Eesti Maaülikool
Estonian University of Life Sciences

EPIDEMIOLOGY OF AFRICAN SWINE FEVER IN ESTONIA AND CHARACTERIZATION OF ONE VIRUS STRAIN

SIGADE AAFRIKA KATKU EPIDEMIOLOOGIA EESTIS JA ÜHE VIIRUSTÜVE ISELOOMUSTUS

IMBI NURMOJA

A Thesis
for applying for the degree of Doctor of Philosophy
in Veterinary Science

Väitekiri
filosoofiadoktori kraadi taotlemiseks
loomaarstiteaduse erialal

Tartu 2021

Eesti Maaülikooli doktoritööd

Doctoral Theses of the Estonian University of Life Sciences

Eesti Maaülikool
Estonian University of Life Sciences

EPIDEMIOLOGY OF AFRICAN SWINE FEVER IN ESTONIA AND CHARACTERIZATION OF ONE VIRUS STRAIN

SIGADE AAFRIKA KATKU EPIDEMIOLOOGIA EESTIS JA ÜHE VIIRUSTÜVE ISELOOMUSTUS

IMBI NURMOJA

A Thesis
for applying for the degree of Doctor of Philosophy
in Veterinary Science

Väitekiri
filosoofiadoktori kraadi taotlemiseks
loomaarstiteaduse erialal

Tartu 2021

Institute of Veterinary Medicine and Animal Sciences
Estonian University of Life Sciences

According to verdict Nº 3 of May 12, 2021, the Doctoral Committee of Veterinary and Food Science of Estonian University of Life Sciences has accepted the thesis for the defence of the degree of Doctor Philosophy in Veterinary Medicine and Food Science.

Opponent: **Dr. Linda Dixon**, PhD
African Swine Fever Virus Group
The Pirbright Institute
Woking, Surrey, United Kingdom

Supervisors: **Prof. Dr. Arvo Viltrop**, DVM PhD
Institute of Veterinary Medicine and Animal Sciences
Estonian University of Life Sciences

PD. Dr. Sandra Blome, DVM PhD
Institute of Diagnostic Virology
Friedrich-Loeffler-Institut, Germany
Federal Research Institute for Animal Health

Dr. Klaus Robert Depner, DVM PhD
Institute of International Animal Health/One Health
Friedrich-Loeffler-Institut, Germany
Federal Research Institute for Animal Health

Defence of the thesis:
International video conference in the BigBlueButton web room at 11.00, June 15, 2021.

The publication of this dissertation is supported by the Estonian University of Life Sciences.

Language editors:
Robert B. Davis (English)
Urve Ansip (Estonian)

ISSN - 2382-7076
ISBN 978-9949-698-89-9
ISBN 978-9949-698-90-5 (pdf)

A journey of a thousand miles begins with a single step.

/Lao Tzu/

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on three original publications (I-III). The articles are referred in the text using Roman numerical.

I 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. Sci. Rep. 7, 12562. doi.org/10.1038/s41598-017-12952-w.

II Nurmoja, I., Petrov, A., Breidenstein, C., Zani, L., Forth, J.H., Beer, M., Kristian, M., Viltrop, A., Blome, S., 2017. Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. Transbound. Emerg. Dis. 64, 2034–2041. doi.org/10.1111/tbed.12614.

III Nurmoja, I., Mõtus, K., Kristian, M., Niine, T., Schulz, K., Depner, K., Viltrop, A., 2020. Epidemiological analysis of the 2015–2017 African swine fever outbreaks in Estonia. Prev. Vet. Med. 181, 104556. doi.org/10.1016/j.prevetmed.2018.10.001.

The contribution of the author's to the research papers

Paper	Original idea, study design	Data collection, sample analysis	Data analysis	Preparation of manuscript
I	**IN**, KS, CS, CSL, AV	**IN**, AV	KS, CS, **IN**	All authors
II	SB, **IN**, MB, AV	SB, **IN**, AP, CB, LZ, JF	SB, AP, **IN**	All authors
III	AV, **IN**, KD, KM	KM, **IN**, AV, TN, MK	KM, **IN**, AV, TN	All authors

IN- Imbi Nurmoja, KS- Katja Schulz, CS- Christoph Staubach, CSL- Carola Sauter-Louis, KD- Klaus Depner, SB- Sandra Blome, FC- Franz J. Conraths, AV- Arvo Viltrop, AP- Anja Petrov, CB- Christiane Breidenstein, LZ- Laura Zani, JF- Jan H. Forth, MB- Martin Beer, MK- Maarja Kristian, KM- Kerli Mõtus, TN- Tarmo Niine

ABBREVIATIONS

ASF	African swine fever
ASFV	African swine fever virus
CSF	Classical swine fever
cq	cycle quantification value
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization of the United Nations
FLI	Friedrich-Loeffler-Institut
IPT	indirect immunoperoxydase technique
N or area N	study area North
NAR	National Animal Register of the Estonian Agricultural Registers and Information Board
OIE	World Animal Health Organisation
PCR	Polymerase chain reaction
S or area S	study area South
VFB	Veterinary and Food Board
WAHID	World Animal Health Information System

1. INTRODUCTION

African swine fever (ASF) is a dangerous viral disease of pigs, with a devastating impact on the animal health and pig industry. While diagnosis of the disease has a remarkable influence on the international trade of live pigs and pig products, it is listed as a notable disease of the World Animal Health Organisation (OIE) and European Commission (EC) (OIE, 2017; OIE, 2020; EC, 2002).

The first description of ASF was made in 1921, when British pathologist Eustace Montgomery published the results of comprehensive research conducted in East Africa. A century has passed since then, knowledge about the disease and the causative agent is now much better, but despite this, around half of African countries still suffer the effects of endemically persistent ASF (Penrith *et al.,* 2013; Gallardo *et al.,* 2015c; Mulumba-Mfumu *et al.,* 2019; OIE WAHID, 2020).

The first case of ASF outside of Africa was reported in Portugal in 1957 (Sánchez-Vizcaíno *et al.,* 2009). During the period from 1960 to 1995, several European countries, including Spain, Portugal, France, Italy, Malta, Belgium and the Netherlands experienced new incursions of the virus (Sánchez-Vizcaíno *et al.,* 2009). All these aforementioned countries managed to eradicate the disease, excluding the Italian island of Sardinia. Eradication took decades in some countries; however, as the result of strict measures, they succeeded. Sardinia was first affected in 1978 and ASF is still endemic despite numerous attempts to get rid of it (Mur *et al.,* 2016; Jurado *et al.,* 2018; OIE WAHID, 2020).

In the 1970s, the disease spread across the Atlantic Ocean to South America (Brazil) and the Caribbean (Cuba, Dominican Republic and Haiti), but was successfully eradicated over the course of a decade (Costard *et al.,* 2009). The most recent and worrying episode of ASF's global spread has been to China (People's Republic of), where in August 2018 the first case of ASF in Asia was reported (Zhou *et al.,* 2018; Tao *et al.,* 2020). The subsequent spread of the disease in Asia and Oceania has been enormous, also reaching Mongolia, Vietnam, Cambodia, Hong Kong, North Korea, South Korea, Laos, the Philippines, Timor-Leste, Myanmar, Indonesia, Papua New Guinea and India (OIE WAHID, visited 24. May 2020).

A new introduction of the ASF virus to Europe occurred in 2007, when it was reported in Georgia in spring 2007 (Rowlands *et al.,* 2008; Sanchez-Vizcaino *et al.,* 2012). Following this, rapid spread of ASF virus to neighbouring countries in the North Caucasus region (Azerbaijan, Armenia) and the Russian Federation occurred, where the virus is still circulating and is endemic over large areas (Rosselkhoznadzor, visited 16. April 2020). In 2012, African swine fever virus (ASFV) was reported in Ukraine and in 2013 in Belarus. In 2014, the virus entered the European Union countries neighbouring Belarus – Lithuania, Poland, and Latvia. During the period 2017–2020, the virus entered the Czech Republic, Moldova, Romania, Bulgaria, Hungary, Belgium, Slovakia, Serbia and Greece (OIE WAHID, visited 24. May 2020). All affected EU countries (excluding the Czech Republic), as well as Ukraine and Moldova, are still reporting ASF cases.

The first case of ASF in Estonia was diagnosed in a dead wild boar in September 2014, near to the Latvian border. As a result of the extensive spread of the virus in the Estonian wild boar population, covering 14 counties out of 15, a total of 3,992 ASF-positive wild boar had been found among the 48,384 investigated wild boar by the end of May 2020. The first case of ASF in domestic pigs in Estonia was reported in the middle of July 2015 and was followed by 17 further outbreaks. Six more outbreaks in 2016, and three more in 2017, were confirmed by the veterinary service. No positive cases among domestic pigs were found in the years 2018 or 2019, or in early 2020 (up to 31. May 2020).

In Europe, the disease affects both domestic pigs and European wild boar (*Sus scrofa*) and, in general, the course of the disease does not differ when comparing them (Gabriel *et al.,* 2011; Blome *et al.,* 2013; Pikalo *et al.,* 2019). Therefore, an infected wild boar population holds the constant risk of infecting domestic pigs and vice versa (Sánchez-Vizcaíno *et al.,* 2012).

The long-lasting ASF epidemic in both domestic pigs and wild boar in Eastern Europe, as well as in domestic pigs on the African continent and Asia, poses a continuous infection threat to the rest of the world (Costard *et al.,* 2009; Penrith *et al.,* 2013; Dixon *et al.,* 2020; Tao *et al.,* 2020; Taylor *et al,* 2020). In Europe, the disease is expanding towards the west step-by-step, approaching regions and countries with very large populations of wild boar and a high density of domestic pigs. The

economic impact of such a development could be substantial for many areas and countries.

Even nowadays, control and eradication of ASF is based only on the rapid recognition in the field and diagnosis, followed by implementation of strict sanitary measures and a stamping-out policy. No vaccine or treatment is available, despite the first attempts to develop a vaccine being undertaken already in the 1950s (Penrith *et al.,* 2013; Dixon *et al.,* 2020).

The general goal of the work in this thesis has been to improve our knowledge regarding the epidemiology of African swine fever in both domestic pigs and wild boar in the north-eastern part of Europe.

2. REVIEW OF THE LITERATURE

2.1. Aetiology and clinical manifestation of African swine fever

African swine fever is caused by a large, enveloped, double-stranded DNA virus, which belongs to the genus *Asfivirus* within the *Asfarviridae* family (Alonso *et al.,* 2018). Up to now, 24 genotypes of ASFV has been determined worldwide based on partial nucleotide sequencing of the gene p72 (Boshoff *et al.,* 2007; Achenbach *et al.,* 2016; Quembo *et al.,* 2018). All genotypes are present in Africa, but only two of them – genotypes I and II – have been found on other continents (Bastos *et al.,* 2003; Gallardo *et al.,* 2009; Arias *et al.,* 2018; Le *et al.,* 2019; Mulumba-Mfumu *et al.,* 2019; Zhao *et al.,* 2019). In all affected European countries ASFV genotype II is circulating, excluding the island of Sardinia in Italy, where genotype I is circulating (Bastos *et al.,* 2003; Rowlands *et al.,* 2008; Malogolovkin *et al.,* 2012; Torresi *et al.,* 2020). All currently affected Asian countries have reported findings of ASFV genotype II (Zhou *et al.,* 2018; Le *et al.,* 2019; Kim *et al.,* 2020).

ASF can have an acute, subacute or chronic disease course depending on virus factors and the host. Based on virulence, the strains of the virus are divided into three main groups: highly virulent, moderately virulent and low virulent strains. Highly virulent ASFV strains produce peracute or acute forms of the disease, moderately virulent strains produce acute or subacute forms of the disease, and low virulent strains produce chronic and asymptomatic forms of the disease (Sánchez-Vizcaíno *et al.,* 2009). The genotype of the isolate is not directly related to its virulence, and isolates with different levels of virulence have even been found for one genotype (Gallardo *et al.,* 2015d; Gallardo *et al.,* 2018a; Gallardo *et al.,* 2018b; Gallardo *et al.,* 2018c; Zani *et al.,* 2018).

ASF is described as a severe, haemorrhagic disease that causes up to 100% morbidity in naive pig herds and can result in very high mortality (Sánchez-Vizcaíno *et al.,* 2009; Costard *et al.,* 2013). The acute cause of ASF is described with a variety of clinical signs including high fever, general depression, anorexia, respiratory and neurological disorders, gastrointestinal signs and haemorrhagic lesions (Sanchez-Vizcaıno *et al.,* 2009). However, the clinical appearance of ASF in both field and experimental conditions is often confined only to unspecific clinical

symptoms like loss of appetite, depression or listlessness, even in cases of severe cause of the disease (Gabriel *et al.,* 2011; Pietschmann *et al.,* 2015; Oelsen *et al.,* 2018b; Gallardo *et al.,* 2018a; Zani *et al.,* 2019; Pikalo *et al.,* 2020; Walczak *et al.,* 2020).

Diseased animals showing clinical symptoms shed the virus in all body secretions (Guinat *et al.,* 2014; Pietschmann *et al.,* 2015; Pikolo *et al.,* 2020), which causes contamination of the environment and may lead to subsequent spread of the virus within the herd or, in the case of wild boar, in the habitat. However, the highest amount of the virus has been found in blood of infected animals (Gabriel *et al.,* 2011; Carvalho Ferreira *et al.,* 2012; Guinat *et al.,* 2014; Gallardo *et al.,* 2015b; Olesen *et al.,* 2017).

2.2. Biological characterization of ASFV genotype II strains in Europe

The ASFV genotype II strains circulating within the EU since 2014 all have a common origin. They have been shown to originate from those genotype II strains causing the ASF epidemic in the Caucasus countries (Malagolovkin *et al.,* 2012; Fraczyk *et al.,* 2014; Gallardo *et al.,* 2014; Fernandez-Pinero, 2018; Pikalo *et al.,* 2020). Although enormous progress has been made in the field of DNA sequencing over the last few decades, just a small number of ASFV whole-genome sequences are currently available for precise characterization of different ASF virus strains. As of May 2020, whole-genome sequences of only 29 ASFV genotype II strains were publicly available (Forth *et al*., 2020). The phylogenetic tree created by Forth *et al.,* (2020) visualizes the relationships between these different ASFV strains and genotypes (Figure 1).

Because of the limited DNA sequence data on ASF virus strains to date, experimental infections, which allow collection of detailed information about the virus strains, are still extremely important. However, such experiments require the availability of special high-containment facilities (L3+), as well as official permission to use live animals; therefore, the number of these studies is very limited.

Experimental infections conducted during the period 2008–2014 showed that the ASFV genotype II strains circulating in Europe are highly virulent and induce an acute form of the disease in both domestic

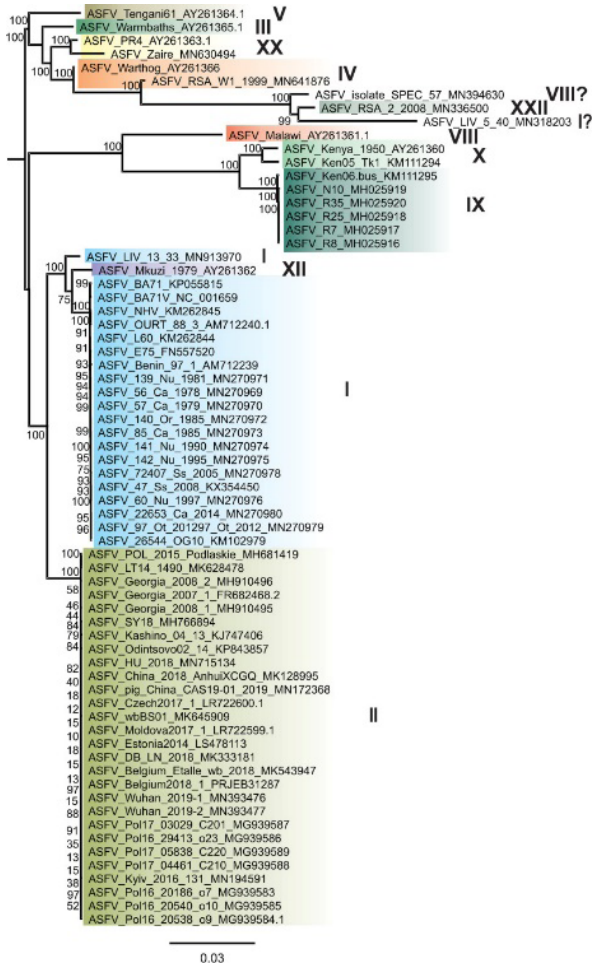


Figure 1. Phylogenetic tree of African swine fever virus strains based on all publicly available whole-genome sequences of African swine fever virus (Forth *et al.,* 2020. Reproduced with permission from the authors)

pigs and European wild boar (Gabriel *et al.,* 2011; Blome *et al.,* 2012; Guinat *et al.,* 2014; Gallardo *et al.,* 2015b; Pikalo *et al.,* 2020). Irrespective of the age of animals, dose of the virus or route of infection, the mortality rate of infected animals was found close to 100% (Gabriel *et al.,* 2011; Blome *et al.,* 2012; Gallardo *et al.,* 2015a; Gallardo *et al.,* 2015b). After an incubation period of 3 to 5 days infected animals started to show clinical symptoms that led to the death of most animals 5 to 13 days post-inoculation (dpi) (Gabriel *et al.,* 2011; Guinat *et al.,* 2014; Gallardo *et al.,* 2015b).

Experimental infections from the period 2015–2019 also describe finding less virulent genotype II virus strains (Gallardo *et al.,* 2018a; Gallardo *et al.,* 2018b; Pershin *et al.,* 2019; Walczak *et al.,* 2020). In these experiments, the recorded mortality rate was between 50 and 100%, and it was observed that the same virus strain may cause various clinical forms of the disease including acute, subacute or chronic forms. Furthermore, in 2017, an attenuated, non-haemadsorbing virus strain was found in a wild boar hunted in Latvia (Gallardo *et al.,* 2019a). The Latvian strain caused chronic or unspecific clinical signs in inoculated pigs, which did not lead to death of the animals, and in in-contact pigs either mild clinical symptoms appeared or they did not develop any detectable clinical symptoms at all (Gallardo *et al.,* 2019a).

2.3. Epidemiology of ASF in Europe

From 1995 to 2007, the only region affected by ASF in Europe was the Italian island of Sardinia, which has been affected since 1978 as the result of an independent incursion. In contrast to the rest of Europe, p72 genotype I is circulating on Sardinia (Bastos *et al.,* 2003; Giammarioli *et al.,* 2011; Jurado *et al.,* 2018; Torresi *et al.,* 2020).

Highly virulent and lethal ASF virus strains of p72 genotype II were introduced to Europe in 2007 (Blome *et al.,* 2012; Gallardo *et al.,* 2015b; OIE WAHID, visited 14. July 2019). During the first two years of the ASF epidemic, the countries in the Caucasus region (Georgia, Azerbaijan and Armenia) and the southern regions of the Russian Federation (Russia) were affected. Since the affected countries were not able to control the disease, the epidemic expanded within these regions and moved towards European Union borders and northern areas of Russia. In 2011, the virus reached the central part of Russia (Gogin *et al.,* 2013; FAO*,* 2013; http://www.fsvps.ru/fsvps/asf, visited 07. March 2019). However, several domestic pig outbreaks had already been diagnosed earlier in the north-west of Russia (St. Petersburg region) in the period 2009–2012; the closest of these to Estonia was reported about 160 km away from the border (FAO*,* 2013). Ukraine reported its first ASF case in 2012, and Belarus in 2013.

In January 2014, the first ASF case in the Baltics, in fact in the European Union, was reported by Lithuania (Gallardo *et al.,* 2015b; Mačiulskis *et al.,* 2020). In February, Poland reported its first case (Smietanka *et al.,*

2016). Latvia confirmed its first ASF cases in June 2014 (Olševskis *et al.,* 2016). Several other countries have followed: Moldova in 2016, the Czech Republic and Romania in 2017, Hungary, Bulgaria and Belgium in 2018, Slovakia and Serbia in 2019, and Greece in 2020 (OIE WAHID, visited 24. May 2020).

In total, since 2007 up the end of May 2020, the disease has spread over large areas of Europe and has been diagnosed in 17 European countries (OIE WAHID, visited 5. July 2020). Figure 2 shows the countries in Europe and the rest of the world, which have experienced ASF outbreaks in domestic pigs and cases in wild boar the period 2015-2020.

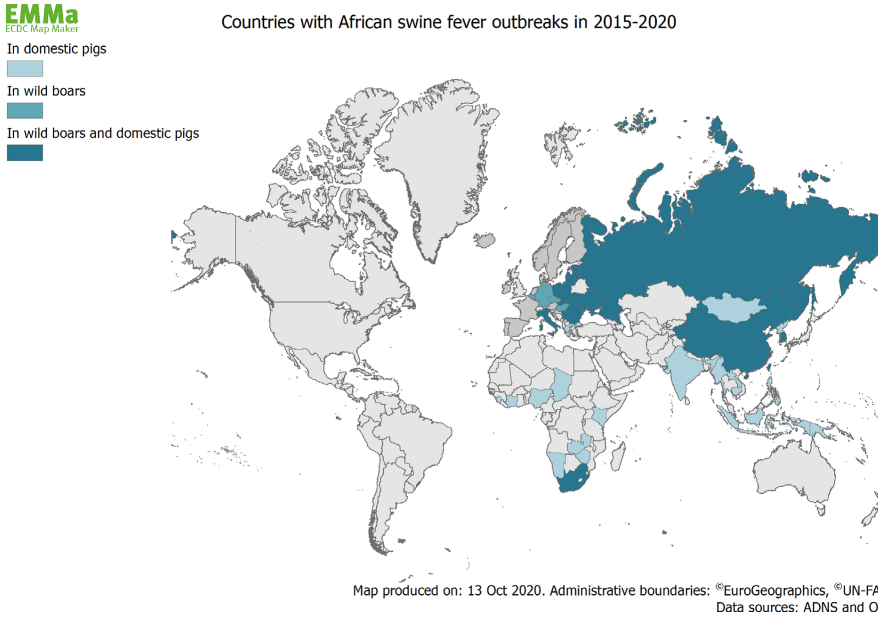


Figure 2. Countries with African swine fever outbreaks in domestic pigs and wild boar, 2015–2020 (EFSA, 2021)

2.3.1. Epidemiology of ASF in European wild boar

2.3.1.1. Transmission of the virus

In Europe, the transmission cycles of the virus are somewhat different compared to Africa. The ancient sylvatic cycle of virus transmission is absent in Europe. Here in Europe there is only one wild Suidae species present – the European wild boar *(Sus scrofa)* – and soft ticks from the

genus *Ornithodorus* spp. inhabit only limited areas of the continent. *Ornithodorus erraticus* is the only species of tick found in Europe that has been identified as a reservoir and biological vector of ASFV (Sánchez-Vizcaíno *et al.,* 2009). They have been found in Mediterranean countries (Portugal, Spain, Italy, Turkey), in the Caucasus (Georgia, Armenia, Azerbaijan), and in Moldova, Ukraine and Romania. From the affected Baltic countries (Estonia, Latvia, Lithuania) and Poland, there are no reports of the occurrence of *Ornithodorus* ticks in nature or their role in transmission of the virus (Sánchez-Vizcaíno *et al.,* 2009; Costard *et al.,* 2013). All ticks belonging to the *Ornithodoros* genus live in open and dry habitats, commonly associated with rodent burrows. They feed mainly on animal species living in burrows, such as rodents and reptiles, as well as Suidae in Africa. While wild boar in Europe do not live in burrows, but are surface animals, they can be only accidental hosts. Therefore, transmission involving soft ticks is not considered to play an active role in the geographical spread and transmission of the virus in Europe (Dixon *et al.,* 2020). However, ticks played an important role during a long-term epidemic on the Iberian Peninsula in the second half of the 20th century (Bech-Nielsen *et al.,* 1995; Sánchez-Vizcaíno *et al.,* 2009; Boinas *et al.,* 2011; Costard *et al.,* 2013).

ASFV can be shed by oral and nasal secretions, urine, faeces, and secretions from the genital tract of infected animals. However, far the best body fluid to shed the virus is blood, as contains large amounts of the virus (Gabriel *et al.,* 2011; Carvalho Ferreira *et al.*, 2012; Guinat *et al.,* 2014; Gallardo *et al.,* 2015b; Guinat *et al.,* 2016; Olesen *et al.,* 2017; Gallardo *et al.,* 2018; Gallardo *et al.,* 2019b). Transmission of the virus in the habitat of wild boar may occur by direct transmission between infected and susceptible wild boar, or by indirect transmission via carcasses. Habitat contamination originates from ASFV-positive wild boar carcasses or from offal left in the forest after hunting (Chenais *et al.*, 2018). However, in the habitat there are many other factors that may influence transmission of the virus, in particular possible intra-species scavenging, the stage of carcass decomposition, climate, season, the nature of the soil, and fauna in the area (Probst *et al.,* 2017). Wild boar-habitat transmission dominates in those areas where the climate is colder than in previously affected areas (in Africa, the Mediterranean and Caucasus region), the wild boar population is large, no natural reservoirs exist and domestic pigs are kept predominantly indoors (e.g. the Baltic countries). The role of contaminated habitat is far from being completely

understood, but it was described as an independent transmission cycle and published for the first time by Chenais *et al.* (2018) (Figure 3).

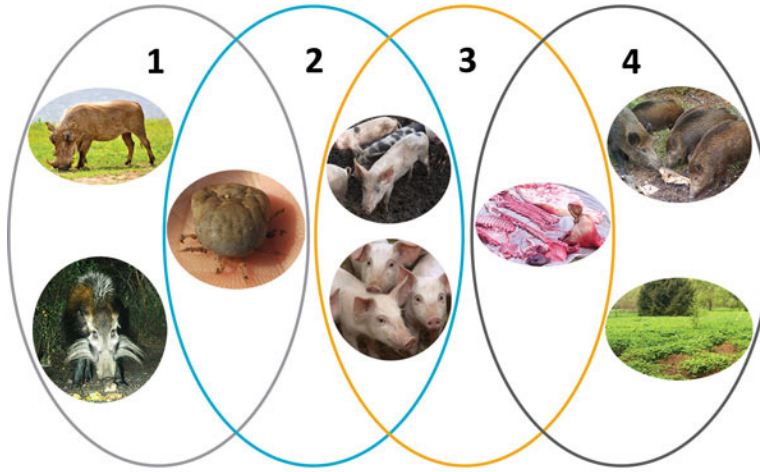


Figure 3. The epidemiological cycles of African swine fever and main transmission agents. 1) Sylvatic cycle: includes common warthog (*Phacochoerus africanuus*), bushpig (*Potamochoerus larvatus*), and soft ticks of *Ornithodoros* spp. 2) Tick–pig cycle: includes soft ticks and domestic pigs. 3) Domestic cycle: includes domestic pigs and products originating from pigs (for example: pork, lard, fat, blood, bones, hides). 4) Wild boar–habitat cycle: includes wild boar; pig and wild boar products and carcasses; and the habitat (Chaines *et al.,* 2018. Reproduced with permission from the authors)

The importance of wild boar as the main reservoir of ASFV was revealed after the virus reached the Baltic countries and Poland between 2014 and 2015 (Olševskis *et al.,* 2016; Pejsak *et al.,* 2018; Mačiulskis *et al.,* 2020). Before this time, when the virus was circulating in Russia and the Caucasus region, the domestic pig sector was considered the main reservoir of the virus (FAO, 2013; Oganesyan *et al.,* 2013). Unauthorized movement of live animals, extensive illegal movement of infected pork and pork products from affected regions, and feeding of food waste to pigs without prior heat treatment in the region supported this (Gogin *et al.,* 2013; FAO, 2013).

Transmission of the virus into disease-free areas including "long-distance jumps" may occur through fomites. Insufficiently cleaned and disinfected livestock trucks and vehicles entering farm territories to transport goods and offer different services have been recognized an important route of virus transmission. Active movement and travelling of people between regions, countries and continents poses a transmission

risk as well. Personal luggage of passengers, which may contain infected pork products, has been identified as one of the biggest contributors to the risk of virus introduction to new areas. This risk is particularly high in the case of ASF since the survival time of the ASF virus in different pork products can be very long – weeks or even months (Petrini *et al.,* 2019; Olesen *et al.,* 2020). Contaminated fomites and movement of people and goods pose a risk of introduction equally to both the wild boar population and domestic pig farms.

2.3.1.2. Contagiousity, morbidity and mortality

Collection of trustworthy field data to measure ASF frequency in a wild boar population is almost impossible, and therefore the information collected in animal experiments and printed in textbooks is crucial. Up to now, in textbooks, ASF is predominantly described as a highly contagious disease with high mortality, especially in cases where the disease is caused by highly virulent strains of the virus (Sánchez-Vizcaíno *et al.,* 2009; Blome *et al.,* 2012; Pietschmann *et al.,* 2015; Pikalo *et al.,* 2020). While high mortality of animals has been described in many experimental studies, this may easily lead to the wrong conclusion regarding the contagiousity of ASF. However, particularly under field conditions, several other factors influence the cause of the disease and mortality rate significantly, such as dose and route of exposure. If many animals get a high dose of the virus at the same time, as often in experimental conditions, getting trustworthy information about its contagiousity is problematic. The same may also occur under field conditions, in the case of high-dose oral infection by feed. However, moderate contagiousity of a highly virulent ASF virus strain has been reported by Pietschmann *et al.* (2015). The authors concluded that very low doses of virus exposure oro-nasally may be linked to moderate contagiousity. Furthermore, low contagiousity of ASF has been reported under field conditions in several domestic pig outbreak farms in Latvia (Olševskis *et al.,* 2016; Lamberga *et al.,* 2018).

Following the results of recent experimental infections, it can be hypothesized that similar scenarios occur under field conditions in both domestic pig herds and the wild boar population. Due to the presumed oral transmission of the virus, and particularly with low-dose exposure, the initial mortality within a herd or group is rather low even if the virus is highly virulent.

2.3.1.3. Risk factors in wild boar

Experimental studies conducted using European ASFV isolates have demonstrated that European wild boar are as susceptible to ASF as domestic pigs (Gabriel *et al.,* 2011; Blome *et al.,* 2012; Pietschmann *et al.,* 2015; Pikalo *et al.,* 2020). Initially, both often develop only non-specific clinical symptoms and, depending mostly on the virulence of the virus strain, animals either die or recover. The age and sex of an animal do not influence the course of the disease.

The role of habitat as a reservoir of the virus is far from being completely understood since the versatility of habitats makes comparison and collection of data difficult, even in neighbouring areas. The long survival time of the ASF virus in blood, excretions and other substances originating from infected animals, as well as in the probably contaminated soil underneath and next to carcasses, supports the long persistence of the disease in affected areas (Probst *et al.,* 2017). Certain climatic conditions, such as chilly and damp weather, and a long winter period with temperatures below 0°C are also considered contributing factors that influence the viability of the virus. The results of a comprehensive study to clarify possible intra-species scavenging conducted by Probst *et al.* (2017) suggest that about one third of wild boar visits led to direct contact with dead conspecifics. Although intra-species scavenging was not observed during the study, frequent reports of sniffing and poking of carcasses, as well as of chewing bare bones originating from carcasses, represent long-term risks of transmission. So, the high viability of ASF virus in the habitat and infected carcasses can be considered even more important than direct contact with live, infected animals (Chenais *et al.,* 2018).

The density of wild boar is also a factor that can substantially influence the spread of the ASF virus. High density affords more contact between animals within the group, as well as between groups, supporting further spread of the disease. Conversely, low density of wild boar may contribute to localization of the disease, especially in cases with a highly virulent circulating virus and high mortality. Direct contact between wild boar, because of their high density has been reported in the Caucasian countries and in Russia (Gogin *et al.,* 2013). Figure 4 shows density of wild boar in Europe in 2015.

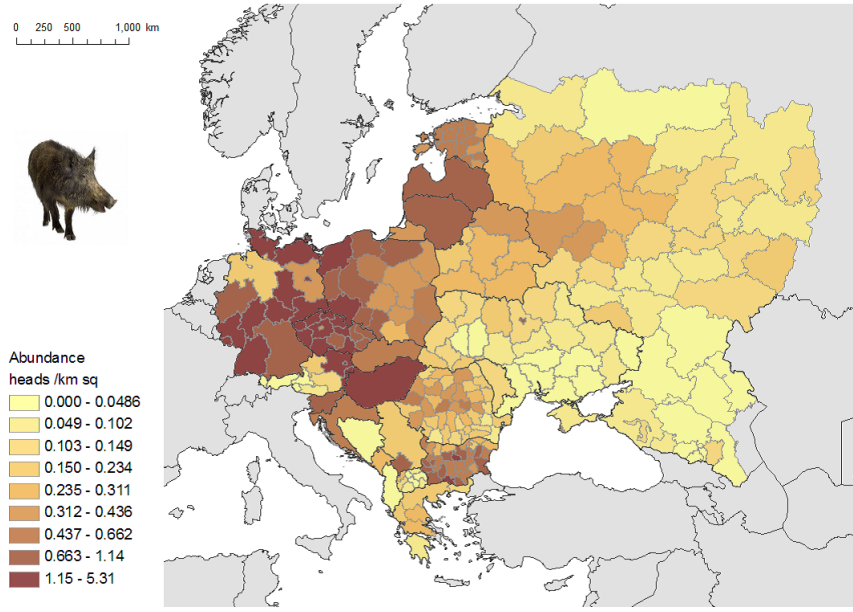


Figure 4. Wild boar population abundance (head per km^2) in Europe based on available population estimates (EFSA, 2015)

Hunting practices have a significant impact as a causal factor in the ASF epidemic in the wild boar population. However, this depends on hunting intensity and type, such as whether dogs are used, whether it is a driven hunt etc. Intensive hunting supports the active movement of animals and, thereby, the spread of the disease to new, previously disease-free areas. Elements of biosecurity related to hunting, including the cleaning and disinfection of hunting equipment and vehicles, and removal of leftovers and carcasses from the forest, have been identified as critical factors in the control of ASF.

The possible carrier status of recovered (survived) animals has been frequently under discussion, and there is still no single position among researchers. Several animal experiments have demonstrated that ASFV or the DNA of the virus can persist in tissues of clinically recovered animals for up to six months (Gallardo *et al.,* 2019b). Thus, it can easily be concluded that these animals are carriers. However, the results of other animal trials have demonstrated that recovered animals do not infect healthy, susceptible animals after commingling (Gallardo *et al.,* 2018a; Petrov *et al.,* 2018). To clarify this question, Stahl *et al.* (2019) conducted a comprehensive review finding that a clear definition for ASF virus carriers is lacking, and therefore it has been quite common

for any survivor or seropositive animal to be referred to as a carrier. The authors concluded that not enough evidence has been reported to prove the existence of such carriers and their significant role as a reservoir of the virus.

Human behaviour has been identified as an important risk factor for the spread of ASF. Short-distance spread of ASFV (1–5 km/month) can be associated with direct (natural) contact between animals (Podgorski *et al.,* 2018; Chenais *et al.,* 2019; Niine *et al.,* 2019), whereas unexpected long-distance spread cannot apparently be explained by direct contact between animals alone. The most important mechanisms causing the spread of the disease over large areas are human induced. In the EU, recent examples of such long-distance spreading have taken place in the Czech Republic, Poland, Hungary and Belgium (Chenais *et al.,* 2019; Linden *et al.,* 2020). Each of these infected areas was several hundred kilometres away from previously known infected regions. The main threat to the wild boar population is probably related to food waste left in the forest or public places by tourists, truck drivers or workers coming from affected areas (Pejsak *et al.,* 2018).

2.3.2. Epidemiology of ASF in domestic pigs in Europe

2.3.2.1. Transmission of the virus

Infected animals shed the virus via most secretions of the body, and therefore direct contact between infected and susceptible animals has been found as the most effective source of infection for herds (Guinat *et al.,* 2014; Gallardo *et al.,* 2015b; Guinat *et al.,* 2016; Gallardo *et al.,* 2018a; Gallardo *et al.,* 2019b). However, under field conditions, such direct contact can be rare, in particular in areas where domestic pigs are kept mostly indoors, such as in the Baltic countries. Direct pig-to-pig or wild-boar-to-pig transmission dominates in those regions (the Caucasian countries, the Russian Federation, Moldova, Ukraine, Romania, Bulgaria, as well as Sardinia) where there is a large backyard farm sector, outside keeping is prevalent, and swill-feeding habits exist (Sánchez-Vizcaíno *et al.,* 2009; Costard *et al.,* 2013; Gogin *et at.,* 2013).

Whether or not the virus is transmitted to farms via animal feed has been unclear for a long time. However, a recent comprehensive study confirms the efficient oral transmission of ASFV via feed (Niederwerder

et al., 2019), and also reports the potential infectious doses of different types of feed. Some field reports from Latvia, Lithuania and Poland describe feeding of contaminated fresh grass or hay as a source of infection (Jazdzewski, 2017), but in general, knowledge is lacking about the role of plant-based and liquid feed consumption. However, feeding of swill and kitchen waste is a well-known transmission route of the virus to farms, especially in the backyard sector.

Despite the fact that in Northern Europe there are no natural vectors, it is still important to identify possible mechanical vectors and clarify their role in transmission of ASFV. The European stable fly (*Stomoxys calcitrans*) has been shown able to mechanically transmit ASFV to pigs up to two days post-infective-meal (Baldacchino *et al.,* 2013; de Carvalho Ferreira *et al.,* 2014). Oelsen *et al.* (2018a) reported that ingesta of *S. calcitrans* flies after a blood meal containing ASFV may be a source of infection for pigs. The authors found it unlikely that blood-sucking files would be a common route for transmission of the virus to a farm. Nevertheless, the results indicate that transmission of the virus over short distances is possible, in particular by larger flies, such as blood-feeding horse flies (family *Tabanidae*). The role of mechanical vectors in transmission of the virus is still not clear and needs further investigation.

Many ASF outbreak investigations from recent years have not identified a clear source of the virus infection or transmission route to the farm. It mostly appears that the virus is introduced to farms by indirect transmission routes by means of contaminated fomites (vehicles, people, tools etc). Low awareness about the disease among pig owners and hunters, inadequate or non-existing biosecurity on pig farms, and illegal movement of infected pigs, pork and pork products, all supported by an uncontrolled increase in the wild boar population, may easily lead to the transmission of the virus by fomites to pig farms. Thus, an infected wild boar population in an area poses a constant infection risk to domestic pigs (Sánchez-Vizcaíno *et al.,* 2012).

2.3.2.2. Contagiousity, morbidity and mortality

ASF is often described as a severe, haemorrhagic and highly contagious disease of pigs with a high mortality (Fenner's, 2017; Veterinary Medicine, 2017; Sánchez-Vizcaíno and Arias, 2012). Morbidity rate and mortality in the herd depend on the virulence of the virus strain, as well as the

route and dose to which the herd is exposed. In the case of an acute form of the disease, morbidity may range from 40 to 85% (Sánchez-Vizcaíno and Arias, 2012), and it may be even higher in cases where it is caused by highly virulent virus strains. Highly virulent virus isolates may cause mortality rates of 90–100%, moderately virulent strains 20–40% in adults and 70–80% in young animals, and low virulent strains result in a mortality of 10–30% (Sánchez-Vizcaíno and Arias, 2012).

From outbreak farms in Russia (2007–2012), Oganesyan *et al.* (2013) reported mean values of mortality and morbidity as 72.4% (64.3–80.4) and 37.8% (28.9–46.6), respectively. Based on field observations in Latvia from 2014, Olševskis *et al.* (2016) reported that on several holdings only one or a few diseased or dead animals were present at the time of suspicion of the disease. Other pigs living in the same stable were clinically healthy. The authors concluded that virus transmission from one animal to the next is a rather delayed process confirming a moderate contagiousity. Another case report from a Latvian ASF outbreak farm with 5,000 pigs also clearly demonstrated the slow spread of the virus within the farm (Lamberga *et al.,* 2018). During the first week after exposure of the farm to the virus, there was no observed increase in the usual mortality rate for the farm; in total, it took over a month until ASF was suspected, even despite the presence of ASF in the region. In Lithuania, ASF was diagnosed on a large commercial farm in 2014 (Anonymous, 2014). On this farm, which had close to 20,000 pigs, 18 weaners died in one unit showing symptoms of feed poisoning, and just over a week later severe symptoms appeared in a sow unit. Thus, these results also indicate low contagiousity of the disease on the farm. Such a slow spread and moderate contagiousity have also been described for a Bulgarian backyard farm with seven pigs (Zani *et al.,* 2019).

Evaluation of ASF frequency parameters on affected backyard farms is not relevant in most cases, because every single diseased or dead animal influences the results significantly. Therefore, we have to take into account that results from backyard farms may not correctly reflect the occurrence of the disease on larger farms. Following available field reports from different affected counties, it can be hypothesized that on larger production farms operating at higher biosecurity levels, morbidity and mortality rates can be low, especially in the first week or weeks after introduction; this is concordant with a low or moderate contagiousity.

It is an important difference between farm sizes, which may influence recognition of the disease especially on large farms.

2.3.2.3. Risk factors in domestic pigs

Risk factors for the introduction of ASF to a pig farm may differ markedly between regions, while climate, farming traditions, landscape, socio-cultural and economical background, as well as many other factors, vary greatly.

Farm size can be considered a risk factor for ASF, although reports may often be contradictory in this regard. It is widely assumed and frequently reported, that backyard and small-scale production farms have a higher probability of becoming infected. Reports from Russia and the Caucasian countries from the period 2007–2013 found that backyard farms are most susceptible to ASF introduction (Oganesyan *et al.,* 2013). Despite this, in 2012, Russia reported a large number of outbreaks on large commercial farms with a high level of biosecurity. Authors of one study concluded that this is possibly due to the underreporting of outbreaks in the backyard sector because of weak or non-existing supervision by veterinary authorities (Gogin *et al.,* 2013). Furthermore, numerous owners of small pig farms in Russia are poor and therefore dependent on income from pig sales. On top of this, when outbreaks occur, an important part of the costs associated with outbreak elimination have to be covered by the pig owner; this may also easily lead to hiding of the disease and underreporting (Gogin *et al.,* 2013; FAO, 2013). Olševskis *et al.* (2016) reported from Latvia that in 2014, 30 out of 32 outbreaks were confirmed on backyard (up to 10 pigs) or small-scale production farms (11–50 pigs). In total, during the period from June 2014 to May 2018, Latvia confirmed that only three of 53 outbreaks (6%) were on large commercial farms (Lamberga *et al.,* 2018). From Lithuania it has been reported that during the period 2014–2017, ASFV mostly affected backyard holdings (Pautienius *et al.,* 2018). In detail, they reported to the EFSA (2018) that in 2017, 28 out of 30 outbreaks were confirmed in backyard farms, and in 2018, 43 out of 49 outbreaks were on backyard farms. In 2017, Poland reported that 85% of outbreaks occurred on those farms with fewer than 50 pigs, and only 4% were on farms keeping over 500 pigs (Jazdzewski, 2017). In July 2019, Romania reported that all 210 ASF outbreaks between January and July 2019 were diagnosed on backyard or small commercial farms (Anonymous, 2019). However,

within European Union countries, there are only a few well recorded ASF case reports published up to now (Lamberga *et al.,* 2018; Zani *et al.,* 2019; Lamberga *et al.,* 2020).

It is not scientifically proven that the type of farm (multiplier, farrow-to-finish, fattening) is a significant risk factor for ASF. However, it can be assumed that farms with breeding sows are more susceptible, because of more human interactions and the lower immunity of pregnant or nursing sows. Lamberga *et al.* (2018) reported an outbreak on a large breeding farm in Latvia, where the disease appeared first in pregnant sows. In 2014, Lithuania reported an outbreak on a large farrow-to-finish farm with breeding sows (Anonymous, 2014). In Bulgaria, an outbreak of ASF on a backyard farm in 2018 also started with the death of a pregnant sow (Zani *et al.,* 2019). However, contrary to this, in Sardinia it seems that the number of open fattening farms is a risk factor for the occurrence of ASF. This may be associated with the more frequent movement of new animals to the herds, a higher density of animals, as well as the management practices on these farms (Martínez-López *et al.,* 2015). The vast majority of ASF outbreaks still occur on backyard or small-scale production farms without clear division of herds based on production type.

Outside or free-range keeping of domestic pigs is a significant risk factor for ASF. Pigs kept outside can easily be in direct contact with wild boar, and this has subsequently been identified as an important transmission pathway for domestic pig herds (Gulenkin *et al.,* 2011; Gogin *et al.,* 2013). Over large areas in the Caucasus region, southern Russia, and the Mediterranean countries, outside keeping of pigs is a widely practiced tradition (Gogin *et al.,* 2013). Since the early stage of the ASF epidemic, all the Baltic countries prohibited the outside keeping of domestic pigs. As a control measure, free-range keeping was banned in the Caucasian countries and in Sardinia. In Romania, the free-range keeping of domestic pigs is strongly discouraged. Contrary to expectations, Martínez-López *et al.* (2015) found in Sardinia that the number of closed farms was associated with a higher risk of ASF outbreak occurrence compared to open farms. However, it was concluded that this might be related to better notification of outbreaks by owners, or a consequence of management practices on these farms, particularly on small-scale farms where there is generally low biosecurity and a swill-feeding tradition.

On Sardinia only a limited number of closed farms are large production farms with a high biosecurity level.

In Europe, the infected wild boar population is an important risk factor for infection of domestic pig herds. During the period 2007–2013, there were some reports from initially affected countries (Armenia, Russia) about the role of wild boar in ASFV transmission. The Caucasian countries and southern Russia reported the role of infected wild boar as the source of infection for domestic pigs (Gogin *et al.,* 2013). However, the situation changed remarkably, after the virus entered EU countries in 2014. All the Baltic countries and Poland reported that the affected wild boar population posed a major threat to domestic pigs. Olševskis *et al.* (2016) analysed domestic pig outbreaks in Latvia in 2014 and concluded that 12 out of 32 outbreaks could be linked to a persistent infection in the wild boar population. In the EFSA scientific report (2018) Poland presented results of a statistical analysis of outbreaks, which indicated a relationship between the presence of ASFV in the wild boar population and the occurrence of the disease in domestic pigs; it was shown that almost all (95%) of ASF outbreaks in domestic pigs occurred in those areas where ASF had already been found in wild boars. Similar results from Poland were also reported by Wozniakowski in 2017 and 2018. In most affected EU countries the first ASF cases were diagnosed in wild boar, and then subsequently in domestic pigs (Olševskis *et al.,* 2016; Smietanka *et al.,* 2016; Pautienius *et al.,* 2018). The situation seems to be different in Sardinia, where results of studies suggest that the role of wild boar as a source of ASF outbreaks is not crucial on the island. On the contrary, it has been suggested that wild boar are often infected from domestic pigs in open grazing areas (Martínez-López *et al.,* 2015). However, the potential role of wild boar as reservoir of ASFV in Sardinia is not clearly known, and therefore wild boar data are not neglected in risk factor analyses.

Feeding of non-heat-treated swill to pigs is one of the most frequently described risk factors for domestic pigs. In conjunction with poor or almost non-existent biosecurity measures on a farm, it may easily lead to outbreaks in herds. The use of food waste to feed pigs has been identified as the main route of ASF introduction to small farms in Sardinia (Martínez-López *et al.,* 2015). Together with generally low biosecurity levels on most Sardinian small farms, ASFV access to farms is easy this way. It has been concluded that swill feeding has been one of

the most important factors why the ASF control programme in Sardinia has failed in the long term (Martínez-López *et al.,* 2015). The Russian Federation reports that use of non-heat-treated swill to feed domestic pigs is a common practice in the domestic pig sector all over the country; therefore, contaminated swill is the most important source of ASFV infection for domestic pigs there (FAO, 2013; Gogin *et al.,* 2013; Kolbasov *et al.,* 2018). Since the Russia is not an exporter of pigs and pig products out of the country, the backyard sector in particular is not motivated to invest in the improvement of biosecurity measures on farms (Gogin *et al.*, 2013). Gogin *et al.* (2013) also reported that the presence of the virus in southern Russia in 2008 and 2009 was linked with feeding of leftovers of meat and meat products from infected pigs. Swill feeding as a source of ASF introduction has also been reported from Latvia (Olševskis *et al.,* 2016), Lithuania (EFSA, 2018), Poland (Jazdzewski, 2017; Woziakowski, 2018) and Romania (Boklund *et al.,* 2020). All the aforementioned countries have also reported low, non-existent or improper biosecurity measures on backyard farms (Jazdzewski, 2017). The FAO report summary from 2013 says that backyard farms in most Eastern European countries operate at a very low biosecurity level, and awareness of the sector regarding the disease and biosecurity in general is very poor.

The illegal trade of live pigs and pig products is a possible risk factor for introduction of the virus to regions and farms. This has been described in many regions, particularly in remote areas where people follow more traditional lifestyles and farming practices. Reports from Sardinia, the Caucasian countries, Russia, Poland, and other countries describe the importance of such a risk (FAO, 2013; Gogin *et al.,* 2013; Martínez-López *et al.,* 2015; Jazdzewski, 2017; Kolbasov *et al.,* 2018). However, to estimate this as a risk factor is difficult, as not much reliable information is available. Costard *et al.* (2015) used mathematical modelling to estimate the risk of release of ASF from backyard and small-scale farms via the emergency sale of infected pigs. An emergency sale is defined as a situation when farmers do not report the suspected disease to the authorities and sell pigs without apparent clinical signs at market or to traders with the aim of reducing economic losses. The authors of the study concluded that emergency selling is a risky practice for farmers and contributes to further spread of the disease. Russia has reported that large-scale spread of the disease, including "long-distance jumps" within the country, were caused by unauthorized movement of infected

animals and/or pork sales (Gogin *et al.,* 2013; Kolbasov *et al.,* 2018). Infected pork products from affected regions were purchased by food supply and catering companies and the food waste was, without prior heat treatment, fed to pigs (Gulenkin *et al.,* 2011; Gogin *et al.,* 2013). Poland has reported that illegal movement of infected live pigs and infected meat and sausages is a source of new outbreaks (Jazdzewski, 2017; Wozniakowski, 2017 and 2018).

A lack of cooperation between different authorities and services responsible for disease eradication can also be defined as a risk factor for ASF introduction. Slow, insufficient or inadequate control measures taken by the authorities have been reported by Russia as a contributing factor of the disease spread (Gogin *et al.,* 2013).

Finally, season is a common risk factor for ASF in Eastern Europe. Olševskis *et al.* (2016) reported from Latvia that most outbreaks in 2014 occurred in the period from July to August. A similar trend is described in Lithuania, where very clear seasonality was reported in 2018; all confirmed outbreaks occurred in the period from June to August (EFSA, 2018). The EFSA scientific report from 2018 summarizes input from all the Baltic countries and Poland, and shows that 88% (367) of all outbreaks for the period 2014–2018 occurred in summer, 9% (38) in autumn, and 3% (12) in winter and spring combined. A seasonal increase in outbreaks in the period from May to October was also noted by Russia in the period 2009–2012 (Oganesyan *et al.,* 2013).

3. AIMS OF THE STUDY

The general aim of this work was to analyse the epidemiology of ASF and the course of the epidemic in the Estonian wild boar population (I, II), as well as in domestic pigs (III).

The specific aims were:

1. To analyse the differences in the development of the ASF epidemic among wild boar populations in the north-eastern and southern areas of Estonia (Study I).

2. To clarify the biological characteristics of the virus strain circulating among wild boar in the north-east of Estonia during the year 2014 (Study II).

3. To describe quantitatively the epidemic of ASF in domestic pigs, and to identify herd-level risk factors for infection in Estonian pig herds, as well as to evaluate the clinical manifestation of the disease in field conditions (Study III).

4. MATERIALS AND METHODS

This dissertation consists of three independent studies. This chapter presents an abridged overview of the materials and methods of each of them. A complete description of the materials and methods can be found in the original articles (I, II and III), which are presented in the corresponding section of this thesis.

4.1. Study setting and data collection

4.1.1. Study I

Study area. We defined two study areas to compare the characteristics of the epidemic in southern and north-eastern Estonia according to the hypothesis that the characteristics of the epidemic (e.g. spread dynamic, mortality, seroprevalence) in these two affected areas were different at the start of the epidemic.

The southern area (area S) comprised four counties: Valga (2,044 km²), Viljandi (3,422 km²), Võru (2,305 km²) and Tartu (2,993 km²). The infected area in the north-east (area N) included one county, Ida-Viru (3,364 km2) (Figure 5).

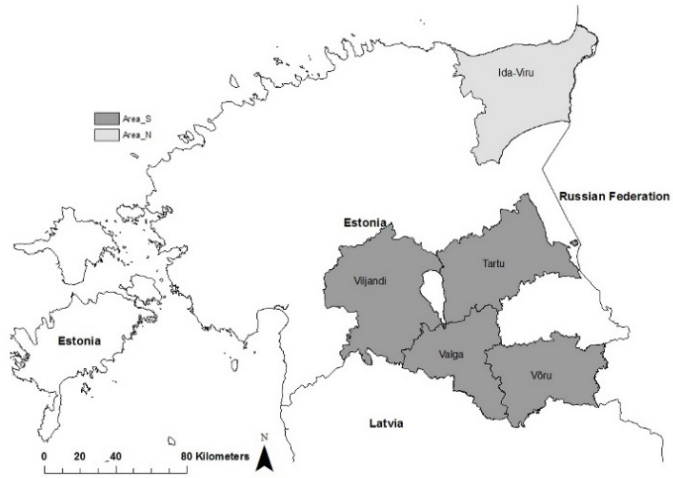


Figure 5. The study areas of the south and north-east of Estonia. Highlighted areas illustrate the four counties included in the southern area (area S) and the one county, which constituted the north-eastern area (area N)

Study period. We analysed surveillance data collected from 01. September 2014 until 30. September 2016 (25 months). We analysed the ASF virus- and seroprevalences on a monthly basis for the entire study period, as well as separately for the first 12 months and the following 13 months. Surveillance data from 2014 were obtained from the database of the Estonian Veterinary and Food Laboratory. Data from 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 set that we finally used for the analyses included information on the location (county and municipality level), year and month of sampling, the age of the sampled animal, the type of the sampled wild boar (hunted or found dead), the virological and serological test results, and the population density of wild boar in the sampled municipalities.

Wild boar population data. These data were provided by the Estonian Environment Agency (Nature department). Population data were available for the hunting years 2012/13, 2013/14 and 2014/15. The number of wild boar was recorded before the breeding season began (observation dates: March 2014, 2015 and 2016). The population density was estimated at the municipality level. Initial population data were available as integer numbers at hunting-ground level. To use the data for analyses, we aggregated the hunting ground data at the municipality level utilizing the software ArcGIS ArcMap 10.3.1 and calculated the wild boar density per km^2 for each administrative unit (municipality).

Wild boar sampling data. Wild boar were sampled according to the Estonian ASF control programme, which included sampling of both wild boar found dead and hunted animals. Passive surveillance of wild boar included animals found dead, as well as animals killed in road traffic accidents or shot when sick. Active surveillance was based on sampling of hunted wild boar.

4.1.2. Study II

The animal trial was conducted in the high-containment facilities (L3+) of the Friedrich-Loeffler-Institut (FLI), Germany. The experiment was approved by the competent authority of Germany under reference number 7221.3-2-023/15.

Animals. The first stage of the study included a total of ten and the follow-up study a total of three European wild boar from the breeding unit at the FLI. The wild boar were approximately 4 months old at the start of the experiment.

Virus strain. The virus strain used in the challenge experiment (Est 14/WB) was isolated from a wild boar found dead in the north-east of Estonia (Ida-Viru County) in 2014.

Preparation of the inoculation material. A spleen suspension from infected animals was prepared for inoculation of experimental animals. To obtain the spleen suspension we intramuscularly inoculated three young wild boar with an organ homogenate in a standard cell culture medium that had been prepared from weakly PCR-positive organ samples obtained in the field. After appearance of clinical signs and confirmation of infection by real-time PCR, the animals were euthanized, and blood and organ samples were collected during necropsy. A pooled spleen suspension with a titre of $10^{4.5}$ haemadsorbing units (HAU) units per ml was prepared for inoculation.

Inoculation. The animals were inoculated oronasally with 2 ml of pooled spleen suspension with an ASFV titre of $10^{4.5}$ HAU per ml.

Design of the experiment. We challenged ten wild boar with the virus strain Est 14/WB. The animals were monitored until death or euthanasia except for one surviving animal, which was used in the follow-up experiment. The aim of the follow-up experiment was to assess if the recovered survivor animal was able to transmit the virus to susceptible healthy animals. The survivor animal was commingled with three sentinel wild boar on day 50 post-inoculation (dpi) and they were kept together until day 96 dpi. On this day, all the animals in the experiment were euthanized and subjected to necropsy as described below.

Data collection. All animals were assessed for clinical parameters every day using a harmonized scoring system, which has been previously described by Pietschmann *et al.* (2015). Throughout the course of the trial, level of viremia, virus distribution, virus shedding, and antibody responses were assessed. For this purpose, we collected blood samples, and oropharyngeal and faecal swabs at days 0, 4, 7 and 10 dpi, and on the day of necropsy.

We performed necropsies on all animals and collected tissue samples (lymph nodes, spleen, tonsil, salivary gland, lung and liver), blood (EDTA, serum) and swab samples for reference purposes.

4.1.3. Study III

For this study we used data collected during the epidemiological investigations on outbreak farms.

Outbreak definition. We defined an outbreak farm as a holding having an individual identification number in the NAR and meeting the criteria of infected herd as defined in Council Directive 2002/60/EC (European Commission, 2002). In accordance with the EU diagnostic manual, all our ASF outbreaks were confirmed by virus genome detection (European Commission, 2003).

Outbreak investigations. Epidemiological information was collected from all farms on which an ASF outbreak was reported during the period 2015–2017 (18 farms in 2015, six farms in 2016 and three farms in 2017). However, as a positive diagnosis of ASF was not confirmed in follow-up investigations for one of the herds in 2015, we excluded this farm from further analysis (on this farm all 15 pigs were tested after culling and were found to be negative for ASF).

Epidemiological investigations were conducted either by the local veterinary officers responsible for management of the outbreaks or by the epidemiology team of the Estonian University of Life Sciences, in compliance with Council Directive 2002/60/EC (European Commission, 2002), using a structured questionnaire.

Defining the biosecurity level of outbreak farms. The biosecurity level of outbreak farms was evaluated based on interview data and data collected during farm visits. The final assessment of biosecurity level for every single farm was a two-step consensus decision by a group of three experts. The first step involved evaluating the farms based on their compliance to basic biosecurity requirements enforced by national legislation, and classifying them as compliant or non-compliant (Riigi Teataja, 1999; Riigi Teataja, 2004). In the second step, the experts divided the herds into five categories based on the predefined criteria.

Defining the high-risk period (HRP) of outbreak farms. The HRP was defined as the length of time that ASF virus may have existed on a farm before its presence was suspected. An HRP was established for every outbreak farm based on mortality data, as well as clinical and laboratory findings.

Domestic pig herd data. A database on Estonian pig herds for the period 2015–2017 was compiled using the information available from the National Animal Register (NAR) of the Estonian Agricultural Registers and Information Board and from the Veterinary and Food Board (VFB). The final database, which we used for analyses, included all farms and households that had kept pigs during the years of observation. The total number of pigs in a herd was counted as the largest number registered in one of the source databases (NAR or VFB).

An epidemiological unit was defined as a group of pigs kept in one building or area (one outdoor herd) that had an individual identification number in the NAR. It was possible for one owner to have more than one production unit (i.e. herd) registered in the NAR. Herds belonging to the same owner were considered as connected herds.

Holdings were grouped into four size categories according to the total number of pigs (piglets, weaners, growers, fatteners, gilts, sows and boars) in an epidemiological unit: 1–10 pigs (G1); 11–100 pigs (G2); 101–1000 pigs (G3); > 1000 pigs (G4). G1 holdings were classified as backyard or non-commercial farms where pigs were kept mainly for domestic consumption. G2, G3 and G4 holdings were classified as commercial farms. The herd type was determined as either farrow-to-finish, multiplier, fattener, or grower based on the information available from the NAR. The final database also included the type of pigs kept on a farm (domestic pigs, wild boar or crosses), as well as the location of the farm (including the coordinates).

Wild boar surveillance and hunting data. ASF surveillance data for wild boar originated from the VFB. This data set covered the period from September 2014 until the end of 2017 including date and location (coordinates) of each ASF case in wild boar. For the year 2015, data on ASF wild boar cases in northern Latvia were drawn from the Animal Diseases Notification System database (ADNS, 2017).

We identified the date and location of the closest wild boar case(s) to each outbreak farm, and we recorded the Euclidean distance between each affected farm and the closest wild boar case occurring a maximum of one year before the outbreak. This made it possible to characterize the infection pressure from wild boar.

Additionally, the Estonian Environment Agency (Nature department) provided wild boar hunting data, and data regarding number of hunters, number of feeding sites and hunting hounds. These data were based on regular reports submitted by regional hunting societies to the Environmental Board.

4.2. Laboratory analytical methods

In studies I and III, real-time PCR was used for ASFV genome detection and was performed according to the protocol published by Tignon, *et al.* (2011). Enzyme-linked immunosorbent assay (ELISA) and the indirect immunoperoxidase technique (IPT), were both used for antibody detection. ELISA tests were performed using a commercially available blocking ELISA (Ingezim PPA COMPAC, Ingenasa, Madrid, Spain) according to the manufacturer's instructions. In the case of an inconclusive ELISA result, the sample was retested using the IPT for confirmation. For IPT, a protocol provided by the European Union Reference Laboratory for ASF was used (CISA-INIA, 2014; Gallardo *et al.,* 2015a). All ASF laboratory analyses were conducted at the Estonian Veterinary and Food Laboratory, which is the National Reference Laboratory for ASF in Estonia.

In study II, the virus was isolated in PBMC-derived (peripheral blood mononuclear cell) macrophages. Blood for the preparation of cells was collected from healthy domestic donor pigs. PBMCs were grown according to the standard laboratory protocol. We performed a haemadsorption test (HAT) according to a slightly modified standard procedure for detecting the virus in serum and tissue samples (Carrascosa *et al.,* 2011).

For viral DNA detection, we extracted viral nucleic acid for qPCR using either the QIAamp® RNA Viral Mini Kit (Qiagen) or the NucleoMag Vet Kit (MACHEREY NAGEL), and the KingFisher® extraction platform (Thermo Scientific). The nucleic acid extraction was performed

with 150 µl of organ homogenate or swab material, and 75 µl of whole blood. Subsequently, we performed qPCR according to the protocol published by King *et al.* (2003) with slight modifications.

For the detection of antibodies against ASFV we used two commercial enzyme-linked immunosorbent assays (Ingezim PPA COMPAC, Ingenasa; ID SCREEN African swine fever virus INDIRECT, IDvet). Both assays were carried out following the manufacturers' instructions.

4.3. Statistical analysis

4.3.1. Study I

We estimated prevalences stratified over time and space and calculated and compared odds ratios. Their confidence intervals were calculated according to Clopper and Pearson (1935). A p-value of ≤ 0.05 was considered statistically significant. Statistical analyses were conducted in R (http://www.r-project.org).

Using the whole data set we performed a Fisher's exact test to test for statistically significant associations between presumed risk factors and positive virological or serological test results for ASF at the animal level. Accordingly, the association between age and the laboratory test results was evaluated. We attributed animals to the age classes "juvenile" (< 1 year) and "adult" (> 1 year). Furthermore, we examined associations between carcass categories ("hunted" or "found dead") and laboratory test results and analysed the age distribution within the two carcass categories (hunted, found dead).

We tested data for associations between the population density and positive ASF laboratory test results. For this purpose we categorized the municipalities as the variable of interest depending on their test results (i) 0 = only negative test results within the study period, (ii) 1 = at least one positive test result within the study period. We averaged population densities over the reported years and assigned to each municipality. For analysing this, a Mann-Whitney U test was used.

We examined our hypothesis that the age and carcass-type distribution were different between the study areas using a Fisher's exact test. The

same test was used to examine associations between the study areas and the virological or serological status of wild boar.

To test for a temporal and spatial effect within the two study areas, we used a hierarchical Bayesian space–time model (Staubach *et al.,* 2002; Staubach *et al.,* 2011). However, this model was only applied to examine seroprevalence. Variables that we identified as statistically significant via univariate analysis we included as fixed effects, whereas space and time were treated as random effects. For both study areas we conducted separate analyses at the municipality level using BayesX 2.0.1 (http://www.uni-goettingen.de/de/bayesx/550513.html). To estimate the parameters of the model we applied a Markov Chain Monte Carlo algorithm (MCMC).

4.3.2. Study III

To calculate herd incidences of ASF we conducted a survival analysis in Stata MP14®. Our data set included all pig farms recorded in source databases (NAR, VFB) in 2015, 2016 and 2017. For the herds in the database, the observation period started from 1st January. The observation period lasted either until the end of the year (right censoring), until the day that production ceased (removal of pigs from the farm), or until the outbreak of ASF occurred.

We calculated mortality risk (cumulative incidence) for each outbreak herd and affected group within the herd for the period including the HRP and the timespan from notification to culling. The affected group was defined as a physically separated unit in a stable containing one type of pig (sows, fatteners, weaners etc.).

A Cox proportional-hazard random-effect model was applied to detect significant differences in ASF infection hazard across farm types, herd-size categories and the three study years. In the multivariable model we retained variables that were significantly associated with the event of interest ($p < 0.05$). We used Akaike information criterion (AIC) values to compare model quality (Dohoo et al., 2009).

We checked the assumption of proportional hazards by creating log–log plots of survival, and with a statistical test using Schoenfeld residuals (Dohoo *et al.,* 2009).

To assess the association between the occurrence of ASF cases in wild boar and ASF outbreaks in domestic pigs we used a hierarchical Bayesian spatio-temporal model (Varewyck *et al.,* 2017). The response variable was 'ASF outbreak in domestic pigs in hunting district' (set as binary). Covariates included by month were as follows: 'total no. of ASF-PCR-positive wild boars' (from September 2014 to November 2017), and 'total no. of wild boars hunted' (from March 2015 to November 2017). Covariates included by year (2014 to 2017) were as follows: 'total no. of hunters', 'total no. of wild boar feeding sites', and 'total no. of hunting hounds'. We chose these last three covariates because we expected they may reflect hunting intensity in a hunting district. The model was checked for convergence.

5. RESULTS

5.1. Study I. Course of the ASF epidemic in wild boar populations in two affected areas in Estonia

The observation period of this study was 25 months (September 2014 – September 2016). In total, we used 7,015 data records for analysis. The number of virologically (n = 7,015) and serologically (n = 6,306) tested animals is presented in Tables 1 and 2.

Table 1. Number of African swine fever virus genome-positive and -negative wild boar samples from study areas North and South, and averaged prevalences for different time periods

Area[a]	Number of samples	Number of negative samples	Number of positive samples	Averaged prevalence during the observation period (%)	95% CI for averaged prevalence
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

[a] The study areas and observation periods (N = study area North, N1 = first 12 months of the observation period, N2 = second 13 months of the observation period; S = study area South, S1 = first 12 months of the observation period, S2 = second 13 months of the observation period)

Table 2. Number of African swine fever antibody-positive and -negative wild boar samples from study areas North and South, and averaged prevalences for different time periods

Area[a]	Number of samples	Number of negative samples	Number of positive samples	Averaged prevalence during the observation period (%)	95% CI for averaged prevalence
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

[a] The study areas and observation periods (N = study area North, N1 = first 12 months of the observation period, N2 = second 13 months of the observation period; S = study area South, S1 = first 12 months of the observation period, S2 = second 13 months of the observation period)

5.1.1. Factors influencing the course of the ASF epidemic in the wild boar population

5.1.1.1. Results of statistical analysis

A statistically significant association was found between age and the positive laboratory test results for both real-time PCR and serology by ELISA/IPT ($p < 0.001$). The probability of detecting an ASFV- or antibody-positive animal was higher in the group of 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). In addition, we found a statistically significant association ($p < 0.001$) with regard to the carcass category (hunted or found dead). For animals found dead there was a higher probability of finding a real-time-PCR- or antibody-positive result (real-time PCR: OR = 69.60, 95% CI = 56.89–85.15; serology: OR = 4.53, 95% CI = 2.83–7.25). We did not detect a statistically significant difference ($p = 0.420$) in the distribution of the two age classes within carcass categories. There were more old animals than young animals in both categories (Figure 6).

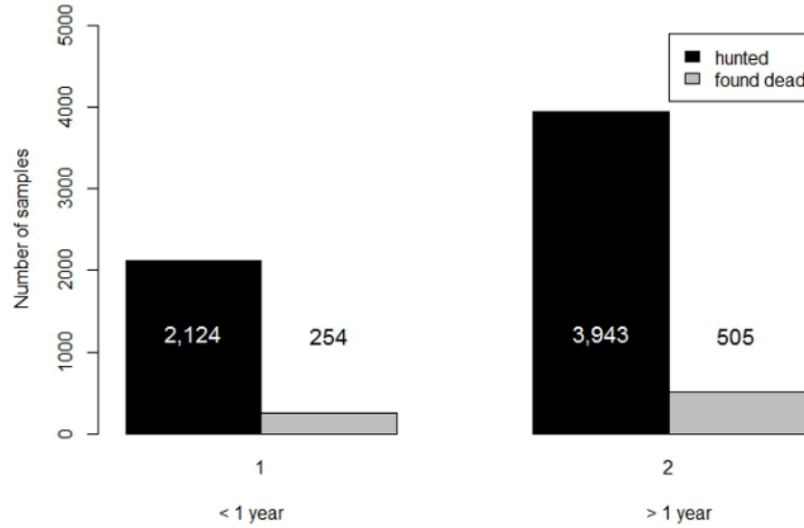


Figure 6. Number of samples from animals hunted or found dead (carcass categories) stratified by age category

We detected a significant association between the wild boar population density and the test results regarding both ASFV genome detection and serology (real-time PCR: $p < 0.001$; serology, $p = 0.009$). In the ASFV-positive municipalities we found a higher wild boar population density. (Figure 7).

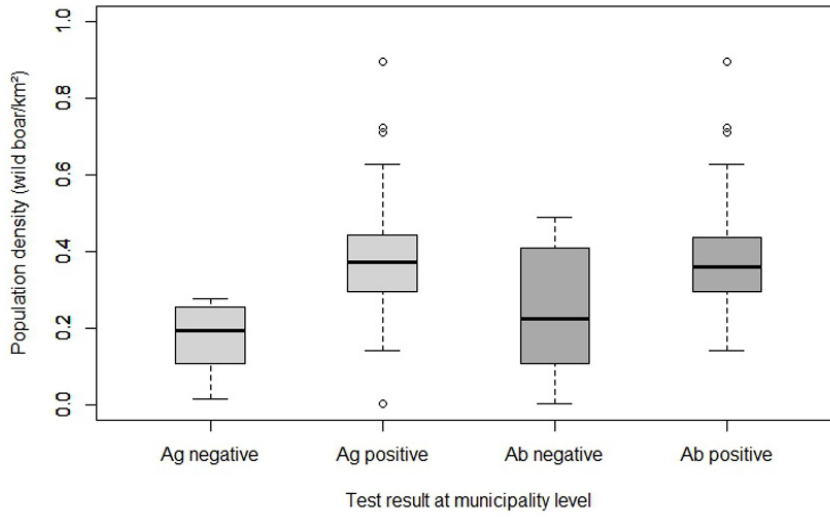


Figure 7. Population density (number of wild boar/km²) in the municipalities of the study areas stratified by virological and serological test results at the municipality level. Ag: African swine fever virus genome detection, Ab: antibody detection

The age distribution of sampled animals was similar in both areas ($p = 0.566$) (Figure 8). However, the distribution of hunted animals and wild boar found dead was different ($p < 0.001$). In area S, there was a significantly higher proportion of animals found dead than in area N (Figure 9). In area S, there was, in addition, a significantly higher population density compared to area N ($p < 0.001$) (Figure 10).

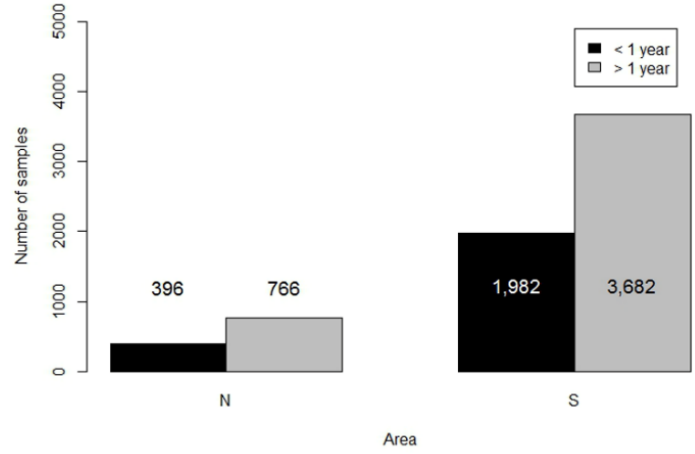


Figure 8. Number of samples of juvenile (< 1 year) and adult (> 1 year) animals stratified by study area North (N) and South (S)

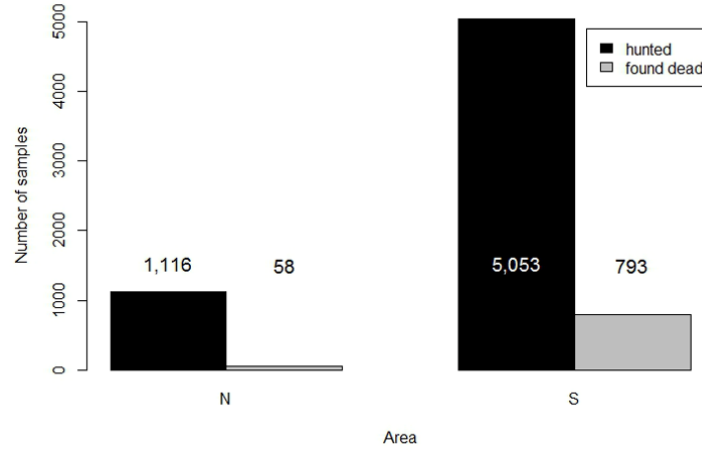


Figure 9. Number of samples from animals hunted or found dead (carcass categories) stratified by study area North (N) and South (S)

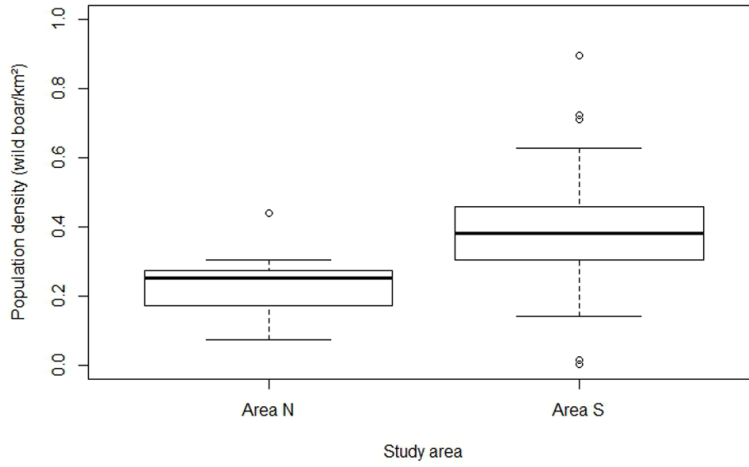


Figure 10. Population density (number of wild boar/km²) in the study areas North (N) and South (S)

We found the prevalence of ASFV-genome-positive wild boar to be significantly higher in study area S compared to area N ($p < 0.001$). We did not detect a significant difference in seroprevalence between the areas ($p = 0.728$).

5.1.1.2. Results of model analysis

We included carcass category, age and population density as fixed effects in the hierarchical Bayesian space–time model, because we found a significant association between these factors and the results of serological tests using univariable analyses.

In area N, age and population density showed a significant effect on the serological test result, but carcass category did not (Table 3). In area S, age and carcass category showed a significant effect on the serological test result, but population density did not (Table 4).

Table 3. Parameter estimates obtained from the hierarchical Bayesian space–time model for analysed risk factors (carcass category, age, population density) in area North

Variable	Mean	SD	Median (95% BCI)	Mean/SD[a]
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

DIC (deviance information criterion): 323.82; Deviance: 291.558; pD (posterior distribution): 16.135; BCI (Bayesian credible intervals)

[a] Mean/SD (standard deviation) > 1.96 indicates statistical significance

Table 4. Parameter estimates obtained from the hierarchical Bayesian space–time model for analysed risk factors (carcass category, age, population density) in area South

Variable	Mean	SD	Median (95% BCI)	Mean/SD[a]
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

DIC (deviance information criterion): 1,344.465; Deviance: 1,269.215; pD (posterior distribution): 37.625; BCI (Bayesian credible intervals)

[a] Mean/SD (standard deviation) > 1.96 indicates statistical significance

The sample sizes differed considerably over time among municipalities in both study areas (Figures 11 and 12). Spatial analysis confirmed a different trend in seroprevalences within study areas. In area N, the highest prevalences over the entire observation period were detected in one municipality located in the western part of Ida-Viru County. In 2015 (data for 12 months), the prevalences were higher in some other municipalities located farther east, but in 2014 (data for four months) it was not possible to obtain reliable prevalence estimates for these municipalities as the sample sizes were too small. In 2016 (data for nine months), the infection also spread to municipalities located in the southern part of area N (Figure 11).

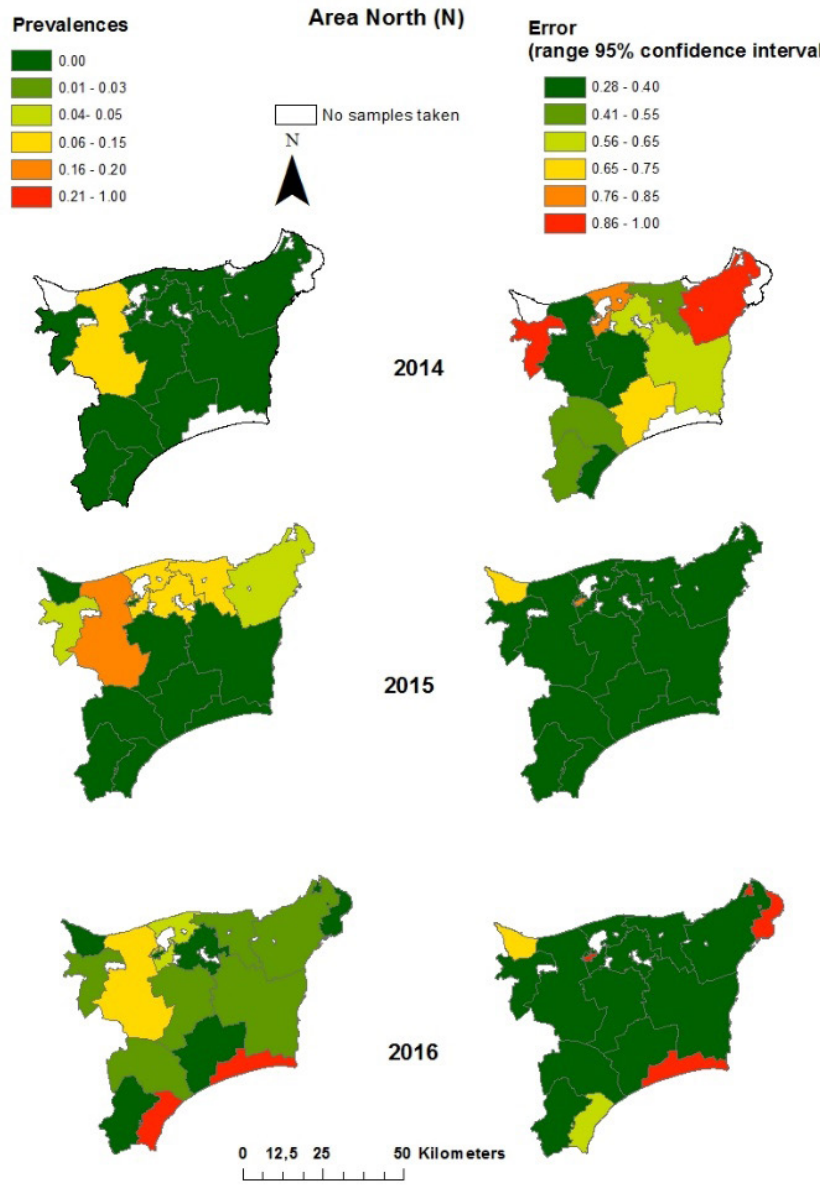


Figure 11. Seroprevalences and 95% confidence intervals for sampled wild boar per municipality in study area North in 2014 (Sept–Dec), 2015 (Jan–Dec) and 2016 (Jan–Sept)

In area S, over 25 months, the infection spread within the wild boar population. In 2014, we detected high prevalences in some municipalities bordering Latvia, during the following years, the disease spread northwards and over the entire study area (Figure 12).

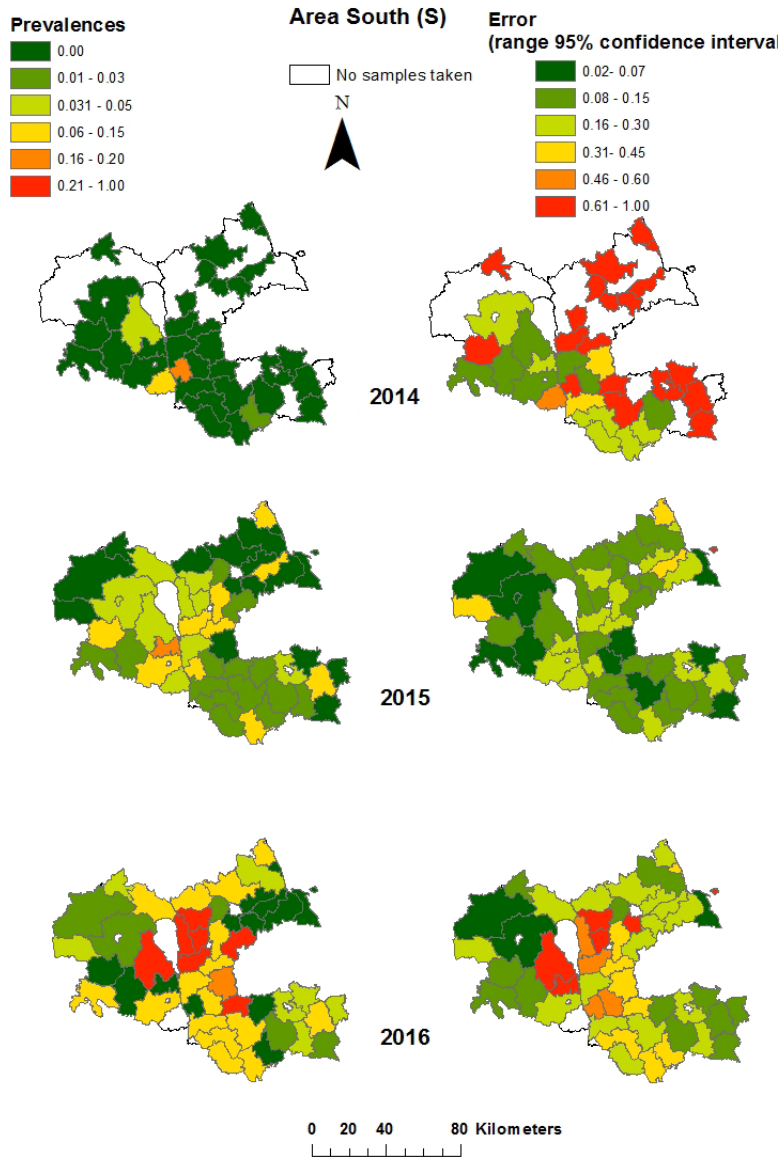


Figure 12. Seroprevalences and 95% confidence intervals for sampled wild boar per municipality in study area South in 2014 (Sept–Dec), 2015 (Jan–Dec) and 2016 (Jan–Sept)

The spatial analyses showed a clear median spatial effect on the logit prevalence per municipality in the northern part of area N, however, in some municipalities we found a negative spatial effect. The wild boar population density was higher in the western part of study area N throughout entire observation period (Figure 13).

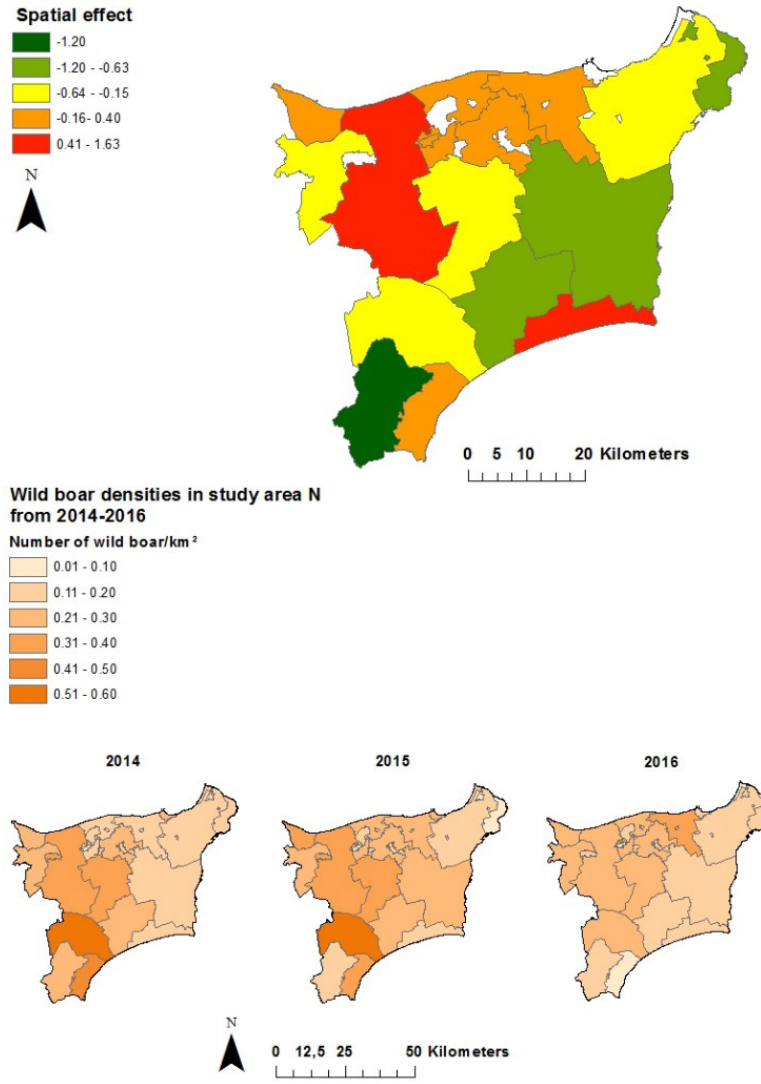


Figure 13. Median-structured spatial effect on the logit prevalence per municipality in study area North for the observation period of 25 months from 2014 to 2016. Maps in the lower row show the population density for each municipality in study area North

In area S, we observed a different infection dynamic between municipalities, shown by a structured spatial effect (Figure 14). The strongest infection dynamics were found in some municipalities bordering Latvia, as well as those located further north (Figure 14). In both areas (S and N), we observed a decrease in the wild boar population density over time (Figures 13 and 14).

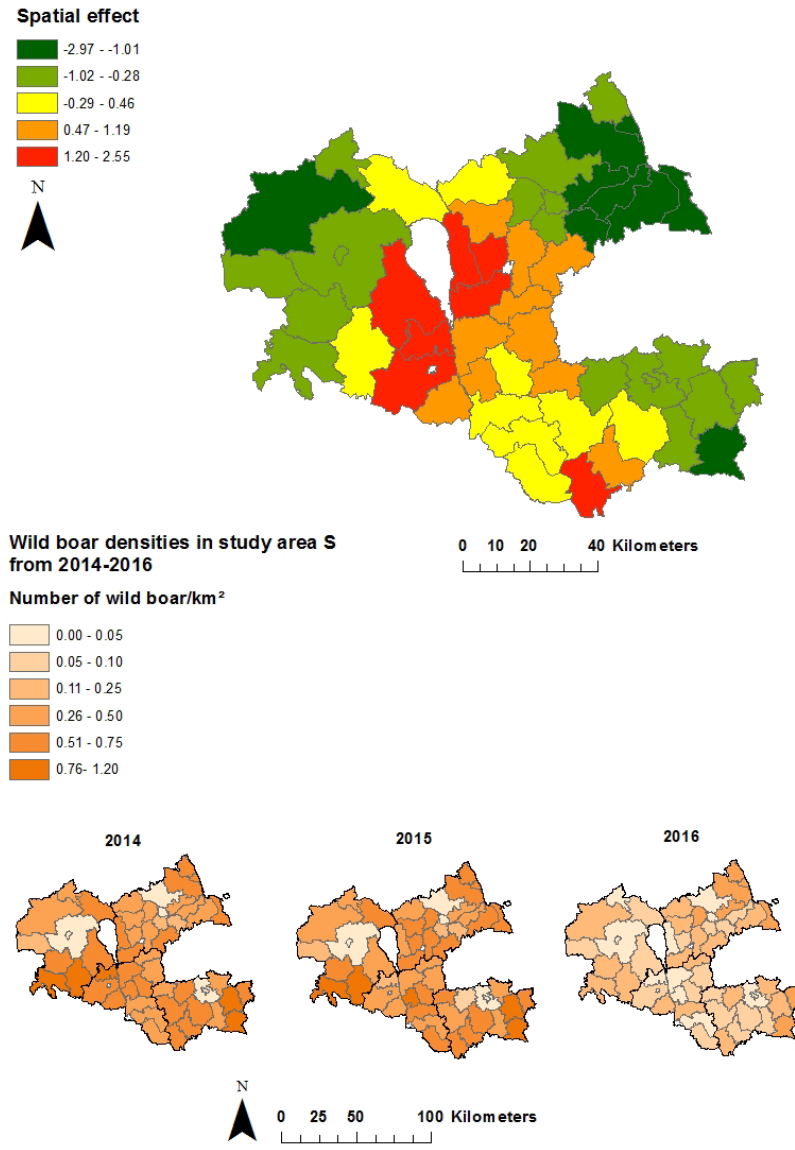


Figure 14. Median-structured spatial effect on the logit prevalence per municipality in study area South for the observation period of 25 months from 2014 to 2016. Maps in the lower row show the population density for each municipality in area South

The results of temporal analyses showed a significant difference in the median temporal effect on the logit prevalence between the two study areas. In area S, we found a significant increasing trend over the whole 25-month observation period. In area N, we did not observe a temporal effect at all during the observation period (Figure 15).

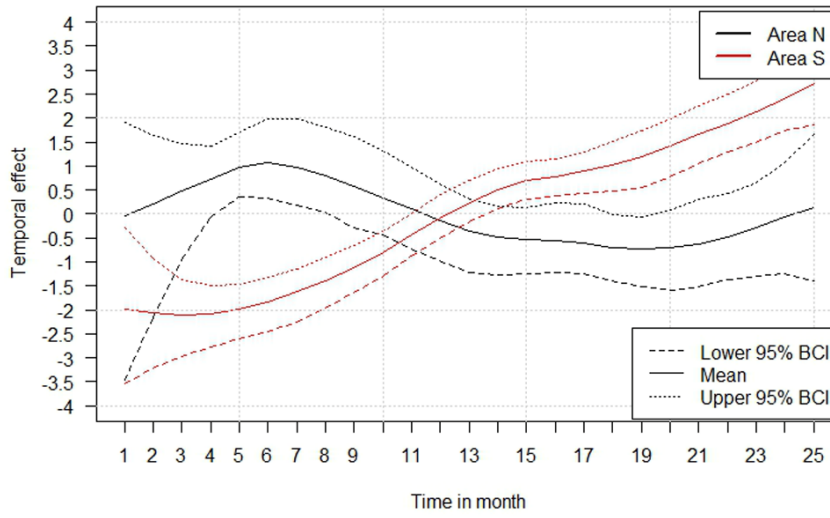


Figure 15. Median temporal effect on the logit seroprevalence in area North (N) and in area South (S) for the observation period of 25 months from 2014 to 2016. 95% Bayesian credible intervals (BCI) are included

5.2. Study II. Biological characteristics of the ASF virus strain Est 14/WB

5.2.1. Clinical course of the disease and pathomorphological findings in wild boar

During the trial, all ten wild boar inoculated in the first stage of the experiment using the virus strain Est 14/WB developed unspecific clinical signs starting from 4 to 6 dpi, including lack of appetite, general depression, respiratory distress and huddling. Between 7 and 13 dpi, nine out of ten inoculated animals showed worsening clinical signs with dyspnea and ataxia, and were euthanized in a moribund state or died overnight (#16). One remaining wild boar (#19) showed decreasing severity of clinical signs starting approximately 14 dpi and recovered completely over the following week.

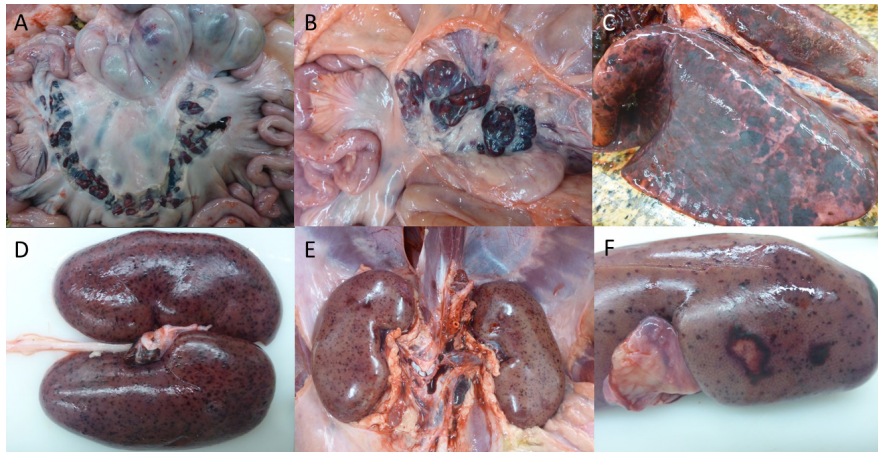


Figure 16. Pathological findings observed during necropsy of acutely, lethally infected wild boar following infection with the African swine fever virus strain Est 14/WB. (a) Haemorrhagic intestinal lymph nodes and striate bleedings in the gut. (b) Ebony-coloured, haemorrhagic lymph nodes in the gastrohepatic area. (c) Lung oedema, fibrinous pleuritis and haemorrhages. (d) and (e) Petechiae in the kidney. (f) Kidney petechiae and infarction (Photos: Sandra Blome)

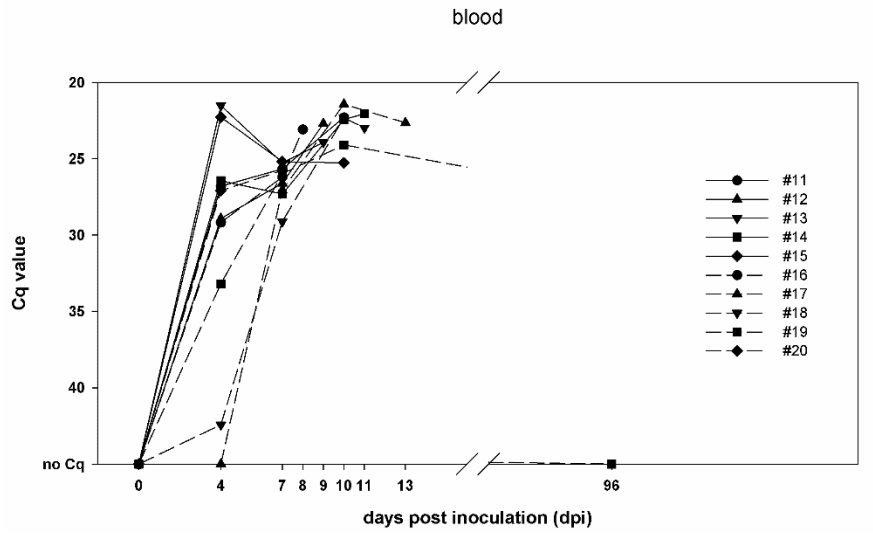
During necropsy we observed typical ASF lesions in all animals that succumbed to infection (Figure 16). Severity of lesions increased with time. Lesions ranged from slight lung oedema and ebony-coloured gastrohepatic lymph nodes to multiple haemorrhages in several organs, haemorrhagic and oedematous lymph nodes in all parts of the body and severe lung oedema. In addition, we observed infrequent findings including renal infarction, gall bladder oedema, arthritis and gastritis.

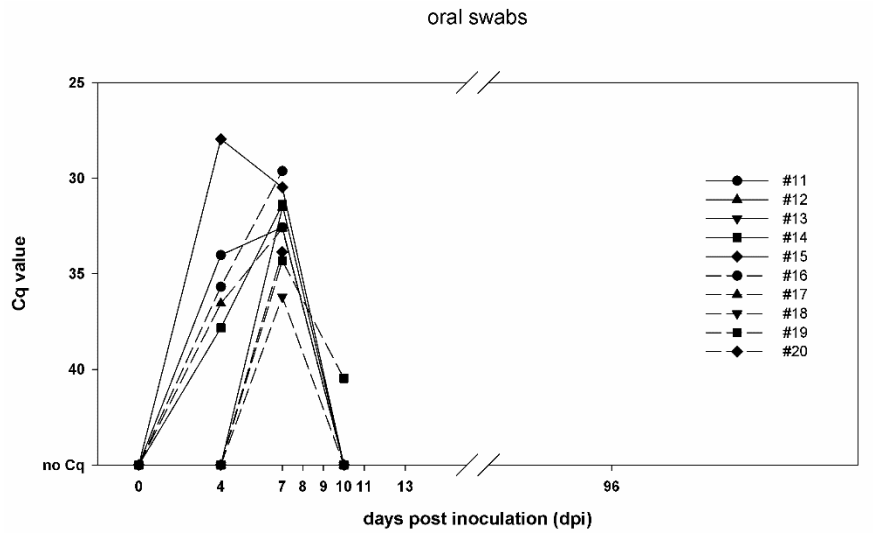
The wild boar (#19) that survived infection and recovered completely was commingled for the follow-up study with three sentinel pigs at 50 dpi. Neither sentinels nor survivor developed clinical signs. All animals remained in good health until the end of the trial at 96 dpi. No ASF-related lesions were observed any of these animals during necropsy.

5.2.2. Development of viremia in challenged animals

At 4 dpi, seven out of ten animals were ASFV-positive in a qPCR from the blood, five animals from oropharyngeal swabs, and also five animals from faecal swabs (Figure 17). At 7 dpi, all available blood and swab samples gave a positive qPCR result (Figure 17). At 10 dpi, all remaining animals gave strong positive qPCR results from blood samples (Figure

17a), and only one oropharyngeal swab sample (#19) gave any positive result, but this was weak (Figure 17b). Samples collected during necropsy (spleen, tonsil, lung, salivary gland and lymph nodes) all gave positive results in qPCR (see Table 5), and all spleen samples gave positive results in HAD tests.





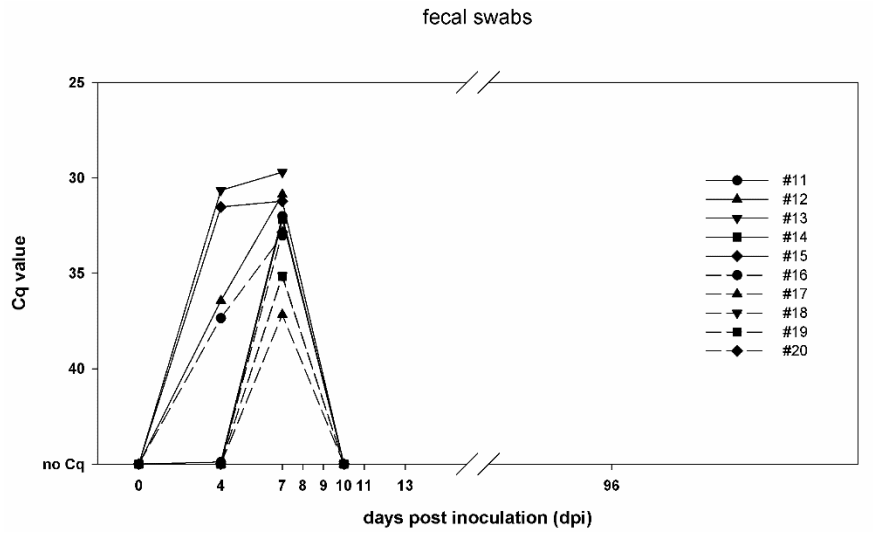


Figure 17. Detection of African swine fever genome by qPCR from samples collected from animals after inoculation. (a) Blood. (b) Oral swabs. (c) Fecal swabs. Results are depicted as cycle quantification (cq) values

During necropsy (at 96 dpi), nine different lymph nodes – mandibular, parotideal, lung-associated, renal, gastrohepatic, intestinal (from both the large and small intestines), inguinal, popliteal – were collected from the survivor (#19) and the sentinel pigs. All collected samples gave negative result for the viral genome (in qPCR) and ASF virus (in HAD test) (Table 5).

5.2.3. Development of immune response in challenged animals

During the entire experiment, we found three animals that were antibody-positive (#14; #17; #19) and two that showed doubtful results (#11; #13); all remaining animals, including sentinels, were found to be antibody-negative. A summary of antibody detection results in both inoculated and sentinel animals is presented in Table 5.

Table 5. Course of the disease, and detection of viral genome and antibodies following oronasal inoculation of ten wild boar with African swine fever virus strain Est 14/WB, and the respective data from the sentinel animals that were commingled with the surviving animal (#19) from 50 to 96 days post-inoculation (dpi)

					Virus detection in organs (cq)[b]												
Animal[a]	Course	End day	Viraemia (qPCR)	Ab detection	Tonsil	Sal Gland	Mand Ln	Lung	Spleen	Ln Par	Ln Lu	LnGa Hep	LnS Int	Ln Lln	Ln Kd	Lnl ng	Ln Popl
11	AL	10	4-10 dpi (ED)	doubtful at 10 dpi	31	28	27	26	30								
12	AL	9	4-10 dpi (ED)	no antibodies	35	33	28	30	29								
13	AL	9	4-10 dpi (ED)	doubtful at 9 dpi	26	24	29	20	27								
14	AL	11	4-10 dpi (ED)	from 10 dpi	35	36	36	29	31								
15	AL	10	4-10 dpi (ED)	no antibodies	29	30	25	31	28								
16	AL	8	4-10 dpi (ED)	no antibodies	29	33	28	28	27								
17	AL	13	4-10 dpi (ED)	at 13 dpi	27	33	30	24	30								
18	AL	10	4-10 dpi (ED)	no antibodies	31	32	29	30	28								
19	AT	96	Transient[c]	from 10 dpi	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq
20	AL	7	4-10 dpi (ED)	no antibodies	25	29	23	24	21								

					Virus detection in organs (cq)[b]												
Animal[a]	Course	End day	Viraemia (qPCR)	Ab detection	Tonsil	Sal Gland	Mand Ln	Lung	Spleen	Ln Par	Ln Lu	LnGa Hep	LnS lnt	Ln Lln	Ln Kd	Ln lng	Ln Popl
1					No	No	No	No	No	No	No	No	No	No	No	No	No
SE	na	96	negative	no antibodies	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq
2					No	No	No	No	No	No	No	No	No	No	No	No	No
SE	na	96	negative	no antibodies	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq
3					No	No	No	No	No	No	No	No	No	No	No	No	No
SE	na	96	negative	no antibodies	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq	cq

SE- sentinel; ED - end day; AL - acute lethal; AT- acute transient; na - not applicable; Ab - antibody; SalGland - salivary gland; MandLn - mandibular lymph nodes; LnPar - parotideal lymph nodes; LnLu - lung lymph nodes; LnGaHep - gastrohepatic lymph nodes; LnSlnt - lymph nodes from the small intestine area; LnLInt - lymph nodes from the large intestine area; LnKd - renal lymph nodes; LnIng - inguinal lymph nodes; LnPopl - popliteal lymph node

[a] Animals: 11-20- included to the initial oronasal inoculation; SE1-SE3- commingled with the surviving animal (#19) from 50 to 96 dpi

[b] Genome detection in organs is presented as cycle quantification (cq) value

[c] Detected positive at 4, 7 and 10 dpi; negative 96 dpi (no other samples)

5.3. Study III. Epidemiological characteristics and risk factors of ASF for domestic pigs in Estonia

5.3.1. Reporting and laboratory findings

ASF was immediately suspected on 12 out of the 26 outbreak farms, while on the other 14 farms the first suspicion was some other disease (Table 6). The reason for reporting was sickness (n = 19) or death (n = 7) of one or several animals.

Table 6. First suspicions on 26 African swine fever outbreak farms in Estonia, 2015–2017

First suspicion	No. of farms
ASF	12
Feed poisoning	7
Erysipelas	2
Pneumonia	2
Heat or stress	2
Salmonellosis	1

All outbreaks were confirmed by virus genome detection (PCR). ASF-virus-specific antibodies were detected in animals on seven farms using ELISA. All antibody-positive animals were also PCR-positive.

The estimated high-risk period varied in time span from seven to 20 days with a median of 11 days (Figure 18).

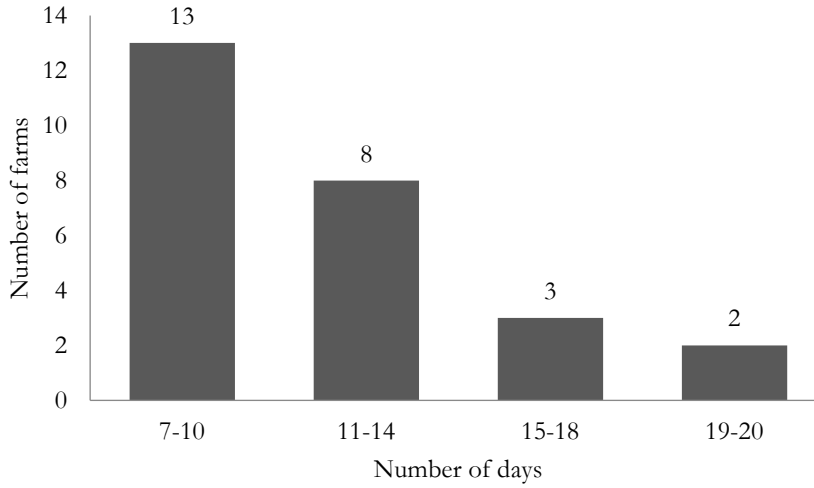


Figure 18. Length of estimated high-risk period (the length of time that African swine fever virus may have existed on the farm before it was suspected) on 26 pig farms affected by African swine fever in Estonia, 2015–2017

5.3.2. Characteristics of affected farms

Table 7 shows the number of outbreaks across farms of different size and type categories.

Table 7. Distribution of Estonian African swine fever-positive domestic pig farms across herd type and size, 2015–2017

	Herd-size category (no. of pigs)				
Production type	G1 (1–10)	G2 (11–100)	G3 (101–1000)	G4 (> 1000)	Total
Multiplier	0	0	1	2	3
Farrow-to-finish	1	1	3[a]	5	10
Fattening	7	0	1	5	13
Total	8	1	5	12	26

[a] Two herds with crosses of wild boar and domestic pigs (one kept outdoors) and one organic pig farm

5.3.3. Clinical signs in pigs and virus spread within farms

The first clinical signs in animals were mostly mild and not specific to ASF. A severe course of the disease was recorded on 13 farms, mostly after longer circulation of the virus on the farm. A summary of recorded clinical signs in pigs on affected farms before and after reporting is given in Table 8.

Table 8. Clinical symptoms in pigs recorded before and after reporting on 26 African swine fever outbreak farms in Estonia, 2015–2017

Clinical manifestation	No. of farms
Loss of appetite	19
Listlessness	19
Sudden death without prior signs in animal	14
Skin haemorrhages or cyanosis	11
Fever[a]	10
Recumbency	10
Incoordination	7
Abortions	5
Respiratory disorders	5
Other[b]	5

[a] On six farms, fever was not detected; on 10 farms, temperature was not measured

[b] Vomiting (n = 2); decrease in milk yield of sows (n = 1); diarrhoea (n = 1); blood in urine (n = 1)

Table 9 presents the observed mortality estimates. The average mortality was lowest in the largest herd-size category (0.7%) and highest in the smallest one (29.7%), being strongly dependent on the herd size.

Table 9. Estimated African swine fever mortality in affected domestic pig herds in Estonia, 2015–2017

Herd-size category		Mortality in the herd			Mortality in the affected group		
(no. of pigs)	n	Average	Min	Max	Average	Min	Max
G1 (1–10)	8	29.7%	0.0%	100.0%[a]	NA	NA	NA
G2 (11–100)	1	25.0%[b]	NA	NA	NA	NA	NA
G3 (101–1000)	5	7.5%	0.4%	25.0%	13.8%	3.8%	25.0%[c]
G4 (> 1000)	12	0.7%	0.04%	2.5%	7.2%	0.1%	43.6%[d]

NA – not applicable as pigs were kept in one group

[a] Mortality on a backyard farm with one pig

[b] At the moment of outbreak there were four pigs on the farm

[c] Herd of 126 crosses kept in one group

[d] Mortality in a group of 39 nursing sows

5.3.4. Probable routes of virus entry into farms and biosecurity level of the outbreak farms

We found that on all 26 outbreak farms, the virus was most likely introduced by some indirect transmission pathway, though we could not verify any specific route of introduction. On all eight non-commercial farms that experienced an outbreak we defined the cause of introduction as "lack of/insufficient biosecurity measures". For commercial herds, possible virus introduction pathways were identified more specifically by our epidemiology team. The results of the analysis are shown in Table 10.

Table 10. Most probable pathways of African swine fever virus introduction to commercial pig farms (n = 18) in Estonia, 2015–2017

	Herd-size category (no. of pigs)			
Introduction pathways	G2 (11–100)	G3 (101–1000)	G4 (> 1000)	Total
Multiple errors in execution of biosecurity procedures (introduction by fomites)	1	0	4	5
Inadequate disinfection of vehicles	0	0	2	2
Minor errors in execution of biosecurity procedures (introduction by fomites)	0	0	2	2
Movement of people or vehicles from an infected farm (secondary outbreak)	0	1	1	2
Contamination of cereal feed during storage or processing	0	3	2	5
Feeding of grass	0	1	0	1
Contamination of bedding material	0	0	1	1
Total	1	5	12	18

From the presented data, it appears that on the majority of commercial farms (n = 11), the virus was most likely introduced via contaminated fomites (people, vehicles, tools) as a result of errors in the execution of biosecurity procedures. The biosecurity levels of all outbreak farms across different herd-size categories are presented in Table 11.

Table 11. Biosecurity levels of Estonian African swine fever outbreak farms according to herd size, 2015–2017

Herd-size category (no. of pigs)	very high	high	moderate	low	very low
G1 (1–10)	0	0	0	1	7
G2 (11–100)	0	0	0	0	1
G3 (101–1000)	0	0	1	0	4
G4 (> 1000)	2	1	6	2	1
Total	2	1	7	3	13

5.3.5. Incidence of ASF outbreaks in pig herds

Tables 12 and 13 present the data on the occurrence of ASF outbreaks, as well as the cumulative herd incidence (presented as outbreak risk estimates), for the years 2015 and 2016 by farm size and type categories. In 2017, all outbreaks occurred in large commercial (G4) herds (outbreak risk = 4.5%, 95% CI 1.5-12.4). The overall outbreak risk in 2017 for all herd-size categories was 2.0% (95% CI 0.7-5.6).

Table 12. Number of African swine fever outbreaks and cumulative herd incidence (outbreak risk) for different farm types and herd-size categories in Estonia in 2015

	Herd-size category					Outbreak risk (CI 95%)
Production type	G1 n herds/ n outbreaks	G2 n herds/ n outbreaks	G3 n herds/ n outbreaks	G4 n herds/ n outbreaks	Total n herds/ n outbreaks	
Multiplier	18/ 0	11/ 0	1/ 1	5/ 2	35/ 3	8.6% (3.0–22.4)
Farrow-to-finish	13/ 0	44/ 1	22/ 3	31/ 3	110/ 7	6.4% (3.1–12.6)
Fattening	456/ 4	39/ 0	13/ 1	46/ 2	556/ 7	1.3% (0.6–2.6)
Total	488/ 4	95/ 1	36/ 5	82/ 7	701/ 17	2.4% (1.5–3.8)
Outbreak risk (CI 95%)	0.8% (0.3–2.1)	1.1% (0.2–5.7)	13.9% (6.1–28.7)	8.5% (4.2–16.6)	2.4% (1.5–3.8)	

Number of pigs: G1 (1–10); G2 (11–100); G3 (101–1000); G4 (> 1000)
CI- confidence intervals

Table 13. Number of African swine fever outbreaks and cumulative herd incidence (outbreak risk) for different farm types and herd-size categories in 2016

Production type	Herd-size category					Outbreak risk (CI 95%)
	G1 n herds/ n outbreaks	G2 n herds/ n outbreaks	G3 n herds/ n outbreaks	G4 n herds/ n outbreaks	Total n herds/ n outbreaks	
Multiplier	8/ 0	9/ 0	1/ 0	3/ 0	21/ 0	0.0% NC
Farrow-to-finish	6/ 1	24/ 0	17/ 0	28/ 1	75/ 2	2.7% (0.7–9.2)
Fattening	80/ 3	21/ 0	11/ 0	40/ 1	152/ 4	2.6% (1.0–6.6)
Total	94/ 4	54/ 0	29/ 0	71/ 2	248/ 6	2.4% (1.1–5.2)
Outbreak risk (CI 95%)	4.2% (1.7–10.4)	0.0% NC	0.0% NC	2.8% (0.8–9.7)	2.4% (1.4–5.2)	

Number of pigs: G1 (1–10); G2 (11–100); G3 (101–1000); G4 (> 1000)

NC-not calculated; CI- confidence intervals

The total herd-incidence rates obtained from survival analysis are presented for the whole three-year period, as well as per year, in Table 14.

Table 14. The herd-incidence rates of African swine fever outbreaks among domestic pig herds in Estonia for the years 2015–2017

Year	No. of outbreaks	No. of herd-years	Incidence rate (outbreaks per 100 herd-years)	95 % Confidence interval
2015	17	646.7	2.6	1.6–4.2
2016	6	229.8	2.6	1.2–5.8
2017	3	140.2	2.1	0.7–6.6
2015–2017	26	1,016.7	2.6	1.7–3.8

The overall yearly incidence rates did not differ significantly ($p > 0.05$) from each other.

The final Cox proportional-hazard random-effect model included county as a random variable and the only evaluated variable was 'herd-size category'. Larger herds (G3, G4) had a significantly higher risk of becoming infected with the ASF virus, compared to the two smaller herd-size categories (G1, G2) (Table 15).

Table 15. Results of the Cox proportional-hazard random-effect model showing the effect of herd size on the incidence of African swine fever outbreaks in Estonian domestic pig herds for the period 2015–2017. 'County' was included as a random variable

Herd-size category (no. of pigs)	N[a] (no. of outbreaks)	Hazard ratio (HR)	P	95% Confidence interval for HR
G1 (1–10)	607 (8)	1	X	X
G2 (11–100)	185 (1)	0.36	0.342	0.05–2.92
G3 (101–1000)	90 (5)	4.22	0.013	1.36–13.14
G4 (> 1000)	220 (12)	4.31	0.002	1.72–10.80

Wald Chi squared = 14.71 ($p = 0.002$)

[a] Number of herds after splitting the observation period into three years

5.3.6. Spatial and temporal distribution of outbreak farms

The geographical locations of outbreak farms changed during the period 2015–2017. As shown in Figure 19, domestic pig outbreaks appeared in those areas where ASF virus was circulating actively in the wild boar population.

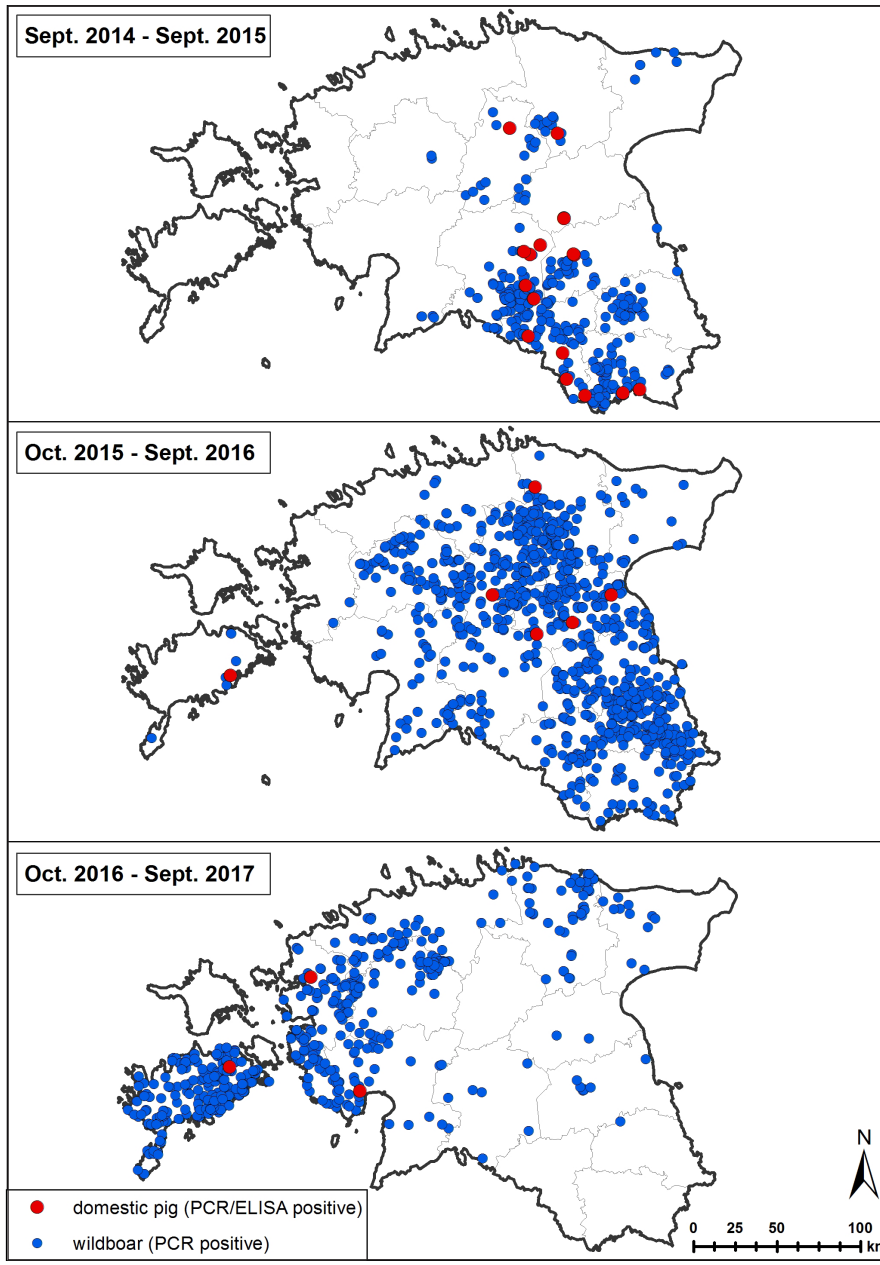


Figure 19. Location of African swine fever domestic pig outbreak farms and virus-positive wild boar cases in Estonia in 2015, 2016 and 2017

The distances between the outbreak farm and their nearest case of ASF in wild boar no more than a year before an outbreak are presented in Figure 20.

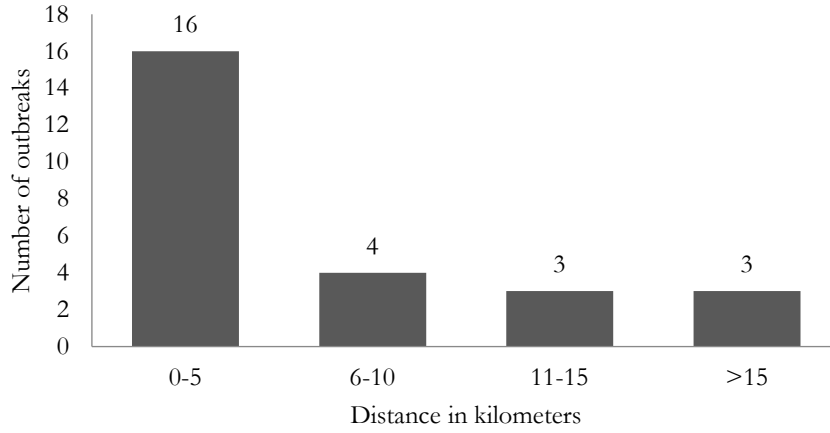


Figure 20. The distance between domestic pig outbreak farm and the closest tested African swine fever-positive wild boar case no more than a year before the outbreak in Estonia, 2015–2017

Each year, all ASF outbreaks were seen in the warm summer period, between June and September (Figure 21).

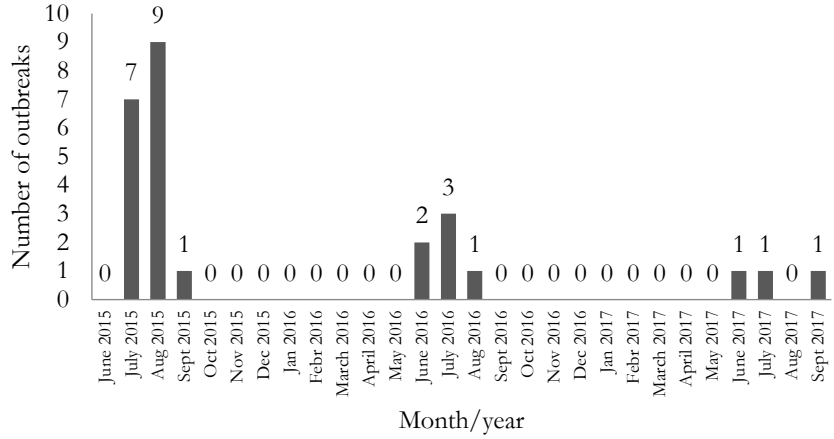


Figure 21. Occurrence of African swine fever outbreaks in Estonia from June 2015 to September 2017

5.3.7. Results of the hierarchical Bayesian spatio-temporal analysis

The results of the Bayesian model analysis indicate a significant positive association with the total number of ASF-positive wild boars detected per month in a hunting district. The total number of feeding sites, hunted wild boar, number of hunters and hunting hounds in a hunting district were not significantly associated with outbreaks in domestic pigs (Table 16).

Table 16. Fixed estimated parameters of the hierarchical Bayesian spatio-temporal model on a natural logarithmic scale

			Prediction interval (quantile)		
Variable	Mean	SD	2.5%	50%	97.5%
Intercept	-6.775	0.41	-7.598	-6.764	-6.012
No. of wild boar hunted (monthly)	-0.024	0.026	-0.081	-0.022	0.022
No of ASF-PCR-positive wild boar detected (monthly)	**0.132**[a,b]	**0.058**	**0.002**	**0.138**	**0.230**
No. of hunters in a district (yearly)	0.012	0.009	-0.006	0.012	0.029
No. of feeding sites (yearly)	0.015	0.024	-0.036	0.016	0.058
No. of hunting hounds (yearly)	0.015	0.067	-0.122	0.017	0.141

[a] Prediction intervals in bold indicate statistically significant parameter

[b] Mean effect of African swine fever-positive wild boar detection in a hunting district on the occurrence of a domestic pig outbreak on the territory of a hunting district was estimated to be 0.132. This means that for a one unit increase in African swine fever-positive wild boar detection, the log odds of having a domestic pig outbreak increases by 0.132 (95% prediction interval = 0.002–0.230)

SD- standard deviation

6. DISCUSSION

Up to the year 2014, when ASF reached EU member states, it was widely assumed that the reservoir of ASFV in Eastern Europe was the domestic pig sector and that wild boar played only a secondary role (Laddomada *et al.,* 1994; Mur *et al.,* 2012; FAO*,* 2013; Oganesyan *et al.,* 2013; Sánchez-Vizcaíno *et al.,* 2013; EFSA, 2014). Already within the first year of the ASF epidemic in Estonia, there was enough field evidence suggesting that the role of wild boar was much more substantial compared to previously affected areas. In addition, the course of the epidemic in wild boar appears to differ markedly in different European regions as well as within the countries. In relation to domestic pig outbreaks, on our medium size and large commercial farms we observed low or very low mortality as well as low contagiousity, both of which were in contrast with previous reports (Sánchez-Vizcaíno *et al.,* 2009; Costard *et al.,* 2013). To fill some knowledge gaps related to the epidemiology of ASF in both wild boar as well as domestic pigs these extensive studies were conducted. The intention was to collect scientific evidence and provide new knowledge for the international researcher's community and EU decision makers.

6.1. Differences between the areas regarding the course of the epidemic

The first ASF-positive wild boar in Estonia was found dead on 02. September 2014 in Valga County near the Latvian border. A week later, the virus was detected in wild boar in Viljandi County, bordering both Valga County and Latvia. The wild boar cases found close to the southern border of Estonia were most likely epidemiologically linked with the epidemic in northern Latvia, where circulation of the virus had been confirmed several weeks before (Olševskis *et al.,* 2016). On 14. September 2014, an ASF-positive wild boar was found in the north-east of Estonia (Ida-Viru County) not far from the border with the Russian Federation, in an area more than 200 km away from the affected area in the south.

During the first year of the ASF epidemic in Estonia (2014-2015) the observed epidemiological characteristics (e.g. mortality, case fatality, etc.) in the wild boar population apparently varied considerably between the

infected areas. In the southern affected area, high mortality (up to 16 dead animals found in one place) was reported and mainly ASF-virus positive animals were found. In the north-eastern affected area, mortality among wild boar was low or almost non-existent, and among hunted wild boar, clinically healthy, antibody-positive animals were found. Detection of ASF-virus or viral genome in the north-eastern area was rare. Moreover, the spread of the disease in the southern area appeared to be more rapid compared to in the north-east, where the infection seemed to remain within one area.

The results of analyses support the field observations. We found that in the south the proportion of the sampled wild boar found dead was significantly higher compared to the north-east. In addition, in the south, there was a significantly higher ASFV genome prevalence and higher chance of animals being detected as ASFV-genome-positive.

The Bayesian model was applied only to analyse serology, as the virus is detectable only over a limited period of time (Gallardo *et al.,* 2015d) and because no measurable memory effect was available. A trend analysis was not feasible with regard to the results of ASFV genome detection.

Despite the fact that we adjusted space and time data for the model, the results we obtained using the univariable analyses and Bayesian modelling differed only slightly. Also, for the univariable analyses, we used the whole data set independently of the study area, whereas for the Bayesian model we analysed the data for area North and area South separately. Still, we were able to confirm a significant association between age and serological result in both areas. In area South, we found a significant association between carcass category (found dead or hunted) and serology, which might be due to the higher relative number of animals found dead in the area. In area North, population density showed a significant effect on seroprevalence, but not in area South. This may be explained by the larger size of area South compared to area North and the associated heterogeneity of the population densities in the municipalities.

The spatial effect on the logit prevalence indicates a difference between the courses of the epidemics in the study areas. In area North the infection seemed to be stable in one limited area. In area South, in 2014, the prevalences were high in the areas bordering Latvia and the infection

seemed to move over time to the north. The spread of the virus in area South was most probably supported by the higher population density of wild boar, which made a higher transmission rate likely (Depner *et al.,* 2016). The findings of the spatial analysis also support the hypothesis that the infection was already present in area North for a longer period of time, whereas it was still spreading in area South at the time that the study was conducted.

The average seroprevalence did not differ significantly between the areas over the study period of 25 months; however, the result of the temporal trend analysis showed a significant difference in the course of infection. In area South we observed an increase in the temporal logit prevalence, which led us to assume that ASF was newly introduced into the area, naive animals became infected, and the proportion of animals developing antibodies subsequently grew. By contrast, in area North we could not see a temporal effect. Our assumptions were supported by the results of the descriptive analyses. In area South, the average seroprevalence showed an increase over time, whereas in area North, the average prevalence of antibody-positive wild boar was even lower in the second part of the study period. We therefore hypothesized that the infection may have been present in area North for a longer time, and it could have been there even before the first case of the disease was officially confirmed in the country. This hypothesis is supported by the fact that in the neighbouring St. Petersburg area (Russian Federation) several outbreaks of ASF had occurred between 2009 and 2012 (FAO*,* 2013). Furthermore, the very small sample sizes at the beginning of the epidemic (September 2014) and in the period before ASF was officially detected in Estonia (2012–2014) made earlier detection of the disease virtually impossible. So, if an undetected epidemic had started in the north-east of Estonia earlier, this may explain the different courses of the epidemics in the north-east and in the south. However, in both study areas, the small sample sizes have to be considered when interpreting the results.

In the follow-up study, the course of the ASF epidemic in the Estonian wild boar population over 44 months was analysed. This study included an additional 22 months (October 2016–July 2018), as well as a larger affected area (nine additional counties). The result of this study demonstrated the decrease in temporal logit antibody prevalences in those areas that were affected shortly after the start of the epidemic.

At the same time, in those areas that were affected in the later stages of the epidemic, we were able to observe an increase in antibody-positive wild boar (Schulz *et al.,* 2019a). Thus, in the first three to four years of the ASF epidemic we observed an increasing prevalence of antibody-positive wild boar, and subsequently this started to decrease, suggesting a decrease in the amount of ASF virus circulating. As of now (23. June 2020), the most recent ASFV-positive wild boar in Estonia was found in February 2019, and from August 2018 to February 2020 the decrease in antibody prevalence in wild boar continued across the country (Schulz *et al.,* 2020).

6.2. Factors influencing the course of the epidemic in wild boar

We found that the probability of detecting an ASF-positive animal (both genome- or antibody-positive) was higher in the young age group of wild boar. This finding was in contrast to results of several experimental studies where no age-dependency for infection was observed (Blome *et al.,* 2012; Pietschmann *et al.,* 2015; Zani *et al.,* 2018; Pikalo *et al.,* 2020). However, as the field results from Latvia correlate with Estonian findings (Schulz *et al.,* 2019b; Olševskis *et al.,* 2020), this may suggest that despite no age-dependency of infection the chance of becoming infected is higher in young wild boar. Young animals need more food for rapid growth, and therefore they are probably more attracted to carcasses (including infected ones) as well as to feeding sites in the habitat, which might be contaminated. Probst *et al.* (2017) describe wild boar having a significantly higher interest in approaching carcasses of dead wild boar during the summer period because young animals have a higher need for protein-rich food to help growth. Since, in this period, young animals still live in the same group together with sows, the infection can easily be transmitted within the group. The second explanation may be that in the summer period the size and density of the wild boar population is at its highest, which support contact between animals, spread of the virus and mortality. Cukor *et al.* (2020) conducted a carcass experiment in the Czech Republic during the winter period. They observed that wild boar had direct contact with the carcass in 81% of all recorded visits and cannibalism was recorded in 9.6% of all recorded visits. An age-dependency of wild boar approaching the carcasses was not detected.

The age distribution of hunted wild boar was the same in both study areas. This similar structure of hunted animals may be the result of similar hunting practices in use all over the country.

There was a higher probability of finding ASF-positive wild boar found dead than hunted. This finding is in accordance with later results from other affected EU countries (Schulz *et al.,* 2019b; Frant *et al.,* 2020; Mačiulskis *et al.,* 2020; EFSA, 2020; Olševskis *et al.,* 2020). This is probably because of the high virulence of circulating virus strains and high lethality of ASF. Such a strong association between animals found dead and a positive ASF result emphasizes the importance of passive surveillance (Schulz *et al.,* 2017; EFSA, 2020), which includes rapid finding of carcasses and immediate disposal of them. This is crucial and one of the most effective measures for successful eradication of ASF in the wild boar population.

Most experts agree that a low population density of wild boar reduces the risk of ASF spread (Smietanka *et al.,* 2016; Jurado *et al.,* 2018; EFSA, 2018; Mur *et al.,* 2018; Podgórski *et al.,* 2019). Our study demonstrates a positive association between population density of wild boar and the municipality status regarding ASF (by ASFV genome detection or serology). Direct contact between animals promotes the transmission of the virus (Gallardo *et al.,* 2015d; Depner *et al.,* 2016; Guinat *et al.,* 2016). Thus, it can be assumed that in densely populated areas contact between wild boar is more frequent, the transmission rate is higher, and this supports the spread of the virus.

6.3. Characteristics of circulating virus strains

The ASF virus strains circulating in Eastern Europe since 2007 are of genotype II and in experimental conditions have shown mostly high virulence for both domestic pigs and European wild boar (Gabriel *et al.,* 2011; Blome *et al.,* 2012; Guinat *et al.,* 2014; Vlasova *et al.,* 2014; Gallardo *et al.,* 2015b; Pietschmann *et al.,* 2015; Olesen *et al.,* 2017; Pikalo *et al.,* 2020). This means, under field conditions, high mortality and obvious clinical signs can be expected as the virus enters a disease-free area. Interestingly, as described earlier (section 6.1), during the real epidemic situation, there was almost no mortality in wild boar in the north-eastern part of Estonia. This led to the hypothesis of circulation of an attenuated virus strain in the area. To clarify how virulent the local virus strain (Est

14/WB) isolated from an infected wild boar in Ida-Viru County was, an animal experiment was conducted. The results demonstrated that this ASFV strain was still highly virulent for young wild boar; nevertheless, one animal recovered completely. While we compared some parameters with previous experimental studies (Gabriel *et al.,* 2011; Blome *et al.,* 2012; Pietschmann *et al.,* 2015; Tauscher *et al.,* 2015), we found genome loads to be slightly lower and detectable antibody responses to be more frequent. However, these differences could also be due to variability in extraction methods and slight differences between PCR machines. The clinical course of infection and the pathomorphological signs did not differ for the animals that succumbed to infection.

6.4. Contagiousness and transmission of the virus

The virological data collected during the experiment indicated that at least one animal (#17) became infected later. This suggests that oral infection can be error prone and therefore needs a quite high dose for infection. Based on previously reported data, virus titres > 10^4 HAU are usually necessary for oral infection and the ratio of viral titres needed for infection of a susceptible animal via the intramuscular/intravenous inoculation *versus* the oral/nasal route is 1:140.000, with less than 1 HAU for the parenteral route (McVicar, 1984). The fact that for oral infection a relatively high dose of the virus is needed might explain why the natural spread of the epidemic in Europe is rather slow (EFSA, 2017; Niine *et al.,* 2019). One supporting factor for the slow spread of the disease may also be moderate or low contagiousity of the disease, which has been described in some experiments (Pietschmann, *et al.,* 2015) and in the field (Lamberga *et al.,* 2018).

Recovery and survival of one infected animal gave the opportunity to study the long-term fate of recovered animals and clarify their potential role in transmission of the virus on a limited scale. Prior to the current study, reliable data were missing regarding this issue. It was widely supposed that survived animals might remain as virus carriers (Bech-Nielsen *et al.,* 1993; Sanchez-Vizcaıno *et al.,* 2012; Gallardo *et al.,* 2019b) and so contribute to the long-term persistence of ASF in a region. The results of the experimental study did not confirm this hypothesis. The survivor did not shed the virus, and did not transmit this to sentinels, even under conditions with low-intensity hierarchical fights upon introduction of new animals. Thus, carrier status is not guaranteed for

all surviving animals, as also concluded in a recent, thorough review (Stahl *et al.,* 2019).

6.5. Virulence of the virus

The results of the animal experiment did not explain why the disease dynamics differ between defined regions in Estonia. Moreover, no evidence of attenuation of the virus strain was found. However, subsequent animal trials using the same virus strain (Est 14/WB) for the infection of mini pigs and domestic pigs have demonstrated a less severe course of the disease (Zani *et al.,* 2018). Both domestic pigs and mini pigs showed mostly mild and non-specific clinical signs characteristic of a subacute or chronic form of the disease. In that study, all five domestic pigs and 13 mini pigs included in the trials recovered from the disease completely. Furthermore, based on the sequence data of the viral genome, we were able to report the discovery of the first attenuated genotype II ASFV strain (Est 14/WB) circulating in eastern Europe, starting from 2007 (Zani *et al.,* 2018). The second attenuated genotype II ASFV strain (Lv17/WB-Rie1) in Europe (which was also non-haemadsorbing) was isolated from hunted wild boar in Latvia in 2017 (Gallardo *et al.,* 2019a). The Latvian strain caused chronic or unspecific clinical signs in two inoculated pigs, which did not lead to death of the animals; in two other in-contact pigs mild clinical symptoms appeared, and in two more in-contact pigs no detectable clinical symptoms developed (Gallardo *et al.,* 2019a).

In 2015 and 2016, in different regions in Estonia (Figure 22), three additional moderately virulent genotype II ASFV strains were isolated from wild boar (Est15/WB-Valga-6; Est15/WB-Tartu-14; Est16/WB-Viru-8) (Gallardo *et al.,* 2018a; Gallardo *et al.,* 2018b). The recorded mortality rate in all these experiments was 66.7%; however, survivors were recorded only within groups of in-contact pigs. Pershin *et al.* (2019) recently reported a probable change in the virulence of ASFV strains in the Russian Federation. This comprehensive paper summarizes the results of 15 experimental infections of pigs with various Russian genotype II ASFV strains isolated the period 2013–2018. In five out of 15 challenge experiments there were survivors and the reported mortality under 100% (50–90%). Similar results are reported from Poland (Walczak *et al.,* 2020), where different parameters of the disease were investigated and

it was found that the same virus strain (Pol18_28298_O111) may cause various clinical forms of ASF (acute, subacute, chronic).

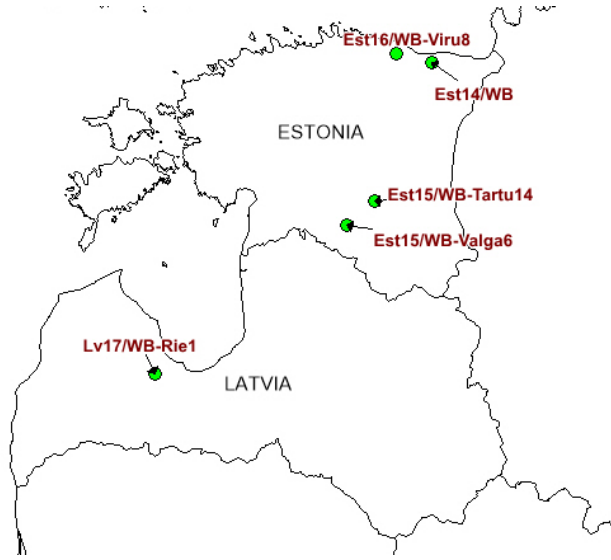


Figure 22. Locations where moderately virulent and attenuated African swine fever virus strains isolated from wild boar in Estonia and Latvia were found (2014–2017)

In summary, based on the results of these experimental studies, we can assume that changes in the virulence of the virus strains of genotype II are not as rare as previously expected. Reduction in the virulence of the virus is an important event, since virus strains with moderate or low virulence, or attenuated strains may induce less severe forms of the disease. Circulation of such strains may cause a decrease in mortality and may lead to hidden circulation of the virus in the wild boar population, as well as in domestic pig herds. However, it is also possible that such virus strains just die out naturally as has recently occurred with two Estonian ASFV strains (Est14/WB; Est15/WB-Tartu-14), which circulated regionally for only a limited time and then disappeared (data not published). Thus, we may conclude that virulent strains of ASFV are probably more viable, especially if there are enough susceptible animals in the region.

Furthermore, such an endemic situation may also require a different approach to ASF surveillance. From the perspective of early detection, the most important tool for detection of ASF is virus genome detection (by PCR). The presence of antibodies is a good marker in the later stage of epidemics, especially in case of subacute or chronic infection. So

parallel testing of samples to detect both the virus genome (PCR) and antibodies (ELISA, IPT, IB) should become the norm.

6.6. Detection of the disease on farms

ASF occurrence on domestic pig farms was generally reported at a relatively early stage of an outbreak in Estonia. This can be concluded based on the fact that the spread of the disease within farms was limited, and seroconverting (antibody-ELISA-positive) animals were found only on 27% of outbreak farms. Since all antibody-positive animals were also PCR-positive, this indicates that the virus should not have been present in the herd for more than four weeks (Gallardo *et al.,* 2018a; Petrov *et al.,* 2018; Zani *et al.,* 2018).

One reason for early reporting may be the relatively high awareness of farm owners regarding ASF. Furthermore, it is the habit of animal owners to involve veterinarians in the case of morbidity or mortality of animals. Estimated mortality was generally low in the two largest farm-size categories (medium-size and large commercial farms), at both herd and production-unit levels. However, this finding does not support early detection of an outbreak. In larger herds, the monitoring of general mortality is not sufficient for early detection of an ASF outbreak. In smaller farm-size categories (backyard and small commercial farms), the average mortality was considerably higher, as every death of an animal influenced the mortality estimate substantially. However, we have to take into account that the estimates of mortality reported here are arbitrary because the time periods during which mortality for every affected herd was calculated differed considerably (reporting 0–14 days from first symptoms, culling 1–3 days after reporting). At the same time, case fatality rate was considered high in all farm-size categories, as most of the affected pigs died 1 to 5 days after the appearance of the first clinical signs. Thus, we may conclude that an ASF epidemic can result in high mortality if there is enough time for the virus to spread within the herd.

6.7. Farms at risk

Outbreaks were confirmed in herds of all size categories and production types. However, we observed a tendency for more outbreaks to occur in herds with breeding animals. We can assume differences in the

management of breeding animals compared to growers and fatteners, and presume that breeding animals need more interaction with humans. In addition, pregnant and nursing sows may be more susceptible to the virus due to immune suppression, and thus lower doses of the virus might be able to initiate the infection. Although Latvia has reported a small number of outbreaks on large commercial farms, at least two of them started in a unit of breading sows (Lamberga *et al.,* 2018; Lamberga *et al.,* 2020).

The number of ASF outbreaks in commercial herds exceeded the number of outbreaks on backyard farms in Estonia. This may indicate that large commercial farms are more exposed to the virus due to more frequent and intensive contact with the external environment through movement of vehicles and people. The higher number of outbreaks on commercial farms can also be explained by the rapid reduction in the number of backyard pig holdings in Estonia, which dropped from 696 in 2014, to 25 by 2017 and resulted from strict biosecurity requirements, which are equal for all pig farms. The latest information from other affected European countries does not support our finding that large commercial farms are more exposed. Although, the large commercial farms in other European countries suffer outbreaks (Lamberga *et al.,* 2018; EFSA, 2020; Anonymous, 2020; OIE WAHID, 2020), it seems that backyard farms are more exposed to virus introduction (FAO*,* 2013; Olševskis *et al.,* 2016; Lamberga *et al.,* 2018; Zani *et al.,* 2019; EFSA, 2020; OIE WAHID, 2020). However, so far, a complete overview regarding this issue is missing.

6.8. Clinical course of the disease and spread of the virus on farms

On outbreak farms, we often found ASF cases with mild clinical signs. Severe clinical signs (apart from sudden death), including the haemorrhagic form of the disease, were seldom observed and often limited to a few animals only. This might be result of the relatively early detection of outbreaks. A severe clinical course and higher morbidity were seen in pregnant or nursing sows, or where the virus had been circulating for longer on a farm. In 2018, Lamberga *et al.* described similar findings on a Latvian large breeding farm. Mild and non-specific clinical signs at the beginning of an outbreak could also be one reason

why diseases other than ASF were suspected at first in more than half of the outbreak herds in Estonia.

We observed that the spread of the virus within outbreak farms was generally slow. In most affected farms, the infection was detected only in one unit or even in one pen, and in affected pens some pigs were still ASFV-negative at the time of reporting. Previous studies have shown that the stability of the virus is found to be higher in protein-rich materials (e.g. in blood or in a carcass) when compared to other materials such as urine, faeces, and various other secretions and excretions. High viral load is always detected in blood from infected pigs (Gabriel *et al.,* 2011; Carvalho Ferreira *et al.,* 2012; Guinat *et al.,* 2014; Gallardo *et al.,* 2015b; Olesen *et al.,* 2017). Viral load in swab samples (incl. nasal, oral, conjunctival, urogenital) have been reported to be considerably lower than those detected in blood (Greig & Plowright, 1970; Gabriel *et al.,* 2011; Carvalho Ferreira *et al.,* 2012; Blome *et al.,* 2013; Guinat *et al.,* 2014; Olesen *et al.,* 2017; Walczak *et al.,* 2020). Lower virus load is also observed for urine and faeces, and the excretion of the virus in urine and faeces is reported to be inconsistent (Greig & Plowright, 1970; Gabriel *et al.,* 2011; Davies *et al.,* 2017; Walczak *et al.,* 2020). These findings may explain why the spread of the virus within a pen or unit can be rather slow. Until some infected animals start to show clinical signs with bleeding or they die, the amount of the virus in the pen environment may not be high enough to infect healthy animals. It has been shown that in an epidemiological situation without tick involvement, direct parenteral inoculation is rather unlikely, and for oral infection, virus titres > 10^4 HAU are usually needed (Petrov *et al.,* 2018); in addition, low-dose ASFV infections may lead to prolonged incubation times and altered clinical courses (Pietschmann *et al.,* 2015). Slow spread of the virus on farms has also been reported for Latvian outbreaks (Olševskis *et al.,* 2016; Lamberga *et al.,* 2018; Lamberga *et al.,* 2020).

6.9. Introduction of the virus to farms

The introduction of the virus to outbreak farms is likely to have occurred mainly by indirect transmission routes. Direct contact with potentially infected wild boar could not be completely excluded in two outbreak farms in our investigation (an organic farm using a single fence with a walking area connected to the barn, and an outdoor farm of crosses

with double fencing). However, even in these cases, we considered direct contact unlikely as no direct evidence of damage to the fences was found.

Feeding of contaminated swill has often been considered as one of the main risk factors for indirect transmission of ASF (Mur *et al.,* 2012; FAO, 2013; Gogin *et al.,* 2013; FAO*,* 2013; Oganesyan *et al.*, 2013; Sánchez-Vizcaíno *et al.,* 2013; Olševskis *et al.,* 2016; Kolbasov *et al.,* 2018). In Estonia, feeding of swill to pigs is illegal. We excluded this transmission route for all affected commercial farms. On backyard farms, the feeding of kitchen leftovers could not be completely excluded. However, we did not consider swill feeding as the main possible route of virus introduction, as meat from pigs on these farms is mainly consumed by the owners. Introduction of the virus with purchased meat products from local shops would assume hidden circulation of the virus in Estonia or contamination of imported products. We also considered this scenario unlikely. Furthermore, according to interview results, none of the farmers or farm workers had contact with affected countries. One other possible source of infection could have been contaminated wild boar meat, the uncontrolled consumption of which we cannot completely exclude. However, most likely, the virus entered affected herds by means of contaminated fomites – vehicles, clothing, feed and bedding – due to inadequate biosecurity measures on farms or errors in the implementation of these measures.

Our analysis showed that in most cases there was no single obvious event that could link the introduction of the virus to a farm. On most affected backyard farms, there were several biosecurity inadequacies at the time of virus entry (e.g. no separation of inside and outside zones, lack of functional disinfection barriers, feeding grass to pigs, pet access or housing other farm animals together with pigs, unsafe storage of feed and bedding material etc.). Therefore, it is difficult to single out one particular cause. On commercial farms, which followed relatively high biosecurity protocols, the route of virus introduction was also difficult to trace. Apparently, minor errors in the implementation of (generally adequate) biosecurity procedures must have led to the introduction of the virus.

6.10. Biosecurity on farms

The majority of outbreaks occurred on farms operating at a low biosecurity level. Based on available data, we could not estimate whether herds with low biosecurity were at higher risk or not as we did not have information about the distribution of biosecurity levels for all farms. However, assuming that the biosecurity level is higher on commercial farms than on backyard farms, our data on herd incidence do not support the general opinion that a higher biosecurity level per se ensures a lower risk of ASF introduction. This may mean that the biosecurity measures applied so far (disinfection and physical barriers) are not fully effective against the incursion of ASF infections (Anonymous, 2014). Furthermore, based on the results of our study we may assume that the risk of herds becoming infected depends on the size of the farm. Thus, it may be relevant in future to evaluate the efficiency of biosecurity measures taking into account the size of the farm. However, even farms operating at a high or very high biosecurity level are depending on the human factor, which, to some extent, is not fully predictable. ASF is mostly a long-lasting epidemic, which is a challenge for each farm owner and worker. Based on field observations, we can still assume that a high biosecurity level is the most important tool for preventing ASF introduction to a farm (Bellini *et al.,* 2016).

6.11. Incidence of ASF outbreaks in pig herds

We observed significantly higher herd incidence risk in the group of commercial farms in the years 2015 and 2017, whereas in 2016 it did not differ significantly from the incidence risk on non-commercial (backyard) farms. Since the herd incidence estimates are dependent on the accuracy of reporting, this led us question whether the reporting in the group of backyard farms was as good as for commercial farms. Considering the general socio-cultural background and the usual habits of smallholders to invite a veterinarian to check diseased animals, we assumed only a slightly lower level of reporting within backyard herds compared to commercial farms. Furthermore, surveillance data (PCR and serological testing) of herds located in restriction zones did not reveal any case of undetected infection in domestic pigs (data not shown).

The herd incidence risk in commercial herds (all size groups) decreased in 2016 and 2017 compared to 2015. This is probably due to improvements

in biosecurity measures on farms, as well as more stringent surveillance by the veterinary authorities regarding the fulfilment of the legal biosecurity requirements. However, the small number of outbreaks has to be taken into account as a limitation in interpreting these results. Interestingly, in the period 2015–2017, the total herd incidence across all herd groups did not change significantly.

6.12. Infection pressure from wild boar

The majority of outbreaks in Estonia were confirmed in areas where ASF had been found in wild boar prior to detection of the virus in domestic pigs. In 23 out of 26 outbreaks, the virus had been circulating among wild boar within a radius of 15 km from the affected farm, and in 16 outbreaks, within a radius of 5 km. The results of spatio-temporal analysis indicate that the occurrence of outbreaks in domestic pigs was associated with the intensity of the infection in the wild boar population. The outbreaks occurred in areas where more virus-positive (detected by PCR) cases were registered in wild boar prior to the outbreak. At the same time, we did not find a significant association of outbreaks in domestic pigs with hunting intensity, which can be explained by the minimal interaction between hunters and pig producers. A similar trend was also observed in Latvia in 2014 where all 32 outbreaks in domestic pigs were detected in areas where ASF was present in the wild boar population (Olševskis *et al.,* 2016).

6.13. Seasonality

All outbreaks in Estonia occurred in the warmest period of the year, from June to September (81% in July and August). A similar seasonal trend has also been reported by Latvia and other EU countries (Olševskis *et al.,* 2016; EFSA, 2020). One explanation for this could be the more frequent contact between farms (both people and vehicles) and the surrounding environment at this time of year because of the seasonal nature of field work. The high-risk period for introduction of the virus to domestic pig farms coincides with the harvest and field work period; this is also the period when wild boar move to feed in the fields. In addition, this is the period when the wild boar density is highest (period after breeding season) and the number of infected wild boar is also at its highest level, which represents infection pressure. Thus, all these factors

may increase the probability of transmission of the virus to the farm via contaminated fomites.

The high season of ASF outbreaks also coincides with the high season of blood-sucking insects in Estonia. This might suggest that they have a potential role in the transmission of the virus from wild boar to domestic pigs. However, there is still not enough scientific evidence regarding the capacity of mechanical insect vectors to transmit the ASF virus. Besides, if this would have been an important transmission route, many more outbreaks should have been expected in domestic pig herds, and a faster spread of infection within affected herds should also have been expected. Nevertheless, the role of insect vectors in transmission of the virus is still not clear and needs further investigation.

7. CONCLUSIONS

The pattern of the ASF epidemic among wild boar in the north-east of Estonia was significantly different from the pattern of the disease in the south of Estonia during the first 25 months of the epidemic (I).

The temporal and spatial differences in the course of the ASF epidemic in the wild boar population between the two areas suggest that the first introduction of ASF took place in the north-east of Estonia and not, as reported officially, in the south. Additionally, it was possible that the epidemic in the north-east was caused by a virus strain with different properties (I).

The biological properties of the ASF virus strain spreading in the north-east of Estonia in 2014 did not notably differ from those of other virulent genotype II strains in an experiment with young wild boar; however, one animal survived the infection and recovered completely (II).

The carrier status of this survivor animal (mentioned above) could not be demonstrated. In an experiment, the wild boar surviving ASF did not shed the virus after recovering and did not transmit it to sentinel animals (II).

The spread of ASF virus in wild boar populations surrounding pig farms was the main risk factor for infection of domestic pigs (III).

Introduction of the virus to farms occurred most likely by indirect transmission pathways, such as via contaminated fomites (people, vehicles, tools). This, presumably, was a result of errors in the execution of biosecurity procedures, even in those cases where the general biosecurity level of a farm was high (III).

This study has shown that transmission of ASF virus between herds was rare indicating that the domestic pig transmission cycle of the virus has largely been avoided in Estonia (III).

Large commercial farms, and also possibly farms with breeding animals, were shown to be at higher risk of becoming infected. Thus, biosecurity

measures have to be at the highest possible level on these farms to prevent outbreaks in ASF-affected areas (III).

The first clinical signs of ASF in herds were unspecific. Thus, in ASF-affected and endangered regions, whenever there is the sudden death of a pig with an unclear cause, or an abortion or loss of appetite, even in one pen, ASF should be considered as a possible cause (III).

In this study, the spread of the virus within farms was shown to be slow, which indicates that the contagiousness of the virus was low during the initial phase of the outbreaks. Thus, monitoring only general morbidity and mortality of a herd is not sufficient for early detection of ASF outbreaks (III).

Investigation of sick and dead domestic pigs and wild boar for ASF (passive surveillance) is an essential tool for early detection of the disease (I, III).

8. REFERENCES

Achenbach, J.E., Gallardo, C., Nieto-Pelegrín, E., Rivera-Arroyo, B., Degefa-Negi, T., Arias, M., Jenberie, S., Mulisa, D.D., Gizaw, D., Gelaye, E., Chibssa, T.R., Belaye, A., Loitsch, A., Forsa, M., Yami, M., Diallo, A., Soler, A., Lamien, C.E., Sánchez-Vizcaíno, J.M., 2016. Identification of a new genotype of African swine fever virus in domestic pigs from Ethiopia. Transbound. Emerg. Dis. 64, 1393-1404. doi.org/10.1111/tbed.12511.

ADNS 2017. Animal Disease Notification System Database. Accessible: https://webgate.ec.europa.eu/ADNS/sec/?event=sec.login. Accessed: January 2017.

Alonso, C., Borca, M., Dixon, L., Revilla, Y., Rodriguez, F., Escribano, J.M., 2018. ICTV report consortium. ICTV virus taxonomy profile: *Asfarviridae*. J. Gen. Virol. 99, 613–614. doi.org/10.1099/jgv.0.001049.

Anonymous 2014. African swine fever in Lithuania. Presentation on SCOFCAH meeting. Accessible: https://ec.europa.eu/food/sites/food/files/animals/docs/reg-com_ahw_20140821_pres_asf_lithuania.pdf. Accessed: May 2020.

Anonymous 2019. Epidemiological situation of ASF in Romania. Presentation on SCOPAFF meeting. Accessible: https://ec.europa.eu/food/sites/food/files/animals/docs/reg-com_ahw_20190708_asf_rou.pdf. Accessed: May 2020.

Anonymous 2020. African Swine Fever situation in Bulgaria. Presentation on SCoPAFF meeting. Accessible: https://ec.europa.eu/food/animals/health/regulatory_committee/presentations_en#20200116. Accessed: May 2020.

Arias, M., de la Torre, A., Dixon, L., Gallardo, C., Jori, F., Laddomada, A., Martins, C., Parkhouse, R.M., Revilla, Y., Rodriguez, F.M., Sanchez-Vizcaino, J.M., 2017. Approaches and perspectives for development of African swine fever virus vaccines. Vaccines 5, 35. doi.org/10.3390/vaccines5040035.

Arias, M., Jurado, C., Gallardo, C., Fernández-Pinero, J., Sánchez-Vizcaíno, J.M., 2018. Gaps in African swine fever: analysis and priorities. Transbound. Emerg. Dis. 65, 235–247. doi.org/10.1111/tbed.12695.

Baldacchino, F., Muenworn, V., Desquesnes, M., Desoli, F., Charoenviriyaphap, T., Duvallet, G., 2013. Transmission of pathogens by *Stomoxys* flies (*Diptera, Muscidae*): a review. Parasite 20, 26. doi.org/10.1051/parasite/2013026.

Bastos, A., Penrith, M.L., Crucière, C., Edrich, J.L., Hutchings, G., Roger, F., Couacy-Hymann, E., Thomson, G.R., 2003. Genotyping field strains of African swine fever virus by partial p72 gene characterization. Arch. Virol. 148, 693-706. doi.org/10.1007/s00705-002-0946-8.

Bech-Nielsen, S., Arias, M., Panadero, J., Escribano, J.M., Gomez-Tejedor, C., Perez Bonilla, Q., Sarchez-Vizcaino, J.M., 1993. Laboratory diagnosis and disease occurrence in the current African swine fever eradication program in Spain, 1989-1991. Prev. Vet. Med. 17, 225-234. doi.org/10.1016/0167-5877(93)90031-N.

Bech-Nielsen, S., Fernandez, F., Martinez-Perda, J., Espinosa, Q., Perez Bonilla, Q., Sarchez-Vizcaino, J.M., 1995. A case study of an outbreak of African swine fever in Spain. Br. Vet. J. 151, 203-214. doi.org/10.1016/S0007-1935(95)80012-3.

Bellini, S., Domenico Rutili, D., Guberti, V., 2016. Preventive measures aimed at minimizing the risk of African swine fever virus spread in pig farming systems. Acta Vet. Scand. 58, 82. doi.org/10.1186/s13028-016-0264-x.

Blome, S., Gabriel, G., Dietze, K., Breithaupt, A., Beer, M., 2012. High virulence of African swine fever virus Caucasus isolate in European wild boars of all ages. Emerg. Infect. Dis. 18, 708. doi.org/10.3201/eid1804.111813.

Blome, S., Gabriel, C., Beer, M., 2013. Pathogenesis of African swine fever in domestic pigs and European wild boar. Virus Res. 173, 122-130. doi.org/10.1016/j.virusres.2012.10.026.

Boinas, F.S., Wilson, A.J., Hutchings, G.H., Martins, C., Dixon, L.J., 2011. The persistence of African swine fever virus in field-infected *Ornithodoros erraticus* during the ASF endemic period in Portugal. PLoS ONE 6, e20383. doi.org/10.1371/journal.pone.0020383.

Boklund, A., Dhollander, S., Chesnoiu Vasile, T., Abrahantes, J.C., Bøtner, A., Gogin, A., Gonzalez Villeta, L.C., Gortázar, C., More, S.J., Papanikolaou, A., Roberts, H., Stegeman, A., Ståhl, K., Thulke, H.H., Viltrop, A., Van der Stede, Y., Mortensen, S., 2020. Risk factors for

African swine fever incursion in Romanian domestic farms during 2019. Sci. Rep. 10, 10215. doi.org/10.1038/s41598-020-66381-3.

Boshoff, C.I., Bastos, A.D.S., Gerber, L.J., Vosloo, W., 2007. Genetic characterization of African swine fever viruses from outbreaks in southern Africa (1973–1999). Vet. Microbiol. 121, 45-55. doi.org/10.1016/j.vetmic.2006.11.007.

Carrascosa, A.L., Bustos, M.J., de Leon, P., 2011. Methods for growing and titrating African swine fever virus: field and laboratory samples. Curr. Protoc. Cell Biol. 53, 1-25. doi.org/10.1002/0471143030.cb2614s53.

de Carvalho Ferreira, H.C., Zúquete, S.T., Wijnveld, M., Weesendorp, E., Jongejan, F., Stegeman, A., Loeffen, W.L.A., 2014. No evidence of African swine fever virus replication in hard ticks. Ticks Tick Borne Dis. 5, 582–589. doi.org/10.1016/j.ttbdis.2013.12.012.

Chenais, E., Ståhl, K., Guberti, V., Depner, K., 2018. Identification of wild boar-habitat epidemiologic cycle in African swine fever epizootic. Emerg. Infect. Dis. 24, 810–812. doi.org/10.3201/eid2404.172127.

Chenais, E., Depner, K., Guberti, V., Dietze, K., Viltrop, A., Ståhl, K., 2019. Epidemiological considerations on African swine fever in Europe 2014–2018. Porc. Health Manag. 5, 6. doi.org/10.1186/s40813-018-0109-2.

CISA-INIA (European Unión Laboratory for African Swine Fever. Animal Health Research Center), 2014. African swine fever (ASF) antibody detection. Accessible: http://asf-referencelab.info/asf/en/procedures-diagnosis/diagnostic-procedures. Accessed: June 2017.

Clopper, C.J., Pearson, E.S., 1935. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 26, 404–413. doi.org/10.2307/2331986.

Costard, S., Wieland, B., de Glanville, W., Jori, F., Rowlands, R., Vosloo, W., Roger, F., Pfeiffer, D.U., Dixon, L., 2009. African swine fever: how can global spread be prevented? Phil. Trans. R. Soc. B. 364, 2683–2696. doi.org/10.1098/rstb.2009.0098.

Costard, S., Mur, L., Lubroth, J., Sanchez-Vizcaino, J.M., Pfeiffer, D.U., 2013. Epidemiology of African swine fever virus. Virus Res. 173, 191-197. doi.org/10.1016/j.virusres.2012.10.030.

Costard, S., Zagmutt, F.J., Porphyre, T., Pfeiffer, D.U., 2015. Small-scale pig farmers' behaviour, silent release of African swine fever virus and consequences for disease spread. Sci. Rep. 5, 17074. doi.org/10.1038/srep17074.

Cukor, J., Linda, R., Václavek, P., Mahlerová, K., Šatrán, P., Havránek, F., 2020. Confirmed cannibalism in wild boar and its possible role in African swine fever transmission. Transbound. Emerg. Dis. 67, 1068-1073. doi.org/10.1111/tbed.13468.

Davies, K., Goatley, L.C., Guinat, C., Netherton, C.L., Gubbins, S., Dixon, L.K., Reis, A.L., 2017. Survival of African swine fever virus in excretions from pigs experimentally infected with the Georgia 2007/1 isolate. Transbound. Emerg. Dis. 64, 425–431. doi.org/10.1111/tbed.12381.

Depner, K., Staubach, C., Probst, C., Globig, A., Blome, S., Dietze, K., Sauter-Louis, C., Conraths, F.J., 2016. Die Afrikanische Schweinepest–epidemiologische Betrachtungen und Konsequenzen für die Tierseuchenbekämpfung (African swine fever - epidemiological considerations and consequences for disease control). Tieraerztl. Umsch. 71, 72–78. In German.

Dixon, L., Stahl, K., Jori, F., Vial, L., Pfeiffer, D.U., 2020. African swine fever epidemiology and control. Annu. Rev. Anim. Biosci. 8, 221–246. doi.org/10.1146/annurev-animal-021419-083741.

Dohoo I., Martin W., Stryhn H., 2009. Veterinary epidemiologic research, 2nd ed. VER Inc., Charlottetown, Canada.

EFSA AHAW Panel (European Food Safety Authority, Panel on Animal Health and Welfare), Authie, E., Berg, C., Bøtner, A., Browman, H., Capua, I., De Koeijer, A., Depner, K., Domingo, M., Edwards, S., Fourichon, C., Koenen, F., More, S., Raj, M., Sihvonen, L., Spoolder, H., Stegeman, J.A., Thulke, H.H., Vågsholm, I., Velarde, A., Willeberg, P., Zientara, S., 2014. Scientific opinion on African swine fever. EFSA Journal 12, 3628. doi.org/10.2903/j.efsa.2014.3628.

EFSA AHAW Panel (European Food Safety Authority, Panel on Animal Health and Welfare), Berg, C., Bøtner, A., Browman, H., De Koeijer, A., Domingo, M., Ducrot, C., Edwards, S., Fourichon, C., Koenen, F., More, S., Raj, M., Sihvonen, L., Spoolder, H., Stegeman, J.A., Thulke, H.H., Vågsholm, I., Velarde, A., Willeberg, P., 2015. Scientific opinion on African swine fever. EFSA Journal 13, 4163. doi.org/10.2903/j.efsa.2015.4163.

EFSA (European Food Safety Authority), Depner, K., Gortazar, C., Guberti, V., Masiulis, M., More, S., Olševskis, E., Thulke, H.H., Viltrop, A., Woźniakowski, G., Cortiñas Abrahantes, J., Gogin, A., Verdonck, F., Dhollander, S., 2017. Scientific report on the epidemiological analyses of African swine fever in the Baltic states and Poland. EFSA Journal 15, 5068. doi.org/10.2903/j.efsa.2017.5068.

EFSA (European Food Safety Authority), Boklund, A., Cay, B., Depner, K., Földi, Z., Guberti, V., Masiulis, M., Miteva, A., More, S., Olsevskis, E., Šatran, P., Spiridon, M., Stahl, K., Thulke, H.H,, Viltrop, A., Wozniakowski, G., Broglia, A., Cortinas Abrahantes, J., Dhollander, S., Gogin, A., Verdonck, F., Amato, L., Papanikolaou, A., Gortazar, C., 2018. Scientific report on the epidemiological analyses of African swine fever in the European Union (November 2017 until November 2018). EFSA Journal 16, 5494. doi.org/10.2903/j.efsa.2018.5494.

EFSA (European Food Safety Authority), Miteva, A., Papanikolaou, A., Gogin, A., Boklund, A., Bøtner, A., Linden, A., Viltrop, A., Schmidt, C.G., Ivanciu, C,, Desmecht, D., Korytarova, D., Olsevskis, E., Helyes, G., Wozniakowski, G., Thulke, H.H., Roberts, H., Abrahantes, J.C., Stahl, K., Depner, K., Gonzalez Villeta, L.C., Spiridon, M., Ostojic, S., More, S., Vasile, T.C., Grigaliuniene, V., Guberti, V., Wallo, R., 2020. Scientific report on the epidemiological analyses of African swine fever in the European Union (November 2018 to October 2019). EFSA Journal 18, 5996. doi.org/10.2903/j.efsa.2020.5996.

EFSA AHAW Panel (European Food Safety Authority, Panel on Animal Health and Welfare), Nielsen, S.S., Alvarez, J., Bicout DJ, Calistri, P., Depner, K., Drewe, J.A., Garin-Bastuji, B., Gonzales Rojas J.L., Gortazar Schmidt, C., Herskin, M., Michel, V., Miranda Chueca M.A., Pasquali, P., Roberts, H.C., Sihvonen, L.H., Spoolder, H., Stahl, K., Velarde, A., Viltrop, A., Winckler, C., De Clercq, K., Klement, E., Stegeman, J.A., Gubbins, S., Antoniou, S.E., Broglia, A., Van der Stede, Y., Zancanaro, G., Aznar, I., 2021. Scientific opinion on the assessment of the control measures of the category A diseases of Animal health law: African swine fever. EFSA Journal 19, 6402. doi.org/10.2903/j.efsa.2021.6402.

European Commission, 2002. Council Directive 2002/60/EC of 27 June 2002 laying down specific provisions for the control of African swine fever and amending Directive 92/119/EEC as regards Teschen disease and African swine fever.

European Commission, 2003. Commission Decision 2003/422/EC of 26 May 2003 approving an African swine fever diagnostic manual.

FAO, 2013. African swine fever in the Russian Federation: risk factors for Europe and beyond. Empres Watch 28. Accessible: http://www.fao.org/publications/card/en/c/456c5e1d-f450-524f-a664-a9803efd4208. Accessed: February 2017.

Fernandez-Pinero, J., 2018. Molecular analysis of eastern European African swine fever viruses. Presentation on EURL workshop on laboratory diagnosis and control of CSF and ASF. Not publicly available.

Forth, J.H., Forth, L.F., Blome, S., Höper, D., Beer, M., 2020. African swine fever whole-genome sequencing- quantity wanted but quality needed. PLoS Pathog. 16, e1008779. doi.org/10.1371/journal.ppat.1008779.

Frączyk, M., Woźniakowski, G., Kowalczyk, A., Bocian, L., Kozak, E., Niemczuk, K., Pejsak, Z., 2016. Evolution of African swine fever virus genes related to evasion of host immune response. Vet. Microbiol. 193, 133-144. doi.org/10.1016/j.vetmic.2016.08.018.

Frant, M., Lyjak, M., Bocian, L., Barszcz, A., Niemczuk, K., Wozniakowski, G., 2020. African swine fever virus (ASFV) in Poland: prevalence in a wild boar population (2017–2018). Vet. Med. (Praha) 65, 143–158. doi.org/10.17221/105/2019-VETMED.

Gabriel, C., Blome, S., Malogolovkin, A., Parilov, S., Kolbasov, D., Teifke, J.P., Beer, M., 2011. Characterization of African swine fever virus Caucasus isolate in European wild boars. Emerg. Infect. Dis. 17, 2342-2345. doi.org/10.3201/eid1712.110430.

Gallardo, C., Mwaengo, D.M., Macharia, J.M., Arias, M., Taracha, E.A., Soler, A., Okoth, E., Martin, E., Kasiti, J., Bishop, R.P., 2009. Enhanced discrimination of African swine fever virus isolates through nucleotide sequencing of the p54, p72, and pB602L (CVR) genes. Virus Genes 38, 85–95. doi.org/10.1007/s11262-008-0293-2.

Gallardo, C., Fernández-Pinero, J., Pelayo, V., Gazaev, I., Markowska-Daniel, I., Pridotkas, G., Nieto, R., Fernandez-Pacheco, P., Bokhan, S., Nevolko, O., Drozhzhe, Z., Perez, C., Soler, A., Kolbasov, D., Ariar, M., 2014. Genetic variation among African swine fever genotype II viruses, eastern and central Europe. Emerg. Infect. Dis. 20, 1544-1547. doi.org/10.3201/eid2009.140554.

Gallardo, C., Nieto, R., Soler, A., Pelayo, V., Fernandez-Pinero, J., Markowska-Daniel, I., Pridotkas, G., Nurmoja, I., Granta, R., Simon, A., Perez, C., Martin, E., Fernandez-Pacheco, P., Arias, M., 2015a. Assessment of African swine fever diagnostic techniques as a response to the epidemic outbreaks in eastern European Union countries: how to improve surveillance and control programs. J. Clin. Microbiol. 53, 2555-2565. doi.org/10.1128/JCM.00857-15.

Gallardo, C., Soler, A., Nieto, R., Cano, C., Pelayo, V., Sánchez, M. A., Pridotkas, G., Fernandez-Pinero, J., Briones, V., Arias, M., 2015b. Experimental infection of domestic pigs with African swine fever virus Lithuania 2014 genotype II field isolate. Transbound. Emerg. Dis. 64, 300–304. doi.org/10.1111/tbed.12346.

Gallardo, C., De la Torre, A., Fernández-Pinero, J., Martín, I., Jesús Muñoz, M., Arias, M., 2015c. African swine fever: a global view of the current challenge. Porc. Health Manag. 1, 21. doi.org/10.1186/s40813-015-0013-y.

Gallardo, C., Soler, A., Nieto, R., Sánchez, M.A., Martins, C., Pelayo, V., Carrascosa, A., Revilla, Y., Simón, A., Briones, V., Sánchez-Vizcaíno, J.M., Arias, M., 2015d. Experimental transmission of African swine fever (ASF) low virulent isolate NH/P68 by surviving pigs. Transbound. Emerg. Dis. 62, 612-622. doi.org/10.1111/tbed.12431.

Gallardo C., Nurmoja I., Soler A., Delicado V., Simón A., Martin E., Perez C., Nieto R., Arias M., 2018a. Evolution in Europe of African swine fever genotype II viruses from highly to moderately virulent. Vet. Microbiol. 219, 70-79. doi.org/10.1016/j.vetmic.2018.04.001.

Gallardo, C., Soler, A., Rozde, I., Delicado Losa, V., Nurmoja, I., Woźniakowski, G., Nieto, R., Arias, M., 2018b. Attenuated and non-haemadsorbing African swine fever (ASF) genotype II European isolate induces subclinical to chronic infection. Presentation on 12th Annual Meeting of EPIZONE.

Gallardo C., Soler A., Rodze, I., Nieto R., Cano-Gomez, C., Fernandez-Pinero, J., Arias M., 2019a. Attenuated and non-haemadsorbing (non-HAD) genotype II African swine fever virus (ASFV) isolated in Europe, Latvia 2017. Transbound. Emerg. Dis. 66, 1399-1404. doi.org/10.1111/tbed.13132.

Gallardo C., Fernández-Pinero J., Arias M., 2019b. African swine fever (ASF) diagnosis, an essential tool in the epidemiological investigation. Virus Res. 271, 197676. doi.org/10.1016/j.virusres.2019.197676.

Giammarioli, M., Gallardo, C., Oggiano, A., Iscaro, C., Nieto, R., Pellegrini, C., Dei Giudici, S., Arias, M., De Mia, G.M., 2011. Genetic characterization of African swine fever viruses from recent and historical outbreaks in Sardinia (1978–2009). Virus Genes 42, 377–387. doi.org/10.1007/s11262-011-0587-7.

Gogin, A., Gerasimov, V., Malogolovkin, A., Kolbasov, D., 2013. African swine fever in the North Caucasus region and the Russian Federation in years 2007-2012. Virus Res. 173, 198-203. doi.org/10.1016/j.virusres.2012.12.007.

Greig, A., Plowright, W., 1970. The excretion of two virulent strains of African swine fever virus by domestic pigs. J. Hyg. 68, 673–682. doi.org/10.1017/S0022 17240 0042613.

Guinat, C., Reis, A.L., Netherton, C.L., Goatley, L., Pfeiffer, D.U., Dixon, L., 2014. Dynamics of African swine fever virus shedding and excretion in domestic pigs infected by intramuscular inoculation and contact transmission. Vet. Res. 45, 93–101. doi.org/10.1186/s1356 7-014-0093-8.

Guinat, C., Gubbins, S., Vergne, T., Gonzales, J., Dixon, L., Pfeiffer, D., 2016. Experimental pig-to-pig transmission dynamics for African swine fever virus, Georgia 2007/1 strain. Epidemiol. Infect. 144, 25-34. doi.org/10.1017/S0950268815000862.

Gulenkin, V.M., Korennoy, F.I., Karaulov, A.K., Dudnikov, S.A., 2011. Cartographical analysis of African swine fever outbreaks in the territory of the Russian Federation and computer modelling of the basic reproduction ratio. Prev. Vet. Med. 102, 167-174. doi.org/10.1016/j.prevetmed.2011.07.004.

Jazdzewski, 2017. ASF in Poland. Presentation on OIE and FAO Global Framework for the progressive control of transboundary animal diseases (GF-TAD). Standing group of experts on African swine fever in Europe. Accessible: https://web.oie.int/rr-europe/eng/regprog/en_gf_tads%20%20standing%20group%20asf.htm#SGE8. Accessed: February 2020.

Jori, F., Bastos, A., 2009. Role of wild *Suids* in the epidemiology of African swine fever. Ecohealth 6, 296-310. doi.org/10.1007/s10393-009-0248-7.

Jurado, C., Fernández-Carrión, E., Mur, L., Rolesu, S., Laddomada, A., Sánchez-Vizcaíno, J.M., 2018. Why is African swine fever still present

in Sardinia? Transbound. Emerg. Dis. 65, 557–566. doi.org/10.1111/tbed.12740.

Kim, S.H., Kim, J., Son, K., Choi, Y., Jeong, H.S., Kim, Y.K., Park, J.E., Hong, Y.J., Lee, S.I., Wang, S.J., Lee, H.S., Kim, W.M., Jheong, W.H., 2020. Wild boar harbouring African swine fever virus in the demilitarized zone in South Korea, 2019. Emerg. Microbes Infect. 9, 628–630. doi.org/10.1080/22221751.2020.1738904.

King, D.P., Reid, S.M., Hutchings, G.H., Grierson, S.S., Wilkinson, P.J., Dixon, L.K., Bastos, A., Drew, T.W., 2003. Development of a TaqMan PCR assay with internal amplification control for the detection of African swine fever virus. J. Virol. Methods 107, 53–61. doi.org/10.1016/s0166-0934(02)00189-1.

Kolbasov, D., Titov, I., Tsybanov, S., Gogin, A., Malogolovkin, A., 2018. African swine fever virus, Siberia, Russia, 2017. Emerg. Infect. Dis. 24, 796-798. doi.org/10.3201/eid2404.171238.

Laddomada, A., Patta, C., Oggiano, A., Caccia, A., Ruiu, A., Cossu, P., Firinu, A., 1994. Epidemiology of classical swine fever in Sardinia: a serological survey of wild boar and comparison with African swine fever. Vet. Rec. 134, 183-187. doi.org/10.1136/vr.134.8.183.

Lamberga, K., Seržants M., Oļševskis E., 2018. African swine fever outbreak investigations in a large commercial pig farm in Latvia: a case report. Berl. Munch. Tierarztl. 131. doi.org/10.2376/0005-9366-18031.

Lamberga, K., Oļševskis, E., Seržants, M., Bērziņš, A., Viltrop, A., Depner, K., 2020. African swine fever in two large commercial pig farms in Latvia- estimation of the high risk period and virus spread within the farm. Vet. Sci. 7, E105. doi.org/10.3390/vetsci7030105.

Le, V.P., Jeong, D.G., Yoon, S.W., Kwon, H.M., Trinh, T.B.N., Nguyen, T.L., Bui, T.T.N., Oh, J., Kim, J.B., Cheong, K.M., Van Tuyen, N., Bae, E., Vu, T.T.H., Yeom, M., Na, W., Song, D., 2019. Outbreak of African swine fever, Vietnam, 2019. Emerg. Infect. Dis. 25, 1433-1435. doi.org/10.3201/eid2507.190303.

Linden, A., Licoppe, A., Desmecht, D., 2020. ASF-WB in Belgium, one year after the emergence. Presentation in the final international conference of the COST Action ASF-STOP - Understanding and combating African swine fever in Europe. Accessible: https://www.asf-stop.com/final-conference/. Accessed: February 2020.

Mačiulskis, P., Masiulis, M., Pridotkas, G., Buitkuvienė, J., Jurgelevičius, V., Jacevičienė, I., Zagrabskaitė, R., Zani, L., Pilevičienė, S., 2020. The African swine fever epidemic in wild boar (*Sus scrofa*) in Lithuania (2014–2018). Vet. Sci. 7, 15. doi.org/10.3390/vetsci7010015.

Malogolovkin, A., Yelsukova, A., Gallardo, C., Tsybanov, S., Kolbasov, D., 2012. Molecular characterization of African swine fever virus isolates originating from outbreaks in the Russian Federation between 2007 and 2011. Vet. Microbiol. 158, 415-419. doi.org/10.1016/j.vetmic.2012.03.002.

Martínez-López, B., Perez, A.M., Feliziani, F., Rolesu, S., Mur, L., Sánchez-Vizcaíno, J.M., 2015. Evaluation of the risk factors contributing to the African swine fever occurrence in Sardinia, Italy. Front. Microbiol. 6, 314. doi.org/10.3389/fmicb.2015.00314.

Montgomery, R.E., 1921. On a form of swine fever occurring in British East Africa (Kenya colony). J. Comp. Pathol. Therap. 34, 159-191.

Mulumba-Mfumu, L.K., Saegerman, C., Dixon, L.K., Kapanga, C. Madimba, K.C., Kazadi, E., Mukalakata, N.T., Oura, C.A.L., Chenais, E., Masembe, C., Ståhl, K., 2019. African swine fever: update on eastern, central and southern Africa. Transbound. Emerg. Dis. 66, 1462– 1480. doi.org/10.1111/tbed.13187.

Mur, L., Boadella, M., Martinez-Lopez, B., Gallardo, C., Gortazar, C., Sanchez-Vizcaino, J.M., 2012. Monitoring of African swine fever in the wild boar population of the most recent endemic area of Spain. Transbound. Emerg. Dis. 59, 526–531. doi.org/10.1111/j.1865-1682.2012.01308.x.

Mur, L., Atzeni, M., Martínez-López, B., Feliziani, F., Rolesu, S., Sanchez-Vizcaino, J.M., 2016. Thirty-five-year presence of African swine fever in Sardinia: history, evolution and risk factors for disease maintenance. Transbound. Emerg. Dis. 63, e165-e177. doi.org/10.1111/tbed.12264.

Mur, L., Sánchez-Vizcaíno. J.M., Fernández-Carrión, E., Jurado, C., Rolesu, S., Feliziani, F., Laddomada, A., Martinez-Lopez, B., 2018. Understanding African swine fever infection dynamics in Sardinia using a spatially explicit transmission model in domestic pig farms. Transbound. Emerg. Dis. 65, 123–134. doi.org/10.1111/tbed.12636.

McVicar, J.W., 1984. Quantitative aspects of the transmission of African swine fever. Am. J. Vet. Res. 45, 1535–1541.

Niederwerder, M.C., Stoian, A., Rowland, R., Dritz, S.S., Petrovan, V., Constance, L.A., Gebhardt, J.T., Olcha, M., Jones, C.K., Woodworth, J.C., Fang, Y., Liang, J., Hefley, T.J., 2019. Infectious dose of African swine fever virus when consumed naturally in liquid or feed. Emerg. Infect. Dis. 25, 891-897. doi.org/10.3201/eid2505.181495.

Niine, T., Nurmoja, I., Maasikamäe, S., Viltrop, A., 2019. Velocity of the spatial spread of African swine fever amongst wild boar in Estonia during 2014-2017. Conference poster on Society for veterinary epidemiology and preventive medicine (SVEPM). doi.org/10.13140/RG.2.2.27370.98244.

Oganesyan, A.S., Petrova, O.N., Korennoy, F.I., Bardina, N.S., Gogin, A.E., Dudnikov, S.A., 2013. African swine fever in the Russian Federation: spatio-temporal analysis and epidemiological overview. Virus Res. 173, 204-211. doi.org/10.1016/j.virusres.2012.12.009.

OIE (World Organisation for Animal Health). Terrestrial Animal Health Code, 2017. Chapter 1.2. Criteria for the inclusion of the diseases, infections and infestations in the OIE list. Accessible: https://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_criteria_diseases.htm. Accessed: April 2020.

OIE (World Organisation for Animal Health). World Animal Health Information Database (WAHID). Accessible: http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/diseasehome. Accessed: April 2020.

OIE (World Organisation for Animal Health), World Animal Health Information Database (WAHID), 2020. OIE-Listed diseases, infections and infestations in force in 2020. Accessible: https://www.oie.int/en/animal-health-in-the-world/oie-listed-diseases-2020. Accessed: April 2020.

OIE (World Organisation for Animal Health), World Animal Health Information Database (WAHID) Interface. Country information, reported disease events. Accessible: https://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Countryhome. Accessed: April 2020.

Olesen, A.S., Lohse, L., Boklund, A., Halasa, T., Gallardo, C., Pejsak, Z., Belsham, G.J., Rasmussen, T.B., Bøtner, A., 2017. Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes. Vet. Microbiol. 211, 92-102. doi.org/10.1016/j.vetmic.2017.10.004.

Olesen, A.S., Lohse, L., Hansen, M.F., Boklund, A., Halasa, T., Belsham, G.J., Rasmussen, T.B., Botner, A., Bodker, R., 2018a. Infection of pigs with African swine fever virus via ingestion of stable flies (*Stomoxys calcitrans*). Transbound. Emerg. Dis. 65, 1152–1157. doi.org/10.1111/tbed.12918.

Olesen, A.S., Lohse, L., Boklund, A., Halasa, T., Belsham, G.J., Rasmussen, T.B., Botner, A., 2018b. Short time window for transmissibility of African swine fever virus from a contaminated environment. Transbound. Emerg. Dis. 65, 1024–1032. doi.org/10.1111/tbed.12837.

Olševskis, E., Guberti, V., Seržants, M., Westergaard, J., Gallardo, C., Rodze, I., Depner, K., 2016. African swine fever virus introduction into the EU in 2014: experience of Latvia. Res. Vet. Sci. 105, 28-30. doi.org/10.1016/j.rvsc.2016.01.006.

Oļševskis, E., Schulz, K., Staubach, C., Seržants, M., Lamberga, K., Pūle, D., Ozoliņš, J., Conraths, F.J., Sauter-Louis, C., 2020. African swine fever in Latvian wild boar– a step closer to elimination. Transbound. Emerg. Dis. 67, 2615-2629. doi.org/10.1111/tbed.13611.

Pautienius, A., Grigas, J., Pileviciene, S., Zagrabskaite, R., Buitkuviene, J., Pridotkas, G., Stankevicius, R., Streimikyte, Z., Salomskas, A., Zienius, D., Stankevicius, A., 2018. Prevalence and spatiotemporal distribution of African swine fever in Lithuania, 2014–2017. Virol. J. 15, 177. doi.org/10.1186/s12985-018-1090-8.

Penrith, M.L., Vosloo, W., Jori, F., Bastos, A.D.S., 2013. African swine fever virus eradication in Africa. Virus Res. 173, 228-246. doi.org/10.1016/j.virusres.2012.10.011.

Pershin, A., Shevchenko, I., Igolkin, A., Zhukov, I., Mazloum, A., Aronova, E., Vlasova, N., Shevtsov, A., 2019. A long-term study of the biological properties of ASF virus isolates originating from various regions of the Russian Federation in 2013–2018. Vet. Sci. 6, 99. doi.org/10.3390/vetsci6040099.

Pietschmann, J., Guinat, C., Beer, M., Pronin, V., Tauscher, K., Petrov, A., Keil, G., Blome, S., 2015. Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. Arch. Virol. 160, 1657–1667. doi.org/10.1007/s00705-015-2430-2.

Pejsak, Z., Niemczuk, K., Frant, M., Mazur, M., Pomorska-Mól, M., Ziętek-Barszcz, A., Bocian, L., Łyjak, M., Borowska, D., Woźniakowski, G., 2018. Four years of African swine fever in Poland. New insights into epidemiology and prognosis of future disease spread. Pol. J. Vet. Sci. 21, 835–841. doi.org/10.24425/pjvs.2018.125598.

Petrini, S., Feliziani, F., Casciari, C., Giammarioli, M., Torresi, C., De Mia, G.M., 2019. Survival of African swine fever virus (ASFV) in various traditional Italian dry-cured meat products. Prev. Vet. Med. 162, 126-130. doi.org/10.1016/j.prevetmed.2018.11.013.

Petrov, A., Forth, J.H., Zani, L., Beer, M., Blome, S., 2018. No evidence for long-term carrier status of pigs after African swine fever virus infection. Transbound. Emerg. Dis. 65, 1318-1328. doi.org/10.1111/tbed.12881.

Pikalo, J., Zani, L., Hühr, J., Beer, M., Blome, S., 2019. Pathogenesis of African swine fever in domestic pigs and European wild boar – lessons learned from recent animal trials. Virus Res. 271, 197614. doi.org/10.1016/j.virusres.2019.04.001.

Pikalo, J., Schoder, M.E., Sehl, J., Breithaupt, A., Tignon, M., Cay, A.B., Gager, A.M., Fischer, M., Beer, M., Blome, S., 2020. The African swine fever virus isolate Belgium 2018/1 shows high virulence in European wild boar. Transbound. Emerg. Dis. 67, 1654-1659. doi.org/10.1111/tbed.13503.

Podgórski, T., Śmietanka, K., 2018. Do wild boar movements drive the spread of African swine fever? Transbound. Emerg. Dis. 65, 1588–1596. doi.org/10.1111/tbed.12910.

Podgórski, T., Borowik, T., Łyjak, M., Woźniakowski, G., 2019. Spatial epidemiology of African swine fever: host, landscape and anthropogenic drivers of disease occurrence in wild boar. Prev. Vet. Med. 77, 104691. doi.org/10.1016/j.prevetmed.2019.104691.

Probst, C., Globig, A., Knoll, B., Conraths, F.J., Depner, K., 2017. Behaviour of free ranging wild boar towards their dead fellows: potential implications for the transmission of African swine fever. Roy. Soc. open Sci. 4, 4170054. doi.org/10.1098/rsos.170054.

Quembo, C.J., Jori, F., Vosloo, W., Heath, L., 2018. Genetic characterization of African swine fever virus isolates from soft ticks at the wildlife/domestic interface in Mozambique and identification of a novel

genotype. Transbound. Emerg. Dis. 65, 420–431. doi.org/10.1111/tbed.12700.

R Core Team. R: A language and environment for statistical computing, 2015. R Foundation for Statistical Computing, Vienna, Austria.

Riigi Teataja, 1999. Loomatauditõrje seadus (Animal Disease Control Act). Riigi Teataja 57, 598. In Estonian.

Riigi Teataja, 2004. Sigade klassikalise katku ja sigade Aafrika katku tõrje eeskiri. Riigi Teataja Lisa 150, 2277. In Estonian.

Rosselkhoznadzor. Federal Service for Veterinary and Phytosanitary Surveillance. Accessible: http://www.fsvps.ru/fsvps/asf. Accessed: April 2020. In Russian.

Rowlands, R.J., Michaud, V., Heath, L., Hutchings, G., Oura, C., Vosloo, W., Dwarka, R., Onashvili, T., Albina, E., Dixon, L.K., 2008. African swine fever virus isolate, Georgia, 2007. Emerg. Infect. Dis. 14, 1870–1874. doi.org/10.3201/eid1412.080591.

Sánchez-Vizcaíno, J.M., Martínez-López, B., Martínez-Avilés, M., Martins, C., Boinas, F., Vial, L., Michaud, V., Jori, F., Etter, E., Albina, E., Roger, F., 2009. Scientific report on African swine fever. EFSA Supporting Publications 6, 1-141.

Sanchez-Vizcaıno, J.M., Mur, L., Martınez-Lopez, B., 2012. African swine fever: an epidemiological update. Transbound. Emerg. Dis. 59, 27-35. doi.org/10.1111/j.1865-1682.2011.01293.x.

Sánchez-Vizcaíno, J.M., Arias, M., 2012. Diseases of swine, 10th ed., 396-404. Edited by Zimmermann et al., A John Willey & Sons Inc., Chichester, UK.

Sanchez-Vizcaıno, J.M., Mur, L., Martınez-Lopez, B., 2013. African swine fever (ASF): five years around Europe. Vet. Microbiol. 165, 45–50. doi.org/10.1016/j.vetmic.2012.11.030.

Schulz, K., Peyre, M., Staubach, C., Schauer, B., Schulz, J., Calba, C., Häsler, B., Conraths, F.J., 2017. Surveillance strategies for classical swine fever in wild boar – a comprehensive evaluation study to ensure powerful surveillance. Sci. Rep. 7, 43871. doi.org/10.1038/srep43871.

Schulz, K., Staubach, C., Blome, S., Viltrop, A., Nurmoja, I., Conraths, F.J., Sauter-Louis, C., 2019a. Analysis of Estonian surveillance in wild

boar suggests a decline in the incidence of African swine fever. Sci. Rep. 9, 8490. doi.org/10.1038/s41598-019-44890-0.

Schulz, K., Oļševskis, E., Staubach, C., Lamberga, K., Seržants, M., Cvetkova, S., Conraths, F.J., Sauter-Louis, C., 2019b. Epidemiological evaluation of Latvian control measures for African swine fever in wild boar on the basis of surveillance data. Sci. Rep. 9, 4189. doi.org/10.1038/s41598-019-40962-3.

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. doi.org/10.3390/vaccines8020336.

Śmietanka, K., Woźniakowski, G., Kozak, E., Niemczuk, K., Frączyk, M., Bocian, Ł., Kowalczyk, A., Pejsak, Z., 2016. African swine fever epidemic, Poland, 2014–2015. Emerg. Infect. Dis. 22, 1201-1207. doi.org/10.3201/eid2207.151708.

Ståhl, K., Sternberg-Lewerin, S., Blome, S., Viltrop, A., Penrith, M.L., Chenais, E., 2019. Lack of evidence for long term carriers of African swine fever virus- a systematic review. Virus Res. 272, 197725. doi.org/10.1016/j.virusres.2019.197725.

Staubach, C., Schmid, V., Knorr-Held, L., Ziller, M., 2002. A Bayesian model for spatial wildlife disease prevalence data. Prev. Vet. Med. 56, 75–87. doi.org/10.1016/s0167-5877(02)00125-3.

Staubach, C., Hoffmann, L., Schmid, V.J., Ziller, M., Tackmann, K., Conraths, F.J., 2011. Bayesian space–time analysis of *Echinococcus multilocularis*-infections in foxes. Vet. Parasitol. 179, 77-83. doi.org/10.1016/j.vetpar.2011.01.065.

Tao, D., Sun, D., Wei, S., An, T., Shen, G., Chen, Z., Liu J., 2020. One Year of African Swine Fever Outbreak in China. doi.org/10.21203/rs.3.rs-22016/v1 (preprint).

Tauscher, K., Pietschmann, J., Wernike, K., Teifke, J.P., Beer, M., Blome, S., 2015. Zur Situation der Afrikanischen Schweinepest und der biologischen Charakterisierung aktueller Virusisolate (On the situation of African swine fever and the biological characterization of recent virus isolates). Berl. Munch. Tierarztl. Wochenschr. 128, 169-176. In German.

Taylor, R.A., Condoleo, R., Simons, R.R.L., Gale, P., Kelly, L.A., Snary, E.L., 2020. The risk of infection by African swine fever virus in

European swine through boar movement and legal trade of pigs and pig meat. Front. Vet. Sci. 6, 486. doi.org/10.3389/fvets.2019.00486.

Tignon, M., Gallardo, C., Iscaro, C., Hutet, E., Van der Stede, Y., Kolbasov D., De Mia, G.M., Le Potier, M., Bishop, R.P., Arias, M., Koenen, F., 2011. Development and inter-laboratory validation study of an improved new real-time PCR assay with internal control for detection and laboratory diagnosis of African swine fever virus. J. Virol. Methods 178, 161-170. doi.org/10.1016/j.jviromet.2011.09.007.

Torresi, C., Fiori, M., Bertolotti, L., Floris, M., Colitti, B., Giammarioli, M., Dei Giudici, S., Oggiano, A., Malmberg, M., De Mia, G.M., Belák, S., Granberg, F., 2020. The evolution of African swine fever virus in Sardinia (1978–2014) as revealed by whole-genome sequencing and comparative analysis. Transbound. Emerg. Dis. 67, 1971-1980. doi.org/10.1111/tbed.13540.

Varewyck, M., Verbeke, T., Cortinas Abrahantes, J., 2017. Exploratory analysis for spatio-temporal epidemiological data WEB App (spatial) (Version EU; R). Zenodo. doi.org/10.5281/zenodo.889551.

Vlasova, N., Varentsova, A., Shevchenko, I., Zhukov, I., Remyga, S., Gavrilova, V., Puzankova, O., Shevtsov, A., Zinyakov, N., Gruzdev, K., 2014. Comparative analysis of clinical and biological characteristics of African swine fever virus isolates from 2013 year Russian Federation. Br. Microbiol. Res. J. 5, 203-215. doi.org/10.9734/BMRJ/2015/12941.

Zani, L., Forth, J.H., Forth, L., Nurmoja, I., Leidenberger, S., Henke, J., Carlson, J., Breidenstein, C., Viltrop, A., Höper, D., Sauter-Louis, C., Beer, M., Blome, S., 2018. Deletion at the 5'-end of Estonian ASFV strains associated with an attenuated phenotype. Sci. Rep. 8, 6510. doi.org/10.1038/s41598-018-24740-1.

Zani, L., Dietze, K., Dimova, Z., Forth, J.H., Denev, D., Depner, K., Alexandrov, T., 2019. African swine fever in a Bulgarian backyard farm- a case report. Vet. Sci. 6, 94. doi.org/10.3390/vetsci6040094.

Zhou, X., Li, N., Luo, Y., Liu, Y., Miao, F., Chen, T., Zhang, S., Cao, P., Li, X., Tian, K., Qiu, H., Hu, R., 2018. Emergence of African swine fever in China, 2018. Transbound. Emerg. Dis. 65, 1482-1484. doi.org/10.1111/tbed.12989.

Zhao, D., Liu, R., Zhang, X., Li, F., Wang, J., Zhang, J., Liu, X., Wang, L., Zhang, J., Wu, X., Guan, Y., Chen, W., Wang, X., He, X., Bu, Z.,

2019. Replication and virulence in pigs of the first African swine fever virus isolated in China. Emerg. Microbes Infect. 8, 438-447. doi.org/10.1080/22221751.2019.1590128.

Walczak, M., Zmudzki, J., Mazur-Panasiuk, N., Juszkiewicz, M., Wozniakowski, G., 2020. Analysis of the clinical course of experimental infection with highly pathogenic African swine fever strain, isolated from an outbreak in Poland. Aspects related to the disease suspicion at the farm level. Pathogens 9, 237. doi.org/10.3390/pathogens9030237.

Wozniakowski, 2017. African swine fever in Poland. Presentation on EURL workshop on laboratory diagnosis and control of CSF and ASF. Not publicly available.

Wozniakowski, 2018. African swine fever in Poland. Presentation on EURL workshop on laboratory diagnosis and control of CSF and

9. SUMMARY IN ESTONIAN

Sigade Aafrika katku epidemioloogia Eestis ja ühe viirustüve iseloomustus

Sissejuhatus

Sigade Aafrika katk (SAK) on ohtlik sigade viirushaigus, mis põhjustab tõsiseid tagajärgi nii loomade tervisele kui ka majanduslikku kahju sektorile. Kuna diagnoositud haigus mõjutab märkimisväärselt elussigade ning sealiha ja lihatoodete rahvusvahelist kaubandust, on haigus Maailma Loomatervise Organisatsiooni (OIE) ning Euroopa Komisjoni ohtlike haiguste nimekirjas (OIE, 2017, 2020; EC, 2002).

Sigade Aafrika katku kirjeldas esimesena Briti patoloog Eustace Montgomery 1921. aastal, kui publitseeris Ida-Aafrikas läbi viidud ulatusliku uuringu tulemused. Sajand on möödas ning teadmisi haiguse ja selle tekitaja kohta märksa rohkem, kuid siiski kannatab SAK-i tõttu endiselt umbes pool Aafrika kontinendist (Penrith jt, 2013; Gallardo jt, 2015c; Mulumba-Mfumu jt, 2019; OIE WAHID, 2020).

1957. aastal teatas Portugal SAK-i esmakordsest leiust väljaspool Aafrika mandrit (Sánchez-Vizcaíno jt, 2009). Aastatel 1960–1995 esines SAK mitmes Lõuna-Euroopa riigis, nagu Hispaania, Portugal, Prantsusmaa, Itaalia, Malta, Belgia ja Holland (Sánchez-Vizcaíno jt, 2009). Kõik need riigid, välja arvatud Itaalia saar Sardiinia, suutsid haigusest vabaneda. See nõudis paljudes riikides aastakümneid, kuid tänu karmidele tõrjemeetmetele saavutasid nad edu. Sardiiniasse jõudis viirus 1978. aastal ja see on vaatamata korduvatele katsetele nakkust tõrjuda endiselt endeemiline piirkond (Mur jt, 2016; Jurado jt, 2017; OIE WAHID, 2020).

20. sajandi 70-ndatel aastatel levis haigus üle Atlandi ookeani Lõuna-Ameerikasse (Brasiilia) ning Kariibidele (Kuuba, Dominikaani Vabariik ja Haiti), kuid seal suudeti see kümnendi jooksul likvideerida (Costard jt, 2009). 2018. aasta augustis diagnoositi SAK esimest korda Aasias Hiina Rahvavabariigis (Zhou jt, 2018; Tao jt, 2020). Praeguseks on haigust Aasias ning Okeaanias diagnoositud Mongoolias, Vietnamis, Kambodžas, Hongkongis, Lõuna-Koreas, Põhja-Koreas, Laoses,

Filipiinidel, Timor-Lestes, Myanmaris, Indoneesias, Paapua Uus-Guineal ja Indias (OIE WAHID, vaadatud 24. mai 2020).

SAK leidis uuesti tee Euroopasse 2007. aasta kevadel, kui see diagnoositi Gruusias (Rowlands jt, 2008; Sanchez-Vizcaino jt, 2012). Haigustekitaja levis edasi Põhja-Kaukaasia teistesse riikidesse (Aserbaidžaan, Armeenia) ja Venemaa Föderatsiooni. 2012. aastal diagnoositi SAK Ukrainas ja 2013. aastal Valgevenes. 2014. aastal jõudis viirus Euroopa Liitu (EL) ning haigus tuvastati Leedus, Poolas ja Lätis. Aastatel 2017–2020 on SAK-i viirust (SAKV) leitud veel Tšehhi Vabariigis, Moldaavias, Rumeenias, Bulgaarias, Ungaris, Belgias, Slovakkias, Serbias ning Kreekas (OIE WAHID, vaadatud 24. mai 2020). Kõik viirusest tabandunud EL-i liikmesriigid (v.a Tšehhi Vabariik) ning ka Moldaavia ja Ukraina teavitavad endiselt haiguse leidudest riigis.

Esimene SAK-i juhtum Eestis diagnoositi 2014. aasta septembri alguses Läti piiri lähistelt surnuna leitud metsseal. Perioodil epideemia algusest kuni 2020. aasta mai lõpuni leiti viirust metssigadel 14 maakonnas 15-st. SAK-i suhtes uuritud 48 384 metsseast andsid 3992 positiivse tulemuse. Esimene haiguse puhang kodusigade farmis diagnoositi 2015. aasta juulis, millele järgnesid sama aasta suvel puhangud veel 17 farmis. 2016. aastal kinnitati SAK-i diagnoos kuues ning 2017. aastal kolmes farmis. Aastatel 2018–2020 (kuni 31. mai 2020) ei ole kodusigade farmides SAK-i diagnoositud.

Haiguse vastu puudub ravi ning vaktsiin, mistõttu põhineb selle tõrje kiirel diagnoosimisel, millele järgnevad ranged kontrollimeetmed ning loomade hukkamine.

Kirjanduse ülevaade

Sigade Aafrika katku põhjustab DNA viirus, mis kuulub *Asfarviridae* sugukonna *Asfivirus*'e perekonda (Alonso jt, 2018). SAKV-i geeni p72 osalise nukleotiidide järjestuse põhjal on tuvastatud viiruse 24 erinevat genotüüpi (Boshoff jt, 2007; Achenbach jt, 2016; Quembo jt, 2018). Kõik genotüübid esinevad Aafrikas, aga ainult I ja II genotüüpi on leitud ka teistel kontinentidel (Bastos jt, 2003; Gallardo jt, 2009; Arias jt, 2017; Le jt, 2019; Mulumba-Mfumu jt, 2019; Zhao jt, 2019). Euroopa riikides levib genotüüp II, välja arvatud Sardiinias, kus ringleb genotüüp I (Bastos jt, 2003; Rowlands jt, 2008; Malogolovkin jt, 2012; Torresi jt,

2020). Kõik alates 2018. aastast viirusest tabandunud Aasia riigid on samuti teavitanud ainult II genotüübi leidudest (Zhou jt, 2018; Le jt, 2019; Kim jt, 2020).

SAK kulgeb ägeda, alaägeda või kroonilise haigusena sõltuvalt viiruse iseloomust ja peremehest. Viirustüved on jagatud virulentsuse järgi kõrge, mõõduka või madala virulentsusega tüvedeks. Kõrge virulentsusega tüved põhjustavad üliägeda ja ägeda kuluga haigestumist, mõõduka virulentsusega tüved ägeda või alaägeda kuluga haigestumist ning madala virulentsusega tüved kroonilist ja asümptomaatilist haigestumist (Sánchez-Vizcaíno jt, 2009). Viirustüve genotüüp ei ole otseses seoses selle virulentsusega, sest sama genotüübi sees esineb erineva virulentsusega tüvesid (Gallardo jt, 2015d; Gallardo jt, 2018a; Gallardo jt, 2018b; Gallardo jt, 2018c; Zani jt, 2018).

SAK-i kirjeldatakse käsiraamatutes kui ägeda kuluga hemorraagilist haigust, mis põhjustab kuni 100% haigestumust ning väga suurt suremust (Sánchez-Vizcaíno jt, 2009; Costard jt, 2013). Haiguse ägeda kulu puhul esinevad haigustunnuseid, nagu kõrge palavik, depressioon, anoreksia, hingamis-, seede- ja närvisümptomid ning hemorraagia (Sanchez-Vizcaıno jt, 2009). Siiski kirjeldatakse haiguse avaldumist nii eksperimentaalsetes kui ka puhangu tingimustes sageli vaid ebatüüpiliste kliiniliste tunnustega, nagu isutus, loidus, kurnatus (Gabriel jt, 2011; Pietschmann jt, 2015; Oelsen jt, 2017; Gallardo jt, 2018a; Zani jt, 2019; Pikalo jt, 2020; Walczak jt, 2020).

Haigestunud loomad, kellel avalduvad kliinilised tunnused, eritavad viirust kõigi kehasekreetidega (Guinat jt, 2014; Pietschmann jt, 2015; Pikolo jt, 2020). See põhjustab farmikeskkonna saastumist ja võib viia nakkuse levikuni karjas või metssigade puhul väliskeskkonnas. Siiski on leitud, et kõige suurem viiruskogus esineb nakatunud looma veres (Gabriel jt, 2011; Carvalho Ferreira jt, 2012; Guinat jt, 2014; Gallardo jt, 2015b; Olesen jt, 2017).

SAKV-i II genotüübi tüved, mis ringlevad EL-i riikides alates 2014. aastast, on sama päritolu kui Kaukaasia riikides haigust põhjustanud viirustüved epideemia alguses (Malagolovkin jt, 2012; Fraczyk jt, 2014; Gallardo jt, 2014; Fernandez-Pinero jt, 2018; Pikalo jt, 2020).

Aastatel 2008–2014 läbi viidud loomkatsed näitasid, et Euroopas SAK-i epideemiat põhjustava II genotüübi viirustüved on kõrge virulentsusega ning põhjustavad nii kodu- kui ka metssigadel haiguse ägedat vormi (Gabriel jt, 2011; Blome jt, 2012; Guinat jt, 2014; Gallardo jt, 2015b; Pikalo jt, 2020). Nakatatud loomadel avaldusid kliinilised tunnused pärast 3–5 päeva kestnud inkubatsiooniperioodi ja 5–13 päeva pärast nakatamist loomad surid (Gabriel jt, 2011; Guinat jt, 2014; Gallardo jt, 2015b). Sõltuvalt katseloomade vanusest, viiruse hulgast ning nakatumisteest oli suremus peaaegu 100% (Gabriel jt, 2011; Blome jt, 2012; Gallardo jt, 2015a; Gallardo jt, 2015b).

Aastatel 2015–2019 läbi viidud loomkatsetes leiti II genotüübi viirustüvede hulgas ka vähenenud virulentsusega SAKV-i tüvesid (Gallardo jt, 2018a; Gallardo jt, 2018b; Pershin jt, 2019; Walczak jt, 2020). Nendes katsetes oli suremus 50–100% ja kliiniliselt avaldus haigus ägeda, alaägeda või kroonilise vormina. 2017. aastal isoleeriti Lätis kütitud metsseal atenueerunud viirustüvi (Gallardo jt, 2019), mis põhjustas eksperimentaalsel nakatamisel loomadel haiguse kroonilist või asümptomaatilist vormi. See viirustüvi ei põhjustanud katseloomade surma ja osal kontaktloomadest puudusid kliinilised tunnused täiesti (Gallardo jt, 2019).

SAK-i epideemia kulgeb piirkonniti erinevalt, sõltudes oluliselt viiruse ülekandemehhanismidest ja kaasatud loomaliikidest. Euroopas on ainuke SAK-i haigestuv koduloomaliik kodusiga (*Sus scrofa domestica*) ja samamoodi kulgeb haigus ka metssigadel *(Sus scrofa scrofa)*. Erinev on olukord Aafrikas, kus tüügassiga (*Phacochoerus africanus*) on viiruse looduslik reservuaar ja haiguse suhtes resistentne. Lisaks esinevad Aafrika ja Lõuna-Euroopa teatud piirkondades *Ornithodorus*'e perekonna puugid, kellel võib sõltuvalt liigist olla oluline roll nii viiruse reservuaari kui ka vektorina (Jori and Bastos, 2009; Sánchez-Vizcaíno jt, 2009; Costard jt, 2013). Viirusest tabandunud Balti riikidest (Eesti, Läti, Leedu) ning Poolast ei ole *Ornithodorus*'e perekonna puuke leitud (Sánchez-Vizcaíno jt, 2009; Costard jt, 2013).

Peale viiruse otsese ülekande kodusealt koduseale, metssealt koduseale, aga ka puugilt kodu- või metsseale on viiruse levikus olulised kaudsed tegurid, näiteks viirusega saastunud söödad, sõidukid, inimesed, töövahendid, samuti elukeskkond (Costard jt, 2013; Guinat jt, 2016; Chenais jt, 2018; Niederwerder jt, 2019). Viiruse ülekandemehhanismide

mõistmine on väga tähtis, sest see aitab töötada välja tõhusaid tõrjeprogramme.

Käsiraamatutes kirjeldataks SAK-i enamasti kui väga nakkavat haigust, mis põhjustab eriti kõrge virulentsusega viirustüvede puhul loomade suurt suremust (Sánchez-Vizcaíno jt, 2009; Blome jt, 2012; Pietschmann jt, 2015; Pikalo jt, 2020). Siiski võib nii haiguse kulgu, haigestumust, suremust kui ka nakkavust farmis ja looduses mõjutada märkimisväärselt mitte ainult viirustüve virulentsus, vaid ka selle kogus ning nakatumistee. Kuna suurt suremust kirjeldatakse paljude eksperimentaalsete nakatamiste puhul, siis võib see viia eksliku järelduseni haiguse kõrge nakkavuse kohta. Kummatigi on erinevad autorid kirjeldanud praeguseks ka mõõduka ja madala nakkavusega viirustüvede esinemist (Pietschmann jt, 2015; Olševskis jt, 2016; Lamberga jt, 2018).

Loomkatsed, mis on viidud läbi Euroopas ringlevate SAK-i viiruse II genotüübi tüvedega on näidanud, et metssiga on SAK-ile sama vastuvõtlik kui kodusiga ning looma vanus ega sugu ei mõjuta haiguse kulgu (Gabriel jt, 2011; Blome jt, 2012; Pietschmann jt, 2015; Pikalo jt, 2020). Mõlemal liigil kujunevad kõigepealt välja mittespetsiifilised haigustunnused ja sõltuvalt tüve virulentsusest loomad paranevad või surevad.

Metssigade puhul on leitud mitu riskitegurit, mis soodustavad viiruse populatsioonisisest levikut. Näiteks loomade suur asustustihedus põhjustab rohkem kontakte nii karja sees kui ka karjade vahel, soodustades nakkuse levikut. Loomade küttimine ning jahipidamisviis (nt ajujaht) ja praktikad (nt koerte kasutamine) soodustavad metssigade tavapärasest aktiivsemat liikumist ning viiruse levikut haigusvabadele aladele. Nakkuse leviku tõkestamisel on tähtis roll jahipidamisele kehtestatud bioturvalisuse nõuete täitmisel ning lihakehade ja jäätmete käitlemisel.

Metssigade elupaik kui viiruse võimalik reservuaar on praeguseks veel vähe tuntud riskitegur. Selle üks põhjusi on elupaigatüüpide paljusus, aga ka märgatavad klimaatilised erinevused isegi lähedaste piirkondade vahel. Jahe ja niiske kliima ning pikad talved soodustavad korjuste pikaajalist säilimist keskkonnas ning seeläbi viiruse püsimist.

Siiski loetakse tänapäeval üheks kõige tähtsamaks SAK-i laialdast levikut soodustavaks teguriks inimtegevust ja inimeste käitumist. Viiruse otsese loomalt loomale leviku kiiruseks on hinnatud 1–5 km kuus (Podgorski jt, 2018; Chenais jt, 2019; Niine jt, 2019), mistõttu ei ole võimalik, et uusi, sadu kilomeetreid eemal asuvaid haiguskoldeid põhjustavad ainult loomad. Euroopa Liidus on sellised hiljutised SAK-i kolded avastatud Tšehhi Vabariigis, Poolas (Varssavi ja Lubuskie piirkond), Ungaris ja Belgias (Chenais jt, 2019; Linden jt, 2020). Kõik nimetatud alad olid varem nakatunud aladest mitusada kilomeetrit eemal.

Kodusigade nakatumise riskitegurid erinevad samuti piirkonniti. Erinev on nii kliima, maastik, loomapidamistavad kui ka sotsiaal-kultuuriline ja majanduslik taust. Farmi suurusest lähtuvalt hinnatakse, et kõige vastuvõtlikumad on nakkusele kodumajapidamised ning väikefarmid, kus bioturvalisuse nõuete täitmine ei ole enamasti tagatud. Sigade väljaspidamist loetakse SAK-i levikul tähtsaks riskiteguriks, sest see soodustab otsest kontakti metssigade või vabalt peetavate sigadega (Gulenkin jt, 2011; Gogin jt, 2013). Nakatunud metssigade populatsioon on kodusigadele oluline ja püsiv riskiallikas, seda ka siis, kui loomi peetakse ainult siseruumides. Eriti selgelt on see näha viirusest tabandunud Balti riikides ja Poolas, kus sageli on teatatud nakkuse levikust metssigadelt farmi (Olševskis jt, 2016; Wozniakowski, 2017, 2018; EFSA, 2018).

Kodusigade nakatumise riskitegur on ka kuumtöötlemata toidujäätmete söötmine. Seda on kirjeldatud enamasti kodumajapidamiste ja väikeste farmide puhul piirkondades, kus elatustase on madalam ning inimesed järgivad rohkem traditsioonilist elukorraldust (FAO, 2013; Gogin jt, 2013; Martínez-López jt, 2015; Olševskis jt, 2016; Jazdzewski, 2017; Kolbasov jt, 2018; Wozniakowski, 2018). Sarnaselt metssigadega on ka kodusigadel märkimisväärseks riskiteguriks inimtegevus ja inimeste käitumine, nagu näiteks illegaalne elusloomade ja sealiha müük, mis aitab kaasa nakkuse laialdasele ja kontrollimatule levikule (FAO, 2013; Gogin jt, 2013; Martínez-López jt, 2015; Jazdzewski, 2017; Wozniakowski, 2017, 2018; Kolbasov jt, 2018).

Uurimistöö eesmärgid

Uurimistöö peaeesmärk oli analüüsida SAK-i epidemioloogiat ning epideemia arengut Eesti metssigade populatsioonis (I ja II uuring) ja kodusigadel (III uuring).

Spetsiifilised eesmärgid:

1. Analüüsida SAK-i epideemia arengu erinevusi Eesti kahe erineva piirkonna metssigade populatsioonis, Kirde-Eestis ja Lõuna-Eestis (I).

2. Selgitada Kirde-Eesti metssigade populatsioonis 2014. aastal ringelnud SAK-i viiruse tüve bioloogilisi omadusi (II).

3. Kirjeldada kvantitatiivselt SAK-i epideemiat kodusigadel ja tuvastada nakkuse riskitegurid Eesti seakarjades karja tasandil ning kirjeldada taudiolukorras haiguse kliinilist avaldumist (III).

Materjal ja metoodika

Väitekiri koosneb kolmest uuringust. Esimeses uuringus selgitati SAK-i epideemia kulgu kahes eraldiasuvas nakatunud metssigade populatsioonis ajavahemikul septembrist 2014 kuni septembrini 2016. Uuriti kaht ala: Lõuna-Eesti nakatunud ala (10 764 km^2), mis koosnes neljast maakonnast (Valga, Võru, Viljandi ja Tartu), ning Kirde-Eesti nakatunud ala (3364 km^2), mis hõlmas üht maakonda (Ida-Viru). Kokku analüüsiti 7015 metssea andmeid.

Metssigade uurimine viidi läbi Eestis kehtiva SAK-i tõrjeprogrammi kohaselt, mis hõlmas nii kütitud kui ka surnuna leitud metssigade uuringuid. SAKV-i genoomi tuvastamiseks kasutati reaalaja PCR-i (ik. *real-time polymerase chain reaction*; ek. reaalaja polümeraasi ahelreaktsioon) meetodit (Tignon jt, 2011), viirusvastaste antikehade tuvastamiseks ELISA (ik. *enzyme-linked immunosorbent assay*; ek. ensümaatiline immunosorptsioon analüüs) (Ingezim PPA COMPAC, Ingenasa, Madrid, Hispaania) ning IPT (ik. *indirect immunoperoxydase technique*; ek. kaudne immuunoperoksüdaastest) (CISA-INIA, 2014; Gallardo jt, 2015a) meetodit. Kõik analüüsid viidi läbi Veterinaar- ja Toidulaboratooriumis.

Andmed metssigade populatsiooni tiheduse kohta jahiaastatel 2012/13, 2013/14 ja 2014/15 saadi Keskkonnaagentuurilt. Kuna algseid andmeid kogusid jahindusorganisatsioonid jahiala kohta, siis geograafiliseks analüüsiks teisaldati need ArcGIS ArcMap 10.3.1 tarkvara kasutades omavalitsuse (vald) tasemele.

Statistiliseks analüüsiks kasutati programmi R (http://www.r-project.org). Levimus määrati erinevatel ajaperioodidel ja piirkondades, usaldusvahemikud (UV) ning šansside suhted (ŠS) arvutati Clopperi ja Pearsoni järgi. P-väärtus ≤ 0,05 loeti statistiliselt oluliseks. Fischeri täpset testi kasutati, et hinnata statistilisi seoseid erinevate riskitegurite vahel, nagu vanus ja uuritav loomarühm (kütitud, surnuna leitud) ning laboranalüüsi tulemused (PCR-positiivne (viropositiivne), ELISA-/IPT-positiivne (seropositiivne)). Loomad jagati vanuserühmadesse: noored (< 1 aasta) ja täiskasvanud (> 1 aasta).

Geograafiliste ja ajaliste muutuste hindamiseks uuritavates piirkondades kasutati hierarhilist Bayesi mudelanalüüsi (Staubach jt, 2002; Staubach jt, 2011). Mudel oli kohandatud vaid serolevimusele (ELISA-/IPT-positiivsed). Andmeid analüüsiti omavalitsuse (vald) tasandil mõlemas uuritavas piirkonnas eraldi.

Teises uuringus selgitati Kirde-Eestis 2014. aastal ringelnud SAK-i viirustüve bioloogilisi omadusi. Selleks viidi läbi eksperimentaalne nakatamine Saksamaal (Friedrich-Loeffler-Institut) spetsiaalses kõrge turvatasemega (L3+) hoones. Uuringus kasutatud viirus oli isoleeritud Ida-Viru maakonnast surnuna leitud metssealt ning sellega nakatati oronasaalselt kümme umbes nelja kuu vanust metssiga. Katse käigus hinnati iga päev loomadel avalduvaid kliinilisi näitajaid, kasutades varem kirjeldatud harmoniseeritud hindamissüsteemi (Pietschmann jt, 2015), ja koguti erinevaid proove. Kõik katseloomad lahati ja neilt koguti uurimismaterjali.

Kuna üks metssiga (nr 19) elas nakkuse üle ja tervenes täielikult, viidi sellega läbi jätku-uuring eesmärgiga hinnata viiruse võimalikku ülekannet tervenenud loomalt tervetele loomadele. Selleks pandi tervenenud metssiga (nr 19) 50 päeva pärast esialgset nakatamist (dpi; ik. days post-inoculation) kokku kolme sama vana terve metsseaga. Loomi hoiti koos 96. dpi-ni, mille järel kõik loomad eutaneeriti (hukati heaolu kaalutlustel) ja lahati. Eksperimentaalse nakatamise läbiviimine oli heaks kiidetud Saksamaa pädeva asutuse loaga nr 7221.3-2-023/15.

Loomkatse käigus kogutud proove analüüsiti laboratoorselt. SAK-i viiruse isoleerimiseks kasutati hemadsorbtsiooni testi (Carrascosa jt, 2011), viiruse genoomi tuvastamiseks reaalaja PCR-i testi (King jt, 2003)

ning viirusvastaste antikehade tuvastamiseks ELISA testi (Ingezim PPA COMPAC, Ingenasa; ID SCREEN ASFV INDIRECT, IDvet).

Kolmandas uuringus selgitati SAK-i epidemioloogiat kodusigadel. Selleks viidi epidemioloogiline uuring läbi kõigis (n = 26) seafarmides, kus ajavahemikul 2015–2017 diagnoositi SAK-i puhangud. 2015. aastal kinnitati SAK-i puhang 18 farmis, 2016. aastal kuues ning 2017. aastal kolmes. Kuna ühes 2015. aastal SAK-i diagnoosi saanud seafarmis ei leidnud diagnoos järeluuringute käigus kinnitust (kõiki 15-t farmis olnud siga uuriti, kõik negatiivse tulemusega), siis seda edasisse analüüsi ei kaasatud.

Puhangufarmi defineeriti kui farmi, millel on Põllumajanduse Registrite ja Informatsiooni Ametis (PRIA) identifitseerimisnumber ning mis vastab Euroopa Nõukogu direktiivi 2002/60/EÜ (Euroopa Komisjon, 2002) kriteeriumidele. Epidemioloogiline uuring viidi läbi vastavuses nõukogu direktiiviga 2002/60/EÜ (Euroopa Komisjon, 2002), kasutades struktureeritud küsimustikku. Farmide bioturvalisuse tase määrati farmidest kogutud andmete põhjal lähtuvalt Eesti seadusandluse nõuetest (Riigi Teataja, 1999; Riigi Teataja, 2004).

Iga puhangufarmi puhul määrati kõrgriski perioodi (HRP), defineeritud kui ajavahemik, mille jooksul võis SAK-i viirus olla farmis enne, kui selle esinemist kahtlustati. Hindamisel võeti aluseks suremusnäitajad farmis ning kliinilised ja laborianalüüside tulemused.

Seakarjade andmed pärinesid PRIA andmebaasist ning Veterinaar- ja Toiduametilt (VTA). Analüüsimiseks jagati seafarmid sigade arvu järgi suurusrühmadesse: 1–10 siga (G1), 11–100 siga (G2), 101–1000 siga (G3), > 1000 sea (G4). G1 farme käsitleti mitte tootmisfarmide, vaid kodumajapidamistena, kus loomi peeti isikliku tarbimise eesmärgil ja G2, G3 ja G4 farme kui tootmisfarme, kus loomi kasvatati müügi eesmärgil. Lisaks jagati farmid tootmistüübi (aretusfarm, täistsükliga farm ja nuumafarm) ja peetavate loomade (kodusead, metssead, ristandid) alusel.

Metssigade seireandmed pärinesid VTA-lt ning hõlmasid teavet uuritava rühma (kütitud, surnuna leitud), loomade leidmise/küttimise kuupäeva ja asukoha (koordinaadid) kohta. Koordinaatide põhjal tuvastati igale puhangufarmile kõige lähemal asunud SAK-positiivne metssiga (kuni ühe aasta jooksul enne puhangut) eesmärgiga kirjeldada nakkuse survet

metssigade poolt. Analüüsimisel kasutati lisaks Keskkonnaagentuuri edastatud andmeid metssigade küttimismahtude, jahimeeste arvu, söötmiskohtade arvu ja jahikoerte arvu kohta.

Andmete analüüsiks kasutati programmi Stata (StataCorp, College Station, Texas, USA) ja Bayesi hierarhilist mudelanalüüsi (Varewyck jt, 2017).

Tulemused ja arutelu

SAK-i epideemia esimesel aastal (2014–2015) ilmnes, et kahes eraldi asuvas metssigade populatsioonis (Kirde-Eestis ja Lõuna-Eestis) on haiguse epidemioloogia erinev. Lõuna-Eestis kulges haigus loomade suure suremusega ja peamiselt SAK viirusele positiivsete (reaalaja PCR-i testiga) loomade leidudega. Samal ajal oli Kirde-Eestis suremus väga väike või peaaegu olematu ning kliiniliselt tervete kütitud metssigade uuringutel leiti enamasti seropositiivseid (ELISA/IPT testiga viiruse vastaste antikehadega) loomi. Viropositiivsete metssigade leiud Kirde-Eestis olid harvad. Lisaks võis täheldada, et viiruse levik Lõuna-Eestis oli võrreldes Kirde-Eestiga kiire ja laialdane, samas kui Kirde-Eestis esines haigus vaid ühes väikeses piirkonnas.

Esimese uuringu tulemused kinnitasid varasemaid tähelepanekuid. Selgus, et Kirde-Eesti metssigadelt 25 esimese epideemiakuu jooksul kogutud 1174 proovist olid keskmiselt 2,0% (95% usaldusvahemik (UV) 1,1–3,0%) viropositiivsed, samal ajal kui Lõuna-Eestis 5841-st uuritud loomast oli viropositiivseid 13,7% (95% UV 12,8–14,6%). Veelgi enam, selle perioodi täpsemal analüüsimisel ilmnes, et 12 esimesel epideemiakuul oli viropositiivseid metssigu Kirde-Eestis keskmiselt vaid 0,8% (95% UV 0,2–3,5%) ning järgmisel 13 kuul 2,4% (95% UV 1,5–3,7%). Lõuna-Eestis ei muutunud sel kahel perioodil viropositiivsete hulk, olles vastavalt 13,8% (95% UV 12,5–15,2%) ja 13,7% (95% UV 12,5–14,9%).

Serolevimuse oluline erinevus piirkonniti tuleb välja, kui vaatame eraldi 25 kuu pikkuse perioodi esimest 12 ja järgnevat 13 kuud. Kirde-Eestis oli serolevimus vastavalt 7,4% (95% UV 4,8–10,7%) ja 2,4% (95% UV 1,4–3,7%) ning Lõuna-Eestis 1,5% (95% UV 1,0–2,0%) ja 5,4% (95% UV 4,6–6,3%). Kuigi viiruse laialdasemat levikut Lõuna-Eestis võis soodustada sealne suurem metssigade asustustihedus, toetavad

need tulemused uuringu hüpoteesi, et SAK-i viirus võis Kirde-Eesti metssigade populatsioonis esineda juba varem, kui see Lõuna-Eestis ametlikult diagnoositi (esimest korda diagnoosis VTA Eestis SAK-i 08. septembril 2014).

Statistilise analüüsi tulemusel selgus, et Lõuna-Eestis oli virolevimus oluliselt kõrgem kui Kirde-Eestis ($p < 0,001$), samas ei tuvastatud serolevimuse osas erinevust ($p = 728$). Statistiliselt oluline seos ($p < 0,001$) leiti metssigade vanuse ja SAK-positiivse (nii viropositiivse kui ka seropositiivse) leiu vahel. Tõenäosus tuvastada SAK-positiivne metssiga oli kõrgem noorte (vanus < 1 aasta) (viropositiivne: šansside suhe (ŠS) = 1,57, 95% UV 1,35–1,83; seropositiivne: ŠS = 1,89, 95% UV 1,45–2,47) ning surnuna leitud loomade rühmas (viropositiivne: ŠS = 69,60, 95% UV 56,89–85,15; seropositiivne: ŠS = 4,53, 95% UV 2,83–7,25).

Statistiliselt olulist erinevust ($p = 0,420$) ei tuvastatud loomarühmade uurimisel vanuserühmiti ehk nii kütitud kui ka surnuna leitud metssigade seas oli täiskasvanud loomade osakaal veidi suurem. Uuritavate loomade vanuseline jaotus oli nii Kirde- kui ka Lõuna-Eestis sarnane ($p = 0,566$). Kütitud ning surnuna leitud loomade jaotus oli Kirde- ja Lõuna-Eestis oluliselt erinev ($p < 0,001$). Lõuna-Eestis oli surnuna leitud metssigade osakaal märksa suurem kui Kirde-Eestis, ka oli seal palju suurem loomade asustustihedus. Leidsime statistiliselt olulise seose metssigade asustustiheduse ja positiivse testitulemuse vahel, vastavalt viropositiivne $p < 0,001$ ja seropositiivne $p = 0,009$.

Bayesi mudelanalüüsi tulemusel ilmnes, et Kirde-Eestis avaldasid seroloogilisele uurimistulemusele olulist mõju loomade vanus ja populatsioonitihedus ning Lõuna-Eestis loomade vanus ja uuritav loomarühm (kütitud/surnuna leitud). Uuritavate proovide geograafilise jaotumise analüüs näitas, et nii Kirde- kui ka Lõuna-Eestis erines proovide arv piirkonna sees omavalitsuste kaupa märgatavalt. Mudelanalüüs kinnitas erinevat serolevimuse suundumust ajas piirkonniti ning piirkondade sees. Kui Kirde-Eestis esines kõrge serolevimus kogu uuringuaja jooksul peamiselt vaid ühes omavalitsuses, siis Lõuna-Eestis esines see mitmetes omavalitsustes. Siiski tuleb tulemusi tõlgendades pidada silmas suhteliselt väikest proovide arvu mõlemas piirkonnas. Seroloogiliste tulemuste ajaline analüüs näitas, et mediaanaja mõju levimusele oli oluliselt erinev uuritavates piirkondades. Kui Kirde-Eestis

ei tuvastatud aja mõju serolevimusele uuritava 25 kuu jooksul, siis Lõuna-Eestis võis kogu perioodi jooksul näha levimuste olulist tõusu.

Uurimistulemused näitasid, et SAK-i epideemia kulges esimesel 25 kuul kahes eraldiasuvas metssigade populatsioonis erinevalt. Lõuna-Eestis võis täheldada klassikalist nakatumise algstaadiumit, kus haiguse leviala laienes, surnud ja haiged loomad olid peamiselt viropositiivsed ning seropositiivsete loomade leidude arv suurenes. Samal ajal oli Kirde-Eestis metssigade suremus ning viropositiivsete loomade osakaal väga väike, nakatunud loomi leiti vaid piiratud alalt ja seropositiivsete loomade arv oli kohe epideemia alguses suhteliselt suur. Sellest tulenevalt võib eeldada, et SAK-i viirus ringles Kirde-Eestis enne, kui see Eestis ja piirkonnas ametlikult diagnoositi.

Teises uuringus kontrolliti hüpoteesi, et Kirde-Eestis ringleva SAK-i viiruse tüve virulentsus on vähenenud (tüvi on atenueerunud) ja seetõttu kulges haigus piirkonnas teistmoodi kui Lõuna-Eestis.

Paljud alates 2007. aastast läbi viidud loomkatsed näitasid, et Ida-Euroopas ringlev SAK-i viiruse II genotüüp on nii kodu- kui ka metssigadele kõrge virulentsusega (Gabriel jt, 2011; Blome jt, 2012; Guinat jt, 2014; Vlasova jt, 2014; Gallardo jt, 2015b; Pietschmann jt, 2015; Olesen jt, 2017; Pikalo jt, 2020). Seega oli eelduseks, et haigus kulgeb ka Eestis tervikuna suure suremuse ja ilmsete kliiniliste tunnustega nagu Lõuna-Eestis.

Uuringu raames teostatud loomkatses kujunesid kõigil kümnel nakatatud metsseal 4–6 päeva pärast nakatamist (dpi) mittespetsiifilised kliinilised tunnused. Üheksal nakatunud loomal haigus süvenes ja nad surid või eutaneeriti 7–13 dpi. Üks loom (nr 19) elas haiguse üle. Alates umbes 14 dpi tema haigustunnused taandusid ja ta tervenes täiesti. Kõigilt eksperimendis osalenud loomadelt koguti katse jooksul perioodiliselt proove ja kõik kümme olid SAK-i viiruse suhtes (PCR-i testiga) positiivsed. SAK-i viiruse vastased antikehad (ELISA testiga) tuvastati kolmel loomal ja kahe proovid andsid antikehade suhtes kahtlase tulemuse.

Katses tervenenud metssiga pandi 50 dpi kokku kolme terve sentinell-loomaga (terve loom, kes ei ole enne haigustekitajaga kokku puutunud). Järgmise 46 päeva jooksul ei kujunenud ühelgi neist välja kliinilist haigestumist, samuti ei tuvastatud kogutud proovidest SAK-i viiruset

(kõik proovid PCR-negatiivsed). Kõik kolm sentinell-looma olid katse lõpus ka SAK-i antikehade suhtes (ELISA) negatiivsed.

Loomkatse tulemusel ei tuvastatud ringleva viirustüve atenueerumist ega saadud vastust, miks erineb haiguse dünaamika Kirde-Eesti metssigade populatsioonis. Siiski näitasid järgnevalt sama viirustüvega kodusigadel ja minisigadel läbi viidud eksperimentaalsed nakatamised haiguse vähem ägedat kulgu, mis viitas haiguse alaägedale või kroonilisele vormile (Zani jt, 2018).

Kolmandas uuringus selgitati SAK-i epidemioloogiat Eesti kodusigadel. Uuringu tulemustest ilmneb, et loomade haigestumisest või suremusest teavitati loomapidajate poolt üsna varases staadiumis. Kõrgriski periood jäi farmides vahemikku 7–20 päeva, mediaanaeg 11 päeva. Kõigis 26 puhangufarmis leiti SAK-i viiruse suhtes (PCR) positiivseid loomi, samas vaid 27% farmidest antikehade suhtes (ELISA) positiivseid loomi.

Tabel 1 annab ülevaate nende Eesti seafarmide suurusest ja tootmistüübist, kus aastatel 2015–2017 diagnoositi SAK-i puhangud.

Tabel 1. Sigade Aafrika katku puhangud Eesti seafarmides aastatel 2015-2017 farmide suuruse ja tootmistüübi järgi

	Karja suurusrühm (sigade arv)				
Tootmise tüüp	G1 (1–10)	G2 (11–100)	G3 (101–1000)	G4 (> 1000)	Kokku
Aretusfarm	0	0	1	2	3
Täistsükliga farm	1	1	3[a]	5	10
Nuumafarm	7	0	1	5	13
Kokku	8	1	5	12	26

[a] Kahes karjas peeti mets- ja kodusea ristandeid (neist üks väljaspidamisega) ning üks oli mahekari

SAK-i puhangute arv tootmisfarmides ületas nende arvu kodumajapidamistes. Selle põhjus võib olla, et suurtes farmides toimub rohkem inimeste, kaupade ja teenuste liikumist ning seetõttu on need nakkusele vastuvõtlikumad. Samas võib põhjus olla ka selles, et rangete bioturvalisuse nõuete kehtestamise tõttu vähenes sigu pidavate kodumajapidamiste arv 696 farmilt 2014. aastal 25 farmile 2017. aastal. Siiski on märkimisväärne, et SAK-i esinemus Eesti seafarmides ei

erinenud ($p > 0,05$) märkimisväärselt 2015., 2016. ja 2017. aastal farmide suurusrühmade põhjal, olles vastavalt 2,4%, 2,4% ja 2,0%.

Nakatunud loomadel ilmnenud esmased kliinilised tunnused olid haigusele mittespetsiifilised (isutus, loidus, kurnatus, üksikute loomade äkksurmad, abordid jm) ja leebed. Loomade ägedast haigestumisest teatati vaid 13 farmis ja sedagi enamasti pärast viiruse pikemaajalist ringlemist farmis. Loomade suremus erineva suurusrühma farmides oli erinev. Kui suurtes tootmisfarmides (> 1000 looma) oli keskmine suremus 0,7% (min 0,04; max 2,5%), siis kodumajapidamistes (< 10 looma) 29,7% (min 0,0 ja max 100%). Haiguse ebatüüpiline kliiniline avaldumine ning madal haigestumus ja suremus võisid olla põhjuseks, miks SAK-i kahtlus püstitati esmase diagnoosina vaid 12 farmis.

Kõigi 26 puhangufarmi puhul leiti, et kõige tõenäolisemalt jõudis viirus farmi mõne kaudse ülekandetee (inimesed, sõidukid, vahendid jm) vahendusel. Kaheksa kodumajapidamise puhul hinnati nakkuse farmi toomise põhjuseks väga madalat või olematut bioturvalisuse taset. Tootmisfarmide puhul üritati täpsemalt välja selgitada nakkuse farmi sisenemise teed. Neis hinnati kõige tõenäolisemateks põhjusteks saastunud allapanu (n = 1), saastunud rohusööta (n = 1), teravilja saastumist selle hoiustamisel või töötlemisel (n = 5) ning kõige sagedamini saastunud vahendajaid (inimesed, sõidukid, vahendid jm) (n = 11). Tulemustest ilmnes selgesti, et bioturvalisuse nõuete täitmisel on tähtis osa farmi kaitsmisel SAK-i ja ka muude haigustekitajate sissetungi eest.

Puhangufarmid asusid erinevatel aastatel erinevates maakondades. Leiti, et enamasti diagnoositi haiguspuhangud nende piirkondade sigalates, kus SAKV ringles eelneva aasta jooksul metssigade populatsioonis. Kahekümne kolmel juhul leiti SAK-positiivseid metssigu puhangufarmist kuni 15 km kaugusel, kusjuures neist 16 farmi puhul lähemal kui 5 km. See näitab püsivalt suurt nakatumisriski seafarmides, kus iga väiksemgi viga bioturvalisuse nõuete täitmisel võib viia puhangu tekkimiseni.

Kõik SAK-i puhangud Eesti seafarmides diagnoositi suveperioodil ajavahemikus juunist septembrini (neist 81% juulis ja augustis). Sarnast sesoonsust täheldati ka Lätis ning teistes EL-i riikides (Olševskis jt, 2016; EFSA, 2020). Selle põhjuseks võib olla rohkem kontakte farmide

ja ümbritseva keskkonna vahel, kuivõrd tegemist on kõige intensiivsema põllutööde ajaga.

Järeldused

SAK-i epideemia kulg Kirde-Eesti metssigade populatsioonis erines epideemia 25 esimese kuu jooksul oluliselt selle kulust Lõuna-Eesti metssigade populatsioonis (I).

SAK-i epideemia ajaline kulg ja geograafiline levik kahe erineva Eesti piirkonna metssigade populatsioonis viitab sellele, et SAK-i esmane sissetung Eestisse toimus Kirde-Eestis, mitte Lõuna-Eestis, nagu ametlikult kinnitati. Lisaks oli võimalik, et Kirde-Eestis epideemia oli põhjustatud teistsuguste omadustega viirustüve poolt (I).

Kirde-Eestis 2014. aastal ringelnud SAK-i viirustüve bioloogilised omadused ei erinenud märkimisväärselt teistest SAKV-i II genotüübi suure virulentsusega viirustüvede bioloogilistest omadustest (võttes aluseks loomkatsete tulemused, mis viidi läbi noorte metssigadega). Vaatamata Kirde-Eesti SAKV-i tüve kõrgele virulentsusele elas üks noor metssiga haiguse loomkatses üle ja tervenes täielikult (II).

Haiguse läbi põdenud loomal ei tuvastatud haigustekitaja hilisemat kandvust. Eksperimentaalse nakatamise tulemusel haiguse läbi põdenud loom ei eritanud pärast täielikku tervenemist viirust ega kandnud seda üle tervetele sentinell-loomadele (II).

SAKV-i levik kodusigade farmide ümbruskonna metssigade populatsioonis oli peamine riskitegur kodusigade nakatumiseks (III).

SAKV jõudis farmi suure tõenäosusega kaudse ülekande teel saastunud ülekandjate (inimesed, sõidukid, seadmed ja riistad) vahendusel. Tõenäoliselt oli tegemist vigadega bioturvalisuse nõuete täitmisel, seda ka juhtudel, kui farmi üldine bioturvalisuse tase oli kõrge (III).

SAKV-i ülekanne ühest farmist teise oli erandlik, viidates SAK-i kodusigade tsükli puudumisele Eestis (III).

Suurtes tootmis- ja aretusfarmides oli suurem risk nakatumiseks. Seega peavad farmide bioturvalisuse meetmed olema kõige kõrgemal

võimalikul tasemel, et vältida SAK-i haiguspuhanguid nakatunud aladel (III).

SAK-i esimesed kliinilised tunnused nakatunud karjades olid ebatüüpilised. Seega peaks SAK-i nakatunud või SAK-ist ohustatud aladel iga ebaselge põhjusega äkksurm, abort või ka söögiisu kaotus kas või seafarmi ühes aedikus olema nakkuskahtluse põhjus (III).

Viiruse levik puhangufarmis oli aeglane, viidates, et SAK-i viiruse nakkavus on puhangu algfaasis väike. Seega ei ole loomade üldise haigestumuse ega suremuse näitajate seire seafarmis SAK-i puhangu varaseks avastamiseks piisav (III).

Haigete ning surnud kodu- ja metssigade uurimine SAK-i suhtes (passiivne seire) on tähtis meede haiguse varaseks avastamiseks (I, III).

10. ACKNOWLEDGEMENTS

This thesis is the outcome of a large international cooperation between the Estonian University of Life Sciences and the Friedrich-Loeffler-Institut (FLI), the Federal Research Institute for Animal Health (Germany). At the Estonian University of Life Sciences, the studies were carried out at the Institute of Veterinary Medicine and Animal Sciences in the Chair of Veterinary Bio- and Population Medicine. The Institute of Epidemiology and the Institute of Diagnostic Virology of the Friedrich-Loeffler-Institut were involved in these studies.

Study II was partially funded by the European Research Project ASFORCE ("Targeted research effort on African swine fever" grant agreement 311931), and the FLI Internal African Swine Fever Virus Research Network. Study III was funded through the Estonian University of Life Sciences grant No 170193.

It has been a great honour to have such an excellent team of supervisors. My deep respect and gratitude belong to all of you. You have inspired, motivated and supported me.

The leading supervisor, Prof. Dr. Arvo Viltrop, has been my colleague for many years and this cooperation has always been pleasant, productive and developing. Our discussions about ASF have always been full of interest and passion, often being far more comprehensive than what is covered in this thesis. My co-supervisor, PD Dr. Sandra Blome from FLI, is an extraordinary person. I have the feeling that she knows all about the ASF virus and I have been fortunate to benefit from her willingness to share this knowledge and her talents. My other co-supervisor, Dr. Klaus Depner from FLI, is also a very special person. He introduced me to the exciting world of epidemiology and especially the importance of field epidemiology. Thank you for sharing your knowledge and passion with me.

Since a lot of work related to this thesis has been done at FLI, there are many more people who have contributed. I would like to express my deepest gratitude to colleagues from the Institute of Epidemiology,

especially Dr. Katja Schulz, Dr. Christoph Staubach and PD Dr. Carola Sauter-Louis. I have learned a lot from all of you about epidemiology, statistics, modelling, writing manuscripts, and about science and life in general. You are all great experts as well as very nice people.

In addition, my deepest gratitude belongs to all colleagues who were involved in my studies and have co-authored my manuscripts.

I would like to express my thanks the Head of the Institute of Epidemiology, Prof. Dr. Franz J. Conraths, and the Head of the Institute of Diagnostic Virology, Prof. Dr. M. Beer, who made both this great cooperation and my stay at FLI possible.

My special thanks go to my colleagues from the Veterinary and Food Board – Dr. Maarja Kristian, Dr. Kärt Jaarma, Dr. Harles Kaup – and many other animal health specialists and veterinary officials who have been on the front line combating ASF in Estonia.

I would like to thank Peep Männil from the Estonian Environment Agency for introducing me to the exciting life of wild boar and for always being cooperative and supportive.

My special gratitude goes to Tõnis Korts, Head of the Estonian Hunter's Society, a person who is always full of ideas, enthusiasm and a readiness to cooperate and work together.

I am also happy that young colleagues from the University of Life Sciences – Dr. Kerli Mõtus and Dr. Tarmo Niine – have been involved in this cooperation. Thank you for your contribution.

My colleagues from the Veterinary and Food Laboratory, your contribution has been enormous. It is far more than can be expressed in words!

My dear friends, it is impossible to overestimate your role in my life. Thank you very much for the time, support and patience you have given me.

My deepest gratitude belongs also to my parents and the families of my sister and brother. Your support is something that has been very important for me all through my life.

Finally, dear Andreas, you have been the key person in this process. Thank you very much for your help, support and love. Because of you, I have experienced many nice moments outside of my doctoral studies and work.

Thank you!

I

ORIGINAL PUBLICATIONS

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
doi:10.1038/s41598-017-12952-w

SCIENTIFIC REPORTS

OPEN

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

Received: 8 June 2017
Accepted: 12 September 2017
Published online: 02 October 2017

Imbi Nurmoja[1,2], Katja Schulz[3], Christoph Staubach[3], Carola Sauter-Louis[3], Klaus Depner[3], Franz J. Conraths[3] & Arvo Viltrop[2]

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.

[1]Estonian Veterinary and Food Laboratory (VFL), Kreutzwaldi 30, 51006, Tartu, Estonia. [2]Estonian University of Life Science, Institute of Veterinary Medicine and Animal Sciences, Kreutzwaldi 62, 51014, Tartu, Estonia. [3]Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Epidemiology, Südufer 10, 17493, Greifswald-Insel Riems, Germany. Imbi Nurmoja and Katja Schulz contributed equally to this work. Correspondence and requests for materials should be addressed to K.S. (email: katja.schulz@fli.de)

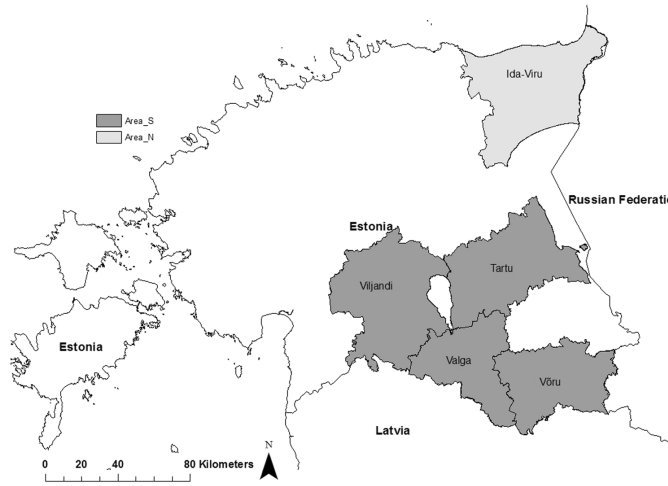


Figure 1. The study areas and the bordering countries in the South and East. Highlighted areas illustrate the four included counties in the South (area S) and the one in the Northeast of Estonia (area N). Map was generated by using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/).

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

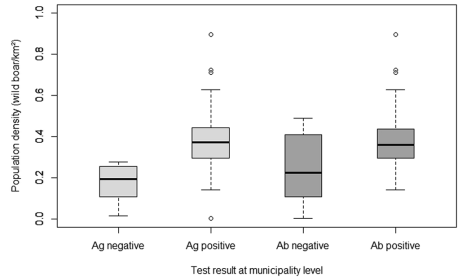


Figure 2. Population density (number of wild boar/km²) in the municipalities of the study areas stratified by the virological and serological test result at the municipality level. Ag: ASFV genome detection, Ab: antibody detection. Figure was generated by using the software package R (http://www.r-project.org)[20].

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 km², whereas the study area in the North consisted only of one county with a size of 3,364 km². 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 km² 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

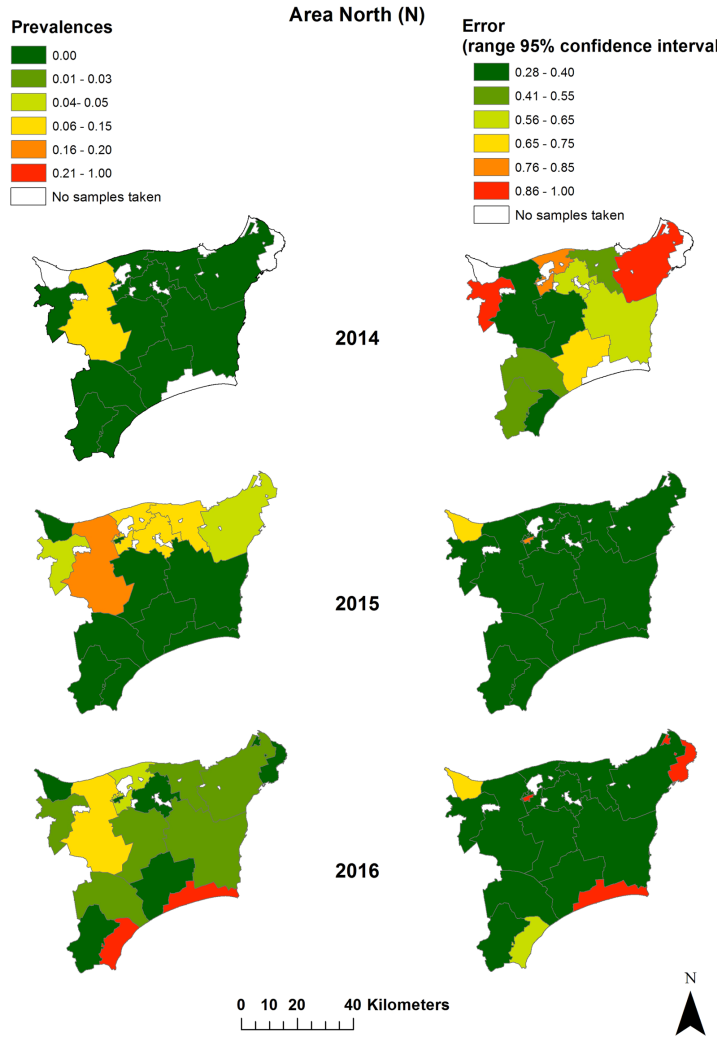


Figure 3. Seroprevalences and 95% confidence intervals for sampled wild boar per municipality in study area N (Ida-Viru county) in 2014 (Sept. – Dec.), 2015 (Jan. – Dec.) and 2016 (Jan. – Sept.). Maps were generated by using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/).

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

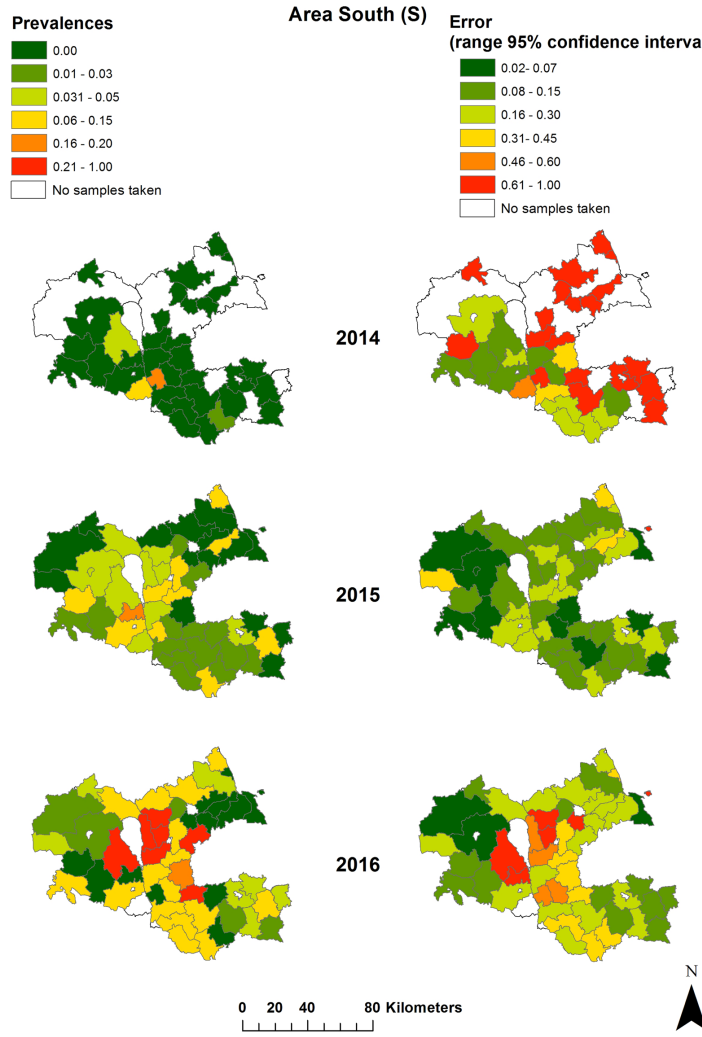


Figure 4. Seroprevalences and 95% confidence intervals for sampled wild boar per municipality in study area S (Viljandi, Tartu, Valga and Voru county) 2014 (Sept. – Dec.), 2015 (Jan. – Dec.) and 2016 (Jan. – Sept.). Maps were generated by using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA, http://www.esri.com/).

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.

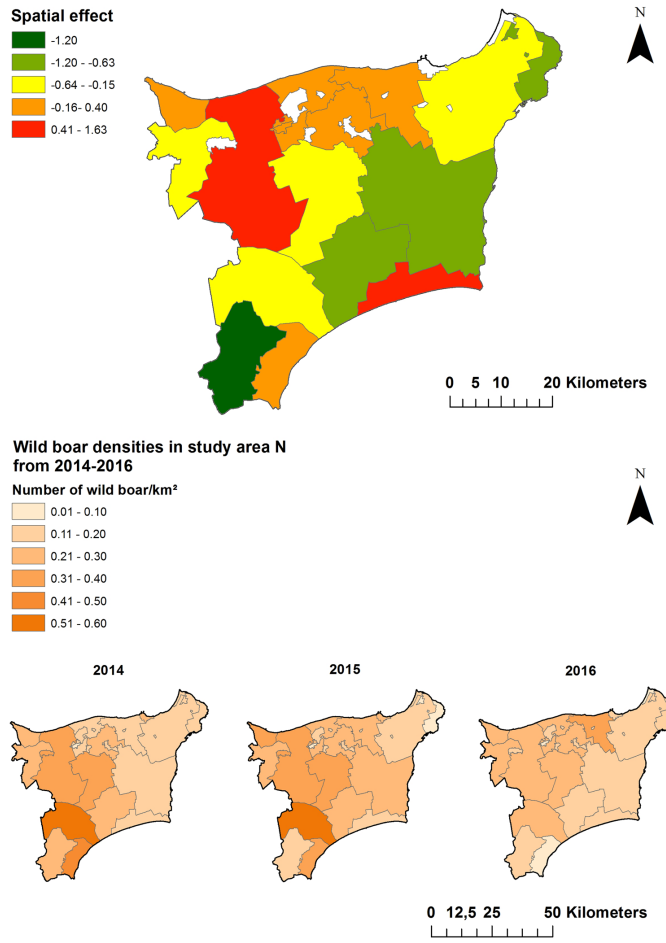


Figure 5. Median-structured spatial effect on the logit prevalence per municipality in study area N (Ida-Viru county) for the study period of 25 months. Maps in the lower row show the population density (number of wild boar/km^2) for each municipality in study area N. Maps were generated by using ArcGIS 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

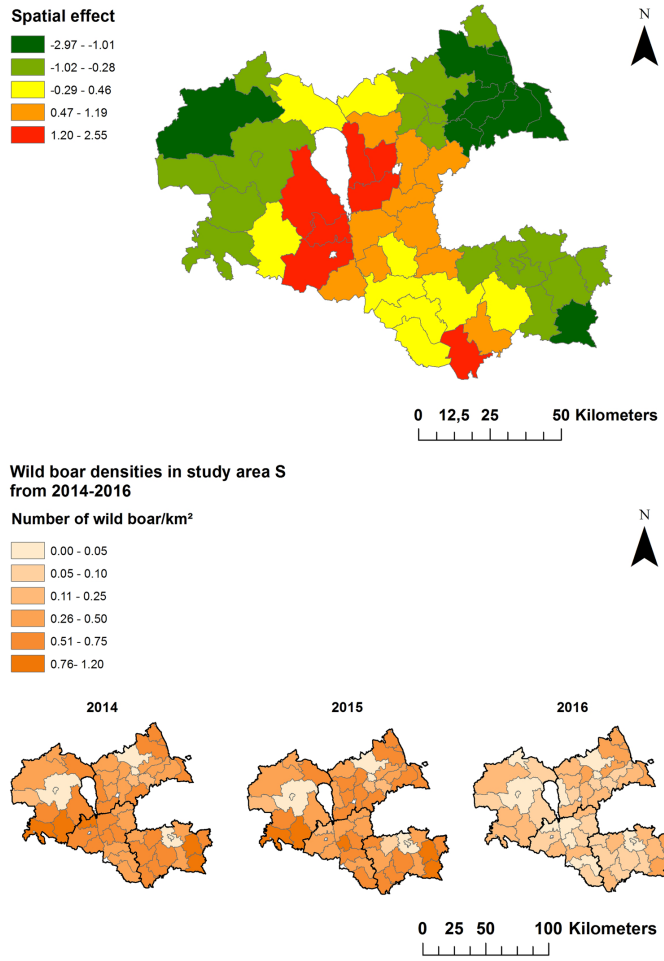


Figure 6. Median-structured spatial effect on the logit prevalence per municipality in study area S (Viljandi, Tartu, Valga and Voru county) for the study period of 25 months. Maps in the lower row show the population density (number of wild boar/km^2) for each municipality in area S. Maps were generated 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

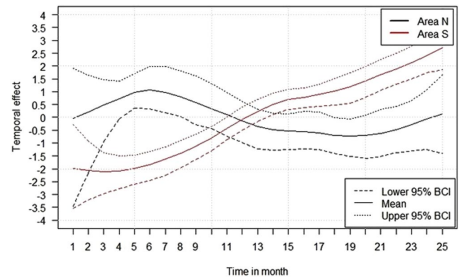


Figure 7. Median temporal effect on the logit prevalence in area North (N) and in area South (S) for the study period of 25 months. 95% Bayesian credible intervals (BCI) are included. Figure was generated by using the software package R (http://www.r-project.org)[20].

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].

References

1. Penrith, M. L. & Vosloo, W. Review of African swine fever: transmission, spread and control. *Journal of the South African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging* **80**, 58–62 (2009).
2. Sanchez-Vizcaino, J. M., Mur, L. & Martinez-Lopez, B. African Swine Fever: An Epidemiological Update. *Transboundary and Emerging Diseases* **59**, 27–35, https://doi.org/10.1111/j.1865-1682.2011.01293.x (2012).
3. Gogin, A., Gerasimov, V., Malogolovkin, A. & Kolbasov, D. African swine fever in the North Caucasus region and the Russian Federation in years 2007-2012. *Virus Research* **173**, 198–203, https://doi.org/10.1016/j.virusres.2012.12.007 (2013).
4. Khomenko, S. *et al.* African Swine Fever in the Russian Federation: Risk Factors for Europe and Beyond. *EMPRES Watch*, 28 (2013).
5. Gallardo, C. *et al.* Genetic Variation among African Swine Fever Genotype II Viruses, Eastern and Central Europe. *Emerging Infectious Diseases* **20**, 1544–1547, https://doi.org/10.3201/eid2009.140554 (2014).
6. Sanchez-Vizcaino, J. M., Mur, L., Gomez-Villamandos, J. C. & Carrasco, L. An Update on the Epidemiology and Pathology of African Swine Fever. *Journal of Comparative Pathology* **152**, 9–21, https://doi.org/10.1016/j.jcpa.2014.09.003 (2015).
7. Olsevskis, E. *et al.* African swine fever virus introduction into the EU in 2014: Experience of Latvia. *Research in Veterinary Science* **105**, 28–30, https://doi.org/10.1016/j.rvsc.2016.01.006 (2016).
8. Kristian, M. personal communication (2014).
9. World Animal Health Organisation. Immediate notification and follow-up reports (2014).
10. World Organisation for Animal Health. Annual Animal Health Report. http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/semestrial/review?year=2014&semester=0&wild=0&country=EST&this_country_code=EST&detailed=1 (2014).
11. Nurmoja, I. *et al.* Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. *Transboundary and Emerging Diseases* **00**, 1–8, https://doi.org/10.1111/tbed.12614 (2017).
12. Tignon, M. *et al.* Development and inter-laboratory validation study of an improved new real-time PCR assay with internal control for detection and laboratory diagnosis of African swine fever virus. *Journal of Virological Methods* **178**, 161–170, https://doi.org/10.1016/j.jviromet.2011.09.007 (2011).

13. Oura, C. African swine fever virus: on the move and dangerous. *Veterinary Record* **173**, 243–245, https://doi.org/10.1136/vr.f5327 (2013).
14. European Unión Laboratory for African Swine Fever. Animal Health Research Center (CISA-INIA). ASF diagnosis, http://asf-referencelab.info/asf/en/procedures-diagnosis/diagnostic-procedures (2016).
15. Gallardo, C. *et al.* Assessment of African Swine Fever Diagnostic Techniques as a Response to the Epidemic Outbreaks in Eastern European Union Countries: How To Improve Surveillance and Control Programs. *Journal of Clinical Microbiology* **53**, 2555–2565, https://doi.org/10.1128/jcm.00857-15 (2015).
16. Estonian Environment Agency. Report on Status of Game populations in Estonia and proposal for hunting in 2014. http://www.keskkonnaagentuur.ee/failid/SEIREARUANNE_2014.pdf (2014).
17. Estonian Environment Agency. Report on Status of Game populations in Estonia and proposal for hunting in 2015. http://www.keskkonnaagentuur.ee/sites/default/files/ulukiseirearuanne_2015.pdf (2015).
18. Estonian Environment Agency. Report on Status of Game populations in Estonia and proposal for hunting in 2016. http://www.keskkonnaagentuur.ee/sites/default/files/seirearuanne_2016.pdf (2016).
19. Riigiteataja, E. H A. https://www.riigiteataja.ee/en/eli/501022016007/consolide (2017).
20. R Core Team. R: A Language and Environment for Statistical Computing. (R Foundation for Statistical Computing, Vienna, Austria, 2015).
21. Clopper, C. J. & Pearson, E. S. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* **26**, 404–413, https://doi.org/10.2307/2331986 (1935).
22. Staubach, C., Schmid, V., Knorr-Held, L. & Ziller, M. A Bayesian model for spatial wildlife disease prevalence data. *Preventive Veterinary Medicine* **56**, 75–87, https://doi.org/10.1016/s0167-5877(02)00125-3 (2002).
23. Staubach, C. *et al.* Bayesian space-time analysis of Echinococcus multilocularis-infections in foxes. *Veterinary Parasitology* **179**, 77–83, https://doi.org/10.1016/j.vetpar.2011.01.065 (2011).
24. Spiegelhalter, D. J., Best, N. G., Carlin, B. R. & van der Linde, A. Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society Series B-Statistical Methodology* **64**, 583–616, https://doi.org/10.1111/1467-9868.00353 (2002).
25. Pietschmann, J. *et al.* Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. *Archives of Virology* **160**, 1657–1667, https://doi.org/10.1007/s00705-015-2430-2 (2015).
26. Blome, S., Gabriel, C., Dietze, K., Breithaupt, A. & Beer, M. High Virulence of African Swine Fever Virus Caucasus Isolate in European Wild Boars of All Ages. *Emerging Infectious Diseases* **18**, 708–708, https://doi.org/10.3201/eid1804.111813 (2012).
27. Blome, S. (personal communication, 2016).
28. Schulz, K., Calba, C., Peyre, M., Staubach, C. & Conraths, F. J. Hunters' acceptability of the surveillance system and alternative surveillance strategies for classical swine fever in wild boar - a participatory approach. *BMC Veterinary Research* **12**, 1–10, https://doi.org/10.1186/s12917-016-0822-5 (2016).
29. Schulz, K. *et al.* Surveillance strategies for Classical Swine Fever in wild boar – a comprehensive evaluation study to ensure powerful surveillance. *Scientific Reports* **7**, doi:10.1038/srep43871 (2017).
30. Depner, K. *et al.* African Swine fever - epidemiological considerations and consequences for disease control. *Tieraerztliche Umschau* **71**, 72–78 (2016).
31. Guinat, C. *et al.* Experimental pig-to-pig transmission dynamics for African swine fever virus, Georgia 2007/1 strain. *Epidemiology and Infection* **144**, 25–34, https://doi.org/10.1017/s0950268815000862 (2016).
32. Gallardo, C. *et al.* Experimental Transmission of African Swine Fever (ASF) Low Virulent Isolate NH/P68 by Surviving Pigs. *Transboundary and Emerging Diseases* **62**, 612–622, https://doi.org/10.1111/tbed.12431 (2015).

Acknowledgements

The authors would like to thank Peep Männil from the Estonian Environmental Agency for providing the Estonian wild boar population and hunting data as well as for a comprehensive overview of hunting practices in Estonia. We thank Katrin Lõhmus for her work in preparation of wild boar database data in the Estonian Veterinary and Food Laboratory. We also thank Dr. Andreas Fröhlich for his support regarding statistical analyses. We are grateful to Patrick Wysocki and Nicole Reimer for their assistance in applying ArcGIS and ArcMap. Finally, we appreciate that Kärt Jaarma from the Estonian Veterinary and Food Board provided us information about official measures taken by the veterinary service related to ASF eradication and control in Estonia.

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

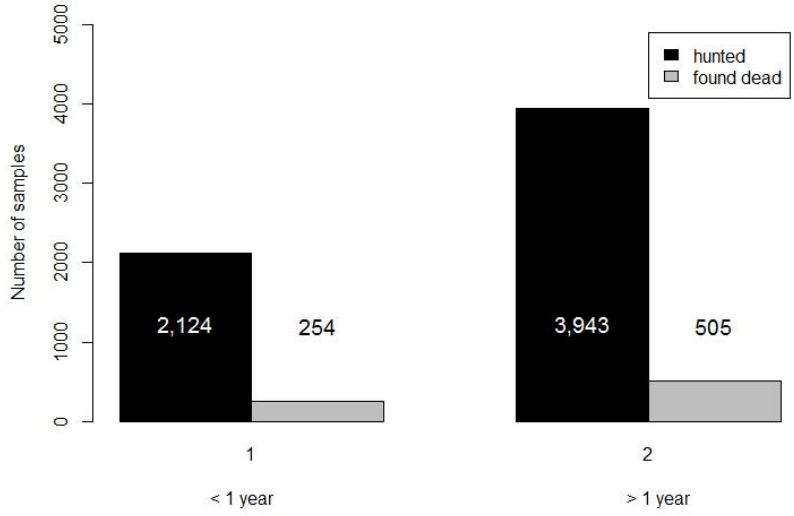
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-017-12952-w.

Competing Interests: The authors declare that they have no competing interests.

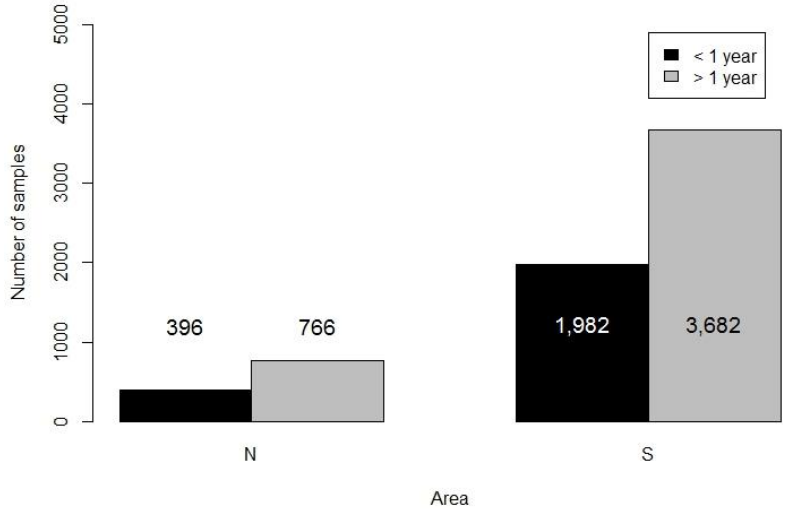
Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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

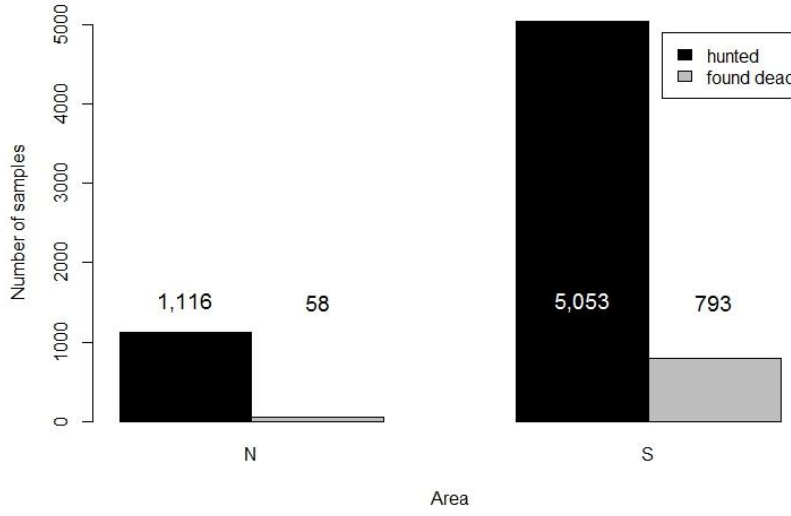
Imbi Nurmoja, Katja Schulz, Christoph Staubach, Carola Sauter-Louis, Klaus Depner, Franz J. Conraths, Arvo Viltrop



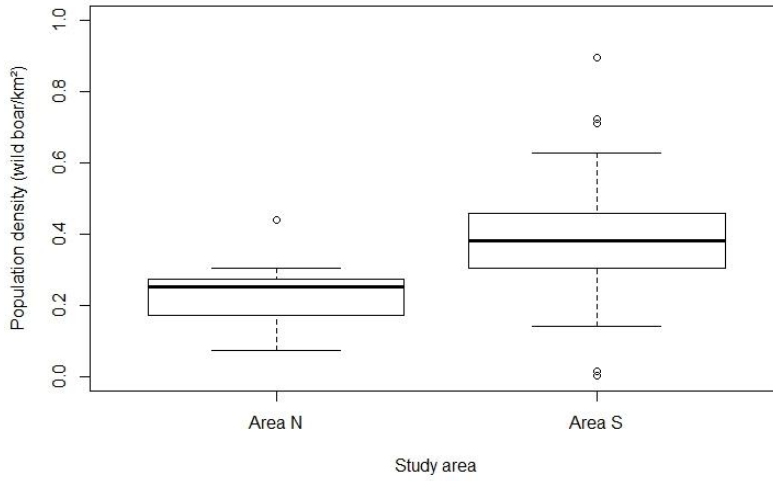
Supplementary Figure S1: Number of samples from animals hunted or found dead (carcass categories) stratified by age category (< 1 year and > 1 year).



Supplementary Figure S2: Number of samples from juvenile and adult animals stratified by study area (area North [N], area South [S]).



Supplementary Figure S3: Number of samples from animals hunted or found dead (carcass categories) stratified by study area (area North [N], area South [S]).



Supplementary Figure S4: Population density (number of wild boar /km²) in the study areas (area North [N], area South [S]).

II

Nurmoja, I., Petrov, A., Breidenstein, C., Zani, L., Forth, J.H., Beer, M., Kristian, M., Viltrop, A., Blome, S., 2017. BIOLOGICAL CHARACTERIZATION OF AFRICAN SWINE FEVER VIRUS GENOTYPE II STRAINS FROM NORTH-EASTERN ESTONIA IN EUROPEAN WILD BOAR. Transboundary and Emerging Diseases, 64, 2034–2041 doi:10.1111/tbed.12614

Received: 11 October 2016
DOI: 10.1111/tbed.12614

ORIGINAL ARTICLE

WILEY Transboundary and Emerging Diseases

Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar

I. Nurmoja[1,2] | A. Petrov[3,4] | C. Breidenstein[3] | L. Zani[3] | J. H. Forth[3] | M. Beer[3] | M. Kristian[5] | A. Viltrop[2] | S. Blome[3]

[1]Estonian Veterinary and Food Laboratory, Tartu, Estonia
[2]Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia
[3]Friedrich-Loeffler-Institut, Greifswald – Insel Riems, Germany
[4]Institute of Virology, University of Veterinary Medicine Hannover, Hannover, Germany
[5]Estonian Veterinary and Food Board, Tallinn, Estonia

Correspondence
S. Blome, Friedrich-Loeffler-Institut, Greifswald – Insel Riems, Germany
Email: sandra.blome@fli.de

Funding information
Seventh Framework Programme, Grant/Award Number: grant agreement 311931; European Research Project ASFORCE; FLI Internal African Swine Fever Virus Research Network.

Summary

Due to its impact on animal health and pig industry, African swine fever (ASF) is regarded as one of the most important viral diseases of pigs. Following the ongoing epidemic in the Transcaucasian countries and the Russian Federation, African swine fever virus was introduced into the Estonian wild boar population in 2014. Epidemiological investigations suggested two different introductions into the southern and the north-eastern part of Estonia. Interestingly, outbreak characteristics varied considerably between the affected regions. While high mortality and mainly virus-positive animals were observed in the southern region, mortality was low in the north-eastern area. In the latter, clinically healthy, antibody-positive animals were found in the hunting bag and detection of virus was rare. Two hypotheses could explain the different behaviour in the north-east: (i) the frequency of antibody detections combined with the low mortality is the tail of an older, so far undetected epidemic wave coming from the east, or (ii) the virus in this region is attenuated and leads to a less severe clinical outcome. To explore the possibility of virus attenuation, a re-isolated ASFV strain from the north-eastern Ida-Viru region was biologically characterized in European wild boar. Oronasal inoculation led to an acute and severe disease course in all animals with typical pathomorphological lesions. However, one animal recovered completely and was subsequently commingled with three sentinels of the same age class to assess disease transmission. By the end of the trial at 96 days post-initial inoculation, all animals were completely healthy and neither virus nor viral genomes were detected in the sentinels or the survivor. The survivor, however, showed high antibody levels. In conclusion, the ASFV strain from north-eastern Estonia was still highly virulent but nevertheless, one animal recovered completely. Under the experimental conditions, no transmission occurred from the survivor to susceptible sentinel pigs.

KEYWORDS
African swine fever virus, Estonia, wild boar, infection experiments, virulence

1 | INTRODUCTION

African swine fever (ASF) is one of the most important and complex notifiable diseases of domestic and wild pigs. It is caused by the eponymous virus which belongs to the genus *Asfivirus* within the *Asfarviridae* family (Takamatsu et al., 2011). Depending on host and virus factors, the disease can run acute, subacute and chronic courses. The former is especially linked to highly virulent virus strains and is characterized by severe clinical signs including high fever, general depression, anorexia, gastrointestinal signs, neurological disorders and haemorrhagic lesions in the final stage of the disease (Sánchez-Vizcaíno et al., 2009). In general, the disease course does not differ when comparing European wild boar and domestic pigs (Blome, Gabriel, & Beer, 2013; Gabriel et al., 2011).

In 2007, a highly virulent genotype II ASF virus (ASFV) was introduced into Georgia and subsequently into several Transcaucasian countries, the Russian Federation and, in 2014, into the European Union (OIE WAHID, visited 18 September 2016). Among the currently affected countries is Estonia. Estonian authorities reported the first outbreaks in wild boar in September 2014, and in this year, a total of 41 ASF cases in wild boar were found in four different counties of fifteen. In the first 4 months of 2015, 52 new wild boar cases were reported from four previously infected counties in the southern (three affected counties) and north-eastern part (Ida-Viru county) of the country (see Figure 1). By December 2015, the number of ASF cases in wild boar had risen to 723, and 11 counties were affected almost all over the territory of Estonia. Apart from the wild boar population, 18 ASF outbreaks were reported from the domestic pig sector in 2015. Interestingly, outbreak characteristics varied considerably between the southern introduction and the north-eastern introduction. While high mortality (up to 16 dead animals found in one place) and mainly virus-positive animals were observed in the southern affected region,

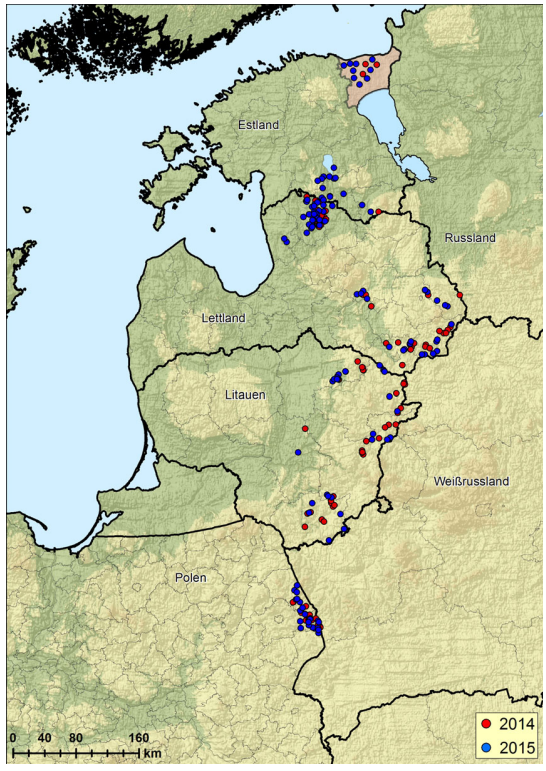


FIGURE 1 ASF cases in Estonia, the other Baltic EU Member States, and Poland from September 2014 to end of April 2015

mortality was low in the north-eastern outbreak area. In the latter, clinically healthy, antibody-positive animals were found in the hunting bag and detection of virus or viral genome was rare. To explain the different behaviour of the virus in the north-east, two hypotheses were phrased: (i) the frequency of antibody detections combined with the low mortality is the manifestation of an older, so far undetected epidemic wave coming from the east; that is, we see its tail represented by surviving animals, or (ii) the virus in this region is attenuated and leads to less severe courses. An attenuated virus could significantly complicate disease detection and may facilitate long-term endemicity.

To test hypothesis (ii) we made an attempt to re-isolate the virus from PCR-positive organ samples from the Ida-Viru region. While isolation in macrophage cultures failed, the virus could be re-isolated by animal passage. Subsequently, the resulting virus was biologically characterized in terms of disease course, virology and serology in ten young wild boar at the Friedrich-Loeffler-Institut (FLI), Isle of Riems, Germany.

2 | MATERIALS AND METHODS

2.1 | Experimental design

To re-isolate the causative ASFV strain from weak PCR-positive organ samples from Ida-Viru, three young wild boar were intramuscularly inoculated with an organ homogenate in standard cell culture medium (no viral growth in macrophage cultures). Upon onset of clinical signs and confirmation of infection by real-time PCR (qPCR), the animals were euthanized and standardized blood and organ samples were collected during necropsy. A pooled spleen suspension with a titre of $10^{4.5}$ haemadsorbing units (HAU) per ml was subsequently used for the trial detailed below.

The main study included a total of ten European wild boar from the breeding unit at the FLI aged approximately 4 month at the start of the trial. The animals were moved from the FLI quarantine stables into the high containment facilities (L3+) where they were kept in one pig pen. All animals were individually ear-tagged with numbers #11 to #20. Over the course of the trial, the animals were fed a commercial pig food with corn and hay–cob supplement and had access to water ad libitum. After an acclimatization phase, the wild boar were inoculated oronasally with 2 ml of the above-mentioned spleen suspension. Clinical parameters of all animals were assessed daily based on a harmonized scoring system as previously described (Pietschmann et al., 2015). In brief, parameters anorexia, recumbency, joint lesions, breathing, ocular discharge, digestive findings and neurological disorders were assigned points according to the severity of findings. The sum of the points was recorded as the clinical score (CS) that was also used to define humane endpoints. Over the course of the trial, levels of viremia, virus distribution, virus shedding and antibody responses were assessed. For this purpose, blood samples were collected along with oropharyngeal and faecal swabs at days 0, 4, 7 and 10 post-inoculation (dpi), and at the day of necropsy. Animals reaching the humane endpoint or that were suffering unacceptably without reaching the endpoint were euthanized through intracardial injection of embutramide (T61, Merck) after deep anaesthesia with tiletamine/zolazepam (Zoletil®, Virbac). Necropsy was performed on all animals, and at the same time, tissue samples (lymph nodes, spleen, tonsil, salivary gland, lung and liver), blood (EDTA, serum) and swab samples were collected for reference purposes.

At the end of the initial trial, one wild boar (#19) had recovered completely. To assess virus transmission to susceptible animals, the survivor was commingled with three sentinel wild boar (#1, #2, #3) from day 50 post-initial inoculation. The sentinels were roughly the same age and were purchased from a game park in Mecklenburg–Western Pomerania. The trial ended at 96 dpi. At this day, the remaining animals were euthanized and subjected to necropsy as described above.

In all trial parts, all applicable animal welfare regulations, including EU Directive 2010/63/EC and institutional guidelines, were taken into consideration. The animal experiments were approved by the competent authority under reference number 7221.3-2-023/15.

2.2 | Cells

Blood for the preparation of peripheral blood mononuclear cell (PBMC)-derived macrophages was collected from healthy domestic donor pigs. In brief, PBMCs were obtained from EDTA-anticoagulated blood using Pancoll animal density gradient medium (PAN Biotech, Aidenbach, Germany). PBMCs were grown in RPMI-1640 cell culture medium with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 10% foetal calf serum (FCS) at 37°C in a humidified atmosphere containing 5% CO_2. The medium was supplied with amphotericin B, streptomycin and penicillin to avoid bacterial and fungal growth. To facilitate maturation of macrophages, GM-CSF (granulocyte macrophage colony-stimulating factor; Biomol, Hamburg, Germany) was added to the cell culture medium at 2 ng/ml.

2.3 | Laboratory investigations

2.3.1 | Processing of samples

Oropharyngeal swabs were soaked in 1 ml of medium (EMEM without addition of FCS), vortexed for approximately 15 s, incubated for 1 hr at room temperature and decanted in microcentrifuge tubes. Serum samples, which were obtained from native blood by centrifugation at 2,500 *g* for 20 min at 20°C, were aliquoted and stored at −80°C until further use. Tissue samples of tonsil, spleen, salivary gland, liver, lung and lymph nodes were collected at necropsy and stored at −80°C. For qPCR and virus isolation (haemadsorption tests), tissue samples were homogenized in 1 ml phosphate-buffered saline (PBS) using a TissueLyser II (Qiagen® GmbH).

2.3.2 | Virus detection

For qPCR, viral nucleic acid was extracted, using the QIAamp® RNA Viral Mini Kit (Qiagen) or the NucleoMag Vet Kit (MACHEREY-

NAGEL) and the KingFisher® extraction platform (Thermo Scientific). Both extraction methods were slightly modified through the addition of an internal control DNA. The nucleic acid extraction was performed with 75 μl of whole blood and 150 μl of organ homogenate and swab material. Subsequently, qPCR was performed according to the protocol published by King et al. (2003) with slight modifications. For confirmatory reason, the virotype ASFV PCR Kit (Qiagen) was employed according to the manufacturer's instructions. Results of both qPCRs were recorded as quantification cycle (cq) values.

To detect ASFV in serum and tissue samples, a haemadsorption test (HAT) was carried out using PBMC-derived macrophages according to the slightly modified standard procedures (Carrascosa, Bustos, & de Leon, 2011). In brief, isolated PBMCs were seeded into a 96-well microplate with a density of app. 1.9×10^6 cells/ml. After 16–24 hr, non-adherent cells were removed and cell culture medium containing GM-CSF was replenished. The culture was then incubated for 24–48 hr to allow initial maturation of macrophages. Subsequently, 20 μl of serum samples and 30 μl of organ homogenate were added to each well. Tests were performed in duplicates. When using organ homogenates, cells were washed after 2 hr of adsorption time using lukewarm PBS, whereas serum was left on the cells until the evaluation of the test. After 24 hr of incubation, 20 μl of homologues 1% erythrocyte suspension was added to each well. For read-out, cultures were analysed for haemadsorption phenomena over a period of 2 days. Virus back-titration was performed by endpoint titration of the diluted spleen suspensions. In this case, the PBMC preparation was seeded into 96-well microplates, the test volume was 100 μl per dilution step, and 20 μl of a 1% homologous erythrocyte suspension was added. These samples were tested in quadruplicate.

2.3.3 | Antibody detection

For the detection of antibodies against African swine fever virus, two commercial enzyme-linked immunosorbent assays (ELISA) were carried out following the manufacturer's instructions (Ingezim PPA COMPAC, Ingenasa; ID SCREEN African swine fever virus INDIRECT, IDvet). The Ingezim PPA ELISA detects antibodies directed against p72 in a competitive format. The ID SCREEN is an indirect ELISA using antigens p32, p62 and p72. All serum samples were tested in duplicate.

All data were recorded and evaluated using Microsoft Excel 2010 (Microsoft Deutschland GmbH) and SigmaPlot for Windows version 11.0 (Systat Software, Inc.).

3 | RESULTS

3.1 | Clinical course and pathomorphological findings

Following oronasal inoculation, all animals developed severe, unspecific clinical signs starting from 4 to 6 dpi including general depression, lack of appetite, huddling and respiratory distress. Three animals reacted with some delay, namely animals #17, #18 and #19. These animals were still very active and interested in food at day 4 and showed only mild signs on day 7. Between days 7 and 13, all but one animal (#19) showed worsening clinical signs with dyspnoea and ataxia and were euthanized in a moribund state or died overnight spontaneously (#16). Wild boar #19 showed decreasing severity of clinical signs starting app. 14 dpi and completely recovered over the following week.

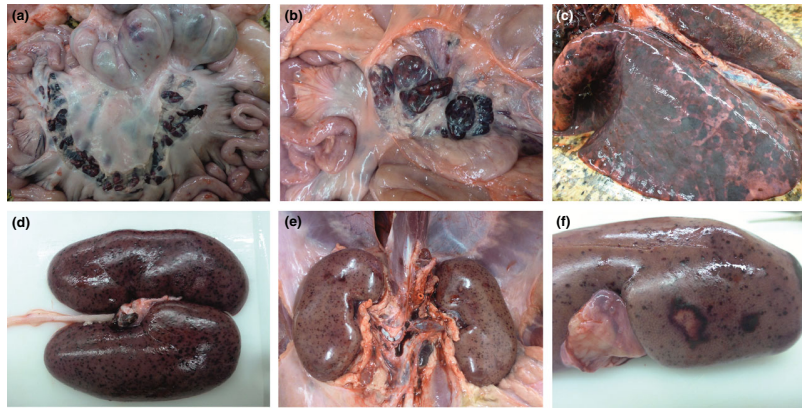


FIGURE 2 Examples of gross pathological findings during necropsy of acute lethally infected wild boar upon infection with the ASFV strain from north-eastern Estonia. (a) Haemorrhagic intestinal lymph nodes and striate bleedings in the gut. (b) Ebony-coloured, haemorrhagic lymph nodes in the gastrohepatic area. (c) Lung oedema, fibrinous pleuritis and haemorrhages; (d) and (e) petechiae in the kidney; (f) kidney petechiae and infarction

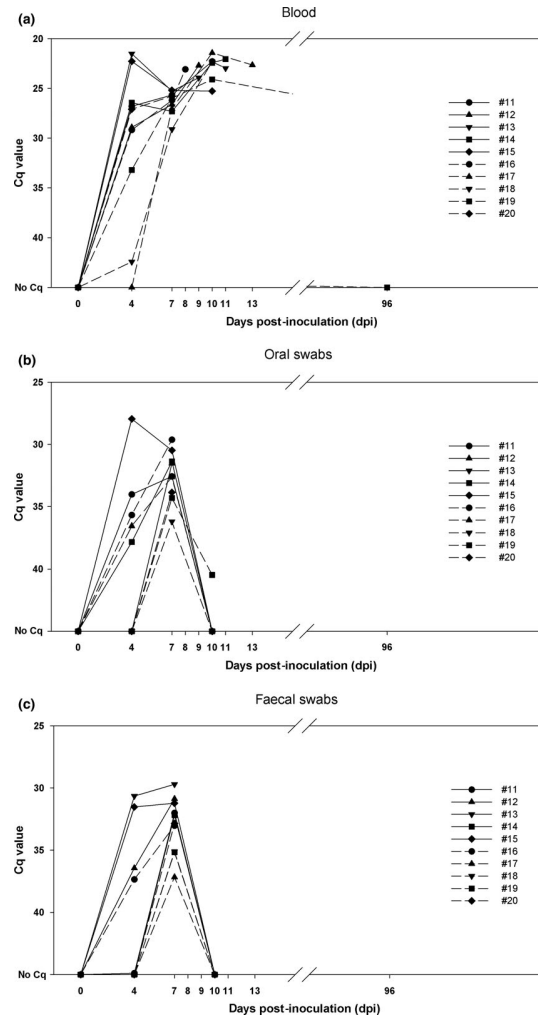


FIGURE 3 Genome detection by qPCR in blood (a), oropharyngeal (b) and faecal swabs (c). Results are depicted as cycle quantification (cq) values

During necropsy, typical ASF lesions of varying severity were observed in all animals that succumbed to infection (for exemplary findings, see Figure 2). Lesions ranged from slight lung oedema and ebony-coloured gastrohepatic lymph nodes to multiple haemorrhages in several organs, haemorrhagic and oedematous lymph nodes in all parts of the body and severe lung oedema. Sporadic findings included gall bladder oedema, renal infarction, gastritis and arthritis. Severity of lesions increased with time in the experiment.

After commingling of the survivor with three sentinels, no clinical signs were observed and all animals stayed in good health until the end of the trial at day 96. No ASF-related lesions were observed during necropsy.

3.2 | Detection of virus and viral genome

At 4 dpi, seven of ten animals were positive in qPCR from EDTA blood with cq values below 30 (see Figure 3a), and two additional animals were weak positive (cq 34 and 41). Animal #17 was still negative at this time. In oropharyngeal swabs, five animals were found to be positive by qPCR with moderate-to-low viral loads (cq 28–38, see Figure 3b). Here, animal #17 was among the weak positives (cq 37), but the two other animals with a low genome load in the blood and with almost no clinical signs were negative (see Figure 3b). The qPCR from faecal swabs also yielded five but not completely congruent positive results (see Figure 3c). Again, viral loads were low (cq values ranging from 31 to 45). Haemadsorption tests from serum were positive for all but animals #17 and #18. At 7 dpi, all available blood and swab samples were positive in qPCR with moderate-to-high genome loads in blood (cq 25–29, see Figure 3a), and moderate-to-low genome loads in swabs (cq 30–37, see Figure 3b and 3c). Here, only five haemadsorption tests were clearly positive, but the positive results included samples from animals #17 and #18. The remaining animals were all strong positive in qPCR from blood at 10 dpi (see Figure 3a), but only one oropharyngeal swab (#19) was very weak positive (cq 41, see Figure 3b). Haemadsorption tests from sera were positive for all animals. Spleen, tonsil, lung, salivary gland and lymph node samples taken during necropsy of animals that succumbed to infection were all positive in qPCR (see Table 1), and all spleen samples reacted positive in haemadsorption tests.

Samples taken from the survivor and the sentinels during necropsy at 96 dpi were all negative for ASF virus and viral genome in two independent qPCR systems (see Table 1). Among the samples were nine lymph nodes from all over the body (mandibular, parotideal, lung-associated, renal, gastrohepatic, intestinal from the large and small intestines, inguinal, popliteal).

3.3 | Detection of antibodies against ASFV

First, positive reactions were seen in both ELISA systems between days 9 and 13 post-inoculation. At 10 dpi, #19 was found positive in both test systems and #14 showed doubtful reactions in the Ingezim PPA and positive reactions in the ID SCREEN African swine fever virus INDIRECT. An additional doubtful result for the serum of animal #11 was found in the Ingezim PPA. At the respective end day, only animal #19 (96 dpi) showed high antibody levels in the Ingezim PPA ELISA. However, several animals were close to the cut-off. In contrast, three animals were found positive (#14, 11 dpi; #17, 13 dpi; #19, 96 dpi) and one doubtful (#13, 9 dpi) in the ID SCREEN African swine fever virus INDIRECT.

4 | DISCUSSION

African swine fever is no longer an exotic disease in several eastern European countries. Since the introduction into the EU in 2014, ASF has spread continuously despite enormous efforts towards controlling the disease. The causative virus strains are of genotype II and showed high virulence for both domestic pigs and European wild boar under experimental conditions (Blome, Gabriel, Dietze, Breithaupt, & Beer, 2012; Gabriel et al., 2011; Gallardo et al., 2015; Guinat et al., 2014; Pietschmann et al., 2015). This would mean that introduction into a free area would be expected to lead to obvious clinical signs and mortality.

While mortality and virus-positive animals were observed in Southern Estonia, this outbreak behaviour was missing in the north-eastern outbreak area. One explanation could be local virus attenuation.

In an attempt to understand the different outbreak characteristics and to investigate the virulence of the local viral variants, an animal trial was conducted with a re-isolated ASFV strain from Ida-Viru.

In a nutshell, the ASFV strain from north-eastern Estonia was still highly virulent for young wild boar, but nevertheless, one animal recovered completely. In direct comparison with previous studies (Blome et al., 2012; Gabriel et al., 2011; Pietschmann et al., 2015; Tauscher et al., 2015), genome loads seemed to be slightly lower and detectable antibody responses were observed more often. However, as only cq values but not exact genome copy numbers could be compared, it cannot be ruled out that the differences were only due to variability of PCR machines and extraction methods. The course of infection and the pathomorphological signs did not differ for the animal that succumbed to infection. The virological data suggest that at least one animal (#17) got infected later. This confirms that oral infection is error prone and needs a quite high dose. It was reported previously that for oral infection, virus titres $>10^4$ HAU are usually necessary and that the ratio of viral titres needed for infection of a susceptible animal via the intramuscular/intravenous inoculation versus the oral/nasal route is 1:140.000 with less than 1 HAU for the parenteral route (McVicar, 1984). The high dose needed for oral infection and the moderate contagiosity of ASF without blood contact could be part of the explanation why the epidemic in eastern Europe spreads rather slowly.

The survival of one animal gave us the opportunity to study the long-term fate of recovered animals and their potential of transmitting the virus on a limited scale. So far, solid data are missing regarding this issue and are needed to estimate the long-term effects of ASF in the wild boar population. It was suggested that survivors will become virus carriers (Sánchez-Vizcaíno, Mur, & Martínez-López, 2012) and thus contribute to the long-term persistence of ASF in a region. At least under our experimental conditions, the single survivor was able to eliminate the virus, and it did not transmit to sentinels, even under conditions with slight hierarchical fights upon introduction of the new animals. Consequently, a carrier state is not an inescapable outcome for all surviving animals.

Hence, we did not find a clear explanation for the different disease dynamics in north-eastern Estonia. Additional data on viral sequences, viral behaviour upon animal passaging and epidemiological drivers are needed.

TABLE 1 Disease course, viral genome and antibody detection upon oronasal (o.n.) inoculation of ten wild boar with an ASFV strain from north-eastern Estonia (ASFV EE). The sentinel animals were commingled with the surviving animal #19 from 50 to 96 days post-inoculation (dpi). Genome detection in organs is presented as cycle quantification value (cq)

Animal	Inoculation	Course	End day	Viraemia qPCR)	Ab detection	Virus detection in organs												
						Tonsil	SalGland	MandLn	Lung	Spleen	LnPar	LnLu	LnGaHep	LnSInt	LnLInt	LnKd	LnIng	LnPopl
11	ASFV EE o.n.	AL	10	4–10 dpi (ED)	Doubtful at 10 dpi	31	28	27	26	30								
12	ASFV EE o.n.	AL	9	4–9 dpi (ED)	No antibodies	35	33	28	30	29								
13	ASFV EE o.n.	AL	9	4–9 dpi (ED)	Doubtful at 9 dpi	26	24	29	20	27								
14	ASFV EE o.n.	AL	11	4–11 dpi (ED)	From 10 dpi	35	36	36	29	31								
15	ASFV EE o.n.	AL	10	4–10 dpi (ED)	No antibodies	29	30	25	31	28								
16	ASFV EE o.n.	AL	8	4–8 dpi (ED)	No antibodies	29	33	28	28	27								
17	ASFV EE o.n.	AL	13	7–13 dpi (ED)	At 13 dpi	27	33	30	24	30								
18	ASFV EE o.n.	AL	10	4–10 dpi (ED)	No antibodies	31	32	29	30	28								
19	ASFV EE o.n.	AT	96	Transient[a]	From 10 dpi	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq
20	ASFV EE o.n.	AL	7	4–7 dpi (ED)	No antibodies	25	29	23	24	21								
1	Sentinel	na	96	Negative	No antibodies	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq
2	Sentinel	na	96	Negative	No antibodies	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq
3	Sentinel	na	96	Negative	No antibodies	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq	No cq

ED, end day; AL, acute lethal; AT, acute transient; na, not applicable; Ab, antibody; SalGland, salivary gland; MandLn, mandibular lymph nodes; LnPar, parotideal lymph nodes; LnLu, lung lymph nodes; LnGaHep gastrohepatic lymph nodes; LnSInt, lymph nodes from the small intestine area; LnLInt, lymph nodes from the large intestine area; LnKd, renal lymph nodes; LnIng, inguinal lymph nodes; LnPopl, popliteal lymph node.

[a]Detected positive at 4, 7 and 10 dpi; negative 96 dpi (no other samples).

ACKNOWLEDGEMENTS

The authors would like to thank Ulrike Wilhelm, Ulrike Kleinert and Holger Freese for excellent laboratory work, and the animal caretakers, especially Frank Klipp, Steffen Brenz and Harald Manthei, for their constant help and support. Moreover, we would like to acknowledge Dr. Christoph Staubach and Dr. Kerstin Wernike for helping us with the design and creation of figures. This work has been partially funded by the European Research Project ASFORCE ("Targeted research effort on African swine fever," grant agreement 311931) and the FLI Internal African Swine Fever Virus Research Network.

REFERENCES

Blome, S., Gabriel, C., & Beer, M. (2013). Pathogenesis of African swine fever in domestic pigs and European wild boar. *Virus Research*, *173*, 122–130.

Blome, S., Gabriel, C., Dietze, K., Breithaupt, A., & Beer, M. (2012). High virulence of African swine fever virus caucasus isolate in European wild boars of all ages. *Emerging Infectious Diseases*, *18*, 708.

Carrascosa, A. L., Bustos, M. J., & de Leon, P. (2011). Methods for growing and titrating African swine fever virus: Field and laboratory samples. *Current Protocols in Cell Biology*. doi:10.1002/0471143030.cb2614s53

Gabriel, C., Blome, S., Malogolovkin, A., Parilov, S., Kolbasov, D., Teifke, J. P., & Beer, M. (2011). Characterization of african Swine Fever virus caucasus isolate in European wild boars. *Emerging Infectious Diseases*, *17*, 2342–2345.

Gallardo, C., Soler, A., Nieto, R., Cano, C., Pelayo, V., Sánchez, M. A., … Arias, M. (2015). Experimental infection of domestic pigs with African swine fever virus Lithuania 2014 genotype II field isolate. *Transboundary and Emerging Diseases*, *64*, 300–304.

Guinat, C., Reis, A. L., Netherton, C. L., Goatley, L., Pfeiffer, D. U., & Dixon, L. (2014). Dynamics of African swine fever virus shedding and excretion in domestic pigs infected by intramuscular inoculation and contact transmission. *Veterinary Research*, *45*, 9.

King, D. P., Reid, S. M., Hutchings, G. H., Grierson, S. S., Wilkinson, P. J., Dixon, L. K., … Drew, T. W. (2003). Development of a TaqMan PCR assay with internal amplification control for the detection of African swine fever virus. *Journal of Virological Methods*, *107*, 53–61.

McVicar, J. W. (1984). Quantitative aspects of the transmission of African swine fever. *American Journal of Veterinary Research*, *45*, 1535–1541.

Pietschmann, J., Guinat, C., Beer, M., Pronin, V., Tauscher, K., Petrov, A., … Blome, S. (2015). Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. *Archives of virology*, *160*, 1657–1667.

Sánchez-Vizcaíno, J. M., Martínez-López, B., Martínez-Avilés, M., Martins, C., Boinas, F., Vial, L., … Roger, F. (2009). *Scientific report on African swine fever* (pp. 1–141) (Submitted).

Sánchez-Vizcaíno, J. M., Mur, L., & Martínez-López, B. (2012). African swine fever: An epidemiological update. *Transboundary and Emerging Diseases*, *59*(Suppl. 1), 27–35.

Takamatsu, H., Martins, C., Escribano, J. M., Alonso, C., Dixon, L. K., Salas, M. L., & Revilla, Y. (2011). Asfarviridae. In: A. M. Q. King, M. J., Adams, E. B. Carstens & E. J. Lefkowitz (Eds.), *Virus taxonomy*, pp. 153–162. Oxford: Elsevier.

Tauscher, K., Pietschmann, J., Wernike, K., Teifke, J. P., Beer, M., & Blome, S. (2015). On the situation of African swine fever and the biological characterization of recent virus isolates. *Berliner und Munchener Tierarztliche Wochenschrift*, *128*, 169–176.

How to cite this article: Nurmoja I, Petrov A, Breidenstein C, et al. Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. *Transbound Emerg Dis*. 2017;00:1–8. doi:10.1111/tbed.12614.

III

Nurmoja, I., Mõtus, K., Kristian, M., Niine, T., Schulz, K., Depner, K., Viltrop, A. 2020.
EPIDEMIOLOGICAL ANALYSIS OF THE 2015–2017 AFRICAN SWINE FEVER OUTBREAKS IN ESTONIA.
Preventive Veterinary Medicine, 181, 104556
doi:10.1016/j.prevetmed.2018.10.001

Preventive Veterinary Medicine 181 (2020) 104556

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

SVEPM: 2018

Epidemiological analysis of the 2015–2017 African swine fever outbreaks in Estonia

Imbi Nurmoja[a,b,*], Kerli Mõtus[a], Maarja Kristian[c], Tarmo Niine[a], Katja Schulz[d], Klaus Depner[d], Arvo Viltrop[a]

[a] Estonian University of Life Science, Institute of Veterinary Medicine and Animal Sciences, Kreutzwaldi 62, 51014 Tartu, Estonia
[b] Estonian Veterinary and Food Laboratory (VFL), Kreutzwaldi 30, 51006 Tartu, Estonia
[c] Estonian Veterinary and Food Board (VFB), Väike-Paala 3, 11415 Tallinn, Estonia
[d] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Epidemiology, Südufer 10, 17493 Greifswald, Insel Riems, Germany

ARTICLE INFO

Keywords:
Domestic pig
Epidemiology
Outbreak investigation
Risk factor analysis
Survival analysis
Spatio-temporal analysis

ABSTRACT

African swine fever (ASF) was first detected in the Estonian wild boar population in September 2014, while the first domestic pig farm was affected in July 2015. In the present study, we aimed to analyse, retrospectively, the epidemiology of the disease in all 26 outbreaks in domestic pig herds that occurred in Estonia during the period 2015–2017. Formal interviews were conducted to estimate the high-risk period for every farm, and to identify the possible origin of the ASF virus and the mode of virus introduction. Furthermore, the clinical manifestation of the disease as well as the course of the disease within the farm were investigated. Survival analysis was used to calculate herd incidence and to estimate outbreak risk. A hierarchical Bayesian space–time model was used to analyse the associations between outbreaks and ASF occurrence in wild boar. The spatial and temporal distribution of outbreaks was analysed to characterise the ASF epidemic in the Estonian domestic pig population from 2015 to 2017.

The estimated high-risk period varied from seven to 20 days with a median of 11 days. On most of the affected farms, the first clinical signs were mild and not specific to ASF despite the high virulence of the circulating virus. Morbidity and mortality were often limited to a single pen or unit of the farm. The highest mortality (29.7%) was seen on backyard farms with 1–10 pigs and the lowest (0.7%) on large commercial farms (> 1000 pigs). The spread of the virus within affected farms has been slow and the contagiousness of the virus has been relatively low. Farms of all sizes and types have been at risk, including large commercial farms operating at a high biosecurity level. In none of the affected farms could the specific route of introduction be verified. However, the findings suggested that virus introduction occurred via indirect transmission routes due to insufficient biosecurity. The total herd incidence of outbreaks was similar across all three years, being 2.4% in 2015 and 2016, and 2.0% in 2017. All outbreaks occurred from June to September, during the warmest period of the year. The results suggest that the increase in ASF cases in local wild boar populations is the main risk factor leading to the infection of farms; 88% of outbreaks occurred in areas where ASF virus was detected in wild boar prior to the outbreak, within a radius of 15 km from the outbreak farm.

1. Introduction

Due to its serious impact on animal health and the pig industry, African swine fever (ASF) is considered one of the most important and dangerous viral diseases of pigs and wild boar. Highly virulent and lethal ASF virus strains from genotype II have been circulating in Eastern Europe since 2007, and in EU countries from 2014 (Blome et al., 2012; Gallardo et al., 2015b; OIE and WAHID, 2017). However, not enough reliable and comprehensive epidemiological field data about domestic pig outbreaks is available. Although in recent decades, different epidemiological data from ASF endemic countries in Africa (Fasina et al., 2012; Penrith et al., 2013), the Italian island of Sardinia (Mur et al., 2018), as well as from the Iberian Peninsula (1960–1995) have been collected and made available, these results are often not valid for Eastern and Northern Europe. The genotype of the virus, and climatic, socio-economic and environmental conditions, as well as the

* Corresponding author at: Estonian Veterinary and Food Laboratory, Kreutzwaldi 30, 51006 Tartu, Estonia.
E-mail addresses: imbi.nurmoja@vetlab.ee (I. Nurmoja), Kerli.Motus@emu.ee (K. Mõtus), Maarja.kristian@vet.agri.ee (M. Kristian), Tarmo.Niine@emu.ee (T. Niine), Katja.Schulz@fli.de (K. Schulz), Klaus.Depner@fli.de (K. Depner), Arvo.Viltrop@emu.ee (A. Viltrop).

https://doi.org/10.1016/j.prevetmed.2018.10.001
Received 17 May 2018; Accepted 1 October 2018

Table 1
Number of detected ASF cases in wild boar and ASF outbreaks in domestic pig herds in Estonia from 1st September 2014 to 31st December 2017.

	2014[a]		2015		2016		2017	
County	WB[b] cases	DP[c] outbreaks	WB cases	DP outbreaks	WB cases	DP outbreaks	WB cases	DP outbreaks
Harju	0	0	0	0	46	0	87	0
Hiiu	0	0	0	0	0	0	0	0
Ida-Viru	4	0	36	0	40	0	14	0
Jõgeva	0	0	60	2	192	3	15	0
Järva	0	0	102	1	117	1	9	0
Lääne	0	0	0	0	58	0	119	1
Lääne-Viru	0	0	91	1	198	1	64	0
Põlva	0	0	233	0	190	0	14	0
Pärnu	0	0	27	0	95	0	87	1
Rapla	0	0	6	0	203	0	90	0
Saare	0	0	0	0	98	1	305	1
Tartu	0	0	124	2	192	0	40	0
Valga	13	0	124	4	24	0	8	0
Viljandi	47	0	174	5	61	0	9	0
Võru	9	0	118	2	56	0	6	0
Total	73	0	1095	17	1570	6	867	3

[a] From 1st September.
[b] Wild boar.
[c] Domestic pig.

structure of the pig industry and farming traditions are considerably different from the aforementioned countries and regions. In Estonia, pig production is highly industrialised and concentrated on large farms, whereas the backyard sector and the number of smallholders became relatively small in number and significance during the last decade. Pigs are kept predominantly inside in weatherproof facilities and keeping them outdoors has been the exception rather than the rule.

The first case of ASF in Estonia was diagnosed in a wild boar found dead near the Latvian border at the beginning of September 2014. In the Latvian wild boar population, ASF had already been present since June 2014 (OIE and WAHID, 2017; Olševskis et al., 2016). In the following years, the virus spread through the entire wild boar population in Estonia, leaving only some islands free of infection. The first ASF outbreak in domestic pigs in Estonia occurred in July 2015 and was followed by 16 outbreaks during the following nine weeks. Six outbreaks were notified in 2016 and three in 2017. An overview of Estonian ASF outbreaks in domestic pig herds and wild boar cases is given in Table 1 (see also Fig. 2).

The aim of the present study was to analyse, retrospectively, the epidemiology of ASF in domestic pigs, based on data from all Estonian outbreak farms. More specifically (i) to estimate the high-risk period and mortality risk, (ii) to analyse the characteristics of the affected herds, (iii) to clarify clinical manifestation of the disease as well as spread of the virus within the farms, (iv) to assess the virus transmission and introduction pathways, (v) to estimate herd incidence and outbreak risks, (vi) to assess temporal and spatial patterns of outbreaks, and (vii) to analyse associations between the occurrence of ASF in wild boar and domestic pig outbreaks.

2. Materials and methods

2.1. ASF outbreak detection

An outbreak farm was defined as a holding having an individual identification number in the National Animal Register (NAR) and meeting the criteria of infected herd as defined in Council Directive 2002/60/EC (European Commission, 2002). All ASF outbreaks were confirmed by virus genome detection in accordance with the EU diagnostic manual (European Commission, 2003). Tissue and blood samples were collected from all or selected dead or sick animals, depending on the clinical course of the disease on the farm in question. The laboratory analyses were performed at the Estonian Veterinary and Food Laboratory, which is also the national reference laboratory for ASF (NRL).

The ASF virus genome was detected by real-time PCR according to the protocol published by Tignon et al. (2011). In addition, the presence of ASF-virus-specific antibodies was analysed using a commercial blocking ELISA (INGEZIM, PPA COMPAC K3, INGENASA, Madrid, Spain) and/or indirect immunoperoxidase technique (IPT) provided by the European Union reference laboratory for ASF (Gallardo et al., 2015a; European Unión Laboratory for African Swine Fever et al., 2014).

2.2. Outbreak investigations

Epidemiological investigations were conducted on all farms in which an ASF outbreak had been reported (18 farms in 2015, six farms in 2016 and three farms in 2017). However, a positive diagnosis of ASF was not confirmed in follow-up investigations of one of the herds in 2015, where all 15 pigs tested after culling were found to be negative for ASF.

Epidemiological investigations were conducted either by the local veterinary officers responsible for management of the outbreaks or by the epidemiology team of the Estonian University of Life Sciences in compliance with Council Directive 2002/60/EC (European Commission, 2002). In principle, epidemiological enquiries dealt with the following: (i) the length of time that the ASF virus may have existed on the holding before the disease was notified or suspected, (ii) the possible origin of the ASF virus at the holding and mode of introduction, (iii) the identification of other holdings at which pigs may have become infected from the same source.

Formal interviews using a structured questionnaire were conducted with farm managers, farm veterinarians and farm workers, focussing on farm management, herd data, animal movements, vehicle movements, feeding and bedding management, biosecurity measures and human activities, all of which might have facilitated virus introduction and spread. Furthermore, investigations were conducted focussing on clinical and pathological data and laboratory findings.

2.3. Biosecurity level of outbreak farms

The level of farm biosecurity was judged by a group of three experts as a consensus judgment based on interview data and from observations made during farm visits. The first step involved evaluating farms based

Table 2
Basic criteria for assessment of farm biosecurity level in ASF outbreak herds in Estonia, 2015–2017.

Criteria	Biosecurity level				
	Compliant				Non-compliant
	very high	high	moderate	low	very low
Indoor keeping[a]	+	+	+	+	One or more require-ments not fulfilled
Fence surrounding the farm boundary[b]	+	+	+	+	
Disinfection barriers at entry points to the farm boundary for vehicles and humans	+	+	+	+/-[c]	
Disinfection barriers at entrances to farm buildings for humans and vehicles	+	+	+	+/-	
No swill and/or grass feeding	+	+	+	+	
No other farm and/or pet animals in pigsties	+	+	+	+	
Number of deficiencies in biosecurity procedures[d]	0	1	2	> 3	

[a] One outdoor farm had special permission to keep pigs in a double-fenced area and was not automatically classified as "very low" – assessment was based on evaluation of all aspects of biosecurity.
[b] Farms without a fence were not automatically classified as "very low" – assessment was based on evaluation of all aspects of biosecurity.
[c] Partly fulfilled.
[d] Functional infrastructure and procedures for disinfection; adequate procedures for entry of animals, humans, vehicles, equipment and materials; secure storage and handling of feed, and bedding material; existence of biosecurity plan.

on their compliance to basic biosecurity requirements enforced by national legislation, and classifying them as compliant or non-compliant (Teataja, 1999, 2004). In the second step, the herds were divided into five categories based on their biosecurity level as shown in Table 2.

2.4. High-risk period of outbreak farms

The length of time that ASF virus may have existed on a farm before it was suspected (high-risk period: HRP) was estimated based on mortality data, and clinical and laboratory findings. In cases where antibody-positive animals (detected by ELISA test) were found in an infected herd, it was concluded that the virus had been circulating in the herd for at least two weeks. In cases where sampled animals were only virus-positive, the time of virus circulation was considered to be one week or less. By combining mortality data, and clinical and laboratory findings, the HRP was established.

2.5. Pig herd data

A database on pig herds in Estonia for the period 2015–2017 was compiled using the information available from the NAR of the Estonian Agricultural Registers and Information Board and from the Veterinary and Food Board (VFB). According to Estonian law, all pig herds must be registered in the NAR and the number of animals in the herd must be reported by owners at least once a year by 1st May. However, during the second half of the years 2015 and 2016, the VFB conducted an inspection of all pig holdings and updated the NAR database with actual number of pigs in herds at the time of inspection. Where needed, the VFB added the holdings not yet registered in the NAR to the database or removed those holdings that no longer kept pigs. The final database included all farms and households that had kept pigs during the year of observation; the total number of pigs in a herd was counted as the largest number registered in one of the source databases (NAR or VFB).

An epidemiological unit was defined as a group of pigs kept in one building or area (one out-door herd) and having an individual identification number in the NAR. One owner may have one or several production units (herds) registered in the NAR. Herds belonging to the same owner were considered as connected herds (epidemiological units).

Holdings were grouped into four size categories according to the total number of pigs (piglets, weaners, growers, fatteners, gilts, sows and boars) in an epidemiological unit: 1–10 pigs (G1); 11–100 pigs (G2); 101–1000 pigs (G3); >1000 pigs (G4). G1 holdings were classified as backyard or non-commercial farms where pigs were kept mainly for the farmers own consumption. G2–G4 holdings were classified as commercial farms.

The herd type (farrow-to-finish, multiplier, fattener or grower) was identified based on the information available from the NAR. Herds consisting of only breeding animals and piglets (up to weaning age) were considered to be multiplier herds, herds with fatteners or growers were classified as fattening herds, and herds with all categories of pigs as farrow-to-finish herds.

The type of pigs kept on a farm (domestic pigs, wild boar, or crosses), as well as the location of the farm (including the coordinates), were taken from source datasets and included in the final database.

The total number of herds and pigs in different herd-size categories are presented in Table 3.

2.6. Wild boar ASF surveillance and hunting data

ASF surveillance data for wild boar from September 2014 until the end of 2017, including date and location (coordinates) of each ASF case, were obtained from the VFB. For the year 2015, data on ASF wild boar cases in Latvia were drawn from the Animal Diseases Notification System database (ADNS, 2017). For 2016 and 2017, Latvian ASF cases were not relevant for the analyses as all Estonian outbreaks in domestic pig farms occurred further away from the Latvian border.

The date and location of the closest wild boar case(s) to each

Table 3
Total number of pig herds and pigs in Estonia in the period 2015–2017.

	2015		2016		2017	
Herd-size category	No. of herds	No. of pigs	No. of herds	No. of pigs	No. of herds	No. of pigs
G1 (1–10)	488	1626	94	418	25	83
G2 (11–100)	94	2560	54	1665	37	735
G3 (101–1000)	37	15,034	29	12,498	24	7516
G4 (>1000)	82	360,307	71	320,511	67	278,572
Total	701	379,527	248	335,092	153	286,906

outbreak farm were identified. The Euclidean distance between each affected farm and the closest wild boar case within a year before the outbreak was recorded, to characterise the infection pressure from wild boar.

Wild boar hunting data, as well as data regarding number of hunters, feeding sites and hunting hounds, were provided by the Estonian Environment Agency (Nature department) and based on regular reports submitted by regional hunting societies to the Environmental Board.

2.7. Statistical analysis and maps

2.7.1. Herd incidence and outbreak risk estimates

Survival analysis was used to calculate herd incidences. The outbreak risk estimates were based on incidence values.

The dataset included all pig farms recorded in source databases in 2015, 2016 and 2017. The observation period started from 1st January each year for those herds that were in the database. The date of start of pig keeping in new herds registered during the year of observation was not known, and such herds were not included in the analysis of the respective year. The observation period lasted either until the day that production ceased (removal of pigs from the farm), the end of the year (right censoring), or until the outbreak of ASF.

The data were declared as survival-time data by specifying the start of the observation period as the '*enter*' option in the '*stset*' command in Stata MP14®. The event of interest was the outbreak of ASF in a domestic pig herd and was specified as the '*failure*' option in the '*stset*' command. Incidence rate, together with 95% confidence intervals, was calculated for each of the study years as well as for the period between 1st January 2015 until 31st December 2017 using the '*strate*' command.

A Cox proportional hazard random-effect model was applied to detect significant differences in ASF infection hazard across farm types, herd-size categories and the three study years. A Cox regression model ('*stcox*' command in STATA®) was applied to detect the significance of the association between variables and the event of interest. The model specified a Breslow method for handling ties, and also included county as a random effect in the '*shared*' option.

Variables significantly associated with the event of interest ($p < 0.05$) were retained in the multivariable model. Akaike information criterion (AIC) values were used to compare the models in terms of their quality (Dohoo et al., 2009).

The assumption of proportional hazards was checked graphically by creating log–log plots of survival, and by a statistical test using Schoenfeld residuals (Dohoo et al., 2009).

2.7.2. Mortality calculations

Mortality risk (cumulative incidence) was calculated for the following: (i) for each outbreak herd, and (ii) for affected groups within the herd for the period including the HRP and the timespan from notification to culling. The affected group was defined as a physically separated unit of a building containing one type of pig (sows, fatteners, weaners etc.).

2.7.3. Spatio-temporal analysis

A hierarchical Bayesian spatio-temporal model (Varewyck et al., 2017) was used to assess the association between the occurrence of ASF cases in wild boar and ASF outbreaks in domestic pigs. No additional time or space–time interaction effects were included in the model; thus, priors were considered to be uninformative. Temporal resolution of the model was set at one month. Spatial resolution for the analysis was based on hunting district (an area allocated to one hunting club for hunting, n = 344) as this was the lowest spatial unit for which covariate data was available. Areas that shared boundaries were considered to be neighbouring, and the model assumed dependency of values between them. One hunting district (334EE–Naissaar) was dropped from the spatio-temporal analysis as it did not have any observations. The implications of this exclusion were considered minimal as it is a small islet off the northern coast, with no direct connections to any other hunting districts.

The response variable was 'ASF outbreak in domestic pigs in hunting district' (set as binary). Covariates included by month were: 'total no. of ASF PCR-positive wild boar' (from September 2014 to November 2017), and 'total no. of wild boar hunted' (from March 2015 to November 2017). Covariates included by year (2014–2017) were: 'total no. of hunters', 'total no. of wild boar feeding sites', and 'total no. of hunting hounds'. These latter three covariates were chosen as they were expected to reflect hunting intensity in a hunting district. The model was checked for convergence.

2.7.4. Maps

Descriptive maps were generated using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA).

3. Results

3.1. Reporting and laboratory findings

ASF was immediately suspected on 12 out of the 26 farms, while on the other farms the first suspicion was feed poisoning (n = 7), erysipelas (n = 3), pneumonia (n = 3), salmonellosis (n = 1) and heat or stress (n = 2). The reason for reporting was sickness (n = 19) or death (n = 7) of one or several animals. In addition to outbreak farms, ASF was suspected and samples were submitted to the NRL for analyses from 18 other farms in 2015, from 28 other farms in 2016 and from 38 other farms in 2017.

On all outbreak farms, PCR-positive animals were detected. In addition, on seven farms, animals with ASF-virus-specific antibodies were detected by ELISA. All antibody-positive animals were also PCR-positive.

The estimated HRP varied from seven to 20 days with a median of 11 days (Fig. 1).

3.2. Characteristics of affected farms

The number of outbreaks across farms of different type and size categories is shown in Table 4.

Twenty-four outbreaks were classified as primary outbreaks, while two outbreaks were considered to be secondary outbreaks due to close contact with infected herds (common ownership and movements of farm workers, vehicles and equipment between farms). There was no movement of animals between these connected outbreak farms during the high-risk period.

3.3. Clinical signs and virus spread within farms

The first clinical signs in pigs were often mild and not specific to ASF. Cases of a severe course of the disease (excluding sudden deaths)

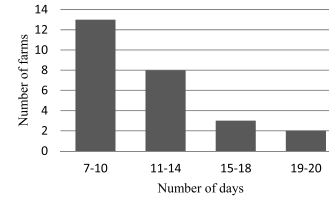


Fig. 1. Length of estimated high-risk period (the length of time that ASF virus may have existed on the farm before it was suspected) on 26 pig farms affected by ASF in Estonia, 2015–2017.

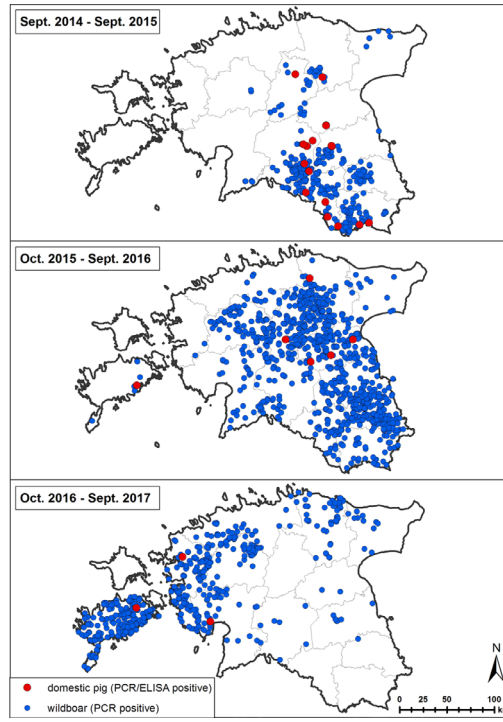


Fig. 2. Location of ASF domestic pig outbreak farms and virus-positive wild boar cases in Estonia in 2015, 2016 and 2017.

were recorded on 13 farms, often after longer circulation of the virus on the farm. On nine out of 12 farms where sows were kept, morbidity occurred firstly among pregnant or nursing sows. Skin haemorrhages or cyanosis were reported in pigs on 11 farms and sudden death on 14 farms, often occurring in a few animals only. A summary of recorded clinical signs in pigs on affected farms before and after reporting is given in Table 5.

In Table 6, the observed mortality estimates are presented. The average mortality was strongly dependent on the herd size, being the lowest in the largest herd-size category (0.7%) and the highest in the smallest one (29.7%).

3.4. Probable routes of virus entry into farms and biosecurity level of the outbreak farms

On all 26 outbreak farms, the virus was most likely introduced by some indirect transmission pathway. In none of the affected farms could the specific route of introduction be verified. However, the findings suggest that on two farms (one commercial outdoor herd and one non-commercial herd with an outdoor walking area), direct (through fence) contact with infected wild boar could not be completely excluded. On eight non-commercial farms with no or very low biosecurity, virus introduction might have occurred via several pathways (e.g. via

Table 4
Distribution of Estonian ASF-positive domestic pig farms across herd type and size, 2015–2017.

	Herd-size category (no. of pigs)				
Production type	G1 (1–10)	G2 (11–100)	G3 (101–1000)	G4 (>1000)	Total
Multiplier	0	0	1	2	3
Farrow-to-finish	1	1	3[a]	5	10
Fattening	7	0	1	5	13
Total	8	1	5	12	26

[a] Two herds with crosses of wild boar and domestic pigs (one kept outdoors) and one organic pig farm.

Table 5
Clinical signs in pigs recorded before and after reporting on 26 ASF outbreak farms in Estonia, 2015–2017.

Clinical manifestation	No. of farms
Loss of appetite	19
Listlessness	19
Sudden death without prior signs in animal	14
Skin haemorrhages or cyanosis	11
Fever[a]	10
Recumbency	10
Incoordination	7
Abortions	5
Respiratory disorders	5
Other[b]	5

[a] On six farms, fever was not detected; on 10 farms, temperature was not measured.
[b] Vomiting (n = 2); decrease in milk yield of sows (n = 1); diarrhoea (n = 1); blood in urine (n = 1).

contaminated feed, grass, clothing, vehicles, other farm animals or pets on the farm, and kitchen waste). The cause of virus introduction for these herds was defined as "lack of/insufficient biosecurity measures".

For commercial herds, possible pathways of virus introduction were identified more specifically by the epidemiology team who analysed the data collected during outbreak investigations. The results of the analysis are presented in Table 7.

It appears from the presented data that on all affected commercial farms the virus was introduced by indirect transmission routes. On the majority of commercial farms (n = 11), the virus was most likely introduced by means of contaminated fomites (vehicles, people, tools) as a result of errors in execution of biosecurity procedures.

The biosecurity levels of affected farms across herd-size categories are shown in Table 8.

The biosecurity measures required by national legislation as described in Table 2, at least at a minimum level, were in place for 13 (50%) outbreak herds. In 10 herds (38%), the measures were implemented at least at a moderate level, and in three outbreak herds (12%) a high or very high biosecurity level was in place.

The biosecurity level on all eight non-commercial (G1) farms was low or very low. On commercial farms (G2–G4), the biosecurity level was generally higher. Biosecurity level of six (33%) commercial farms was estimated as very low because of multiple deficiencies in the fulfilment of biosecurity requirements presented in Table 2.

3.5. Herd incidence

The data on occurrence of outbreaks, as well as the cumulative herd incidences (presented as outbreak risk estimates), for the years 2015 and 2016 per farm type and size category are given in Tables 9 and 10. In 2017, all outbreaks occurred in G4 herds (outbreak risk = 4.5%, 95% CI 1.5; 12.4), and the overall outbreak risk in all herd-size categories was 2.0% (95% CI 0.7; 5.6).

The total herd incidence rates per year and for the whole three-year period obtained from survival analysis are presented in Table 11.

The overall yearly incidence rates did not differ significantly ($p > 0.05$) from each other.

In a univariable Cox proportional hazard random-effect model (including county as a random effect), the multiplier and farrow-to-finish herds had a significantly higher hazard of experiencing an outbreak compared to fattening herds in 2015 (data not presented). In the model that included the data from three years (2015–2017), a similar trend could be observed although the association was not statistically significant ($p = 0.064$). Including the variable 'year' in the model did not improve the model fit. Thus, only the variable 'herd-size category', adjusted for the random effect 'county', was included in the final model. Compared to the two smaller herd-size categories (G1, G2), larger herds (G3, G4) had a significantly higher risk of becoming infected with the ASF virus (Table 12).

3.6. Spatial and temporal distribution of outbreak farms

The geographical locations of outbreak farms changed during the epidemic. As shown in Fig. 2, domestic pig outbreaks appeared in those areas where ASF virus was circulating actively in the wild boar population.

Of 26 outbreaks, 23 occurred in regions where the disease was also present in the wild boar population within a radius of 15 km from the affected farm. The distances between the outbreak farm and the nearest case of ASF in wild boar within a year before an outbreak are shown in Fig. 3. In ten cases, the closest wild boar case was found less than one month before the outbreak, in six cases between one and four months before the outbreak, and in seven cases over four months before the outbreak.

All ASF outbreaks were detected during the warmest period of the year, between June and September. Most of the outbreaks (81%) were detected in July and August (See Fig. 4).

3.7. Results of the hierarchical Bayesian spatio-temporal model

The results of the model analysis are presented in Table 13.

The results of the analysis indicate a significant positive association with the total number of ASF-positive wild boar detected per month in a hunting district. The total number of wild boar hunted, number of hunters, feeding sites and hunting hounds in a hunting district were not significantly associated with outbreaks in domestic pigs.

4. Discussion

4.1. Reporting and laboratory findings

ASF occurrence on Estonian domestic pig farms was generally

Table 6
Estimated ASF mortality in affected domestic pig herds in Estonia, 2015–2017.

Herd-size category		Mortality in the herd			Mortality in the affected group		
	n	Average	Min	Max	Average	Min	Max
G1 (1–10)	8	29.7%	0.0%	100.0%[a]	NA	NA	NA
G2 (11–100)	1	25.0%[b]	NA	NA	NA	NA	NA
G3 (101–1000)	5	7.5%	0.4%	25.0%	13.8%	3.8%	25.0%[c]
G4 (>1000)	12	0.7%	0.04%	2.5%	7.2%	0.1%	43.6%[d]

NA – not applicable as pigs were kept in one group.
[a] Mortality in a backyard farm with one pig.
[b] At the moment of outbreak there were four pigs on the farm.
[c] Herd of 126 crosses kept in one group.
[d] Mortality in a group of 39 nursing sows.

Table 7
Most probable pathways of ASF virus introduction to commercial pig farms in Estonia, 2015–2017.

Introduction pathways	Herd-size category (no. of pigs)			
	G2 (11–100)	G3 (101–1000)	G4 (>1000)	Total
Multiple errors in execution of biosecurity procedures (introduction by fomites)	1	0	4	5
Inadequate disinfection of vehicles	0	0	2	2
Minor errors in execution of biosecurity procedures (introduction by fomites)	0	0	2	2
Movement of people or vehicle from an infected farm (secondary outbreak)	0	1	1	2
Contamination of cereal feed during storage or processing	0	3	2	5
Feeding of grass	0	1	0	1
Contamination of bedding material	0	0	1	1
Total	1	5	12	18

Table 8
Biosecurity levels of Estonian ASF outbreak farms according to herd size, 2015–2017.

Herd-size category (no. of pigs)	very high	high	moderate	low	very low
G1 (1–10)	0	0	0	1	7
G2 (11–100)	0	0	0	0	1
G3 (101–1000)	0	0	1	0	4
G4 (>1000)	2	1	6	2	1
Total	2	1	7	3	13

reported within the first week after the appearance of clinical signs, therefore at a relatively early stage of the outbreak. This was confirmed by the fact that no seroconverting (antibody ELISA positive) animals were found in most herds and the spread of the disease within farms was limited. In seven cases, reporting was delayed for two weeks after appearance of the first disease signs in animals. In these herds, ASF antibody ELISA positive pigs were present. However, all these animals were PCR-positive as well, which indicates that the virus should not have been present in the herd for more than four weeks (Nurmoja et al., 2017; Gallardo et al., 2018; Zani et al., 2018). The speed of reporting was not dependent on whether the herd was commercial or not.

In more than half of the outbreak herds, diseases other than ASF were suspected at first. This can mainly be explained by non-specific signs of ASF at the beginning of the outbreak, particularly due to a lack of characteristic pathological post-mortem findings (data not presented).

4.2. Characteristics of affected farms

Outbreaks occurred in herds of all production types and size categories. The proportion of herds with breeding animals among outbreak farms (50%) exceeded the proportion of these herds in the general population (28%), and there was a trend in the data towards a higher risk for outbreaks in herds with breeding animals. This may be explained by differences in the management of breeding pigs compared to growers and fatteners (more human interaction with breeding pigs). Furthermore, pregnant and nursing sows may be more susceptible to the virus due to immune suppression, and so lower doses of the virus might be able to initiate the infection. Sows in heat may also attract male wild boar (including infected ones), and as a consequence the surrounding environment of breeding farms may become more contaminated with the virus, increasing the likelihood of transmission with fomites onto the farms.

The number of ASF outbreaks in commercial herds exceeded the number of outbreaks in backyard farms. This can partly be explained by the rapid reduction of backyard pig holdings due to strict biosecurity requirements, which are equal for all pig farms in Estonia. This brought the number of backyard pig farms down from 696 in 2014, to 25 by 2017. On the other hand, it may also indicate that large commercial farms are more exposed to the virus due to more frequent and intensive contact with the external environment through movement of people and vehicles.

4.3. Clinical findings and spread of the virus on farms

Although ASF is described as a severe, haemorrhagic disease that causes up to 100% morbidity in naive pig herds and can result in very high mortality (Sánchez-Vizcaíno et al., 2009; Costard et al., 2013), under field conditions we often found ASF cases with mild clinical signs. Severe clinical signs, as well as the haemorrhagic form of the disease, were seldom observed, and often limited to a few animals only. This can be explained by the relatively early detection of outbreaks, as most were reported within seven days of the first observation of disease signs. A severe clinical course and higher morbidity were seen in pregnant or nursing sows, or in the case of longer virus circulation on a farm.

The spread of the virus within affected herds was generally slow,

Table 9
Number of ASF outbreaks and cumulative herd incidence (outbreak risk) in different farm types and herd-size categories in Estonia in 2015.

Production type	Herd-size category					Outbreak risk (CI 95%)
	G1 n herds/ n outbreaks	G2 n herds/ n outbreaks	G3 n herds/ n outbreaks	G4 n herds/ n outbreaks	Total n herds/ n outbreaks	
Multiplier	18/ 0	11/ 0	1/ 1	5/ 2	35/ 3	8.6% (3.0–22.4)
Farrow-to-finish	13/ 0	44/ 1	22/ 3	31/ 3	110/ 7	6.4% (3.1–12.6)
Fattening	456/ 4	39/ 0	13/ 1	46/ 2	556/ 7	1.3% (0.6–2.6)
Total	488/ 4	95/ 1	36/ 5	82/ 7	701/ 17	2.4% (1.5–3.8)
Outbreak risk (CI 95%):	0.8% (0.3–2.1)	1.1% (0.2–5.7)	13.9% (6.1–28.7)	8.5% (4.2–16.6)	2.4% (1.5–3.8)	

Table 10
Number of ASF outbreaks and cumulative herd incidence (outbreak risk) in different farm types and herd-size categories in 2016.

Production type	Herd-size category					Outbreak risk (CI 95%)
	G1 n herds/ n outbreaks	G2 n herds/ n outbreaks	G3 n herds/ n outbreaks	G4 n herds/ n outbreaks	Total n herds/ n outbreaks	
Multiplier	8/ 0	9/ 0	1/ 0	3/ 0	21/ 0	0.0% NC
Farrow-to-finish	6/ 1	24/ 0	17/ 0	28/ 1	75/ 2	2.7% (0.7–9.2)
Fattening	80/ 3	21/ 0	11/ 0	40/ 1	152/ 4	2.6% (1.0–6.6)
Total	94/ 4	54/ 0	29/ 0	71/ 2	248/ 6	2.4% (1.1–5.2)
Outbreak risk (CI 95%):	4.2% (1.7–10.4)	0.0% NC	0.0% NC	2.8% (0.8–9.7)	2.4% (1.4–5.2)	

Table 11
The herd incidence rates of ASF outbreaks among domestic pig herds in Estonia for the years 2015–2017.

Year	No. of outbreaks	No. of herd-years	Incidence rate (outbreaks per 100 herd-years)	95 % Confidence interval
2015	17	646.7	2.6	1.6–4.2
2016	6	229.8	2.6	1.2–5.8
2017	3	140.2	2.1	0.7–6.6
2015–2017	26	1016.7	2.6	1.7–3.8

Table 12
The results of the Cox proportional hazard random-effect model showing the effect of herd size on the incidence of ASF outbreaks in Estonian domestic pig herds in the period 2015–2017. 'County' was included as a random variable.

Herd-size category (no. of pigs)	N[a] (no. of outbreaks)	Hazard ratio (HR)	P-value	95% Confidence interval for HR
G1 (1–10)	607 (8)	1	X	X
G2 (11–100)	185 (1)	0.36	0.342	0.05–2.92
G3 (101–1000)	90 (5)	4.22	0.013	1.36–13.14
G4 (>1000)	220 (12)	4.31	0.002	1.72–10.80

Wald Chi squared = 14.71 (p = 0.002).
[a] Number of herds after splitting the observation period into three years.

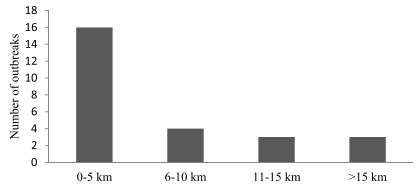


Fig. 3. The distance between domestic pig outbreak farms and the closest tested ASF-positive wild boar case within a year before an outbreak in Estonia, 2015–2017.

meaning that the contagiousness of the virus was low. Even in affected pens, some pigs were still ASF-virus-negative at the time of reporting, and in most outbreaks the infection was detected only in one unit or even in one pen. Similar findings were reported by Olševskis et al. (2016) in Latvia.

The estimates of mortality risk reported here are arbitrary as the time-periods for calculation of the mortality risk for every affected herd differed considerably (reporting 0–14 days from first symptoms, culling 1–3 days after reporting). Nevertheless, in the two largest farm-size categories (G3, G4), the herd-level and production-unit-level mortality risks were generally low. This indicates that in larger herds (G3, G4) the monitoring of general mortality is not suitable for early detection of an ASF outbreak. In smaller herds (G1, G2), the average mortality risk was considerably higher, as every case of death influenced the risk estimate markedly. However, the case fatality rate can be considered high, as most of the affected pigs died 1–5 days after the appearance of the first clinical signs, which means that an ASF epidemic may result in high mortality if there is enough time for the virus to spread within the herd.

Nevertheless, in affected and endangered regions, every sudden death of a pig with an unclear cause should be considered a possible case of ASF, and "high mortality" should not be expected at the start of an outbreak.

4.4. Probable routes of virus entry into farms and biosecurity level of the outbreak farms

Based on the collected epidemiological information, the introduction of the virus into domestic pig herds is likely to have occurred mainly by indirect transmission routes. None of the outbreaks could be linked to the introduction of infected pigs. Direct contact with potentially infected wild boar could not be completely excluded in two herds – one outdoor farm of crosses with double fencing, and one organic farm using a single fence with a walking area connected to the barn. However, even in these herds, direct contact was considered unlikely. The fencing of the outdoor farm was checked during the outbreak investigation and no damage was discovered. The organic farm was located in an open area (no forest nearby) and no direct signs or evidence of wild boar entering the farm could be identified.

Feeding of contaminated swill has generally been considered one of the main risk factors for indirect transmission of ASF (FAO, 2013; Gogin et al., 2013; Oganesyan et al., 2013). In Estonia, the feeding of swill to pigs is illegal and could be excluded as a route of virus introduction on all affected commercial farms. On backyard farms, the feeding of kitchens leftovers could not be excluded. However, swill feeding was not considered the main possible route of virus introduction, as the owners mainly consumed pig meat from their own pigs. Introduction of the virus to these farms with purchased meat products (ham, sausages etc.) from local shops would assume hidden circulation of the virus in Estonia or contamination of imported products. This was considered unlikely. According to the interview results, none of the farmers or farm workers had contacts with affected non-EU countries. Thus, the introduction of contaminated pig meat or products from these countries to outbreak farms was also considered unlikely. Another possible source of infection is contaminated wild boar meat. Limited circulation and use of uncontrolled wild boar meat cannot be excluded in Estonia.

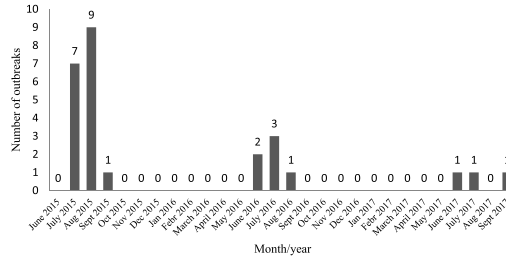


Fig. 4. Occurrence of ASF outbreaks in Estonia from June 2015 to September 2017.

Table 13
Fixed estimated parameters of the hierarchical Bayesian spatio-temporal model on a natural logarithmic scale.

			Prediction interval (quantile)		
Variable	Mean	SD	2.5%	50%	97.5%
Intercept	−6.775	0.41	−7.598	−6.764	−6.012
No. of wild boar hunted (monthly)	−0.024	0.026	−0.081	−0.022	0.022
No of ASF PCR-positive wild boar detected (monthly)	**0.132**[a,b]	**0.058**	**0.002**	**0.138**	**0.230**
No. of hunters in a district (yearly)	0.012	0.009	−0.006	0.012	0.029
No. of feeding sites (yearly)	0.015	0.024	−0.036	0.016	0.058
No. of hunting hounds (yearly)	0.015	0.067	−0.122	0.017	0.141

[a] Prediction intervals in bold indicate statistically significant parameters.
[b] Mean effect of ASF-positive wild boar detection in a hunting district on the occurrence of a domestic pig outbreak on the territory of a hunting district was estimated to be 0.132. It means that for a one unit increase in ASF-positive wild boar detection the log odds of having a domestic pig outbreak increases by 0.132 (95% prediction interval = 0.002–0.230).

However, evidence of the use of wild boar meat in affected backyard herds could not be established except for in one case, where the owner was a hunter. Thus, most likely, the virus has entered affected herds by means of contaminated fomites – clothing, vehicles, feed and bedding material – due to inadequate biosecurity measures or errors in the implementation of these measures.

For most outbreaks, there was no single obvious cause or event that could be linked with the introduction of the virus. In most affected backyard farms, there were several biosecurity gaps at the time (e.g. lack of functional disinfection barriers, no separation of inside and outside zones, pet access or housing other farm animals together with pigs, feeding grass to pigs, unsafe storage of bedding material and feed etc.). It is difficult to single out one particular cause. In commercial herds, which followed relatively high biosecurity protocols, the route of virus introduction was difficult to trace. Seemingly minor errors in the implementation of (generally adequate) disinfection procedures must have led to the introduction of the virus.

The majority of outbreaks occurred on farms with either a low or very low biosecurity level. However, looking at commercial farms separately, it appears that those farms with at least a moderate biosecurity level experienced outbreaks to the same extent as those with low and very low biosecurity levels. It is generally assumed that low biosecurity level farms are at higher risk of introduction of infections. Based on available data, it was not possible to estimate whether herds with a low biosecurity level were at higher risk or not as information about the distribution of biosecurity levels for the whole population is lacking. However, assuming that the biosecurity level is in general higher on commercial farms than on backyard farms, our data on herd incidence do not support the general opinion that a higher biosecurity level ensures a lower risk of ASF introduction (see below). This may mean that the biosecurity measures applied so far (physical and disinfection barriers) are not fully effective in protecting against the incursion of ASF virus.

4.5. Herd incidence

The herd incidence estimates are dependent on the accuracy of reporting. The observed herd incidence risk was significantly higher in the group of commercial herds in years 2015 and 2017 and did not differ significantly from the incidence risk in non-commercial (backyard) herds in 2016. One may question whether the reporting in the group of backyard farms was as good as for commercial farms or not. Considering the availability of veterinary services in Estonia (there are veterinarians available for every animal keeper), and the usual habits of smallholders to invite a veterinarian to check diseased animals, we would assume, at worst, only a slightly lower level of reporting in backyard herds compared to commercial farms. Surveillance (including serological and PCR testing) of herds located in restriction zones (areas where infection in wild boar or domestic pigs has been detected) has not revealed any case of undetected infection in domestic pigs (data not shown).

The observed herd incidence risk in commercial herds (G2–G4) decreased significantly in 2016 and 2017, compared to 2015. This is likely the result of improvements in biosecurity measures on farms, and more stringent surveillance by the veterinary authorities regarding the fulfilment of legal requirements on biosecurity. Interestingly, the total herd incidence across all herds did not change significantly. However, we might expect that there was some reporting bias for the group of backyard herds (G1) in 2015 as the owners might have not recognised or reported the disease if it was limited to the sudden death of just one or two pigs.

4.6. Spatial and temporal distribution of outbreak farms and associations between ASF outbreak farms and wild boar

Similarly to Latvia in 2014 (Olševskis et al., 2016), the vast majority of outbreaks in Estonian domestic pigs occurred in areas where ASF had been found in wild boar prior to detection of the virus in domestic herds. In 23 outbreaks, the virus had been circulating among wild boar within a radius of 15 km from the affected farm, and in 16 outbreaks, within a radius of 5 km from the affected farm. On the island of Saaremaa, the infection was first discovered in a domestic pig herd. However, a couple of days after the reporting of this case in domestic pigs, two infected wild boar carcasses were found 3 km and 10 km respectively from the outbreak farm. The age of these carcasses indicates that the virus was present in the wild boar population for some time before the outbreak in domestic pigs occurred.

According to the spatio-temporal analysis, the occurrence of outbreaks in domestic pigs was associated with the intensity of the infection in the wild boar population – the outbreaks occurred in areas where there were more virus-positive (as detected by PCR) cases in wild boar registered prior to the outbreak. There was no significant association with hunting intensity; this might be since there is minimal interaction between hunters and pig producers.

The introduction of ASF virus into domestic herds has been strictly seasonal in Estonia and associated with the warmest period of the year – June to September. Most of the outbreaks (81%) were detected in July and August. A similar seasonal trend has also been observed in other infected EU countries (Olševskis et al., 2016; EFSA (European Food Safety Authority) et al., 2017). One explanation for this seasonality might be that during the summer months, contact between farms (people and vehicles) and the wild boar in the surrounding environment is much more frequent because of the seasonal nature of field work. The high-risk period for introduction of the virus into domestic pig herds coincides with the harvest period, when wild boar also move to feed in the fields. This is also the period when wild boar density is highest (period after breeding season), and additionally, the number of infected wild boar is also at its highest, which indicates infection pressure. All these factors may increase the probability of transmission via contaminated fomites.

The high season of ASF outbreaks in domestic herds also coincides with the high season of blood-sucking insects in Estonia, suggesting their potential role in transmitting the virus from wild boar to pigs. However, there is very little scientific evidence regarding the capacity of mechanical insect vectors to transmit the ASF virus. Furthermore, if this was to be an important transmission route, many more outbreaks should be expected in domestic herds, as should a faster spread of the infection within herds. Nevertheless, the role of insect vectors in transmission of the virus is still not clear and needs further investigation.

5. Conclusions

The results of this study suggest that the presence of ASF virus in wild boar populations is the main risk for domestic pig farms becoming infected. Farms of all sizes and types are at risk, including large commercial farms operating at a high biosecurity level. Farms with breeding animals seem to be at higher risk of becoming infected. Despite the high virulence of the circulating virus strain, the clinical manifestation of the disease has initially been unspecific and mild in most herds. The spread of the virus within farms has been slow, and the contagiousness of the virus has been relatively low.

Conflict of interests

The authors of this research paper have no financial or personal interests that could have influenced this paper.

Acknowledgements

This work was funded through the Estonian Universisty of Life Sciences grant No 170193. The authors would like to thank the veterinary officials and members of the local disease control teams, farmers, field veterinarians and diagnosticians, who provided data on the outbreaks. We thank Olle Antson, from the NAR, for promptly responding to all our data requests. We thank Peep Männil, from the Estonian Environmental Agency, for providing data regarding hunting and hunting practices. We thank also Katrin Lõhmus, from the Estonian Veterinary and Food Laboratory, for her work in preparation of laboratory data. Furthermore, we would like to thank Andrey Gogin and José Cortinas Abrahantes, from the European Food Safety Authority (EFSA), for help with spatio-temporal modelling.

References

ADNS, 2017. Animal Disease Notification System Database. (Accessed: January 2017). https://webgate.ec.europa.eu/ADNS/sec/?event=sec.login.

Blome, S., Gabriel, G., Dietze, K., Breithaupt, A., Beer, M., 2012. High virulence of African swine fever virus Caucasus isolate in European wild boars of all ages. Emerg. Infect. Dis. 18, 708. https://doi.org/10.3201/eid1804.111813.

Costard, S., Mur, L., Lubroth, J., Sanchez-Vizcaino, J.M., Pfeiffer, D.U., 2013. Epidemiology of African swine fever virus. Virus Res. 173, 191–197. https://doi.org/10.1016/j.virusres.2012.10.030.

Dohoo, I., Martin, W., Stryhn, H., 2009. Veterinary Epidemiologic Research, 2nd ed. VER Inc., Charlottetown, Canada.

EFSA (European Food Safety Authority), Depner, K., Gortazar, C., Guberti, V., Masiulis, M., More, S., Olševskis, E., Thulke, H.H., Viltrop, A., Woźniakowski, G., Cortiñas Abrahantes, J., Gogin, A., Verdonck, F., Dhollander, S., 2017. Scientific report on the epidemiological analyses of African swine fever in the Baltic States and Poland. EFSA J. 15, 1–59.

European Commission, 2002. COUNCIL Council DIRECTIVE Directive 2002/60/EC of 27 June 2002 Laying Down Specific Provisions for the Control of African Swine Fever and Amending Directive 92/ 119/EEC as Regards Teschen Disease and African Swine Fever.

European Commission, 2003. Commission Decision 2003/422/EC of 26 May 2003 Approving an African Swine Fever Diagnostic Manual.

European Unión Laboratory for African Swine Fever, Animal Health Research Center, (CISA-INIA), 2014. African Swine Fever (ASF) Antibody Detection. (Accessed: June 2017). http://asf-referencelab.info/asf/en/procedures-diagnosis/diagnostic-procedures.

FAO, 2013. African Swine Fever in the Russian Federation: Risk Factors for Europe and Beyond. Empres Watch, 28, May, Rome. .

Fasina, F.O., Agbaje, M., Ajani, F.L., Talabi, O.A., Lazarus, D.D., Gallardo, C., Thompson, P.N., Bastos, A.D., 2012. Risk factors for farm-level African swine fever infection in major pig-producing areas in Nigeria, 1997-2011. Prev. Vet. Med. 107, 65–75. https://doi.org/10.1016/j.prevetmed.2012.05.011.

Gallardo, C., Nieto, R., Soler, A., Pelayo, V., Fernandez-Pinero, J., Markowska-Daniel, I., Pridotkas, G., Nurmoja, I., Granta, R., Simon, A., Perez, C., Martin, E., Fernandez-Pachhero, P., Arias, M., 2015a. Assessment of African Swine Fever Diagnostic Techniques as a Response to the Epidemic Outbreaks in Eastern European Union Countries: How To Improve Surveillance and Control Programs. J. Clin. Micr. 53, 2555–2565. https://doi.org/10.1128/JCM.00857-15.

Gallardo, C., Soler, A., Nieto, R., Cano, C., Pelayo, V., Sánchez, M.A., Pridotkas, G., Fernandez-Pinero, J., Briones, V., Arias, M., 2015b. Experimental infection of domestic pigs with African swine fever virus Lithuania 2014 genotype II field isolate. Transbound. Emerg. Dis. 64, 300–304. https://doi.org/10.1111/tbed.12346.

Gallardo, C., Nurmoja, I., Soler, A., Delicado, V., Simón, A., Martin, E., Perez, C., Nieto, R., Arias, M., 2018. Evolution in Europe of African swine fever genotype II viruses from highly to moderately virulent. Vet. Microbiol. 219, 70–79. https://doi.org/10.1016/j.vetmic.2018.04.001.

Gogin, A., Gerasimov, V., Malogolovkin, A., Kolbasov, D., 2013. African swine fever in the North Caucasus region and the Russian Federation in years 2007–2012. Virus Res. 173, 198–203. https://doi.org/10.1016/j.virusres.2012.12.007.

Mur, L., Sánchez-Vizcaíno, J.M., Fernández-Carrión, E., Jurado, C., Rolesu, S., Feliziani, F., Laddomada, A., Martinez-Lopez, B., 2018. Understanding African swine fever infection dynamics in Sardinia using a spatially explicit transmission model in domestic pig farms. Transbound. Emerg. Dis. 65, 123–134. https://doi.org/10.1111/tbed.12636.

Nurmoja, I., Petrov, A., Breidenstein, C., Zani, L., Forth, J.H., Beer, M., Kristian, M., Viltrop, A., Blome, S., 2017. Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. Transbound. Emerg. Dis. 64 (December (6)), 2034–2041. https://doi.org/10.1111/tbed.12614. 2017, Epub 2017 Jan 24.

Oganesyan, A.S., Petrova, O.N., Korennoy, F.I., Bardina, N.S., Gogin, A.E., Dudnikov, S.A., 2013. African swine fever in the Russian Federation: spatio-temporal analysis and epidemiological overview. Virus Res. 173, 204–211. https://doi.org/10.1016/j.virusres.2012.12.009.

OIE, WAHID, 2017. World Animal Health Information Database. (Accessed: in December

2017). http://web.oie.int/wahis/public.php?page=home.
Olševskis, E., Guberti, V., Seržants, M., Westergaard, J., Gallardo, C., Rodze, I., Depner, K., 2016. African swine fever virus introduction into the EU in 2014: experience of Latvia. Res. in Vet. Sci. 105, 28–30. https://doi.org/10.1016/j.rvsc.2016.01.006.
Penrith, M.-L., Vosloo, W., Jori, F., Bastos, A.D.S., 2013. African swine fever virus eradication in Africa. Virus Res. 173, 228–246. https://doi.org/10.1016/j.virusres.2012.10.011.
Sánchez-Vizcaíno, J.M., Martínez-López, B., Martínez-Avilés, M., Martins, C., Boinas, F., Vial, L., Michaud, V., Jori, F., Etter, E., Albina, E., Roger, F., 2009. Scientific report on African swine fever. EFSA Support. Publ. 6, 1–141.
Teataja, Riigi, 1999. Loomatauditõrje seadus (Animal Disease Control Act). Riigi Teataja 57, 598 (in Estonian).
Riigi Teataja, 2004. Sigade klassikalise katku ja sigade Aafrika katku tõrje eeskiri. Riigi Teataja Lisa, 150, 2277 (in Estonian).
Tignon, M., Gallardo, C., Iscaro, C., Hutet, E., Van der Stede, Y., Kolbasov, D., De Mia, G.M., Le Potier, M., Bishop, R.P., Arias, M., Koenen, F., 2011. Development and inter-laboratory validation study of an improved new real-time PCR assay with internal control for detection and laboratory diagnosis of African swine fever virus. J. Vir. Meth. 178, 161–170. https://doi.org/10.1016/j.jviromet.2011.09.007.
Varewyck, M., Verbeke, T., Cortinas Abrahantes, J., 2017. Exploratory Analysis for Spatio-Temporal Epidemiological Data WEB App (Spatial). Zenodohttps://doi.org/10.5281/zenodo.889551.
Zani, L., Forth, J.H., Forth, L., Nurmoja, I., Leidenberger, S., Henke, J., Carlson, J., Breidenstein, C., Viltrop, A., Höper, D., Sauter-Louis, C., Beer, M., Blome, S., 2018. Deletion at the 5'-end of Estonian ASFV strains associated with an attenuated phenotype. Sci. Rep. 8, 6510. https://doi.org/10.1038/s41598-018-24740-1.

CURRICULUM VITAE

First name Imbi
Surname Nurmoja
Date of birth 09.12.1968
E-mail imbi.nurmoja@vetlab.ee / imbi.nurmoja@gmail.com

Institution and occupation

2012-….	Veterinary and Food Laboratory, deputy director on animal health (1.00)
2008-2009	Veterinary and Food Laboratory, adviser on animal health (1.00)
2003-2008	Veterinary and Food Laboratory, deputy director on animal health (1.00)
1997-2002	Veterinary and Food Laboratory, head of the regional laboratory (1.00)
1993-1997	Veterinary and Food Laboratory, veterinarian-microbiologist (1.00)

Education

2015-2021	Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Doctoral studies
1987-1992	Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, degree of veterinary medicine
1980-1987	14th Secondary School of Tartu (Gymnasium)
1976-1980	10th Secondary School of Tartu

R&D related managerial and administrative work

2012-….	The responsible specialist of NRL for African and Classical swine fever in Estonia
2012-….	A member of the State Animal Disease Control Committee

2013-….. OIE (The World Organisation for Animal Health) National Focal Points for Veterinary Laboratories

Participation in scientific projects

No 862874- Horizon 2020 project "A safe DIVA vaccine for African Swine Fever control and eradication" (VACDIVA). Duration: 10.2019 – 09.2023. Project leader: Centre for Veterinary Health Surveillance (VISAVET) of the Complutense University of Madrid (UCM), Spain, Prof. José Manuel Sánchez-Vizcaíno. https://vacdiva.eu/about-vacdiva/

No 773830- Horizon 2020 OneHealth EJP FBZ2.2 project „Biosecurity practices for pig farming across Europe" (BIOPIGEE). Duration: 01.2020– 06.2022. Project leader: Federal Institute for Risk Assessment (BfR), Berlin, Germany. https://onehealthejp.eu/jrp-biopigee/

CA 15116- COST Action "Understanding and combating African swine fever in Europe" (ASF-STOP). Duration: 05.2016-04.2020. Project leader: National Veterinary Institute, Uppsala, Sweden. Prof. Dolores Gavier-Widén. https://www.asf-stop.com/

No 3.2-3/200- Collaboration project "Epidemiological investigations of ASF in Estonia and Latvia". Duration: 06.2016-06.2018. Project leader: Friedich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Insel Riems, Germany. Dr Carola Sauter-Louis.

ELULOOKIRJELDUS

Eesnimi	Imbi
Perekonnanimi	Nurmoja
Sünniaeg	09.12.1968
E-post	imbi.nurmoja@vetlab.ee/ imbi.nurmoja@gmail.com

Töökohad ja ametid

2012-….	Veterinaar- ja Toidulaboratoorium, direktori asetäitja loomahaiguste alal (1.00)
2008-2009	Veterinaar- ja Toidulaboratoorium, nõunik loomahaiguste alal (1.00)
2003-2008	Veterinaar- ja Toidulaboratoorium, direktori asetäitja loomahaiguste alal (1.00)
1997-2002	Veterinaar- ja Toidulaboratoorium, piirkondliku laboratooriumi juhataja (1.00)
1993-1997	Veterinaar- ja Toidulaboratoorium, veterinaararst-mikrobioloog (1.00)

Haridustee

2015-2021	Eesti Maaülikool, veterinaarmeditsiini ja loomakasvatuse instituut, doktoriõpe
1987-1992	Eesti Põllumajandusülikool, veterinaaria eriala
1980-1987	Tartu 14. Keskkool
1976-1980	Tartu 10. Keskkool

Teadusorganisatsiooniline ja -administratiivne töö

2012-….	Riikliku referentlaboratooriumi sigade Aafrika katku ja sigade klassikalise katku alal vastutav spetsialist
2012-….	Riikliku loomatauditõrjekomisjoni liige
2013-…..	OIE (Maailma Loomatervise Organisatsiooni) riiklik kontaktisik veterinaarlaborite valdkonnas

Osalemine uurimisprojektides

No 862874- Horisont 2020 projekt " Ohutu DIVA vaktsiin sigade Aafrika katku järelevalveks ning tõrjeks" (VACDIVA). Kestvus: 10.2019 – 09.2023. Projekti juhtpartner: Centre for Veterinary Health Surveillance (VISAVET) of the Complutense University of Madrid (UCM), Hispaania, Prof. José Manuel Sánchez-Vizcaíno. https://vacdiva.eu/about-vacdiva/

No 773830- Horisont 2020 OneHealth EJP FBZ2.2 projekt „Seafarmide bioturvalisuse praktikad Euroopas" (BIOPIGEE). Kestvus: 01.2020–06.2022. Projekti juhtpartner: Federal Institute for Risk Assessment (BfR), Berlin, Saksamaa. https://onehealthejp.eu/jrp-biopigee/

CA 15116- COST Action "Sigade Aafrika katku tundma õppimine ja võitlus selle vastu Euroopas" (ASF-STOP). Kestvus: 05.2016-04.2020. Projekti juhtpartner: National Veterinary Institute, Uppsala, Rootsi. Prof. Dolores Gavier-Widén. https://www.asf-stop.com/

No 3.2-3/200- Koostööprojekt "Sigade Aafrika katku epidemioloogilised uuringud Eestis ja Lätis". Kestvus: 06.2016-06.2018. Projekti juht: Friedich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Insel Riems, Saksamaa. Dr

LIST OF PUBLICATIONS

1.1. Scholarly articles indexed by Web of Science Science Citation Index Expanded, Social Sciences Citation Index, Arts & Humanities Citation Index and/or indexed by Scopus (excluding chapters in books)

Nurmoja, I., Mõtus, K., Kristjan, M., Niine, T., Schulz, K., Depner, K., Viltrop, A., 2020. Epidemiological analysis of the 2015–2017 African swine fever outbreaks in Estonia. Prev. Vet. Med. 181, 104556. doi.org/181.10.1016/j.prevetmed.2018.10.001.

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. Prev. Vet. Med. 182, 105121. doi.org/10.1016/j.prevetmed.2020.105121.

Schulz, K., Conraths, F.J., Staubach, C., Viltrop, A., Olsevskis, 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. Transbound. Emerg. Dis. 67, 1816-1819 doi.org/.10.1111/tbed.13560.

Vilem, A., Nurmoja, I., Niine, T., Riit, T., Nieto, R., Viltrop, A., Gallardo, C., 2020. Molecular characterization of African swine fever virus isolates in Estonia in 2014-2019. Pathog. 9, 582. doi.org/10.3390/pathogens9070582.

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. doi.org/10.3390/vaccines8020336.

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. Sci. Rep. 9, 8490. doi.org/10.1038/s41598-019-44890-0.

Gallardo, C., Nurmoja, I., Soler, A., Delicado, V., Simon, A., Martin, E., Perez, C., Nieto, R., Arias, M., 2018. Evolution in Europe of African swine fever genotype II viruses from highly to moderately virulent. Vet. Microbiol. 219, 70−79. doi.org/10.1016/j.vetmic.2018.04.001.

Zani, L., Forth, J.H., Forth, L., Nurmoja, I., Leidenberger, S., Henke, J., Carlson, J., Breidenstein, C., Viltrop, A., Hoper, D., Sauter-Louis, C., Beer, M., Blome, S., 2018. Deletion at the 5 '-end of Estonian ASFV strains associated with an attenuated phenotype. Sci. Rep. 8, 6510. doi.org/10.1038/s41598-018-24740-1.

Carlson, J., Zani, L., Schwaiger, T., Nurmoja, I., Viltrop, A., Vilem, A., Beer, M., Blome, S., 2018. Simplifying sampling for African swine fever surveillance: assessment of antibody and pathogen detection from blood swabs. Transbound. Emerg. Dis. 65, e165−e172. doi.org/10.1111/tbed.12706.

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. Sci. Rep. 7, 12562. doi.org/10.1038/s41598-017-12952-w.

Nurmoja, I., Petrov, A., Breidenstein, C., Zani, L., Forth, J.H., Beer, M., Kristian, M., Viltrop, A., Blome, S., 2017. Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. Transbound. Emerg. Dis. 64, 2034–2041. doi.org/10.1111/tbed.12614.

Gallardo, C., Nieto, R., Soler, A., Pelayo, V., Fernandez-Pinero, J., Markowska-Daniel, I., Pridotkas, G., Nurmoja, I., Granta, R., Simon, A., Perez, C., Martin, E., Fernandez-Pacheco, P., Arias, M., 2015. Assessment of African swine fever diagnostic techniques as a response to the epidemic outbreaks in eastern European Union countries: how to improve surveillance and control programs. J. Clin. Microbiol. 53, 2555−2565. doi.org/10.1128/JCM.00857-15.

Kramarenko, T., Nurmoja, I., Kärssin, A., Meremäe, K., Hörman, A., Roasto, M., 2014. The prevalence and serovar diversity of *Salmonella* in various food products in Estonia. Food Control 42, 43−47. doi.org/10.1016/j.foodcont.2014.01.032.

Raaperi, K., Nurmoja, I., Orro, T., Viltrop, A., 2010. Seroepidemiology of bovine herpesvirus 1 (BHV1) infection among Estonian dairy herds and risk factors for the spread within herds. Prev. Vet. Med. 96, 74−81. doi.org/10.1016/j.prevetmed.2010.06.001.

1.3. Scholarly articles in Estonian and other peer-reviewed research journals with a local editorial board; peer-reviewed scientific articles in journals important for Estonian culture or scholarly articles in Akadeemia, Looming, Vikerkaar

Nurmoja, I., Kristian, M., Viltrop, A., 2016. Sigade Aafrika katk *(Pestis Africana Suum*). Agraarteadus XXVII, 76−82.

3.2. Articles/chapters in books published by the publishers not listed in Annex

Nurmoja, I., Mõtus, K., Kristian, M., Schulz, K., Depner, K., Viltrop, A., 2018. African swine fever in domestic pigs in Estonia 2015-2017: epidemiological analysis of outbreaks. Proceedings of the Society of veterinary epidemiology and preventive veterinary medicine annual meeting, 231−243. Edited by Brennan, M.L., Lindberg, A. Society for veterinary epidemiology and preventive medicine.

3.4. Articles/presentations published in conference proceedings not listed in Section 3.1

Raaperi, K., Viltrop, A., Orro, T., Must, K., Mähar, K., Nurmoja, I., 2008. Seroprevalence of bovine herpesvirus 1 (BHV1) infection among Estonian dairy cattle. Proceedings of the 9th Middle European Buiatrics Congress, 202-205. Edited by Szenci, O., Brydl, E. A/3 Press Ltd.

3.5. Articles/presentations published in local conference proceedings

Nurmoja, I., Viltrop, A., Kristian, M., 2020. Five years of African swine fever in Estonia: how close we are to freedom? Final international conference of the COST Action ASF-STOP - Understanding and Combating African swine fever in Europe, 29-30 January 2020, Brescia, Italy. In abstract book, 40.

Schulz, K., Urner, N., Mõtus, K., Nurmoja, I., Schulz, J., Staubach, C., Conraths, F.J., Sauter-Louis, C., 2020. A participatory approach to support the control of African swine fever in wild boar. Final international conference of the COST Action ASF-STOP-Understanding and Combating African Swine Fever in Europe, 29-30 January 2020, Brescia, Italy. In abstract book, 24.

Schulz, K., Oļševskis, E., Staubach, C., Nurmoja, I., Blome, S., Lamberga, K., Seržants, M., Viltrop, A., Conraths, F.J., Sauter-Louis, C., 2019. Is the African swine fever epidemic in Estonian and Latvian wild boar subsiding? Epidemiological analyses of surveillance data. 13th EPIZONE Annual Meeting, 26-28 August 2019, Berlin, Germany. In abstract book, 101.

Nurmoja, I., Mõtus, K., Kristian, M., Niine, T., Schulz, K., Depner, K., Viltrop, A., 2019. African swine fever in domestic pigs-Epidemiological insights from Estonia. 13th EPIZONE Annual Meeting, 26-28 August 2019, Berlin, Germany. In abstract book, 67.

Gallardo, C., Soler, A., Rozde, I., Losa, Veronia D., Nurmoja, I., Woźniakowski, G., Nieto, R., Arias, M., 2018. Attenuated and non-haemadsorbing African swine fever (ASF) genotype II European isolate induces subclinical to chronic infection. 11th International Congress for Veterinary Virology – ESVV 2018, jointly with the 12th Annual Meeting of EPIZONE, 27-30 August 2018, Vienna, Austria. In abstract book, 41.

Depner, K., Viltrop, A., Evert, E., Tummeleht, L., Nurmoja, I., Knoll, B., Globig, A., Guberti, V., Conraths, F.J., Probst, C., 2018. Behaviour of wild boar towards soil underneath and close to wild boar carcasses – potential epidemiological relevance for the local perpetuation of

African swine fever. 4th Annual GARA Scientific Workshop, 11-13 April 2018, Cagliari-Sardinia, Italy. In abstract book, 13.

Gallardo, C., Soler, A., Rodze, I., Delicado, V., Nurmoja, I., Wozniakowski, G., Simon, A., Martin, E., Perez, C., Nieto, R., Arias, M., 2018. Evolution in Europe of African swine fever genotype II viruses from highly to non haemadsorbing (non-HAD) attenuated strains. 4th Annual GARA Scientific Workshop, 11-13 April 2018, Cagliari-Sardinia, Italy. In abstract book, 26.

Nurmoja, I., Vilem, A., Riit, T., Nieto, R., Gallardo, C., Viltrop, A., 2018. Molecular characterization of African Swine Fever Virus (ASFV) strains circulating in Estonia. 4th Annual GARA Scientific Workshop, 11-13 April 2018, Cagliari-Sardinia, Italy. In abstract book, 51.

Niine, T., Nurmoja, I., Viltrop, A., 2018. Sigade Aafrika katku puhangute kodusigadel tekkimise seos haigusjuhtude arvuga metssigadel, jahipidamise ja metsaraie intensiivsusega. Terve loom ja tervislik toit 2018. Toim. Marko Kass. Tartu: Eesti Maaülikool, 52−56.

Carlson, J., Zani, L., Henke, J., Leidenberger, S., Nurmoja, I., Tummeleht, L.,; Viltrop, A., Beer, M., Blome, S., 2017. Soil as a vector for African swine fever virus in wild boar populations. 11th EPIZONE Annual Meeting, 19-21 September 2017, Paris, France. In abstract book, 72.

Zani, L., Kauer, L., Forth, J. H., Leidenberger, S., Carlson, J., Nurmoja, I., Viltrop, A., Beer, M., Höper, D., Blome, S., 2017. Deletion at the 5'-end of Estonian ASFV strains associated with an attenuated phenotype? 11th EPIZONE Annual Meeting: EPIZONE, 19-21 September 2017, Paris, France. In abstract book, 69.

Cano-Gómez, C., Nieto, R., Soler A., Nurmoja I., Pridotkas G., Arias M., Gallardo C., Fernández-Pinero J., 2017. Full genome sequencing of African swine fever virus using targeted sequence capture technology. 18th WAVLD symposium, 7-10 June 2017, Sorrento, Italy.

Nurmoja, I., Kristian, M., Mõtus, K., Viltrop, A., 2016. African swine fever in domestic pigs in Estonia in 2015 and 2016: epidemiological analysis of outbreak investigations. ASF-STOP International Launch

Conference, 6-8 December 2016, Pulawy, Poland. In abstract book, 41-42.

Zani, L., Petrov, A., Nurmoja, I., Forth, J.H., Beer, M., Blome, S., 2016. Experience from recent animal trials using recent genotype I and II ASFV strains – implications for epidemiology and control. ASF-STOP International Launch Conference, 6-8 December 2016, Pulawy, Poland. In abstract book, 23.

Gallardo, C., Soler, A., Delicado, V., Nurmoja, I., Simon, A., Nieto, R., Briones, V., Arias, M., 2016. *In vivo* experimental studies of genotype II African swine fever virus (ASFV) isolates currently circulating in two Estonian counties. 10th EPIZONE Annual Meeting, 27-29 September 2016, Madrid, Spain. In abstract book, 80.

Nieto, R., Soler, A., Nurmoja, I., Pelayo, V., Pridotkas, G., Rozde, I., Kowalczyk, A., Markowska-Daniel, I., Pérez, C., Simón, A., Martín, E., Fernandez-Pinero, J., Arias, M., Gallardo, C., 2016. Molecular Characterization of African swine fever virus (ASFV) isolates circulating in the eastern European Union countries 2014-2016. 10th EPIZONE Annual Meeting, 27-29 September 2016, Madrid, Spain. In abstract book, 164.

Zani, L., Nurmoja, I., Breidenstein, C., Leidenberger, S., Beer, M., Blome, S., 2016. First evidence of an attenuated phenotype of genotype II African swine fever virus in Estonia. 3rd Annual GARA Scientific Workshop, 6-8 September 2016, Ploufragan, France. In abstract book, 18.

Nurmoja, I., Kristian, M., Blome, S., Gallardo, C., Viltrop, A., 2016. African swine fever virus spread in Estonia: the preliminary results of epidemiological analysis. 3rd Annual GARA Scientific Workshop, 6-8 September 2016, Ploufragan, France. In abstract book, 32.

5.2. Conference abstracts that do not belong to section 5.1

Vilem, A., Nurmoja, I., Riit, T., Viltrop, A., 2019. Sigade Aafrika katku viiruse Eestis ringlevate tüvede molekulaarne iseloomustus. Konverentsi „Terve Loom ja Tervislik Toit" artiklite kogumik, 88−91. Toimetaja Kass, M. Vali Press OÜ.

Niine, T., Nurmoja, I., Viltrop, A., 2018. Estimating Africa swine fever outbreaks in domestic pigs by using Bayesian hierarchical models. 36th SVEPM Annual Conference, 21-23 March 2018, Tallinn, Estonia.

Nurmoja, I., 2015. Sigade Aafrika katk Eestis. Konverents "Veterinaarmeditsiin", 30-31 October 2015, Tartu, Estonia.

6.3. Popular science articles

Nurmoja, I., Kristian, M., 2014. Sigade Aafrika katku levik ning raskused selle ennetamisel ja tõrjel Ida-Euroopas aastatel 2007-2014. Eesti Loomaarstlik Ringvaade 2, 10−14.

Nurmoja, I. Kristian, M. Viltrop, A., 2013. Lammaste katarraalse palaviku serotüüp 14 vaktsiinitüve levik Venemaal, Baltikumis ja Poolas. Eesti Loomaarstlik Ringvaade 2, 14−17.

6.6. Articles in other journals and newspapers

Nurmoja, I., Viltrop, A., Kristian, M., 2018. Sigade Aafrika katkust. Eesti Jahimees 3, 61-63.

VIIS VIIMAST KAITSMIST

MARIANA MAANTE-KULJUS
VIINAPUU (*VITIS* SP) SAAGI KÜPSUSNÄITJAD
MATURITY PARAMETERS OF GRAPEVINE (*VITIS* SP) YIELD
Professor **Kadri Karp**, dotsent **Leila Mainla**, dotsent **Ele Vool**
24. mai 2021

BURAK ÖĞLÜ
KLIIMAMUUTUSTE JA TEISTE ÖKOLOOGILISTE TEGURITE MÕJU VALITUD KALALIIKIDE POPULATSIOONIDELE JA KALAPÜÜGILE EESTI SUURJÄRVEDES
IMPACT OF CLIMATE CHANGE AND OTHER ECOLOGICAL FACTORS ON SELECTED FISH POPULATIONS AND FISHERY IN ESTONIAN LARGE LAKES
Tanel Kaart, Külli Kangur, Fabien André Daniel Cremona
28. mai 2021

TARMO PILVING
KOOSTÖÖ EESTI MAATURISMIS
COLLABORATION IN ESTONIAN RURAL TOURISM
Vanemteadur **Ants-Hannes Viira**, professor **Tiiu Kull**, nooremprofessor **Monika Suškevičs**
11. juuni 2021

MARIKA KOSE
RANNANIIDUD: SÄILIMINE, TAASTAMINE JA TAASTUMINE
COASTAL MEADOWS: MAINTENANCE, RESTORATION AND RECOVERY
Vanemteadur **Karin Kauer**, vanemteadur **Kadri Tali**
11. juuni 2021

ELINA KARRON
ERINEVATE KASVATUSTEHNOLOOGIATE MÕJU FUSARIUM SPP. ESINEMISELE JA MÜKOTOKSIINIDE TEKKIMISELE TERAVILJADEL
INFLUENCE OF CULTIVATION TECHNOLOGIES ON PATHOGENIC FUSARIUM SPP. OCCURRENCE AND PRODUCTION OF MYCOTOXINS IN CEREALS
Dotsent **Eve Runno-Paurson**, professor **Ülo Niinemets**, emeriitdotsent **Enn Lauringson**
15. juuni 2021

ISSN 2382-7076
ISBN 978-9949-698-89-9 (trükis)
ISBN 978-9949-698-90-5 (pdf)