
3.97	Selective hunting of wildboar/Reduction of wild boar population	1
3.05	Bait feeding + shooting	0.92
2.56	Incentives for hunting	0.62
2.36	Selective increased hunting of female wild boar	-0.03
0.7	Ban of additional feeding	-0.5
0.17	Increase carcass searching and removal	-0.43
0.08	Fencing	-0.83
0	Ban of hunting	-1
0	Army/police	-1

Fig. 5. Control measures ranked by liking and trust in their suitability for controlling ASF, as stated by hunters in Estonia during focus group discussions.

of ASF. Incentives for hunted wild boar were well accepted and trusted regarding effectiveness, although the currently paid incentives were perceived as too low due to the high costs of hunting. Paying higher amounts than the arising costs for hunting was discussed as a question of ethics, as money was not regarded as a respectable reason to hunt. Selectively increased hunting of female wild boar was likewise a highly discussed control measure. Although perceived as effective to control ASF and the wild boar population, it was regarded as unethical due to the possible creation of orphans during the summer farrowing season. Selective hunting of females in autumn and winter was preferably accepted. Vaccination was favoured by all focus groups that mentioned this control measure. Yet, vaccination was never involved in the proportional piling, as there is no vaccine currently available (Sanchez-Vizcaino et al., 2013; Barasona et al., 2019).

Control measures that hinder hunting (ban of hunting and additional feeding) were generally disliked. Furthermore, fencing was seen as a "waste of money and time", without any trust in effectiveness. Spatially limiting effects on all species were seen as negative and the spread of ASF through humans was believed to be not preventable by a fence. In addition, the involvement of army and police was strongly rejected. It was argued that hunters had a profound knowledge of their hunting grounds and the wildlife in these areas over a long period of time, therefore army and police were recognized as untrained in hunting and animal behaviour. Hunters judged the involvement of army and police in hunting as unethical, as a "massacre and genocide and destructive for the forest". Increasing targeted carcass search and removal of carcasses from the environment was perceived solely as a preventive measure and not suitable in the case of an ongoing outbreak. The trust in effectiveness and the acceptance of this measure was low.

3.3.2. Acceptability of passive surveillance

3.3.2.1. a. Impact diagram. Hunters regarded the listed consequences of passive surveillance mostly as negative. Fig. 6 shows the eight consequences ranked by their impact on the hunters. Some detailed consequences, such as the transportation of carcasses from the forest and disinfection process, were judged as highly important for the participants, followed by general consequences like additional work, labour and financial costs.

Additional paperwork, disinfection and transportation was stated to

Rank	Trust (Piling)		Consequence
1	2.34	Neg	Taking the carcass out of woods
2	1.74	Neg	Additional work/time
3	1.73	Neg	Disinfection of the car and equipment (ensure biosecurity)
4	1.71	Neg	Financial cost
5	1.27	Neg	Emotional considerations (disgust/sorrow)
6	0.92	Pos	Improvement of the cooperation and communication between hunters/landowners
7	0.59	Neg	Taking a sample
8	0.38	Pos	Elimination of one infection source

Fig. 6. Consequences of finding a dead wild boar as mentioned by the hunters participating in focus groups in Estonia, ranked by their impact.

have a huge impact on the hunters due to difficulties in finding assistance and covering additional material expenses without reimbursement.

A selection of focus groups struggled to think of any positive consequences of finding dead wild boar. If a possible positive consequence was identified, it was considered of minor importance (Fig. 6). The same applied for financial compensation, mentioned by two groups.

3.3.2.2. b. List of motivation options to increase passive surveillance. The most motivational options described by the participants in the focus group discussions were divided in two scenarios. If the work of reporting and removal of the carcass remains the task of hunters, the increase of currently paid incentives will represent the most efficient motivational option. If the task of the hunters is to report a carcass sighting only, while other stakeholders with extra resources (veterinarians, army/police, voluntaries were listed) fulfil the task of removal and disinfection, this would be the second-most trusted motivation (Fig. 7). Furthermore, reduction of infection pressure in their hunting area was regarded as a motivation for reporting, as the hunters expressed their personal commitment to protect and preserve their hunting areas. Detailed feedback from the veterinary services was considered as the option with the least motivational potential.

4. Discussion

The willingness of hunters to support defined control measures and motivational tools is based on the experience of applicability in the daily life of the participating hunters. The decrease of ASF cases in Estonian wild boar population (Schulz et al., 2019b) may also suggest that the acceptance and collaboration of hunters were good in Estonia, which could make their opinions, knowledge and experience even more valuable for others.

In veterinary epidemiology, participatory methods have so far only rarely been used in developed countries (Catley et al., 2012; Calba et al., 2014; Calba et al., 2015a; Schulz et al., 2016), although participatory epidemiology has shown major advantages, especially in evaluation of disease control and surveillance measures in developing countries (Allepuz et al., 2017). The advantages of combining conventional focus group discussions (FGD) and tools of PE in this study were to collect numerical data (comparable to questionnaires) on opinions and strategies to base our analyses on data that is not prone to loss of information due to translation (translation bias). Furthermore, we wanted to give the hunters room for discussions to obtain information or suggestions on

Rank	Motivation tools	Trust (Piling)
1	Increase of currently paid incentives	3.54
2	Only reporting, no further work	2.63
3	Reduction of infection pressure within the wild boar population	2.18
4	Detailed feedback	0.85

Fig. 7. Motivation tools, ranked by the median trust perceived by hunters in motivating them to report dead wild boar as mentioned during focus group discussions in Estonia.

ASF control strategies (comparable to conventional FGD).

The implemented methods were adapted from Schulz et al. (2016) and Calba et al. (2015a). The structure of the study and the used tools (e.g. proportional piling, smileys and diagrams) were similar. In the analysis, ranks were assigned to smileys and arrows as described, but proportional piling was adapted. To take the different numbers of listed options (e.g. stakeholders, control measures) within the groups into account, weighted averages were calculated. Due to differences in the research questions, the combination and the order of the applied methods were adapted accordingly. The results of Schulz et al. (2016), which yielded a very low acceptability of passive surveillance in addition to its importance in the control of ASF (Nurmoja et al., 2017b; Schulz et al., 2019b), led us to focus on passive surveillance and options to increase the motivation of hunters to support this task.

We also wanted to establish participatory methods in research in this particular context, as PE can be seen as a novel method in veterinary epidemiology in industrialised countries. To set standards for participatory work and bypass avoidable biases of subjectivity in qualitative analyses, the focus group discussions with participants have to be as comprehensive as possible. However, the participatory methods and the quantitative analyses embody an objective basis for the subsidiary qualitative analysis, which otherwise might lead to potential biases and limitations.

To obtain a wide variation of opinions and balance possible sociocultural and economical differences between regions as well as variation caused by different starting times of the epidemic in the region (e.g. different control measures in force), hunters from different regions (Fig. 1) were recruited. Mostly men participated in the interviews. While this may represent gender distribution among Estonian hunters, a possible gender bias must be recognized in the present study. The direct contact and recruitment of the hunters for the focus groups was done by contact persons mostly named by the Estonian Hunters' Society (EHS), leading to a possible preference of hunters affiliated with the EHS or the contact persons. People generally more interested in cooperation and discussions may have had an increased chance of becoming members of the focus discussion groups. This selection bias can unfortunately be present in many participatory studies and could not be completely eliminated in our study (Calba et al., 2015a; Schulz et al., 2016). Furthermore, there is the possibility of a potential bias due to the impression that the hunters' answers were monitored and might have negative consequences for them, e.g. if rules were broken. However, this bias could be seen as minor as the facilitator was a scientist from the Estonian University of Life Sciences and not involved in any supervision by authorities.

At first sight, the sample size of 46 hunters (0.3% of Estonian hunters) seems to be low, however, analysis of the interview results clearly indicate that the principal of theoretical saturation was fulfilled (Glaser et al., 1967). Nevertheless, we cannot rule out that we may have missed some observations made by hunters who did not receive the chance to join the discussion groups. To some extent, the small sample size may also weaken the credibility of the results. In several tasks we used consensus opinions and analysed group averages instead of considering every individual view stated, therefore taking the risk of losing perceptions and opinions possibly differing from the average or leading opinion. To minimise this loss, we integrated all statements in the descriptive analyses. Another limitation of the study that increases the potential loss of information was due to translation/information bias. The audio recording was translated into English by a qualified independent third party and in cases of ambiguous translations the facilitator was consulted. By collecting numerical data in this study it was possible to focus the analyses mainly on the quantitative analyses. However, as mentioned above, discussions and arguments were still included as much as possible, substantiating and complementing the results of the quantitative analyses.

Despite potential bias and other limitations that cannot be avoided in such studies, the use of participatory methods offered the unique possibility to learn about hunters' perceptions and allows the inclusion of the gained knowledge in the further planning and implementation of ASF control and surveillance.

Policies and regulations for hunters should take the interests and the expertise of stakeholders into account. Reflection of their priorities, needs and problems is therefore essential. Lack in understanding community needs may lead to conflicts of interests followed by unwillingness to implement ASF control measures (Allepuz et al., 2017). PE has the ability to fill this gap. The importance of establishing the trust of hunters in working with veterinary services, their lack of knowledge in the mechanisms of disease spread and the importance of early detection shows the need for participatory approaches (Catley et al., 2012; Allepuz et al., 2017; EFSA, 2018a). One possible reason for the fact that there are only few participatory studies in developed countries might be that veterinarians and epidemiologists in government, academic and research centres tend to regard participatory epidemiology as a "soft" approach (Catley et al., 2012). However, visualisation tools in focus group discussions, such as the described and used proportional piling, produce "hard" numerical data that can be analysed using conventional statistical methods. In addition, another advantage of proportional piling as a scoring method is its higher sensitivity in comparison to ranking methods, thus allowing the use of weighing participants' responses (Catley et al., 2012). In contrast to conventional approaches where participants are expected to devote time and effort into topics that are possibly of limited advantage to them, participatory studies focus specifically on local or topic-related issues, generally in the interest of the participating stakeholders.

An important issue for discussion is the possibility of PE to challenge existing disease control by involving stakeholders and the limited options of authorities to react to findings of participatory studies (Catley et al., 2012). An example for the limited margins of authorities might be to react to the demand for higher financial incentives. Funding (direct costs and payroll costs) might not be completely at the discretion of the authorities as budgets might be limited and funding subject to particular conditions imposed by funding agencies.

Assuming that satisfactory relationships with trustworthy stakeholders within the network of the hunters increase the acceptability of control measures and the willingness to work with or for these stakeholders, the acquired knowledge of the network can be of great assistance when implementing measures. Given the neutral relationship and absent trust in officials, the good relationship and trust in the hunting authorities can be used to communicate with hunters on directives and obligations to implement control measures. As the hunting authorities were regarded as a gathering facility for the voices of hunters and had insights into both the bureaucracy and the work in the field, directives openly discussed with the hunting authorities are more likely to be accepted. The neutral satisfaction regarding the relationship with local veterinarians and the Veterinary and Food Board and the limited trust in them could be improved by an enhanced will to work together. Joined meetings and workshops may not only improve the relationship, but also satisfy the hunters' interest in further scientific information while at the same time overcoming the lack of knowledge of veterinarians regarding hunting issues perceived by the hunters. This can be of great use seeing as the quantity of contact between these stakeholders is already high.

Increased acceptance of control measures is achieved by understanding alongside trust and support for the control measures. The prepared control measures were of interest as they are already in place in Estonia or are highly discussed to have an effect on controlling ASF. The quantitative analyses and the interviews showed the clear preference of intensified hunting as the most effective measure in the hunters' opinion. Supportive measures as baiting or incentives were also perceived as effective. In accord with this, any measure constraining hunting, such as ban of hunting, ban of additional feeding or involving army and police and thus replacing hunters, were not accepted by the hunters. Consequently, they do not trust these measures, deny that they have an impact on ASF control or find it unethical to hunt in this way (untrained army or shooting wild boar trapped in a cage). Convincing hunters to build fences will represent a challenge when taking their limited trust in the efficacy of fences in controlling ASF due to the huge workload, time consumption and the hindrance of movement of other species, in particular other wildlife into account. Obviously, Estonian hunters are convinced that wild boar are less a problem in spreading the disease than humans. The discrepancy in the neutral liking and the high trust in selective increased hunting of females can possibly be explained as the risk to produce orphans by shooting sows in the farrowing season, was regarded as unethical. Restricting this measure to autumn, winter and early spring could lead to a higher acceptance.

As passive surveillance is not only important for early detection, but also to reduce the risk of further disease spread (i.e. as a control measure) (Nurmoja et al., 2017b; Schulz et al., 2019b), it was included in the evaluation of the acceptability of different control measures. The low trust and the lack of support for increasing carcass search and removal activities can be explained by a lack of hunters' knowledge on the huge impact of passive surveillance to control ASF. Moreover, the additional workload and the absence of any benefit for hunters were seen as impediments to support passive surveillance. These results correspond to those of other studies, in which a poor acceptance of passive surveillance has also been observed (Schulz et al., 2016). Further information on the advantages of passive surveillance provided to the hunters might increase their acceptance of these measures. Due to its huge importance of passive surveillance in controlling ASF in wild boar, we focused on it with separate PE methods in addition to the general approach regarding control measures. Due to previous study results, which showed the low acceptability to support passive surveillance activities (Schulz et al., 2016) and as hunters are the primary stakeholder when it comes to implementing passive surveillance, we considered it as crucial to investigate motivational options for hunters to increase the reporting and sampling of found wild boar carcasses. Understanding the perceived consequences and identifying those with the biggest impact can help to recognize the consequences that should be further developed or improved. Nearly all consequences perceived as negative by the hunters can be categorized into three groups - additional work, time consumption and financial costs. It was often mentioned in the discussions that the hunters were willing to help if all costs were covered, hence a reimbursement system may solve this problem. Alternatively, hunters could be equipped with all items needed for safe carcass removal, an option mentioned in some discussions. Communication between hunters does not stop at national borders. This may be particularly important with respect to incentives paid for hunting wild boar or finding and removing wild boar carcasses.

Reducing the workload for hunters most likely represents one of the most effective motivational tools. Hunters would not feel replaced in hunting matters but appreciated for their knowledge of the forest and their capability to find carcasses, which would both benefit the stated motivation options. A trained, well-equipped group (army, police, others) could undertake the duty of transportation of the carcass and the following disinfection. In this case, hunters expressed that they would even accept reduced incentives. The high amount of trust in the motivational option to reduce the infection pressure in wild boar can be seen as a solid foundation for the work with hunters, as they are motivated by their commitment to maintaining and fostering the wild boar population.

We are not aware of any studies that assessed the benefit of including participatory methods into conventional epidemiological studies in comparison to studies without PE. However, we assume that obtaining insider knowledge from external stakeholders systematically might only be possible by including methods of PE into the respective projects. The feedback of participating hunters in our study was positive. The hunters felt that they were heard and they were grateful for being involved in the study. The focus of this study on addressing the acceptance of control measures and passive surveillance could be a first step in designing recommendations to change the strategy of controlling ASF. To implement newly gained knowledge, follow-up studies should focus on the perspective of other stakeholders involved in controlling ASF. Furthermore, direct involvement of hunters in defining plans for future ASF control policies could be taken into consideration.

5. Conclusion

This study provides an insight in the perception of hunters regarding ASF control measures and passive surveillance in wild boar. Options to improve ASF control and the motivation to report dead wild boar are discussed by taking the acceptance of various measures into account. The success of control measures or surveillance strategies is highly related to the hunters' willingness and capability to implement the measures. Further communication and teamwork between all stakeholders could ultimately lead to improved ASF control in wild boar. Thus, these findings are highly significant and informative for other countries when planning or adjusting surveillance and measures to control ASF.

Ethics approval and consent to participate

All hunters participated voluntarily in the group discussions and were informed about the objectives of the study. They approved the anonymous publication of the results. Personalized information was not recorded. Formal consent or ethics approval was not required for this study, as it included neither clinical trials in humans, for which ethical approval might have been required, nor animal experiments.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2020.105121.

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4.2.2.2 Hunters’ view on the control of African swine fever in wild boar. A participatory study in Latvia.

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Highlights

- The hunters’ trust in the Food and Veterinary Service and the State Forest Service was high
- The trust of hunters in vaccination for ASF control emphasised the need to constantly communicate with hunters to avoid any false conclusions or hope
- Hunters expressed the desire for more support regarding the sampling and removal of wild boar carcasses
- The increased hunting of female wild boar was considered as unethical and will not be supported

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Hunters' view on the control of African swine fever in wild boar. A participatory study in Latvia

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ABSTRACT

African swine fever (ASF) has massively spread in recent years and threatens the global pig industry. ASF has been present in Latvia since 2014. Hunters play a major role in the implementation of measures to control ASF and in passive disease surveillance. The probability to detect an ASF-positive wild boar is much higher in animals found dead than in hunted animals. Thus, the willingness and the motivation of hunters to support passive surveillance is of utmost importance. Using participatory methods, this study aimed to assess the acceptability of control measures for ASF in wild boar among hunters. In addition, new approaches to increase hunters' motivation to report wild boar found dead were investigated.

A total of ten focus group discussions with hunters from different regions in Latvia were conducted. To assess the quantity and quality of contacts between hunters and stakeholders involved in the control of ASF, relation diagrams were used. Using ranking tools, the trust of the participants in stakeholders to implement control measures successfully was evaluated. Defined control measures were presented to the hunters and their acceptability investigated. An impact diagram and a list of defined motivation options for passive surveillance were offered to identify new ways to increase the willingness of hunters to support passive surveillance actively.

A satisfactory and regular relationship was identified between the hunters, the Food and Veterinary Service (FVS) and the State Forest Service (SFS). The hunters' trust in these authorities was high. Although there is no vaccine against ASF, hunters were convinced of the potential of vaccination in controlling ASF. However, building fences was considered as useless and ineffective. To increase the willingness of hunters to support passive surveillance, reducing the infection pressure in the forests was regarded as most motivating. Furthermore, hunters would appreciate a decrease in their costs and workload.

The study provides new insight into the concerns and experiences of hunters. Including their views and expectations in the further design and implementation of control and surveillance activities may help to improve current efforts to control ASF in wild boar populations. Although representing the perceptions of Latvian hunters, the main conclusions may be adaptable to adjust ASF control and surveillance in other countries.

1. Introduction

African swine fever (ASF) was introduced into Georgia in 2007 (Sanchez-Vizcaino et al., 2013). With the exception of Sardinia, where the disease has been endemic since 1978, this introduction was the first one in Europe since the eradication of ASF in 1995 (Sanchez-Cordon et al., 2018; Cwynar et al., 2019). Since then, ASF has spread widely. In 2014, the disease reached Poland and Lithuania. Latvia was the third member state of the European Union (EU), where ASF was introduced in June 2014. The first ASF cases emerged in wild boar in the eastern part of the country (Oļševskis et al., 2016; Schulz et al., 2019). Over time, the disease spread towards the West, so that most of the country has now

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been affected by ASF.

Infected wild boar constitute a major threat for domestic pig holdings. Controlling ASF in these animals is much more difficult than in domestic pigs, but it is of vital importance for the pig industry (Depner et al., 2016; European Food Safety Authority, 2018; Schulz et al., 2019). In Latvia, several control measures have been applied for defined periods. Monetary incentives were paid to hunters for shooting wild boar, particularly for animals older than one year and for female wild boar. Driven hunts and baiting were restricted. In addition, the collection and safe disposal of wild boar carcasses was enforced (Schulz et al., 2019). Schulz et al. (2019) found that the implemented control measures had little effect on the prevalence of ASF virus (ASFV)-positive wild boar in the short term. However, more recent findings indicate a decline in the incidence of ASFV-positive animals, which may suggest that the control measures could have had an impact on the course of ASF in wild boar in the long term (Oļševskis et al., 2020).

Passive surveillance, i.e. the reporting or sampling of wild boar found dead is not only an important requirement for control measures to hinder the spread of the virus, but also the most important surveillance tool for the early detection of ASF (Oļševskis et al., 2016; Smietanka et al., 2016; European Food Safety Authority et al., 2017; Nurmoja et al., 2017). The probability to detect an ASFV-positive wild boar among animals found dead is significantly higher as compared to hunted wild boar (Nurmoja et al., 2017; European Food Safety Authority, 2018, 2019; Schulz et al., 2019).

Neither ASF control measures nor passive surveillance in wild boar can be effectively implemented without the support of hunters. Hunters play a major role in wildlife management and therefore also in the implementation of measures to control diseases in the wild boar population (Schulz et al., 2016; Quiros-Fernandez et al., 2017). However, in Latvia, hunting is just a hobby and any associated activities occur on a voluntary basis. Accordingly, hunters' willingness to support control measures and passive surveillance for ASF in wild boar is vital.

Participatory methods can help to explore the rationales, experiences and concerns of key players concerning the design and implementation of control measures and passive surveillance (Catley et al., 2012; Calba et al., 2016; Schulz et al., 2016; Allepuz et al., 2017). For a long time, participatory methods were almost exclusively used in settings in developing countries. However, by recognising the advantages of including these methods into conventional epidemiological research, the application of these methods, also in developed countries, increased recently (Calba et al., 2015, 2016; Duval et al., 2016; Schulz et al., 2016). In contrast to traditional epidemiology, participatory methods involve the affected persons or groups in defining relevant problems and in the process of finding solutions that are readily applicable and satisfy all affected parties (Catley et al., 2012; Mariner et al., 2014). Various tools are used to ease communication and to understand the rationales of the persons and stakeholders involved. The results of the studies can be used to improve surveillance activities and disease control. The acceptability of certain measures or activities is defined as the willingness of key players to participate actively and support activities (Meynard et al., 2008). Often, acceptability is assessed by applying participatory methods (Bronner et al., 2014; McKerr et al., 2015).

In the present study, we used participatory methods to assess the acceptability of control measures for ASF in wild boar among Latvian hunters. Furthermore, we investigated the willingness of hunters to support passive surveillance in order to identify the best options for motivating hunters to increase passive surveillance. The results may help to improve ASF control measures and to increase the effectiveness of passive surveillance.

2. Materials & methods

2.1. Focus group discussions

The method of focus group discussions was chosen as a suitable method for data collection to allow for expression of a variety of views and perceptions of hunters. This was combined with visualization, ranking and scoring methods to gather numerical data. All participating hunters were informed about the aims of the study, the principles of participatory epidemiology and logistic details of the meetings. After anonymity had been assured, all participating hunters agreed that the meetings could be recorded and the results published. The discussions were held and recorded (by a typist) in Latvian. For further analyses, the transcript was translated into English by the professional translator company 'Skrivanek Baltic' (https://skrivanek.lv/en/).

The discussions were divided into two parts. First, the acceptance of ASF control measures was addressed. In the second part, impediments or motivation options were identified that might influence hunters' support for passive surveillance.

The participatory methods used in the present study were adapted from Calba et al. (2015) and Schulz et al. (2016). They were also applied in the study of Urner et al. (2020).

All meetings were guided by the same facilitator. The facilitator was trained in the applied methodology and was advised to ensure similar participation of all hunters in the discussions. The facilitator was a female employee of the Latvian Food and Veterinary Service who had no prior contact with hunters, ASF and its monitoring before the meetings. Results of the participatory methods were analysed in a semi-quantitative fashion and the qualitative results of the recorded discussions were included descriptively.

2.2. Recruiting hunters

For the focus group discussions (FGD), three to six hunters per meeting were recruited between April and July 2019. In close cooperation with the two largest Latvian hunting organisations ("Latvian Hunter's association" and "Latvian Hunter's Union"), hunters from five (Kurzeme, Latgale, Pierīga, Vidzeme and Zemgale) out of six administrative regions of Latvia were recruited to cover the whole territory of Latvia (Fig. 1). Therefore, in each region, two FGD were conducted. The hunting organisations provided a list of potential contact persons on a regional or local level, mostly leaders of regional or local hunting clubs. The leading hunters were contacted by phone, invited to participate and asked to identify further hunters who might be willing to participate voluntarily in the study. The criterion for participation of the hunters was their willingness to participate. These hunters were then contacted and invited to the focus group discussions.

2.3. Assessment of control measures

A relation diagram was applied to assess hunters' satisfaction with the perceived network of stakeholders involved in controlling ASF. The hunters first listed stakeholders, whom they considered as relevant in the network of control measures. The quantity of contacts (from 'no contact' to 'daily contact') between the hunters and each listed stakeholder was assessed by arrows (Fig. 2). Each contact was classified as outgoing from the hunters reaching out to the stakeholder and vice versa. Within each focus discussion group, consensus had to be reached for each arrow. Subsequently, each hunter received three smileys, which were used to rate the quality of the contacts individually (Fig. 2). The individual rating could be seen by each of the participants. Hunters' trust in the stakeholders' performance in ASF control was assessed by 'proportional piling'.

For the process of proportional piling, the participants had to achieve consensus in their discussion and to attribute then 100 glass beans in proportion to their trust in the listed stakeholders to implement control measures successfully.

The listed control measures were presented to the participants for evaluation:

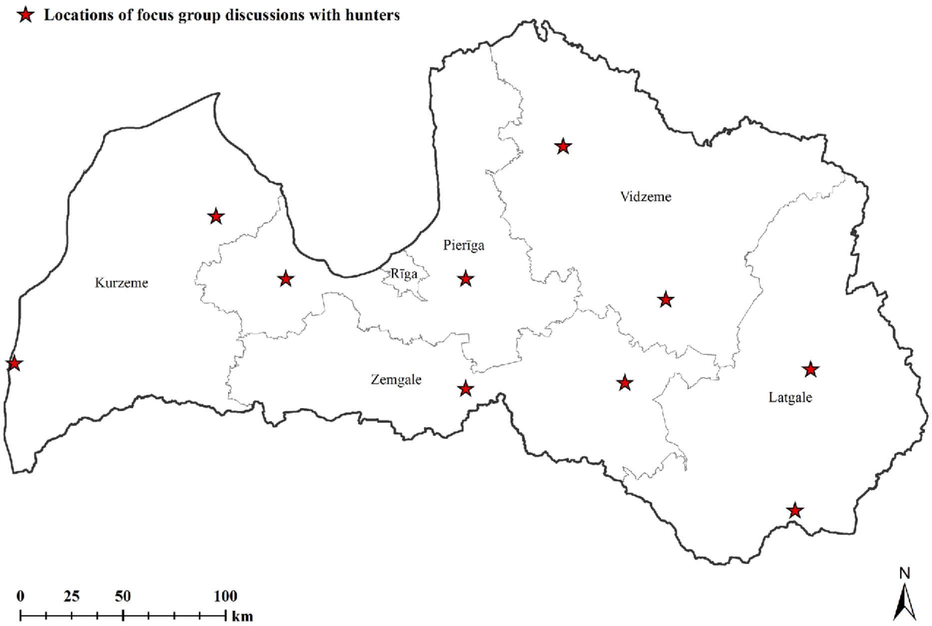

Fig. 1. Locations of the focus group discussions with hunters in five administrative regions in Latvia.

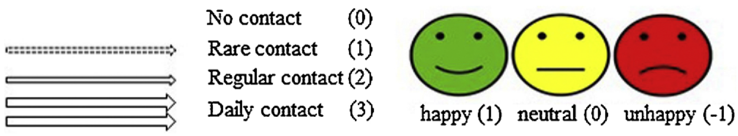

Fig. 2. Arrows, smileys and glass beans and the ranks assigned to them. The number of glass beans attributed to a stakeholder represented the proportion of trust in the respective stakeholder.

- Fencing
- Ban of hunting
- Including professionals for intensive hunting (police or army)
- Increased hunting of female wild boar (WB)
- Incentives for hunting
- Increased carcass search and removal

The hunters had the chance to add further control measures. Each hunter was requested to express his/her personal view on each individual control measure using smileys. To display the hunters' trust in the effectivity of the measures to control ASF, 'proportional piling' was applied.

2.4. Assessment of passive surveillance

An impact diagram was used to assess negative and positive consequences expected by hunters when detecting and reporting a wild boar found dead. After the participants had listed potential consequences, they were requested to reach consensus and attribute 100 glass beans proportionally to the perceived size of impact of the listed consequences. All consequences were thereby included in the same ranking process, irrespective of their character as presumed negative or positive consequences.

To assess the acceptability of possible motivation options to improve participation in passive surveillance, the following items were listed and the hunters asked to add further options:

- Increase of currently paid compensation
- Reduction of infection pressure in the wild boar population (by finding, sampling and removing carcasses)
- Only reporting dead wild boar without any further work for the hunter
- Detailed feedback on the health status of the detected wild boar from the relevant authority to the hunter

'Proportional piling' was used to assess the participants' trust in the listed options to motivate hunters to improve their support for passive surveillance.

2.5. Quantitative analyses

The average of contacts between hunters and stakeholders were calculated by assigning the appropriate rank to the respective arrows (Fig. 2). To determine the quality of contacts, i.e. the satisfaction with the quantity of cooperation with the rated stakeholders, ranks were assigned according to the numbers of smileys given (Fig. 2).The average was calculated for each individual group and for all groups that had mentioned a particular stakeholder.

'Proportional piling' was applied and analysed with a weighted average. For example, the assessment of the trust in the listed control measures was calculated as follows: The average trust T_{CMi} was calculated taking the control measures CM_i i ∈ {1,…,28}, listed by all participants, into account. In the first step, the proportion of glass beans assigned to the control measures P_j was calculated by weighting the number of glass beans GB_{ij} allocated to the control measure CM_j from group G_j with j ∈ {1,…,10} in relation to Z_j^{CM}, the number of control measures CM listed by group G_j.

$$P_j = \frac{Z_j^{CM}}{\sum_{j=1}^{10} Z_j^{CM}}$$

The average trust T_{CMi} for all control measures was calculated with N_{CMi}, the number of groups G mentioning control measure CM_j,

$$T_{CM_i} = \frac{1}{N_{CM_i}} \cdot \sum_{j=1}^{10} P_j \cdot GB_{ij}$$

3. Results

3.1. Recruiting hunters

Between April and July 2019, ten focus group discussions with 50 participants in total, 49 men and one woman, were conducted across Latvia (Fig. 1). In each of the administrative regions of Latvia, two focus group discussions were held (Table 1). The estimated average age of the participants was 50 years.

3.2. Control measures

The participants named 27 stakeholders in total. All ten groups mentioned the Food and Veterinary Service (FVS), the State Forest Service (SFS) and the respective municipal administrations. Eight groups mentioned hunters, seven named the hunting organisations. Three to five groups mentioned the media, landowners, the Ministry of Agriculture (MoA), the "Latvia's State Forests" (a joint stock company), the State Police, scientists of the Institute of Food Safety, Animal Health and Environment "BIOR", farmers, the society and logging companies. Disposal companies were named twice. Each, the hunters' magazine, the Ministry of Interior, the laboratory, veterinarians, individual hunters, State Border Guards, Municipal Police, the European Commission, national media (TV & Radio), farmers' parliament, the State Environmental Service and animal protection organisations were mentioned once (Fig. 3). The participants expressed the highest trust in the proper implementation of control measures against ASF in wild boar by the FVS followed by hunters, hunting organisation and the SFS (Fig. 3).

The largest numbers of contacts of the participants (quantity) were

Table 1
Number of hunters per focus group discussion and administrative region of Latvia.

Administrative Region	Locations of focus group discussions	No of participating hunters
Pieriga	Ropazi	6
Pieriga	Tukums	4
Vidzeme	Valmiera	4
Vidzeme	Madona	4
Zemgale	Barbele	7
Zemgale	Jekabpils	6
Kurzeme	Talsi	5
Kurzeme	Liepaja	5
Latgale	Krastava	5
Latgale	Rezekne	4

with other hunters and stakeholders of the regular routine procedures, i. e. laboratory, FVS, SFS, hunting organisations and the municipal administrations. The hunters had little contact with the national research centre, veterinarians, the police, disposal companies and the MoA (Fig. 4). The quality of contact was higher for stakeholders with a large number of contacts than for stakeholders with a small number of contacts.

Analysis of the translated recordings of the focus discussion groups revealed that the hunters considered their co-operations with the FVS as positive. Feedback was regarded as sufficient and the employees of the FVS were mentioned to be solution-oriented and helpful. The SFS was also perceived as solution-oriented, but a lack of information and communication was reported. At the local level, there were only contacts with the municipal administrations and the police. The participants mentioned the companionable teamwork with other hunters and hunting organisations, based on their perception, that they were the 'real workers' in the control of ASF. The assumed important role of loggers and logging companies in the local spread of ASF was mentioned in connection with the perceived disinterest of these stakeholders in any ASF-related topic. The hunters believed that the focus of the Ministry of Interior, the MoA and BIOR, was exclusively on conducting office and desktop work, without taking the "whole picture" and the daily routines sufficiently into account. The hunters desired more communication with the authorities to have a chance to express their views and share their experience.

Vaccination was the most trusted measure to control ASF and received the highest value for satisfaction, followed by incentives for hunting, intensive hunting and biosecurity measures. Increased hunting of females received lower trust ratings and was not favoured by the participants. Fencing, involvement of police or army and the ban of hunting were the least-trusted control measures and received the lowest values for satisfaction (Fig. 5). Further control measures involved in the weighted average, which were mentioned only three times or less, were for example education of hunters on all ASF-related issues, various hunting methods, restriction of public access to areas with ASF-infected wild boar, poisoning and hunting of birds or small predators (Supplemental material Table 1).

In the discussion, covering hunters' costs that arise from their participation in the control measures during hunting was considered as an absolute requirement. Nevertheless, hunters who only hunt for money were considered as ethically dubious. Education and improved biosecurity to be implemented by everybody working in the forest were seen as beneficial for hunters, veterinarians and loggers in reducing the local spread of ASF. Increased hunting of females was regarded as an appropriate measure to reduce the size of the wild boar population, but not as trustworthy in controlling ASF. Moreover, it was disliked in its implementation, due to issues of hunting ethics. In particular, the risk of producing orphans during the farrowing season was seen as unacceptable. Hunting outside of the farrowing season was mentioned to be more satisfactory. Due to the perceived risk of intensified traffic in the forest, the hunters feared that the involvement of police or army and increased hunting of females might lead to spreading ASF rather than controlling it. Furthermore, the participants expressed concern that the police or the army might kill wild boar in an inappropriate or unethical way. They were perceived as unfamiliar with and untrained in hunting.

3.3. Passive surveillance

The hunters mentioned 31 consequences with respect to passive surveillance. Although several groups were not able to list any positive consequence of passive surveillance, reporting wild boar carcasses was a perceived positive consequence as it might help to control ASF. All other consequences of passive surveillance were regarded as negative (Fig. 6).

A self-inflicted duty and the interest of hunters to do everything in their power to control ASF were mentioned in the focus group discussions. Several problems were identified, including additional work, time

Stakeholder	Mentions by groups	Weighted average trust	Rank
Food and Veterinary Service	10	2.96	1
Hunters	8	2.84	2
Hunting organisation	7	1.83	3
State Forest Service	10	1.42	4
Hunters' magazine	1	0.99	5
Institute BIOR	3	0.97	6
Municipal administration	10	0.7	7
Ministry of Interior	1	0.68	8
Laboratory	1	0.68	8
Farmers	3	0.57	9
Landowners	4	0.41	10
Media	5	0.38	11
Society	3	0.29	12
Veterinarian	1	0.24	13
Ministry of Agriculture	4	0.2	14
Logging companies/ workers	3	0.14	15
"Latvia's State Forests"	4	0.13	16
State Police	4	0.05	17
Individual hunters	1	0	18
State Border Guard	1	0	18
Municipal Police	1	0	18
European Commission	1	0	18
National media (TV & Radio)	1	0	18
Farmers' parliament	1	0	18
State Environmental Service	1	0	18
Disposal company	2	0	18
Animal protection organisations	1	0	18

Fig. 3. Stakeholders listed by the ten focus discussion groups, ranked by hunters' trust in their proper implementation of control measures against ASF in wild boar.

	Quality (Smileys) of contact of the hunters with stakeholders	Quantity (Arrows) of contact of the hunters with stakeholders	Stakeholder	Quantity (Arrows) of contact of the stakeholders with hunters	Quality (Smileys) of contact of the stakeholders with hunters	
MAX	1				1	MAX
	0.98	3	Hunters	3	0.83	
	1	3	Laboratory	3	1	
	0.72	2.8	Food and Veterinary Service	2.8	0.78	
	0.73	2.5	State Forest Service	2.2	0.53	
	0.73	2	Landowners	2	0.48	
	0.62	2	Hunting organisation	1.86	0.62	
	0.4	1.75	Media	1.4	0.36	
	1	1	Hunters' magazine	2	1	
	0.54	1.4	Municipalities	1.3	0.42	
	0.31	2	Logging companies/ workers	0.67	-0.08	
	-0.12	1.67	Institute BIOR	0.67	-0.58	
	0.27	1.33	Farmers	1	0.27	
	-0.17	1	Individual hunters	1	0	
	-1	1	National media (TV & Radio)	1	-1	
	0.75	1	State Environmental Service	1	0.75	
	0.62	1	Society	1	0.57	
	-0.6	1	Veterinarian	1	-0.6	
	0.15	0.75	"Latvia's State Forests"	0.5	0.06	
	-0.12	0.5	State Police	0.75	-0.04	
	0	0	Municipal Police	1	0	
	-0.15	0	Ministry of Agriculture	0.25	-0.23	
	0	0	State Border Guard	0	0	
	0.25	0	European Commission	0	0	
	0	0	Farmers' parliament	0	-0.25	
	-0.3	0	Disposal company	0	-0.2	
	0.4	0	Ministry of Interior	0	0.6	
	0.75	0	Animal protection organisations	0	0.5	
MIN	-1				-1	MIN

Fig. 4. Stakeholders mentioned in the focus discussion groups, ranked by the quantity of hunters' contacts with stakeholders (arrows) and the average quality of the contacts (smileys).

consumption and the costs of burying wild boar carcasses. The participants reported that they felt shocked when they were confronted with the duties imposed on them by national regulations and orders at the beginning of the ASF epidemic in wild boar. Missing information and the inability to bury wild boar carcasses in the forest without help or appropriate technical tools were mentioned. The participants perceived the current financial compensation as insufficient, as the time they were expected to spend on the respective activities (hunting, sampling and disposing carcasses etc.) could be used for "more rewarding" tasks, monetary and non-monetary (e.g. spending time with their families).

A potential reduction of the infection pressure within the wild boar population was perceived as the most motivational tool. This was followed by reducing the current workload to only reporting dead wild boar (i.e. transfer of further activities such as sampling and disposal of the carcasses to others), an increase in the currently paid compensation and detailed feedback on the health status of the detected wild boar

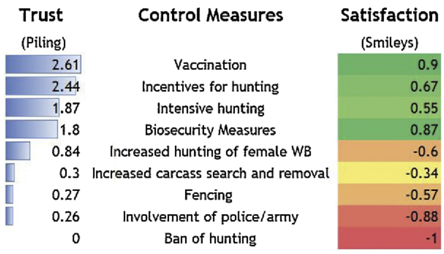

Fig. 5. Control measures mentioned by at least four of the ten focus discussion groups, ranked by trust in the perceived ability of the measures to control ASF efficiently and the hunters' satisfaction to implement the control measures.

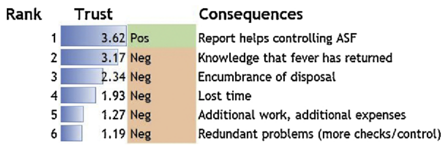

Fig. 6. Perceived consequences of passive surveillance mentioned by at least three focus discussion groups, ranked by the weighted average of glass beans allocated to the consequences in proportion to their perceived impact on the hunters.

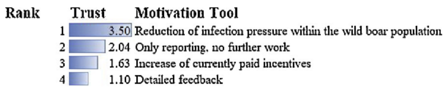

Fig. 7. Motivation tools, ranked by the weighted average of assigned glass beans in proportion to the perceived strength of motivational effect on hunters to participate in passive surveillance.

(Fig. 7). Further tools were included in the calculation of the weighted trust (Supplementary material Table 1).

In the discussions, the hunters explained that they regarded reducing the infection pressure in the wild boar population as their duty. Furthermore, difficulties in burying carcasses without help and fear of doing something in an incorrect way were mentioned. The participants expressed two ideas when discussing motivation tools: They proposed (i) a professional group for carcass removal, sampling and burial and (ii) programming a smart phone app that can be used by citizens or hunters to report the coordinates of found carcasses and to upload pictures. Moreover, the currently paid compensations were found to be too low and an optimised and faster financial flow of the monetary incentives from the authority to the hunters was seen as desirable.

4. Discussion

Participatory methods have so far not been intensively use in developed countries, despite their potential in involving key stakeholders in the design of disease surveillance and control programmes (Catley et al., 2012; Calba et al., 2014, 2015; Schulz et al., 2016). By choosing a participatory approach, stakeholders become involved and feel accepted as partners. Moreover, important information on the application of directives and orders in daily routines and topic-related expertise may become accessible.

However, participatory methods have their limitations as other knowledge-based approaches. One limitation of the present study was the lack of training of the facilitator in participatory methods. A one-day training focusing on this study was the only preparation that the facilitator received prior to the study. The training was supervised by the supervising author of the study, who received preliminary training at CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) (Schulz et al., 2016). The study questions and the implementation of focus group discussion was practiced with volunteers prior to the first implementation in the field. Therefore, some of the basic principles of participatory epidemiology may have remained unclear, which could have had an impact on the quality of the study. Particularly in focus group discussions, it is vital to include all participants. This helps to prevent dominant behaviour of individuals and thus to avoid losing the opinions of less dominant participants (Fischer et al., 2020). Furthermore, the used method of group discussions, in comparison to interviews (face to face or online by a questionnaire), might imply that participants can influence each other. While a dominant behaviour of individuals was controlled by the facilitator, there is the possibility that participants express opinions in the discussion or comment in specific ways to keep a particular social status or avoid to interfere with opinions of others. The moderation of the discussions, their recording and the translation of the transcripts into English involved the risk of information loss. To reduce translation bias, the transcript was checked by the Latvian colleagues and we double-checked all analyses with the facilitator.

In contrast to a similar study that we had conducted in Estonia (Urner et al., 2020), the facilitator was not an independent scientist, but a staff member of the FVS. She had neither been involved in work with hunters previously nor in ASF control management in wild boar. The lack of expert knowledge might have had a positive effect on the willingness of the participants to state their perceptions openly without any fear to disgrace themselves. On the other hand, the fact that the facilitator belonged to the FVS could have increased hunters' reluctance to express their real opinions loudly, particularly in case of a negative statement regarding the FVS. This potential bias may therefore be taken into account when the results are interpreted. However, a study with Estonian hunters, where an independent scientist acted as the facilitator, yielded similar results (Urner et al., 2020).

Hunters across five of the six administrative regions of Latvia were involved in the study to avoid clustered local opinions and experiences. The administrative region of the Latvian capital, Riga, was not included due to its urban character and relatively small area. The selection of participants often represents a limitation in social studies. This is also the case in the present study as the participating hunters may have been selected because they had been in contact with the authorities before the study or were particularly eager to express their opinion. Furthermore, the possibility of the leading hunters to contact only hunters, whom they knew and who might have similar opinions, cannot be excluded as a participation bias. Such a selection bias cannot be completely ruled out in these studies (Calba et al., 2015; Schulz et al., 2016). An obvious gender bias is illustrated by the fact that only a single woman could be recruited. The small number of participating women is likely to represent the small proportion of female hunters within the Latvian hunting community. Nevertheless, increasing the participation of female hunters would certainly be beneficial to expand the insight in the view of hunters.

Moreover, the representativeness of the study population (50 hunters) in relation to the total Latvian hunting community may be questioned. However, according to the observations of the facilitator, no new information was added by the hunters of the last focus groups to the already mentioned queries and perceptions. These observations resemble the systematically documented findings of Guest et al. (2006), in which saturation occurred within the first twelve interviews and basic elements were already present after six interviews. Finally, an age-related bias was present in this study. The estimated average age of the participants was 50 years, which represents the average age distribution in the Latvian hunting community. Nevertheless, an age-bias cannot be excluded.

We chose relation diagrams as a participatory method, assuming that a large quantity and a high quality of contacts between stakeholders in the network of control measures increases acceptance of measures to control ASF in wild boar. FVS, SFS and the hunters do most of the office- and fieldwork related to ASF control in wild boar. Therefore, the described combination of frequent and satisfactory contacts can be seen as beneficial for controlling ASF. As representatives of the hunting organisations are already invited to meetings where relevant information on the epidemiological situation and ASF control is shared, the perceived lack of communication between the SFS and the hunters may have been caused by missing communication between the representatives and every individual hunter.

Another suggestion of the participants was to post regular news on ASF in the media. This may also provide continuous information to the society as a whole, help to raise awareness and maintain it at a high level, if needed.

The contact with logging companies regarding the control of further spread of ASF was perceived as important by the participants. However, communication was regarded as difficult, highlighting the need for improvement, e.g. by sharing relevant information on ASF control measures also with logging companies.

Regarding the control measures assessed by the hunters, vaccination was often mentioned and perceived as the best solution. However, this control measure does unfortunately not exist at the moment as there is no effective vaccine against ASF available (EFSA, 2019). The mind-set of regarding vaccination as the only way to control ASF effectively could weaken the willingness of hunters to implement any of the currently available control measures. Therefore, further information on their effectiveness may lead to higher acceptance of all measures, except vaccination. Moreover, hunters need to be informed that vaccination against ASF is currently not an option. On several occasions, the participants mentioned the desire for continuous education of hunters regarding ASF and on the effects of control measures. Hunting and all other measures that support hunting were highly appreciated. To improve the acceptance of financial incentives, full compensation for the time and materials spent on ASF control need to be taken into consideration.

In addition to the presence of loggers, veterinarians, citizens and hunters in the forest, the participating hunters perceived the possible involvement of the army and the police as a serious biosecurity problem, also because it was expected that intensified carcass search supported by these forces may lead to increased traffic. Implementing educational trainings and discussions on biosecurity during hunting, logging, transportation and intensified carcass search could lead to a better understanding and to problem-oriented solutions that are supported by hunters, veterinarians and loggers.

Modification of control measures regarded as unethical (e.g. hunting of sows or hunting with perceived untrained stakeholders) could also lead to a better acceptance of these measures. The participants suggested limiting the increased hunt of sows to months outside the farrowing season. The perception that involving the army or the police might lead to unethical "killing" of wild boar could be changed by giving hunters a leading role in educating external forces and by hunting jointly.

Fencing was seen as a waste of money and time, because it was perceived as ineffective. Moreover, it was pointed out that fences represent obstacles for all wild animals. Therefore, it seems difficult to overcome these concerns, even if fencing is limited to small areas, if natural borders are used to avoid fences and if fences are constructed in such a way that the effect on non-target species is minimized (height, mesh size etc.). It should be noted that the effectiveness of fences is also controversially disputed in the scientific community (Jori et al., 2020). As poisoning wild boar was only mentioned as a potential control measure by two groups (and highly disliked), this measure may not be appropriate. Moreover, poisoning wild boar is unlikely to be accepted by the general public (Jori et al., 2020).

Passive surveillance in combination with carcass disposal is regarded as one of the most effective measures for controlling ASF in wild boar (Nurmoja et al., 2017; European Food Safety Authority, 2018, 2019; Schulz et al., 2019). Therefore, a high level of acceptance of passive surveillance by hunters, the key stakeholder, is of utmost importance to reduce the risk of ASF spread. The perceived consequences for hunters finding a dead wild boar were analysed under the assumption that knowing the consequences might help to adjust them and thus to increase the motivation of hunters to support passive surveillance.

Similar to the results reported by Calba et al. (2015) and Schulz et al. (2016), most Latvian hunters found it difficult to list any positive consequences, when detecting a dead wild boar. However, the existing commitment of the Latvian hunters to contribute to ASF control was demonstrated by their motivation to reduce the infection pressure within the wild boar population. Therefore, they were willing to help controlling ASF by finding and reporting dead wild boar, although they regarded the consequences as negative (Fig. 6).

The second most important consequence of finding and reporting a wild boar carcass was mentioned along with the comment that this meant that 'the disease has returned'. This illustrates the intrinsic interest of the participating hunters to control ASF. This motivation might originate from the perception of the hunters' duty and the often-stated importance of managing the wild boar population in an ethically acceptable way. To encourage hunters in participating in passive surveillance, the perceived negative consequences should at least be mitigated. The second most trusted tool to motivate hunters to participate in passive surveillance, i.e. only reporting wild boar carcasses without any obligation of sampling or disposal, was complemented by the participants' ideas to programme a smart phone app for reporting and to involve external forces in the search for wild boar carcasses. With respect to a reporting app, it should be noted that such a tool already exists in Germany (https://tierfund-kataster.de/tfk/tfk_erfassung.php).

To increase the acceptance of the involvement of groups like the army or logging companies, clear rules should be set up first, followed by information and biosecurity workshops. To reduce the workload for hunters, a professional group for carcass removal, sampling and burial could be implemented. In this regard, the participants suggested a proportional distribution of financial incentives among the involved stakeholders, depending on the level of their involvement. If hunters remain the stakeholder with the largest number of duties, it will be advisable to increase incentives for them. Although the Latvian hunters were largely satisfied with feedback and communication by the authorities in the country, the motivation tool of detailed feedback may be a relevant issue in other regions that should not be neglected.

5. Conclusions

The results of this study show hunters' views on aspects of ASF control and passive surveillance for the disease. The findings demonstrate a willingness of Latvian hunters to cooperate with the authorities in the control of ASF that may help to ensure the success of the measures. The results presented here offer the chance to include the hunters' views in the control of ASF in wild boar and to improve the measures by taking their acceptability into account. Successful control of ASF in wild boar seems only feasible with the active support of hunters for measures that are acceptable to them.

Ethics approval and consent to participate

All hunters participated voluntarily in the group discussions and were informed about the objectives of the study. They approved the anonymous publication of the results. In consultation with the Food and Veterinary Service formal consent or ethics approval was not required for this study, as it included neither clinical trials in humans, for which ethical approval might have been required, nor animal experiments.

Declaration of Competing Interest

All authors declare that they have no conflicts of interest relevant to this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2020.105229.

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4.2.2.3 A comparison of perceptions of Estonian and Latvian hunters with regard to the control of African swine fever

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Highlights

- The choice and the education of facilitators has to be considered to ensure high quality studies
- Translation processes hold the danger for information loss
- Hunters speak more freely in the absence of any authority members
- The perceptions towards ASF control measures and motivational options to support passive surveillance are very similar in both countries

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A Comparison of Perceptions of Estonian and Latvian Hunters With Regard to the Control of African Swine Fever

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Since the first detected African swine fever (ASF) cases in Lithuanian wild boar in 2014, the virus has occurred in many other member states of the European Union (EU), most recently in Belgium in 2018 and in Germany in 2020. Passive surveillance and various control measures are implemented as part of the strategy to stop disease spread in the wild boar population. Within this framework, hunters perform important activities, such as the removal of carcasses, fencing or hunting. Therefore, the successful implementation of these measures largely depends on their acceptability by hunters. Methods of participatory epidemiology can be used to determine the acceptance of control measures. The use of participatory methods allows the involvement of key stakeholders in the design, the implementation and the analysis of control and surveillance activities. In the present study, two studies that had been conducted using participatory epidemiology with hunters in Estonia and Latvia were compared on the topics recruitment, participants, facilitators, focus group discussion (FGDs) and their contents. The aim was to evaluate similarities and differences in the two studies and to identify a broader spectrum of possibilities to increase the willingness of hunters supporting the fight against ASF. Evaluating all conducted FGDs in both countries showed primarily similarities in the perceptions and opinions of the hunters in Estonia and Latvia. One notable difference was that passive surveillance in Latvia was perceived mostly as topic of duty and ethics rather than an issue driven by incentives. Participatory methods have proven to be an effective tool in the evaluation of the acceptance of established ASF control systems. The results of this study point out further chances for improving the cooperation with hunters in the future. Nevertheless, the importance of gathering and analyzing the opinions of hunters in all ASF affected countries individually is highlighted.

Keywords: African swine fever, participatory epidemiology, control measures, passive surveillance, acceptability, hunter, wild boar

INTRODUCTION

The recent entry of African swine fever (ASF) into Germany in September 2020 showed that the ASF spread in the European Union has not yet come to a hold (1). Since the beginning of the current epidemic in Georgia in 2007, more and more wild boar and domestic pigs have become infected globally (2). The ASF virus emerged in Lithuania, Poland, Latvia and Estonia as the first affected member states in the Eastern part of the EU (3). Currently, there are two main mechanisms, which are deemed to be responsible for the spread of ASF, i.e., trans-regional human mediated virus spread, sometimes over long distances, and local transmission by migrating wild boar (3–5). The potential role of wild boar as a susceptible species in the spread of ASF emphasizes the importance of establishing measures aimed at controlling local wild boar populations (2, 6–10).

Hunters belong to the most important stakeholders in the implementation of ASF control measures in the wild boar population (11, 12). Their regular presence in the forest, their experience and knowledge regarding local wildlife make them valuable partners with regard to control measures and passive surveillance. So far, hunters have been primarily involved in the implementation of mandatory processes, such as wild boar carcass searches, removal of carcasses from the environment and shooting wild boar. However, expert knowledge on the local situation, also with respect to the peculiarities of the wild boar population, is an important basis for the control of the ASF (11, 13). As mentioned by experts, hunters should therefore be included in the decision-making process (2, 14). This can be achieved by using methods of participatory epidemiology (PE) (15, 16). PE allows the involvement of stakeholders, e.g., in data collection or decision making on topics relevant for the community (11, 14, 17, 18). Participatory methods such as focus group discussions (FGDs) in combination with visualization or ranking and scoring tools are widely used in developing countries to support quantitative data generation in rural areas (13, 17, 19–22). Despite its potential in considering issues from different points of view and implementing specific local measures avoiding unpopular approaches, PE has not frequently been used in developed countries so far (17, 23).

To employ the advantages of PE by investigating perceptions of hunters and thus learning more about their motivations or reasons for hindrance to support ASF control in wild boar, two PE studies were conducted in Estonia and Latvia. In both studies, the same methods of FGD and visualization methods were used and regional opinions on the acceptance of ASF control measures and passive surveillance were collected and analyzed (24, 25). In the present study, the results of these two studies were compared, thus assessing similarities and differences. By comparing both studies, we aimed at identifying functioning processes and difficulties (26) in current control strategies against ASF, which may be addressed in future collaboration with hunters to increase the acceptance of passive surveillance and defined ASF control measures.

MATERIALS AND METHODS

Recruitment

Hunters from different areas in Estonia and Latvia were invited to participate. We intended to include a broad range of experiences and perceptions regarding ASF. In co-operation with hunting communities from Estonia and Latvia, leading hunters of regional hunting organizations were contacted. They were informed about PE and the aims of the studies and asked to invite hunters to the FGDs. In Latvia, staff of the "Latvian Food and Veterinary Service" contacted leading hunters. In Estonia, staff of the Veterinary and Food Laboratory contacted potential participants. The Veterinary and Food Laboratory is a facility, to which hunters regularly deliver samples.

Participants

It was planned to form ten FGDs per country with four to six participating hunters per group. The only requirement for participation was the willingness of the hunters to attend the meetings.

Facilitators and Focus Group Discussions

The participatory methods used by Urner et al. (24, 25) were adapted from Calba et al. (13) and Schulz et al. (11). The FGDs were divided into two tasks with regard to control measures and two tasks concerning passive surveillance. In each country, they were moderated by a national facilitator. The facilitators' responsibility was to introduce each task to the hunters and explain issues to avoid misunderstandings. In addition, the facilitators had the function to stimulate discussions and encourage reticent participants to express their views while moderating dominant participants. The facilitators were asked not to express their own personal view or to emphasize any particular opinion. The discussions were transcribed in Estonian and Latvian and translated into English.

Content

Acceptability of Control Measures

For the first task, the participating hunters were asked to enumerate all stakeholders they perceived as being part of the ASF control system. Subsequently, they were motivated to indicate the quantity of contacts from hunters to stakeholders and vice versa with four different arrows (no contact, little contact, normal contact, intensive contact). In addition, they were asked to rate the intensity of contacts qualitatively. To this end, each hunter assessed the contacts using smileys as good, neutral or bad (individual ratings). The last step of the first task was that the hunters were asked to use proportional piling to illustrate their trust in the stakeholders with respect to implementing control measures. For this purpose, the participants were given 100 glass beans, which they had to distribute among all stakeholders in proportion to their trust in the stakeholders to implement control measures appropriately (based on a consensus within the group).

In the second task, a list of six control measures was presented to the hunters [fencing, ban of hunting, including professionals for intensive hunting (police/army), increased hunting of female wild boar, incentives for hunting and increased carcass search and removal]. The participants were then asked to list additional measures, they could think of. All control measures were evaluated based on the hunters' satisfaction in implementing them (individual rating using smileys) and on the trust that the implementation of the measure might help to control ASF (consensus within the group, using proportional piling).

Acceptability of Passive Surveillance and Different Motivation Options

In the third task, the participants were asked to list positive and negative consequences that came to their mind when finding dead wild boar. Thereafter, the participants had to discuss until they had reached consensus and to evaluate the mentioned consequences by distributing 100 glass beans proportionally to the perceived impact the consequence would have on the hunters (proportional piling).

In the fourth task, four options to increase the motivation of hunters to participate in passive surveillance were presented to the hunters (increase of currently paid incentives, passive surveillance achieving the benefit of reduction of infection pressure in the wild boar population, only reporting dead wild boar without any further work for the hunter and detailed feedback from the relevant authority to the hunter). The participants were asked to add further options. Using proportional piling the hunters had to illustrate the potential of the options to motivate them to increase their engagement in passive surveillance.

Analysis

The results of the participatory methods were analyzed semi-quantitatively. To this end, the four different arrows were assigned to the numbers 0, 1, 2, 3 and the smileys to the numbers −1, 0, 1. For each option evaluated by these tools (stakeholders, control measures...), the average for all groups was calculated.

To evaluate proportional piling, a weighted average was calculated for each option (Stakeholder, control measures...). To calculate the trust T_{SHi} for a mentioned stakeholder (a) SH_i, the number of stakeholders mentioned in all groups SH, the number of groups which mentioned stakeholder (a) N_{SHi}, the number of stakeholders in the group in which stakeholder (a) was mentioned C_j^{SH} and the glass beans allocated to stakeholder (a) in each group GB_{ij} were taken into account. Details are described in Urner et al. (24, 25).

$$T_{SH_i} = \frac{1}{N_{SH_i}} \cdot \sum_{j=1}^{10} \frac{C_j^{SH}}{\sum_{j=1}^{10} C_j^{SH'}} \cdot GB_{ij},$$

The trust in a control measure to help control ASF, the impact of possible consequences on the hunters and the potential of an option to motivate hunters to participate in passive surveillance were calculated accordingly.

The results of the discussions were included descriptively.

The data and results from both countries were descriptively compared regarding the topics recruitment, participants, facilitators and FGDs.

RESULTS

Recruitment

The recruitment of participants were done similarly in both studies. A list of contact persons (leading hunters of local hunting clubs) had been provided by the national hunting organizations. These contact persons were contacted by phone or mail and informed about the aims of the study. The only difference was the organization that had contacted leading hunters of regional hunting organizations.

Participants

In total, 96 hunters participated, 46 in Estonia and 50 in Latvia. In each country, one woman participated. The age of the participants was no criteria for participation. To respect their personal rights and to keep the FGDs anonymous, they were not asked for their age. The estimated average age was 50 years.

Facilitator and Focus Group Discussions

Twenty FGDs were organized from May 2019 to July 2019. Ten FGDs took place in each country, with two to seven hunters per meeting. The group size did not differ in the two studies.

In Estonia, the facilitator was a female staff member of the Estonian University of Life Science, who had not worked with hunters previously and had not been involved in ASF control. She participated in a 3-day training school for participatory methods before the PE study started in Estonia. The study design was practiced under the guidance of the supervising author, who received PE training at the French Agricultural Research Centre for International Development (CIRAD) (11). In Estonia, only the facilitator attended the meetings. The discussions were therefore audio-recorded and afterwards transcribed by the facilitator. In Latvia, the facilitator was a female staff member of the Latvian Food and Veterinary Service, who had not worked with hunters previously and had not been involved in ASF control. The Latvian facilitator did not receive formal participatory training, but practiced the procedures during the discussions with the supervising author and the Estonian facilitator. The Latvian facilitator was assisted by two colleagues from the Latvian Food and Veterinary Service. One of them, a male colleague, was present as an observer and provided scientific background for questions regarding wild boar and the other one, a lady, transcribed the discussions. For analysis, the transcriptions were translated into English by the Language Centre of the Estonian University of Life Sciences in Estonia and the professional translator company "Skrivanek Baltic" in Latvia.

Contents

Acceptability of Control Measures

The listings and ratings of the stakeholders involved in controlling ASF of the Estonian and Latvian participants were similar (**Table 1**). In both countries, the minor contact to the research centers (Estonian University of Life Science and Institute BIOR) was perceived as unsatisfactory. Participants in both countries rated the police and the army as the least trustworthy organizations with one of the lowest contact rates.

Several stakeholders in society, such as the media, farmers and animal protection organizations were mentioned only in Latvia.

All hunters rated vaccination and hunting as the most trustworthy measures to control ASF and most satisfactory to implement (**Figure 1**). In Estonia, vaccination was not included in proportional piling by the facilitator as vaccination is currently not an option because there is no functional vaccine (27). Nevertheless, the hunters mentioned in the discussions that they would rate vaccination as the most trustworthy measure. The moral conflict of producing orphans by hunting female wild boar in the farrowing season was mentioned in discussions in both countries. The least trusted control measures in Estonia and Latvia overlapped as well (**Figure 1**). Similar reasons were mentioned, such as the hindrance of all game animals if a fence is built up. Implementing biosecurity measures during hunting was only mentioned in Latvia. It was trusted mediocre in controlling ASF and perceived satisfactory to implement. On the other hand, various hunting methods were mentioned only by Estonian participants. For example, bait feeding and shooting was highly trusted and considered satisfactory to implement.

TABLE 1 | The top five stakeholders rated by the participants to be the most trustworthy to implement control measures in an appropriate manner.

Estonia	Rank	Latvia
Hunters	1	Food and Veterinary Service
Veterinary and Food Laboratory	2	Hunters
Hunting Council of a county	3	Hunting organization
Estonian Hunters' Society	4	State Forest Service
Estonian University of Life Sciences (EMÜ)	5	Institute BIOR

Acceptability of Passive Surveillance and Different Motivation Options

The perceived consequences of finding dead wild boar overlapped in both countries. However, the assessment of the impact for hunters differed.

All participants mentioned consequences such as extra work, lost time, financial costs, recovering and disposing of the carcass. In Latvia, the perceived consequences focused on the fact that ASF can be controlled by searching carcasses (and removing them). This was mentioned as the "hunters' duty" in the discussions. In Estonia, the focus was rather on the negative consequences (**Figure 2**).

Comparing the proposed options to further increase participation in passive surveillance showed that in Estonia, an increase in financial incentives was considered more motivating than mere reporting with no further work. In Latvia, the pure idea of reducing the infection pressure in the wild boar population by searching for carcasses and removing them was considered the most motivating factor (**Figure 3**).

DISCUSSION

The success of ASF control measures and passive surveillance depends on the willingness of hunters to implement them (2, 11, 14). It is therefore of utmost importance that the national and international control of ASF focuses on identifying motivations or obstacles to support control measures and passive surveillance and, if necessary, on increasing the willingness of hunters to participate in these measures actively. To achieve this, PE methods should more frequently be included to complement conventional epidemiological approaches, also in industrialized countries. By integrating key stakeholders, decisions can be made based on extended information from the everyday life of those, who are directly affected and involved. However, this also

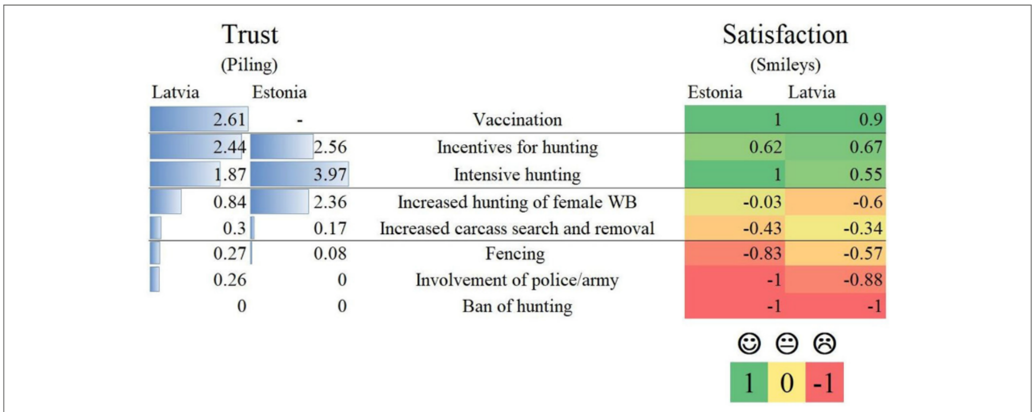

FIGURE 1 | Control measures rated by trust to control ASF and satisfaction in implementing them of Estonian and Latvian participants in ten focus group discussions comparison.

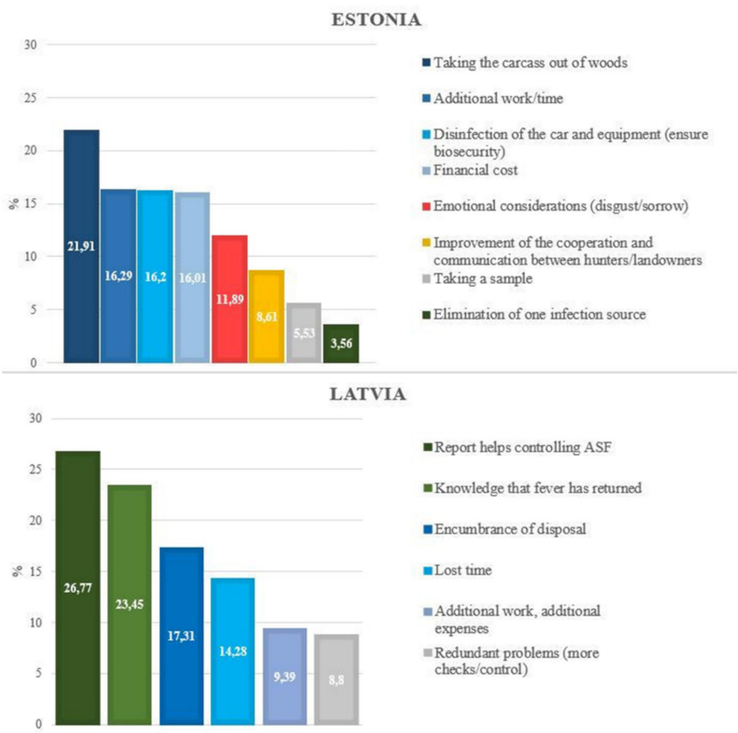

FIGURE 2 | Perceived extent of the impact by hunters of potential consequences when a dead boar is found, expressed as a percentage of all evaluated consequences in Estonia (*n* = 46) and Latvia (*n* = 50). The consequences are colored in green for ethical consequences, blue for consequences on time, work and money and reddish for emotional consequences.

influences the decision-making process by adding new biases, which are present in most participatory studies.

In the studies analyzed here, a potential selection bias may have been present due to the recruitment process (11, 13). Inviting participants through hunting associations holds the danger of recruiting only hunters of the direct social network of the contact person, who may share a common opinion. In addition, it is possible that mainly hunters were recruited, who were highly communicative toward hunting organizations and authorities (28). In addition, contact by the Ministry may have resulted in a situation, where some hunters felt compelled or obliged to participate and others may have been deterred. However, the roughly equal number of participants in both countries suggests that this bias has probably been low. The willingness to participate was therefore generally present and there was no obvious indication that hunters felt compelled to become involved. The total number of 96 participants may question the representability of the results. However, theoretical saturation was found in both studies as described in Glaser et al. (29) and Guest et al. (30). As the results were largely similar in both studies, which included hunters with a very different social background, the participation bias and question of representability may be regarded as minor.

Although the procedures to be followed by both facilitators were identical, a complete consistency cannot be guaranteed. Skills that characterize a good facilitator to get the most unfiltered results in a discussion could not be conveyed in short training provided to the facilitators (31), who also lacked experience in conducting PE studies. Furthermore, the openness of the participants toward an employee of a university (Estonia) might differ from the attitude toward an employee of a national authority (Latvia). In addition, there is the possibility that certain opinions may have been expressed in Latvia, precisely because the authority organized and carried out the FGDs. It seems possible that the hunters wanted to keep or create a certain image when confronted with a representative of a state authority or to

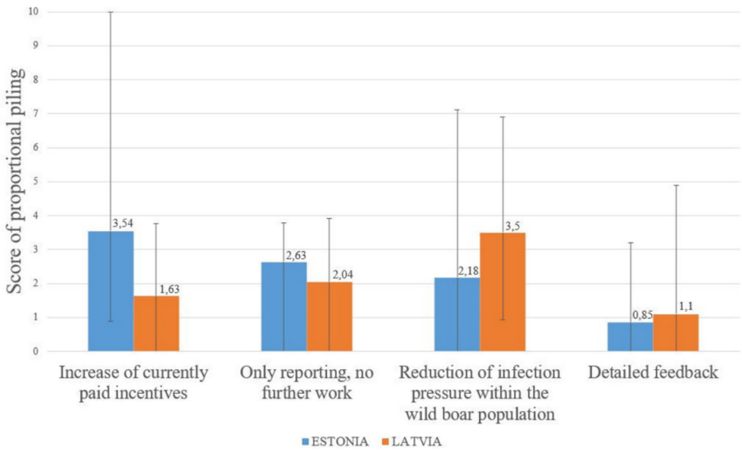

FIGURE 3 | Comparison of the perceived possible effectiveness of tools to increase participation in passive surveillance based on the calculated weighted average of the proportional piling. The average of all groups is displayed along with the range between minimum and maximum value of the weighted piles.

stimulate certain reactions by the authority. As a male employee working in ASF disease control was present in the Latvian FGDs for questions and misunderstandings, this may have influenced hunters' statements. However, the general overlap of the results suggests that this potential bias had little impact on the outcome.

Direct transcription in Latvia instead of recording in Estonia had the advantage that no further transcript had to be made from the audio recording. However, direct transcribing the contents of the FDGs might have led to a loss of information due to subjectivity, as it is very likely that not every spoken word was considered important, so that some statements could have been missed. The translation process of both transcripts into English might have caused some information loss (translation bias).

Diverging extraneous circumstances like substantial differences in ASF control, varying hunting structures and the biases discussed above prevent that a detailed statistical analysis adds value to the conclusions that can be drawn by a simple descriptive comparison. Moreover, several results were only available in a qualitative form, which made a statistical comparison not only extremely difficult, but also and not very telling. We therefore focused on the purely descriptive comparison. Despite these potential biases, the statements of the participants in Estonia and Latvia showed similarities. For some topics, almost identical statements were made. This does not only show the strong and similar opinions of the hunters, but also suggests that these biases can be regarded as minor.

The acceptance of working with stakeholders in the hunters' network strongly overlapped in both studies. This indicated relationships, which may be utilized and improved. Various possible co-operations (e.g., support from the army) should be discussed in advance with the hunters; otherwise they might feel not sufficiently respected in their main competence, i.e., hunting. It could be discussed, for example, that the army/police might only support carcass search and not hunting, which may subsequently lead to a higher acceptance of this measure by the hunters. Furthermore, the dissatisfaction with the small numbers of contacts with the research centers became obvious. This again supports the importance of communication, also with regard to scientific exchange before implementing measures. The differences in the networks of hunters in the two countries appeared to be small. The lack of mentioning various public stakeholders (e.g., animal welfare organizations, media) in Estonia compared to the ones mentioned in Latvia, could be explained by a different perception of the participants, who the relevant stakeholders were, or by a difference in the network of ASF control in Latvia.

The clear trend of acceptance of specific control measures was present in both countries, indicating a similar attitude of hunters, regardless of the individually implemented system of control measures. When interpreting the results, it must be taken into account that the two Baltic States are neighboring countries with a comparable recent history (32). Thus, the broad agreement in the perceptions and views of the hunters might be related to this neighborhood. To allow a more general statement about attitudes of hunters regarding ASF, it may be useful to implement the study in countries with more diverse geographical, historical and political background information.

Controlling ASF with hunting and increasing financial incentives for hunting is likely to find favor with hunters. Furthermore, the general acceptance of increasing incentives underlines the potential need of financial support for arising costs, such as equipment for biosecurity and transport. The same reasons given for not accepting fences (restricting other wildlife) and hunting female wild boar (morally contradictory to produce orphans in the farrowing season) reflect the common concerns of the hunting community and should be solved if these measures are to be implemented. Additionally, the high acceptance of vaccination and low acceptance of increased carcass search show how important scientific exchange is, especially on these specific topics to discuss effectiveness and in the case of vaccination availability (2, 7, 9, 27).

The fact that only in Latvia biosecurity during hunting was mentioned as a measure and only in Estonia several specific hunting methods were listed might show the different prioritization or awareness of control measures in the two countries. Biosecurity was mentioned in Estonia not before discussing passive surveillance and transporting carcasses. Thus, the awareness of hunters that biosecurity is appropriate in any handling of wild boar should be increased accordingly. However, it should also be considered that in Estonia, hunters just forgot to mention biosecurity as a control measure without any indication for the general perceived importance of biosecurity measures in Estonia.

The findings of Calba et al. (13) and Schulz et al. (11) that passive surveillance might not be highly accepted among hunters are supported by the perceptions of the hunters in the compared studies. Negative consequences such as increased workload, costs and time consumption were the focus in both countries. Reducing these hindering factors or even preventing them from occurring in the first place could significantly increase the acceptance of passive surveillance. All participants mentioned the same following approaches in this regard. Accordingly, the increase of financial support and the involvement of the army/police under the guidance of the hunters should be focused. In this respect, according to the participants, the emphasis should be on reducing the obligations of hunters. The implemented feedback systems seem to be sufficient, as additional detailed feedback was perceived not to be highly motivating in both countries. Thereby, increasing the details of feedback would only increase the workload for the veterinary laboratories without achieving higher participation rates in passive surveillance.

Despite the importance of eliminating negative consequences, Latvian hunters were more motivated by their moral obligation to participate in passive surveillance in order to contain ASF. This difference may have been caused by a potential bias of the observer from the Latvian authority. As mentioned before, the presence of the Latvian authority may have motivated the hunters to make statements, which make them look favorable. On the other side, the self-image of hunters in Latvia as workers for nature and wildlife may be different from that in Estonia, as passive surveillance was more often described as "hunters' duty" during FGDs in Latvia. Since the assessment of the motivating options was only comparative, it is possible that the perceived obligation of hunters has a similar status in Estonia, but the lack of financial support was regarded as more significant. These differences emphasize the need to communicate with hunters in each country individually and with regard to their specific views and concerns.

In summary, two main issues could be identified, which should be considered in efforts to improving cooperation with hunters and thus supporting the joint fight against ASF.

First, communication and cooperation with hunters should be increased, especially when it comes to the decision-making process. Communication should also include the dialogue with research centers. Hunters would like to become involved in scientific discussion. This was mentioned by all participants. This will ensure that they are informed about the most recent research results on ASF by the researches themselves. On the other side, through a two-way communication, disease control will benefit from the expert knowledge of hunters in implementing practical and successful control systems. In this context, workshops or training courses may largely support increased communication. These events could be very helpful to explain the reasons and the possible positive effects of measures to the hunters as executive stakeholders, especially regarding passive surveillance. Possible modifications of already implemented measures could also be communicated, discussed and adapted jointly, for example hunting female wild boars only in autumn and winter.

Secondly, loss of time and the increased workload are the main conflicting issues for hunters to contribute to passive surveillance. These issues could be addressed by having other external stakeholders supporting the hunters by taking over the collection and disposal of wild boar carcasses after a hunter has reported the finding. If this is not possible, financial incentives or compensations may be increased to cover costs and time.

This study describes hunters' opinions regarding passive surveillance of ASF and measures to control ASF in two EU member states affected by the disease. In essence, despite different systems of ASF control and the different hunting structures in the EU member states there was broad consensus on a large number of issues in the hunting communities of Latvia and Estonia. The results of this study may be incorporated with caution into future work on ASF control, as they only reflect the opinions of a single stakeholder group. Participatory studies including stakeholders involved in ASF surveillance and control other than hunters should also be conducted or these groups included.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

NU drafted the manuscript for this comparing study. KS, CS-L, CS, and FC provided scientific input and background for the draft of the manuscript and revised it extensively. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.642126/full#supplementary-material

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5 Discussion

African swine fever (ASF) has been a major research topic for almost exactly 100 years, and effective treatments or vaccines are still not available. Although the disease only affects suids, it is of global concern. It is known that the disease can be transmitted between domestic pigs and wild boar. In the past European ASF epidemic on the Iberian Peninsula, the role of wild boar was considered negligible. It was even postulated that virus circulation in wild boar depended on ASF outbreaks in domestic pig holdings and in case of a successful disease eradication in the domestic pig sector, ASFV spread would not be maintained within wild boar populations. Thus, it was assumed that infected domestic pigs rather constitute a threat for wild boar than vice versa (Mur et al., 2012). However, this view was based on the experience of an ASF epidemic in a region with relatively small wild boar population in an environment that clearly differs from eastern and central Europe. Currently, it appears that particularly in Europe, the susceptibility of wild boar combined with the high wild boar population density constitute a serious threat of industrialized pig holdings (Ruiz-Fons et al., 2008, Massei et al., 2015, European Food Safety Authority, 2018). ASF outbreaks in domestic pig holdings usually result in trade restrictions, which consequently entail economic losses (Halasa et al., 2016). These consequences and the lack of a curative treatment or vaccine require reliable surveillance measures and an effective control of ASF, particularly in wild boar. Despite the 100 years of research on ASF, the virus managed and still succeeds to spread globally. Not only the huge wild boar density and the difficulty to reduce it sustainably, but also wild boar behavior and the social structures in wild boar populations impose major challenges regarding the control of ASF. Thus, the aim of this thesis and the included publications was to close knowledge gaps, but also to examine the epidemiology of ASF in wild boar on the basis of comprehensive "real-life" surveillance data. The epidemiological studies served not only to increase the knowledge about ASF, but also to identify weakness in ASF surveillance and control. Consequently, the integration of methods from PE aimed to support the inclusion of insider knowledge and the perceptions of key figures and thus the identification of starting points for a more successful design of surveillance and control measures.

Before Montgomery (1921) classified ASFV as a new virus, the disease was not distinguished from CSF (Penrith, 2013). Despite the subsequent differentiation of the two causative viruses and their features, surveillance and control measures for ASF in Europe were often adapted from the ones designed for CSF. However, comparing the virology, immunology and the epidemiology of the two diseases demonstrated the need to consider the diseases separately from each other **(Publication 1)**. In the review, it was already shown that the spread and transmission rate of ASF seems to be more slowly than that of CSF, which was confirmed in a narrative review regarding the spreading speed of ASF **(Publication 2)**. Several experimental and field studies could disprove the common and still wide-spread belief that ASF is a highly contagious disease. These findings are supported by more recent studies, where slow ASFV spread has been observed within affected farms and wild boar populations, experimentally and using modeling approaches (Lamberga et al., 2019, Zani et al., 2019, Mačiulskis et al., 2020, Marcon et al., 2020, Pepin et al., 2021). Following the increasing evidence that ASF spread is slower than originally assumed, surveillance and control measures have to be adapted. Particularly with regard to the surveillance objective "early detection of disease", the knowledge is vital that an introduction of ASF does not necessarily result in an immediately high mortality. Even a small number of animals that die on a farm or in a wild boar population may indicate an ASFV introduction. Accordingly, to ensure reliable surveillance results, all dead wild boar or domestic pigs should be sampled and examined for ASFV, at least in regions, where ASF must be expected to occur. However, not only in case of early detection, but also when aiming for reduction of virus spread and disease elimination, the detection and sampling of wild boar carcasses is essential. Several studies proved that the ASFV can remain infectious for a long time in carcasses of infected wild boar and their surrounding (Fischer et al., 2020, Mazur-Panasiuk and Woźniakowski, 2020). Furthermore, it was shown that wild boar are interested in their dead conspecifics and the surrounding soil, even when scavenging was not observed (Probst et al., 2017, Masiulis et al., 2019). Using a modeling approach, O'Neill et al. (2020) confirmed that infected carcasses play a major role in the spread of ASF.

Although the spreading speed of ASF is not as fast as assumed, it still can vary considerably. Human activities play a crucial role in the spread of ASF, which can be clearly seen in the individual point entries in Belgium, the Czech Republic, in western Poland (Saegerman, 2018, Mighell and Ward, 2021, Šatrán, 2019), and most recently perhaps also in southern parts of Mecklenburg-Western Pomerania, Germany. Mighell and Ward (2021) and Nurmoja et al. (2018) showed that the spreading speed of ASF in the domestic pig sector was dependent on the number of backyard holdings with a low biosecurity level. The strict ban and the subsequent decrease in backyard holdings in Estonia certainly supported the long period without any reported ASF outbreaks in domestic pigs. In contrast, in Romania, where the density of backyard holdings is very high, the significant spread of ASF between domestic pig holdings is indicative (Boklund et al., 2020). In an interdisciplinary review **(Publication 3)**, the current knowledge regarding ASF in wild boar was summarized and updated, thus working out the challenges in ASF surveillance and control. Not only by reviewing available literature, the difficulties in ASF surveillance and control became evident, but also by analyzing ASF wild boar surveillance data from the Baltic States. ASF reached Lithuania as the first of the three Baltic States in January 2014 (Pautienius et al., 2018). In June of the same year, the disease emerged in Latvia (Oļševskis et al., 2016) and finally in September 2014, also Estonia had to report ASF cases in wild boar **(Publication 4)**. To assess the epidemiological course of ASF in a wild boar population, it is essential to understand and interpret the laboratory test results adequately **(Publication 5)**. ASF research showed that animals that get infected with ASFV usually yield a PCR-positive laboratory test result after approximately 4 days. In case of a survival of the infected animal, ASFV-specific antibodies can be detected around days 7-10 post infection, whereby the seropositivity does not allow any statement about the chances of survival of the infected wild boar (Blome et al., 2020). This holds true at least for the first 100 days of infection, in which usually ASFV genome and antibodies are simultaneously detectable. In experimental studies, it was shown that pigs that survive an ASFV infection for approx. 100 days, usually yield a PCR negative result if sampled, but can still test positive for ASFV-specific antibodies (Nurmoja et al., 2017a, Petrov et al., 2018). Thus, it can be assumed that the few animals that survive ASF, do not shed the virus and accordingly do not pose a risk to further transmit the disease. However, this hypothesis and the role of exclusively seropositive wild boar in the ASF epidemic is controversially discussed (European Food Safety Authority, 2021) **(Publication 12)**. Extensive analyses of ASF in wild boar revealed similar epidemiological courses in the three Baltic countries **(Publications 4, 5, 6, 7, 8)**. The disease was introduced in the East and it spread slowly towards the West, affecting almost the entire countries after approx. 2 years. Also in Poland, the disease spread within 2-3 years to different places within the country (Frant et al., 2020). In these early affected EU member states, ASF was probably introduced by migrating infected wild boar from neighboring countries. Although first ASF cases were reported from the south of Estonia, the results of our study **(Publication 4)** suggested that the virus was first introduced in the northeast of the country. However, the small number of samples that were investigated before the discovery of ASF probably prevented an earlier detection. When studying the recent course of ASF in Estonia with a long absence of ASFV in the wild boar population and the new emergence of ASFV-positive wild boar after 1.5 years, it could also be shown that the number of investigated samples was too small to detect a circulating virus at a low prevalence (European Food Safety Authority, 2021) **(Publication 12)**. However, in 2020, the wild boar population density in Estonia was significantly lower than at the beginning of the epidemic in 2014, further complicating disease detection. In 2014, the new introduction of the disease reached a completely naïve wild boar population, resulting in an increased number of wild boar carcasses and the presence of large amounts of ASFV in the environment. Accordingly, the low awareness of the public and of hunters before the first ASFV introduction in the country might have additionally impeded a timely detection in the north of Estonia. The need to raise awareness among affected stakeholders and thus increasing the chance for a successful disease control was particularly evident in the field of ASF in domestic pig holdings (Dione et al., 2017, Yoo et al., 2020, Bellini et al., 2021, Kurian et al., 2021, Wozniakowski et al., 2021). In the study of Vergne et al. (2016), a lack of awareness was identified as justification for hunters to waive sampling of wild boar carcasses. The European Food Safety Authority et al. (2021) also stressed the urgent need to raise awareness among all key figures involved in the surveillance and control of ASF. Using the participatory “World Café” method to gather expert opinion, these findings could be confirmed **(Publication 16)**, emphasizing the need for transdisciplinary approaches in the control of ASF.

Although the course of ASF in the three Baltic States was similar including a decrease of the detection of ASFV-positive wild boar and an accumulation of surviving animals, showing seropositive and PCR-negative test results, this course was not as obvious in Lithuania as in Estonia and Latvia **(Publications 7, 8)**. In 2017 and 2018, the assumed virus load in the wild boar population was high, leading to a huge number of samples obtained from wild boar carcasses. These numbers also indicated the good cooperation between the involved groups of persons in the control of ASF, highlighting the importance of communication and the willingness to support disease control. Despite the ongoing circulation of ASFV in Lithuania, the number of investigated samples dropped after 2018. Based on a questionnaire, the attitude of Lithuanian hunters towards ASF, its surveillance and control was investigated (Stonciute et al., 2021). It was found that hunters did not favor to support passive surveillance measures, confirming findings from participatory studies (Schulz et al., 2016) **(Publications 18, 19)**. In contrast to the PE studies that explored the rationales behind the unpopularity of passive surveillance measures, the questionnaire only revealed the dissatisfaction of the hunters with these measures. This comparison of the results of the two different methods illustrates the advantages of PE. Although a questionnaire study might be easier to conduct, PE is irreplaceable, if the aim is to obtain the statements of the hunters directly, i.e. for a better understanding and for being able to react by asking for their motives, views and questions. However, the lack of motivation of hunters to support passive surveillance was similar in Estonia and Latvia **(Publications 18, 19)** and is therefore not sufficient to explain the slightly different course of ASF in Lithuania. Over time, Lithuania reported a huge number of ASF outbreaks in domestic pig holdings (Mačiulskis et al., 2020). In contrast to Estonia, small backyard holdings played a substantial role in the ASF epidemic. The at least 300 km long Lithuanian border with Belarus, where the ASF status in wild boar is officially unknown, but a wide-spread occurrence of the disease likely, and wild boar migration across the border as well as human border traffic pose a constant risk of "new" virus introductions. These complex social interactions and the resulting consequences demonstrate that the control of ASF in wild boar and in domestic pig holdings should not be considered separately.

In all three Baltic countries, the decrease in the wild boar population density over time was significant **(Publications 5, 8, 13)**. This was certainly mainly due to ASF itself, but it was probably also supported by the implemented control measures such as increased hunting (Morelle et al., 2020). The estimations of wild boar density are difficult and different methods, their accuracy and their reliability have been a topic of discussion for a long time (European Food Safety Authority, 2018, European Food Safety Authority et al., 2021). It has been assumed that the true wild boar density is often much higher than the reported numbers (O'Neill et al., 2020). In addition to the difficulty of precisely determining wild boar abundance, the methods used in the various countries may differ greatly. Although mostly, density estimates are based on the hunting bag or the hunting index of the population density, the methods, how these figures are recorded, reported and interpreted, differ, thus hampering comparisons (ENETWILD-consortium et al., 2019). However, in the Baltic studies, population densities were compared within the countries and over time. Accordingly, the compared data were obtained by applying the same methods and thus, the trend in the timely course was considered informative. The significant decrease in the wild boar population caused by ASF slowed down the spread of ASF and the number of detected ASF-infected wild boar declined. This decline is obviously welcome as it may suggest that the control measures were successful, however, simultaneously with the decrease in the number of wild boar, the detection probability decreases, thus allowing the virus to spread unnoticed at a low prevalence (European Food Safety Authority, 2021) **(Publication 12)**. This density drop that can develop in the course of an ASF epidemic in wild boar requires a constant vigilance of the involved stakeholders and a long-term and economic allocation of available financial and human resources. It is obvious that stakeholder engagement and interest decreases over time, particularly in the case of an intensive involvement as seen in the three Baltic States at the beginning of the ASF epidemic. The re-emergence of ASFV-positive wild boar in Estonia emphasizes the need of continuous information, involvement and consideration of the hunting associations and their role in the long-term surveillance and control of ASF **(Publication 12)**.

Regardless of the uncertainties concerning the exact determination of wild boar abundance, a high wild boar population density can be seen as a risk factor for a higher probability of ASF introduction, detection and also for a faster virus spread **(Publications 4, 13)** (Food and Agriculture Organization of the United Nations et al., 2019, O'Neill et al., 2020). Although other studies did not confirm a suspected association between population density and the presence of ASF (European Food Safety Authority et al., 2017), the high population density of wild boar is still problematic. This is particularly relevant due to the growing wild boar population throughout Europe and the high complexity in achieving a lasting reduction (European Food Safety Authority, 2018). Thus, joint and increased efforts to reduce the wild boar population should be maintained during all stages of the epidemic, but also to prevent new disease introductions and establishment of ASF in recently affected regions. This emphasizes again the urgent need to have the hunters on board from the beginning and to consolidate a long-term and sustainable cooperation, which can be supported by the integration of participatory methods. Furthermore, several studies identified the intensified hunt of juvenile and female wild boar as the best option to reduce the population density effectively. This is mainly due to the high level of reproduction, which can start at a very young age (Bieber and Ruf, 2005, Toigo et al., 2008, Keuling et al., 2013, European Food Safety Authority, 2018). These management recommendations contradict the usual goals of hunters, namely to bring home large trophies. Hunting juveniles is therefore not very attractive for hunters. Hunters are often also reluctant to kill adult female wild boar. In their eyes, the females play an essential role in the cohesion of the pack and the hunters fear that taking out the leading female will cause the pack to break up (Schulz et al., 2016). In addition, there are ethical concerns about hunting females and leaving suckling piglets without their mother **(Publications 18 and 19)**. Consequently, it is and will be very difficult to motivate hunters to act against their beliefs and ethical values. This is particularly the case when their concerns are justified and "right" solutions are not easy to define. Leaving piglets without their mother is certainly an animal welfare issue, however, the agonizing death of countless wild boar must also be prevented. Accordingly, the animal suffering that one is prepared to accept must be weighed up against each other, quite apart from the economic consequences of ASF, which of course also carry great weight in these discussions.

Thus, it is inevitable to seek a dialogue, to discuss the common opportunities to reduce the wild boar population density sustainably and informing hunters about the differences between hunting and disease control. Hunters have to understand the necessity of reducing the wild boar population density to avoid further disease spread and thus avoiding animal suffering and economic damage. Decision makers should ensure that hunters understand that ASF is a disease that leads to a painful death of wild boar and that their help in combating the disease is irreplaceable. However, the expertise of hunters and their ethical and epidemiological concerns should not only be acknowledged but also seriously considered in the design of control measures. It is not enough to try to convince hunters to cooperate but transdisciplinary discussions should take place at eye level and justified objections towards certain measures should be reviewed and possible alternatives discussed together.

In addition to the population density, it became indicative in all prevalence studies that the probability of detecting an ASF-positive wild boar is much higher in wild boar carcasses than in apparently healthy, hunted animals **(Publications 4, 5, 6, 7, 8, 11, 13).** This result is not surprising as, in contrast to other diseases (e.g. CSF), the case/fatality ratio of ASF is very high **(Publication 1, 2)**. It was found that the probability to detect an ASF-positive wild boar was higher in animals younger than one year **(Publication 4, 13)**. This might be due to a higher risk of young animals to die from ASF. However, in further studies, age was not confirmed as a risk factor for a higher probability to test positive for ASF **(Publication 8)**, thus emphasizing the need to perform sampling independently of the age of the animal.

In February 2019, Estonia reported the last ASFV-positive wild boar for 1.5 years. During these 1.5 years, seropositive wild boar were still hunted. Although the issue of persistent ASFV carriers is controversially discussed **(Publication 12)**, there is no scientific evidence so far that the solely seropositive animals could shed ASFV and might thus play a significant role in the maintenance and further spread of ASF (Nurmoja et al., 2017a, Petrov et al., 2018, Stahl et al., 2019). During the long time of apparent absence of circulating ASFV within the Estonian wild boar population and assuming the negligible role of seropositive wild boar in the spread of ASF, the question arose, when the country might be declared as free from ASF **(Publication 11)**. This question was also intensively discussed by the European Food Safety Authority (2021). They concluded that the current surveillance effort is not sufficient to demonstrate the freedom from ASF in wild boar reliably. Also Gervasi et al. (2020) showed that the probability to detect ASFV in a wild boar population of low density and virus circulation at a low prevalence is almost impossible. In the case of Estonia, this statement and the legal impossibility to declare the country free from ASF in wild boar was certainly correct. The further course of ASF in the country showed that even after 1.5 years, the virus emerged again. A comprehensive study investigating three different hypotheses regarding the cause of this event finally failed to provide a single definitive explanation **(Publication 12)**. However, the study illustrated the urgent need to clarify the role of seropositive wild boar in the epidemiology of ASF. O'Neill et al. (2020), who found using a model, that ASFV persistence in a low-dense wild boar population requires surviving animals that transmit the virus, also suggested this. Furthermore, the study showed that an increased surveillance effort is necessary to eliminate the disease from an affected wild boar population.

In a narrative review, environmental risk factors for ASF were identified **(Publication 9)**. The current findings hold the chance to design risk-based surveillance measures and to allocate available resources cautiously (Stärk et al., 2006). Considering a risk-based surveillance approach particularly matters in epidemiological situations like in the Baltic States, where new virus introduction has to be anticipated constantly due to the ASF situation in neighboring countries.

In Germany, ASF emerged in September 2020 **(Publication 14)**. The epidemic situation in western Poland (Mazur-Panasiuk et al., 2020) led to the hypotheses that the disease was introduced through the immigration of infected wild boar from Poland into Germany. A direct comparison to the epidemiological situations in Belgium and the Czech Republic, where the disease was eliminated from the wild boar population (Saegerman, 2018, Šatrán, 2019), supported this hypothesis **(Publication 15)**. In contrast to the two other European countries, into which the disease was only introduced at one single point, Germany faces constant infection pressure from the East along the border with Poland over a distance of at least 100 km. In some areas along the German-Polish border, attempts are already being made to prevent or reduce border crossings by wild boar by building a fence. However, the effectiveness of fences is controversial both among experts and among hunters **(Publication 16, 18, 19)**. Due to unavoidable interruptions by roads, water bodies or other landscape factors, it is unlikely that a fence could 100% prevent the migration of wild boar and make intensive surveillance measures unnecessary. In addition, the maintenance of a fence requires regular checks and, if necessary, repairs, which can be very time-consuming and costly. Apart from the practical aspects that should be taken into account when planning a fence, it should not be forgotten that fencing between two countries can also be of political importance and damage neighborly relations **(Publication 16)**.

This constant infection pressure from a neighboring country makes it very likely that the German ASF epidemic is and will be rather comparable to the ones in the Baltic States, then to those in Belgium or the Czech Republic Consequently, it is to be feared that the disease will remain in Germany for several years despite the control efforts. Taking these arguments into account, it may be even more important to consider risk-based surveillance approaches. Although the sampling of animals that were killed through an RTA did not yield in a higher probability to detect ASF, it is still recommended to sample these animals and test them for ASF **(Publication 10)**. It can be assumed that the extrication of such animals is usually accompanied by the authorities or by hunters, so that it is uncomplicated to take a sample at the same time. Thus, extra resources can be saved and still a surveillance sample retrieved. Sampling dead wild boar increases the sample size and thus the power of surveillance. This is of particular importance considering the calculated low detection probabilities after a certain period, in which ASF has reduced the wild boar population significantly and in which the prevalence of ASFV-positive wild boar consequently declined (Gervasi et al., 2020, European Food Safety Authority, 2021) **(Publication 12)**. These conclusions seem sobering, particularly with regard to the benefits of implementing participatory methods to motivate hunters to support ASF surveillance. However, in addition to surveillance, also the control of ASF has to be improved. The evaluation of ASF control measures in Latvia failed to yield promising results **(Publication 13)** even more emphasizing the need for a revision of ASF surveillance and control measures in wild boar. Despite the devastating prognosis regarding successful ASF control in wild boar, there is still hope to tackle the problem with a strong transboundary and transdisciplinary approach.

Using a participatory approach such as the “World Café” method showed that a huge amount of transdisciplinary expert knowledge can be interactively collected **(Publication 16)**. The main statements from this study were very similar to the ones by the hunters collected in the FGDs **(Publications 18, 19)** emphasizing the need for decision makers to seriously consider these findings. In all groups and by the experts, passive surveillance was acknowledged as important, but not very popular. This is in accord with previous findings (Calba et al., 2015a, Schulz et al., 2016). However, the vital role of passive surveillance in the control of ASF is scientifically undisputed and thus, motivating factors have to be identified to increase the willingness of hunters to participate.

Despite the potential benefits of including PE in conventional epidemiological studies, the conduction of participatory studies can be challenging. It was found that important aspects like the composition of the group, the language and the balance of power have to be considered **(Publication 17)**. These findings were confirmed by Ebata et al. (2020), who emphasized the need for an adequate implementation of PE. When planning FGDs, one of the most important aspects is the choice of the moderator **(Publication 17)**. A difference in the quality of the performance and the results of a study could be revealed between a study, in which the moderators were trained in the methodology and one, in which the moderator was only briefly informed about the methods **(Publication 20)**. Dealing with power imbalances within a group requires not only the ability to conduct the FGDs appropriately, but also to recognize the imbalances in the first place. Otherwise, there is a risk that unbalanced participation will affect the quality of the discussions and the results. Individual perceptions might be missed, the mood will not be reflected sufficiently and results might be biased. Berglund et al. (2013) identified the increased use of participatory approaches and highlighted the need to train staff in communication before performing participatory studies. In addition to adequate training, it is of utmost importance that one of the most important principles of PE is acknowledged and taken into account. When choosing a facilitator, the perceived relationship to the participants has to be considered. It is only human that the behavior of the participants in the presence of a potential expert (as facilitator) is more reserved. The participants might feel more intimidated and restrained in their free speech than in presence of an equal person **(Publication 17)**. These complex requirements for successful and advantageous discussions emphasize the need to consider more transdisciplinary approaches and the involvement of experts from different fields in all phases of a study.

The evolving integration of PE in veterinary medicine is highly desirable, but unfortunately, the discussed aspects are too often ignored. This does not only endanger the quality of the studies and the reliability of the data, but also the scientific recognition of the potential benefit of including participatory approaches in conventional epidemiology. The scientific community considers qualitative data often not as sufficiently scientific. The reliability, reproducibility and objectivity of collected data and participatory study results has therefore been questioned (Mays and Pope, 2000, Grunenberg, 2004, Patton, 2014, Camerer et al., 2018) and such critical feedback seems justified to some extent.

The studies included in the present thesis have clearly shown the complexity of ASF surveillance and control. Weaknesses have been identified, but at the same time starting points could be named that have the potential of increasing the effectiveness of the measures. Improving communication and motivating hunters to be actively involved in ASF control will be crucial, if there is a chance of eliminating this disease from wild boar. The participatory studies have clearly shown the difficulties that hunters are facing and highlighted specific aspects that may support hunters to be more willing to help. However, due to the partly insufficient implementation of PE in veterinary medicine, but also due to caveats concerning the use of qualitative data in natural sciences as discussed above, participatory studies and the results, they produce, are often ignored and may not be recognized. The methods too often run the risk of being labeled and ridiculed. However, in disease situations as complex as ASF in wild boar, people often wonder, why the disease cannot be controlled, while vast amounts of money are spent for laboratory research and vaccine development. This certainly has its justification, but would it not be worthwhile to dare the step not only resorting to conventional methods, but to include a relatively new approach recognizing the role of transdisciplinary communication? However, before this step can be taken, it has to be clarified, how "participatory" participatory studies are in veterinary medicine (Fischer et al., 2016, Fischer and Chenais, 2019, Catley, 2020). Current studies use participatory methods, still mainly aiming at generating research data instead of involving the affected groups of people in the planning, implementation and analyses (Berglund et al., 2013, Ebata et al., 2020). In social and public health sciences, the origin of participatory methods, the purpose of real participation is clearly the empowerment of affected people (Arnstein, 1969). In the presented PE studies **(Publication 18, 19)**, data were collected to identify concrete approaches to increase the willingness of hunters to support ASF surveillance and control in wild boar, but the next higher level of true participation has not yet been reached. Gain of knowledge is obviously not enough for true participation. The hunters and their expertise should therefore be included at a much earlier stage when planning defined ASF control measures. In the complex situation of the European ASF epidemic in wild boar, courage is needed to move away from the usual top-down approach and to allow at least for a rudimentary bottom-up approach. Transdisciplinarity, joint willingness and engagement to combat the disease may be key for a collective success.

An easy solution for controlling the ASF epidemic in wild boar does not seem to exist and strict regulations and swift actions are necessary to avoid the suffering of animals, economic damage and to ensure the maintenance of trade. To do this, hierarchical structures are indispensable, which in turn limits the unrestricted inclusion of various groups of people, thus hampering real participation processes. Consequently, PE and particularly, participatory approaches that get close to the true meaning of participation, should be integrated at a very early stage of surveillance and in the planning of disease control measures. This offers the chance to control animal diseases, particularly in wildlife, more effectively and at best also to eliminate them on the long-term.

6 Concluding remarks

The conducted studies aimed at closing knowledge gaps regarding ASF in wild boar and in long-term to support more successful surveillance and control measures by integrating PE. Gervasi et al. (2020) pointed out that successful ASF control in case of circulating virus at a low prevalence is almost impossible, thus supporting unhindered slow and yet continuous disease spread. This was confirmed in **Publication 12**. While the studies and the gained knowledge have not yet been able to defeat the disease, they may still promote the evaluation and interpretation of the epidemiology of ASF. With the help of participatory studies, weaknesses in the system could be identified and potential solutions were demonstrated.

Reduction of wild boar population density but also passive surveillance measures are only two examples, for which the vital need to increase the understanding and the willingness of hunters to support them was evidenced. Including the newly gained knowledge and the key figures in surveillance and control in future decisions and measures increases the chance to eliminate ASF from wild boar populations. At some moments and in some disillusioning studies, the task seems to be a “mission impossible”. However, when decision makers are willing to partly change in thinking and allow transdisciplinary approaches, they might sufficiently learn about and understand new methods to have at some point the courage to use them. Thus, elimination of ASF in wild boar may be possible, but only in a joint effort and provided that true transdisciplinary approaches get their chance to prove themselves.

7 Summary

African swine fever (ASF) has been a concern and a research topic for 100 years. However, after the disease reached Georgia in 2007 and spread rapidly, researchers in- and outside of Africa became even more interested. To date, there is neither a curative treatment nor a vaccine against the viral disease available. This makes disease surveillance and control measures particularly important. The role of wild boar in the current epidemic is prominent. They are as susceptible as domestic pigs and therefore pose a threat to the global pig economy due to the risk of disease transmission into domestic pig holdings. The epidemiology of ASF in wild boar was therefore examined in detail and existing knowledge gaps closed. Based on extensive analyses of surveillance data from the Baltic countries, it was found that the disease spread slowly, but steadily within wild boar populations, showing a high case/fatality ratio. The epidemiological patterns of the disease were similar in all three Baltic countries, which suggests that the course of disease is largely independent from external factors. In the advanced course of the epidemic, the number of surviving wild boar, tested positive for ASFV-specific antibodies, accumulated and simultaneously, the prevalence of wild boar tested positive for ASFV decreased, making further disease transmission or new virus introduction unlikely. Thus, it was assumed that the epidemic might fade out. These results illustrated the urgent need to differentiate between different types of laboratory test results, i.e. virus detection and serology when assessing the epidemiological situation in an affected country.

The significant reduction in the wild boar population density in individual countries can complicate surveillance measures and reduces the probability of detection. Based on the studies, further risk factors for a higher probability of detecting ASF in wild boar were identified. These findings may be used to design risk-based measures and to ensure an effective, but sustainable surveillance of the disease situation in a wild boar population. Such an approach might be particularly required in case of a constant infection pressure from ASF-affected

neighbouring countries. These situations, like they are present in the Baltic States and also in Germany call for a prudent use of the available financial and human resources.

The integration of participatory methods in the design and the implementation of surveillance and control measures can help to motivate involved key figures to support those measures. The enclosed participatory studies with hunters helped to identify starting points whose inclusion could help to improve ASF surveillance, make it more effective and thus increase the chance of its successful application. However, only generating the data is not sufficient. In order to exploit the potential of participatory epidemiology, a partial change in the attitude of decision-makers is needed. Above all, the will and the courage to include the knowledge and the concerns of people, who are indispensable in the implementation of measures to control ASF is required. Only in this way, transdisciplinarity has a chance of solving complex problems such as ASF surveillance and control.

8 Zusammenfassung

Die Afrikanische Schweinepest (ASP) beschäftigt Wissenschaftler schon seit 100 Jahren. Nachdem die Tierseuche allerdings im Jahre 2007 Georgien erreichte und sich daraufhin rasant ausbreitete, wurde das Interesse der Forschenden innerhalb und außerhalb Afrikas noch größer. Bisher gibt es weder eine kurative Behandlung noch eine Impfung gegen die Viruserkrankung, weshalb der Krankheitsüberwachung und Bekämpfungsmaßnahmen eine besonders hohe Bedeutung zukommen. Ein besonderes Problem der momentanen Epidemie ist die Rolle der Wildschweine. Diese sind ebenso empfänglich wie Hausschweine und stellen damit durch eine mögliche Übertragung in Hausschweinebetriebe eine Bedrohung für die globale Wirtschaft dar. Aus diesem Grund wurde die Epidemiologie der ASP in Wildschweinen genauer beleuchtet und durch Literaturrecherchen konnten bestehende Wissenslücken geschlossen werden. Anhand umfangreicher Analysen von Überwachungsdaten aus den baltischen Ländern, konnte festgestellt werden, dass sich die Krankheit innerhalb einer Wildschweinpopulation langsam aber stetig ausbreitet und dabei eine hohe Fallsterblichkeit aufweist. Die epidemiologischen Muster der Krankheit waren in allen drei baltischen Ländern ähnlich, was darauf schließen lässt, dass der Krankheitsverlauf weitgehend unabhängig von externen Faktoren ist. Im fortgeschrittenen Verlauf der Epidemie stieg die Zahl der überlebenden Wildschweine, die positiv auf ASP Virus-spezifische Antikörper getestet wurden an, und gleichzeitig nahm die Prävalenz der auf ASPV positiv getesteten Wildschweine ab, was darauf hindeutet, dass eine Weiterverbreitung der Krankheit oder ein neuer Viruseintrag unwahrscheinlich ist. Es wurde daher angenommen, dass die Epidemie am Abklingen sein könnte. Diese Ergebnisse zeigten die dringende Notwendigkeit auf, bei der Bewertung der epidemiologischen Situation in einem betroffenen Land zwischen den unterschiedlichen Laborergebnissen, d.h. zwischen der Virusdetektion und der Serologie, zu unterscheiden.

Die erhebliche Reduktion der Wildschweinepopulation in den einzelnen Ländern kann Überwachungsmaßnahmen verkomplizieren und die Entdeckungswahrscheinlichkeit vermindern. Basierend auf den Studien konnten weitere Risikofaktoren für eine höhere Wahrscheinlichkeit, die ASP zu entdecken, herausgearbeitet werden. Diese Erkenntnisse können genutzt werden, um risikobasierte Maßnahmen zu entwickeln und eine effektive, aber nachhaltige Überwachung der Seuchensituation in einer Wildschweinpopulation zu gewährleisten. Ein solcher Ansatz könnte insbesondere dann erforderlich sein, wenn ein ständiger Infektionsdruck aus ASP-befallenen Nachbarländern besteht. Solche Situationen, wie sie momentan in den Baltischen Staaten und in Deutschland vorliegen, erfordern einen wohlbedachten Einsatz vorhandener finanzieller und personeller Ressourcen.

Die Einbeziehung partizipativer Methoden in die Gestaltung und Umsetzung von Überwachungs- und Bekämpfungsmaßnahmen kann dazu beitragen, die beteiligten Schlüsselpersonen zur Unterstützung dieser Maßnahmen zu motivieren. Die beiliegenden partizipativen Studien mit Jägern haben geholfen, Ansatzpunkte zu benennen, deren Einbeziehung dazu beitragen könnte, die ASP Überwachung zu verbessern, sie effektiver zu gestalten und damit die Chance einer erfolgreichen Anwendung zu erhöhen. Allerdings reicht es nicht aus, nur die Daten zu generieren. Um das Potenzial der partizipativen Epidemiologie auszuschöpfen, bedarf es teilweise eines Umdenkens bei den Entscheidungsträgern. Es braucht vor allem den Willen und den Mut, das Wissen und die Anliegen der Menschen wirklich einzubeziehen, die bei der Umsetzung der Maßnahmen zur Bekämpfung der ASP unverzichtbar sind. Nur so hat Transdisziplinarität eine Chance, komplexe Probleme wie die Überwachung und Bekämpfung der ASP zu lösen.

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10 Appendix

10.1 Theses

1) The spread of ASF is not as fast as originally assumed. The disease is characterized by a high case/fatality ratio, but a moderate transmission rate.
2) The epidemiological patterns and the temporal course of ASF in wild boar in all three Baltic States were similar, suggesting that the course of the disease resembles one another, largely independent of external factors.
3) The increase of the seroprevalence (ASFV-specific antibodies) indicates the accumulation of surviving animals. The simultaneous decrease of the ASFV prevalence suggests a decline in circulating virus.
4) For the demonstration of freedom from ASF in a wild boar population, it is necessary to differentiate, also in the legal assessment, between different types of laboratory test results, i.e. virus detection and serology.
5) ASF leads to a reduction of the wild boar population density, which may hamper further ASF control measures and the detection of virus circulation at a low prevalence.
6) Risk-based ASF surveillance can save resources and thus sustainably maintain long-term disease surveillance. The sampling of wild boar killed in RTAs should be considered to increase the reliability regarding the disease status.
7) There is no evidence that solely seropositive wild boar spread ASF, but it was not possible to prove the opposite.
8) In contrast to an ASFV introduction at one single point, constant infection pressure from ASF-affected areas, makes effective disease surveillance and control nearly impossible without considering the available financial and human resources cautiously.
9) Methods from the field of participatory epidemiological can be useful, but a correct and well-designed application has to be assured. It is not enough, to use participatory methods to generate data. It may be advantageous to use them also to ensure a high acceptance of measures and to improve surveillance and control with the knowledge of stakeholders and the people who implement the measures.
10) Eliminating ASF in wild boar will only be possible using a transdisciplinary approach, seriously including the perceptions and the expertise of key figures (e.g. hunters).

10.2 Declaration

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Insel Riems, den 13.01.2022

Katja Schulz

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