AFRICAN HORSE SICKNESS: THE POTENTIAL FOR AN OUTBREAK IN DISEASE-FREE REGIONS AND CURRENT DISEASE CONTROL AND ELIMINATION TECHNIQUES

LIST OF ABBREVIATIONS

AHS	African horse sickness
AHSV	African horse sickness virus
BT	Bluetongue
BTV	Bluetongue virus
OIE	World Organisation for Animal Health

INTRODUCTION

African horse sickness (AHS) is an infectious, non-contagious, vector-borne viral disease of equids. Possible references to the disease have been found from several centuries ago, however the first recorded outbreak was in 1719 amongst imported European horses in Africa [1]. AHS is currently endemic in parts of sub-Saharan Africa and is associated with case fatality rates of up to 95% in naïve populations [2]. No specific treatment is available for AHS and vaccination is used to control the disease in South Africa [3; 4]. Due to the combination of high mortality and the ability of the virus to expand out of its endemic area without warning, the World Organisation for Animal Health (OIE) classifies AHS as a listed disease. Official AHS disease free status can be obtained from the OIE on fulfilment of a number of requirements and the organisation provides up-to-date detail on global disease status [5].

AHS virus (AHSV) is a member of the genus Orbivirus (family Reoviridae) and consists of nine different serotypes [6]. All nine serotypes of AHSV are endemic in sub-Saharan

Africa and outbreaks of two serotypes have occurred elsewhere [3]. Major epizootics associated with AHSV-9 were reported in the Middle East, western Asia and India [7; 8] in 1959-1961, and in North Africa and Spain in 1965-1966 [9]. A second epizootic occurred in the western Mediterranean region (Spain, Portugal and Morocco) during 1987-1991, this time caused by AHSV-4. [10]. There have been no further outbreaks in Europe. However, there have been recent epizootics caused by AHSV-2, 4, 6, 7, 8 and 9 in eastern and northern parts of Africa [11; 12].

The principal vectors for transmission of AHSV are *Culicoides* biting midges, which are ubiquitous on farms throughout most of the inhabited world [13; 14]. The geographical distribution and seasonal occurrence of AHS are entirely dependent on those of the vector and the dynamics and behaviour of *Culicoides* are therefore essential to understanding the disease [15].

It has been suggested that recent changes in the global distribution of several vector-borne viral diseases may be associated with climate change and the increasing international movement of animals and animal products [16]. This has led to concerns that some vector-borne diseases, including AHS, will increasingly threaten parts of the world currently considered disease-free [17-19]. This review will discuss key aspects of AHS, focusing in particular on the evidence to support concerns that an epizootic may occur in AHS-free countries and the response plans in place at the current time.

DISEASE TRANSMISSION

African horse sickness is not contagious by direct or indirect contact and biological viral transmission occurs during blood-feeding by *Culicoides*. Mechanical transmission by other biting flies may be possible, but is unlikely to play a significant role in disease

transmission [4]. Parenteral inoculation of infected blood has been shown to transmit the virus between horses, although avoiding re-use of needles and syringes and basic biosecurity measures should prevent this from posing a risk [20; 21]. African horse sickness is almost exclusively a disease of equids and is not considered zoonotic, although disease associated with the virus has been described in humans following nasal exposure to virus from broken vaccine vials [22]. Disease has also been reported in dogs (usually, but not exclusively, following ingestion of virus infected meat), which are considered dead-end hosts [23; 24].

Vector infection occurs when *Culicoides* feed on a viraemic vertebrate host. In horses, the viraemic phase typically lasts only 2-8 days; however, reservoir mammalian host species (as detailed below) have a more prolonged period of infectivity [4]. Following ingestion by a vector-competent female *Culicoides*, the virus replicates in the insect gut then translocates and replicates in the salivary glands before infection of the next mammalian host [14].

PATHOPHYSIOLOGY AND CLINICAL SIGNS

Following inoculation during vector feeding, viral replication occurs within the regional lymph nodes of the bite area before haematogenous dissemination throughout the body to the endothelial cells of multiple target tissues [25]. Viral multiplication in these tissues gives rise to a secondary viraemia of varying duration and titre, depending upon a number of host and serotype factors [3]. The underlying pathology of AHS in the target organs is vascular endothelial damage with subsequent effusion, cardiovascular compromise and haemorrhage.

The incubation period of AHS is between 2-10 days, depending on viral load, viral virulence and host factors [4]. Four different clinical forms of AHS are recognised, depending on the target organs and severity of disease [4].

PERACUTE PULMONARY FORM ('Dunkop') – The peracute form is characterised by rapidly progressive respiratory failure and usually occurs when AHSV infects fully susceptible horses. Recovery is the exception with >95% case fatality rates common [4]. Clinical signs include pyrexia (up to 41˚C), severe respiratory distress, forced expiration, profuse sweating and paroxysmal coughing [4]. The onset of dyspnoea can be sudden, with death occurring as little as 30 minutes after the onset of clinical signs (Figure 1).

CARDIAC FORM ('Dikkop'). This form is characterised by oedema, which is usually preceded by 3-4 days of pyrexia. The oedema starts in the supraorbital fossa (Figure 2), before extending to the conjunctiva (Figure 3) and then the remainder of the head and neck. The distal limbs and ventral abdomen are only rarely affected. Dyspnoea, cyanosis, signs of abdominal pain and heart failure also occur. The cardiac form is less clinically severe and more protracted than the pulmonary form, with fatality in > 50% of cases [4].

MIXED FORM – Cases with this form are found to have a combination of pathologies at post-mortem, although this is often not detected clinically. Pyrexia and mild pulmonary or subclinical cardiac disease are followed by oedema, cardiac failure or respiratory failure [4]. The mixed form is the most common and comprised the majority of cases during the 1987-1990 outbreak in Spain [10]. The case fatality rate varies in the mixed form.

HORSESICKNESS FEVER - This form of disease is associated with a mild fever that may be subclinical and is seen only in reservoir species and partially immune horses [3; 26].

DIAGNOSIS

In disease–free countries any suspected cases of AHS must be reported to the State Veterinary Service and is subject to laboratory confirmation [4]. Virus isolation is considered the gold standard for diagnosis, however the World Organisation for Animal Health (OIE) accepts molecular evidence of viral presence by polymerase chain reaction (PCR) and serological evidence of infection via enzyme-linked immunosorbant assays (ELISAs) [27]. Viral isolation is performed by inoculation of various cell cultures or mice cerebral tissue and the process can take several days, which impedes the control of disease outbreak [4]. The use of serology for initial diagnosis in an outbreak situation is limited by the rapid mortality associated with AHS. Historically though, serological testing by complement fixation, virus neutralisation and enzyme-linked immunosorbant assay has been the gold standard for identification of AHSV serotypes [28-32]. Unfortunately these methods are difficult and time consuming, requiring either virus isolation or access to reagents that may pose a potential biosecurity risk. Several PCR tests have demonstrated rapid, sensitive and reliable detection of AHSV genetic material in infected blood, tissue samples, homogenised *Culicoides*, and tissue culture supernatant and these would be essential during a disease outbreak [33-35]. Not all of the PCR methods available have currently been validated by the OIE.

Recently, type-specific PCR assays for the identification of individual AHSV serotypes have been described, which would be potentially useful for guiding appropriate vaccination and control strategies, as well as for the declaration of disease-free status

after an outbreak [36]. Serological testing to use in combination with DIVA vaccines (differentiating infected from vaccinated animals) is also currently under evaluation.

THE ROLE OF RESERVOIR MAMMALIAN SPECIES

No equids that recover from AHS remain as long-term carriers of the virus. The term 'reservoir' refers to the fact that the low mortality rate and prolonged viraemia associated with AHSV infection in these equid species allows the establishment of continuous cycling of the virus [3; 26]. This is key to the ability of AHSV to persist within endemic areas. In areas where the virus is non-endemic, it must be reintroduced (either within *Culicoides* or equids) at the start of each outbreak.

Zebra are an important reservoir host for AHSV and their role in maintaining the disease in South Africa has been well documented [26]. The ability of certain AHSV serotypes to persist intermittently in West Africa and Spain, where there are no zebra herds, suggests that other mammalian species may play a role. Donkeys almost certainly act as reservoir hosts, particularly in northern parts of Africa, and have been shown to become viraemic following inoculation with virulent AHSV strains in the absence of clinical signs [37].

For AHSV to persist in an area there must be a sufficient density of reservoir hosts for continual cycling of the virus, which relies on both climatic and geographic factors [26; 38]. While the minimum size of a reservoir herd is unknown, the incidence of AHSV is much lower in areas of South Africa where zebra herd sizes are less than 100 [26]. It is interesting to note that there were approximately 300 zebra and 10,000 donkeys in the UK in 2009, with over half of the donkeys housed at 8 sites belonging to a single charity [39]. Large donkey herds therefore exist far from AHS-affected regions, which could potentially allow maintenance of a continuous AHSV presence.

158

159 **CULICOIDES BITING MIDGES AND THEIR ROLE IN THE EPIDEMIOLOGY OF AHS**

160

161 *Culicoides* midges are among the world's smallest and most widespread insects. They
162 are considered a biting nuisance to humans and livestock, transmit viral and parasitic
163 diseases and are the major cause of insect bite hypersensitivity (IBH) in horses [40].
164 There are currently over 1400 different species of *Culicoides* identified, with around 30
165 of these thought to be capable of virus transmission and over 50 different viruses
166 isolated from midges worldwide [14; 41; 42]. Comparisons with the arboviral disease
167 bluetongue (BT) are often made when considering AHS, as the viruses share vector
168 *Culicoides* species within Africa and both have made incursions north into Europe [13;
169 16; 43]. The most relevant *Culicoides* species when considering AHSV and BT virus
170 (BTV) are shown in *Table 1*. The life-cycle of *Culicoides* includes the egg, 4 larval stages,
171 the pupa and the adult [44]. As only female adults blood-feed, they are of primary
172 importance when considering virus transmission.

173

174 Light traps are the standard sampling method for collecting *Culicoides* midges when
175 conducting epidemiological investigations and much of the evidence supporting the
176 AHSV and BTV vector roles of certain *Culicoides* species is based on associations
177 between disease occurrence and species abundance as measured by light trapping [45-
178 48]. It is poorly defined how the numbers, species composition and physiological status
179 of light trap catches relate to the *Culicoides* actually feeding on a natural host and
180 alternate methods including CO_2-baited traps and aspiration from hosts require further
181 investigation [49-53].

182

183 In Africa, the most commonly implicated AHSV vectors are *C. imicola*, which makes up
184 over 90% of species caught using light-traps in AHS endemic areas, and *C. bolitinos*

185 which has more recently been recognised as an alternative vector in some regions [41;
186 54]. It is important to consider the evidence available to support the AHSV vector roles
187 of these species. Biting insects have long been suspected to transmit AHSV and the
188 disease was first induced in horses following inoculation with *Culicoides* extract in 1944
189 [13]. The ability of *Culicoides* to actually transmit AHSV was more convincingly
190 demonstrated when the North American BTV vector, *C. variipennis* (now *C. sonorensis*),
191 was shown to be an efficient laboratory vector for AHSV following oral inoculation [55].
192 Remarkably, transmission between live equid hosts has still not been demonstrated for
193 any *Culicoides* species. Epidemiological studies have added some evidence to support
194 this theory by demonstrating spatial and temporal associations between the abundance
195 of *C. imicola* (as caught by light traps) and the incidence of AHS in Spain, Portugal,
196 Morocco and South Africa [45-48].
197
198 Traditionally, *Culicoides* species are identified based on several morphological traits.
199 The wing pattern in particular is very important, with variations in venation, colour,
200 marking pattern and covering by short hairs used for differentiation. Other features,
201 including thoracic colouring, antennae and abdominal spermathecae, are also used [56;
202 57]. Unfortunately, identification of many species requires a specialised knowledge of
203 insect morphology that is no longer readily available [58; 59]. Given the importance of
204 several of these species in arboviral transmission, polymerase chain reaction (PCR)
205 assays have recently been developed to provide rapid and accurate identification [59;
206 60].
207
208 The ability of *Culicoides* to cause outbreaks of AHS is dependent on the production of
209 large numbers of midges that can only occur when the appropriate weather conditions
210 and biotic environment allow the development of large populations [14]. The
211 epidemiology of AHSV is therefore closely linked to climatic and meteorological factors

with seasonal outbreaks occurring in endemic countries almost always following periods of warm, wet weather, which allows maximum larval development and adult survival [14]. In southern Africa, climatic conditions favourable to large epizootics are often triggered by the El Niño Southern Oscillation [61].

Because of difficulties associated with data collection and the lack of transmission of significant human pathogens, *Culicoides* research has been limited compared to that on many other insect vectors. Recent epizootics of *Culicoides* associated arboviral diseases in previously unaffected parts of the world (including those caused by BTV and Schmallenberg virus) have led to a significant increase in knowledge, although there is still much unknown. As effective environmental control of *Culicoides* numbers is impractical, recent research has focused on methods to predict when and where disease outbreaks can occur [14]. A key issue has been the need to identify areas of the world with or without competent vector species and the knowledge of species distribution is now extensive, although incomplete. Significant recent developments have included molecular methods of species differentiation and the development of more advanced modelling systems to predict *Culicoides* distribution and abundance, two critical parameters when examining the risk of AHS [14; 48; 59; 60; 62]. Unfortunately, the significant variation in *Culicoides* abundance found at the local scale limits the applications of these models at present [63; 64].

SCENARIOS FOR AN AHS OUTBREAK IN DISEASE-FREE REGIONS

An outbreak of AHS requires the presence of the virus, suitable equid hosts, competent vector species of *Culicoides* and appropriate climatic and geographical conditions for vector-host interaction [65]. The following five scenarios must be considered when assessing the risk in AHS-free regions:

1 - ALTERED GLOBAL DISTRIBUTION OF KNOWN AHSV VECTOR SPECIES

The effects of climate change may alter the distribution of the known vectors of AHSV. The worldwide distribution of the principal vector, *C. imicola*, is extensive and extends from South Africa to southern Europe and from western Africa to southern China [62; 66]. It is not present in The Americas, northern Europe or Australasia, although the distribution is expanding northwards within Europe and studies estimate that it may reach central Europe by the early part of the 21st century [14; 48; 62]. In addition, most of South America and Southeast Asia, and smaller regions of the USA and Australia are already considered climatically suitable if the species were to be introduced [62]. Vector-species of mosquito have been introduced into Europe in recent years via international tyre and plant trade, although similar movement of *Culicoides* has not yet been demonstrated [67].

2 - VECTOR ROLE OF INDIGENOUS *CULICOIDES* SPECIES (TABLE 1)

Another scenario is that *Culicoides* species indigenous to AHS-free countries might be able to transmit disease if the virus were introduced [68]. This could be due to an inherent ability to transmit the virus or climate change mediated effects on vectorial capacity [16].

Vectorial capacity is the ability of a vector to transmit a pathogen under field conditions and is determined by several factors [69]. Vectorial capacity has been shown to increase with ambient temperatures of 27-30°C and *Culicoides* species traditionally considered non-vectors of AHSV have increased susceptibility to infection if raised under warmer

conditions [70; 71]. It has been predicted that the effects of climate change will result in UK temperatures continuing to rise by at least 0.2°C per decade for the foreseeable future and, while the relationship is by no means straight-forward, this is anticipated to increase the likelihood of competent AHSV vectors being present in the region [72].

Evidence for a potential role of indigenous *Culicoides* species is provided by comparisons with BTV epidemiology. African horse sickness virus and BTV share vector species (including *C. imicola*) and both have made incursions north into Europe [13; 16; 43]. During the recent BT outbreaks in Europe, disease occurred in regions where the known vector species are absent and indigenous *Culicoides* species must therefore have acted as vectors [73]. There is substantial evidence that *Culicoides* species including *C. pulicaris, C. punctatus*, *C. dewulfi, C. obsoletus, C. scoticus* and *C. chiopterus* acted as vectors of BTV in northern Europe from 2006 [74; 75]. Temperatures during this time were among the warmest recorded and this may have significantly increased the ability of these species to act as BTV vectors [19; 76]. These species are therefore considered potential vectors for AHSV in northern Europe and have recently been shown to be the most abundant species on equine premises in the southeast UK [77]. Unfortunately, there is very little empirical evidence available to support this theory. In a single study, AHSV was isolated from mixed pools of *Culicoides* in Spain that did not contain any known vector species, but did contain mainly *C. pulicaris* and *C. obsoletus* [78]. It was also suspected that *C. obsoletus* played a role in AHSV transmission in parts of Morocco [45]. While this is only very poor quality evidence, more convincing data can only be obtained during epizootics, by which time it is too late to implement preventive measures. The evidence is more convincing in the USA, where *C. sonorensis*, the primary North American BTV vector, has been shown to act as an efficient biological vector for AHSV in a laboratory setting [55; 79].

292 *Culicoides sonorensis* is absent from much of Central America and all of South America
293 [14]. Bluetongue virus is endemic in Central America, where *C. insignis* and *C. pusillus*
294 are the vectors of primary importance and it is suspected that the region acts as a source
295 of BTV for both North and South America [80]. Although evidence is limited, BTV has
296 been reported to be present in large parts of South America, where *C. insignis* and *C.*
297 *pusillus* are again thought to be primary vectors [14; 81]. Brazil is of particular current
298 importance, given the upcoming 2016 Olympic Games. Although very little *Culicoides*
299 distribution data is available, a recent study showed that *C. insignis* accounted for 81%
300 of livestock-associated catches in Brazil [82]. This species must therefore be considered
301 of greater potential as an AHSV vector in the region.
302
303 In Australasia *C. fulvus, C. wadai, C. actoni,* and C*. brevitarsis* are important vector species
304 for BTV [14]. It has been suggested that *C. brevitarsis* and *C.imicola* may share a common
305 ancestry and the competency of *C. brevitarsis* for AHSV should therefore be investigated
306 [62; 83]. In Asia, BTV is transmitted by several vector species, although data is limited in
307 many parts of the region. Of particular interest in the region is the presence of *C. imicola*
308 in China [14].
309
310 When considering the current *Culicoides* species of global importance relevant to
311 transmission of BTV and AHSV (as summarised in *Table 1*) it is clear that there is a
312 dearth of basic research on the vector competence of many *Culicoides* species for AHSV.
313 This has led to a reliance on BTV vector knowledge as a reference for AHSV and greater
314 research effort is thus urgently required. In summary, it is possible that the appropriate
315 *Culicoides* species and climatic conditions to support an outbreak of AHS are currently
316 present in many AHS-free countries, although more research is urgently required.
317
318 **3- VIRAL INTRODUCTION WITHIN AN INFECTED VERTEBRATE**

There has been a rapid expansion in the number of international equine events and many horses routinely compete worldwide [84]. The risk of AHS entering OIE disease-free countries via a legally transported horse is considered very low, due to the stringent regulations in place and the rapid severity of the disease [85]. This perceived low risk is supported by a recent quantitative risk assessment for undetected AHS infection in a horse exported from an infected country [86]. Pre-export quarantine in a vector-protected facility and multiple PCR tests prior to export were key factors in managing risk in the models assessed.

There is still concern regarding the possibility of vector exposure during legal transit as horses can be transported via certain AHSV infected countries as long as they remain on the plane [87]. Examples include the transport of horses from South America to the UK via Senegal, which is not AHSV free. The OIE now recommends that insecticide impregnated mesh be placed over containers during transport of horses through regions not free of AHSV [27]. Alphacypermethrin-treated high density polyethylene mesh has been shown to reduce exposure of horses in jet stalls to *C. imicola* and is therefore recommended, although it is not completely protective [88].

The presence of AHSV infection within reservoir species presents a more difficult problem. The importation of infected zebra from Namibia to a safari park near Madrid was considered the cause of the 1987-1991 outbreak in the Iberian Peninsula and Morocco [10]. The longest reported viraemia in zebra is six weeks, thus it may be possible for an infected animal to remain clinically undetected during the required 40-day quarantine period [26]. Failure of compulsory paired serology testing would also have to occur for virus entry. The illegal transport of a reservoir equid (for example a donkey moved from northern Africa into Europe) represents a definite risk that cannot

346 be quantified [87]. The likelihood of the introduction of AHSV to Great Britain via the
347 legal trade of equine semen, ova and embryos, meat and other specified biological
348 products is considered to be negligible [87].
349
350 **4 - VIRAL INTRODUCTION WITHIN INFECTED *CULICOIDES***
351
352 There are two possible ways that a virus-infected *Culicoides* midge could reach a
353 previously unaffected area. The first is within a plane or freight container in transit,
354 especially those containing vegetative materials such as packaged flowers [89; 90].
355 While this is well documented for other vector insects, there is no suitable information
356 available for estimating the risk of AHSV introduction via inadvertent transportation of
357 *Culicoides* [89; 91]. An assessment of the risk of a European BT outbreak caused by
358 *Culicoides* movement via intracontinental transport and trade concluded that large
359 numbers of vectors would have to be transported to pose a significant risk [92]. An even
360 greater number of *Culicoides* would likely have to be transported for an extensive AHS
361 outbreak, as the number of resident equid hosts is generally fewer compared to
362 livestock affecting BTV transmission.
363
364 The second potential method of virus introduction via *Culicoides* is wind dispersal.
365 Although adult *Culicoides* rarely fly further than a few hundred metres from their
366 breeding grounds, they can be passively dispersed over much greater distances if wind
367 patterns are appropriate [14]. The wind dispersal of infected *Culicoides* has been
368 implicated as the cause of the overseas spread of AHSV from Morocco to Spain in 1966
369 and BTV from mainland Europe to the UK in 2007 [93; 94].
370
371 **5 - REVERSION TO VIRULENCE OF VACCINE STRAINS**
372

There is concern that AHSV could be introduced to a disease-free region by reversion to virulence of attenuated vaccine strains. There is a theoretical risk that horses vaccinated with live-attenuated vaccine may be imported into AHS-free regions and pose a risk via vaccine-induced viraemia, although quarantine requirements should preclude this risk. Recently an AHSV strain circulating in The Gambia was thought highly likely to have been derived from a live-attenuated AHSV-9 vaccine strain [95]. The illegal importation and use of live-attenuated vaccines in AHS-free regions also poses a risk. In support of these concerns, both the field transmission and re-assortment of live attenuated vaccine strains of BTV have been demonstrated in Europe [96; 97].

CONSEQUENCES OF AN AHS OUTBREAK IN OIE DISEASE-FREE COUNTRIES AND CURRENT RESPONSE PLANS

Another AHS epizootic would have severe consequences for equine welfare and industry in affected regions. During a three-year outbreak in Asia between 1959-1961 over 300,000 equids died and in Spain 110 horses died as a direct result of AHS from 1987-1990, with a further 900 slaughtered as part of control measures [10; 98]. The economic cost of an outbreak of AHS in the Netherlands has been estimated at 272–516 million Euros [99]. African horse sickness is notifiable in OIE disease-free countries and suspicion must therefore be reported immediately to the relevant authorities. If the virus is confirmed as being present, the immediate priority is to stop the virus from spreading into any potential *Culicoides* vector population. The prevention and control plan for Great Britain is laid out in the 'African horse sickness control strategy for Great Britain', which is freely available online [21]. A summary of the measures that would be taken in response to a disease outbreak in the UK is provided (Figure 4).

CULLING OF HORSES

In Great Britain, culling of horses infected or suspected to be infected with AHSV would be implemented, unless there was proof that the virus was already circulating extensively within the vector population. No compensation would be paid for culled horses. Exclusions from culling would potentially be available for animals of genetic importance if they can be immediately moved to fully operational vector-proofed facilities. In practice, these facilities do not exist outside of quarantine centres and laboratories. In a recent study of several premier equine facilities in the southeast of England, none had vector-proof facilities available [77]. In addition, the rapid mortality and disease severity seen in naïve horses renders debate on moving such horses to a protected facility as hypothetical only. Public concerns on culling would almost certainly be raised and it is anticipated that complex legal situations would quickly arise [100]

TRACKING OF EQUIDS

Detailed information on equid location and movement would be essential during an epizootic. Unfortunately detailed information on the numbers, movements and whereabouts of equids is not currently available throughout most AHS-free countries [101-103]. A new central equine database is being introduced within the European Union in 2016; however there are currently no requirements to record transport of horses within most EU countries and modelling horse movements between countries is very challenging [101]. The USA has developed the National Animal Identification Scheme (NAIS), with the aim of recording all animal identities, premises locations and animal movements. Unfortunately, the scheme has been met with resistance and does not appear to be an active program [104]. A survey conducted in the USA in 2009 revealed that only 47% of questioned equine veterinarians were in favour of the NAIS (although the remaining 53% were almost entirely neutral with only 3.6% opposed to

the scheme) and this was considered very disappointing as 81.6% of the respondents did not have a plan to deal with clients' horses during a disaster [105]. In much of Australia, property identification codes should be registered for equine premises, however there is no national movement database.

VACCINATION

Annual vaccination of horses is the mainstay of controlling AHS in South Africa, with the first highly effective live attenuated vaccine produced in 1936 [3; 4]. This vaccine currently contains live-attenuated forms of seven of the nine AHSV serotypes: AHSV-5 and AHSV-9 were omitted due to safety concerns and regional low prevalence, respectively. *In-vivo* cross-protection between AHSV-6 and AHSV-9 and between AHSV-5 and AHSV-8 has been demonstrated in horses [106]. Vaccinated horses are generally considered well protected, although the vaccine cannot be relied upon to fully protect all horses [4]. A recent study showed that 16% of immunised horses in an AHS endemic area were infected with AHSV over a two-year period [107]. As half of these cases were sub-clinically infected, they could have an impact on disease epidemiology if they were illegally transported while viraemic. It is important to note that the authors could not confirm if the level of viraemia detected in the sub-clinically infected horses would be sufficient to infect *Culicoides* [107].

Outside of endemic regions, vaccination has been successfully used to control outbreaks of AHS, and hundreds of thousands of horses were vaccinated during the 1966 and 1987-1990 outbreaks in the Iberian Peninsula [10]. The availability of vaccines is a cause for concern and suggested European Union vaccine banks have yet to be approved [87]. In addition, the number and feasibility of vaccinations to be effective must be

considered; a recent UK-based study predicted that 85% uptake would be required [102].

As previously discussed, there are concerns about reversion to virulence of attenuated vaccine strains. Thus, alternative vaccine types, including inactivated virus and recombinant vaccines are being developed, with recent studies demonstrating efficacy of recombinant vaccines expressing genes encoding the outer capsid proteins of AHSV [108-112]. These vaccines represent a potentially safer alternative to the live-attenuated types, particularly for use in non-endemic countries, and allow differentiation of infected from vaccinated animals as previously mentioned.

PREVENTION OF CULICOIDES-HORSE INTERACTION

The prevention of *Culicoides* blood-feeding on horses is an essential part of controlling an AHS outbreak. Unfortunately there are very few studies that assess methods used to prevent *Culicoides* from biting horses, making it almost impossible to determine their potential for use during an AHS outbreak [113]. Despite *Culicoides* triggered IBH being one of the most common skin diseases of horses, the only truly effective control method known is complete allergen avoidance [114; 115]. While moving horses to areas devoid of *Culicoides* would be effective for preventing AHSV transfer, it is often highly impractical and would be either inappropriate or forbidden during an epizootic.

In South Africa it has long been observed that stabling of horses at night is an effective method for minimising the risk of contracting AHS [116]. However, the housing must be constructed to clearly defined specifications to prevent *Culicoides* entry and there are various levels of vector proofing attainable. The behaviour of the different *Culicoides* species is very important when considering the effectiveness of housing, depending on

whether they display endophilic or exophilic activity [117]. For example, it has been demonstrated that catches of exophilic *C. imicola* are higher outside open stables, while catches of endophilic *C. bolitinos* are greater inside [118]. This suggests that housing horses in normal stables with open windows and top-doors may actually increase the biting risk from endophilic species, while reducing the risk from exophilic species. When simple vector protection (closed doors and gauzed windows) was applied to equine housing in South Africa, there was a 14-fold reduction in the catch of both endophilic and exophilic species [118]. Covering of entrances with mesh significantly reduced the catches of *Culicoides* in stables in the UK [119]. The use of netting and fans has also been shown to reduce blood-feeding by *Culicoides* on horses in various housing systems in Switzerland [120]. Use of insecticide-impregnated mesh rather than plain gauze is also likely to further reduce the entry of midges into animal housing and thereby reduce the midge attack and biting rate [119; 121; 122]. Insect blankets with both neck and hood covers have been shown to limit the feeding rate of *Culicoides* on horses in The Netherlands, and the authors of this study suggested that this might be helpful to protect horses from bites of AHS-infected *Culicoides* [123].

The most effective time periods during the day to use protective measures must also be considered. As *Culicoides* are crepuscular, with peak activity at dawn and dusk, it is recommended that any protective effects are focused at this time [114; 124]. Unfortunately, many *Culicoides* species have been shown to feed during the day, potentially making this recommendation unsuitable for completely effective disease control [14; 52; 125].

The UK AHS regulations advise that deltamethrin is the most effective insecticidal product to use against *Culicoides*, although they emphasise that it is not licensed in the horse nor specifically against midges in any species [21]. The application of permethrin

507 to horses with IBH significantly improved clinical signs in 86% of 43 horses [126].
508 Other studies in horses do not support the use of topical deltamethrin or permethrin
509 solution as a repellent to prevent *Culicoides* from biting horses [127; 128]. However,
510 these studies did not investigate the possible insecticidal effects of deltamethrin in
511 reducing onward transmission of disease from viraemic horses or the numbers of adult
512 *Culicoides* within an area. This emphasises the important and often poorly defined
513 distinction between insecticides and repellents [127]. Possibly the most direct
514 indication of the effects of the permethrins on the transmission of arboviral disease is a
515 field study conducted in cattle. This study demonstrated that 2-weekly application of
516 topical permethrin did not reduce exposure to BTV as measured by serology [129].
517 Injectable avermectins are used to control ectoparasites in many species, including the
518 horse. Unfortunately their efficacy against different *Culicoides* species varies
519 significantly, with near toxic doses required in some cases and there is no data available
520 on their efficacy against European *Culicoides* species [117].
521
522 N,N-diethyl-3-methylbenzamide (DEET) has been shown to reduce the biting rate of *C.*
523 *impunctatus* in humans [130]. The application of 15% DEET impregnated mesh to
524 vacuum light traps has been shown to significantly reduce *Culicoides* catches when
525 compared to untreated mesh [131]. Unfortunately, there is *in vivo* evidence of adverse
526 effects (including hypersteatosis and dermatosis) occurring in horses when DEET is
527 applied topically at concentrations greater than 15%, although many were only mild
528 [132]. Recent work has demonstrated that a combination of DEET and plant-derived
529 organic fatty acids may provide an effective and long-lasting repellent effect against
530 *Culicoides* [133]. Citronella oil, while known to be an effective mosquito repellent, has
531 been repeatedly shown to have either no repellent effect or potentially an attractant
532 effect on *Culicoides* [131; 134].
533

Other control methods, such as the use of chemo-attractants to bait traps have been trialled in Scotland based on knowledge of host-location for *C. impunctatus* [135]. The host kairomones carbon dioxide and 1-octen-3-ol have been shown to attract *Culicoides* in the UK, although effective use as a control method is not yet possible [136]. In Scotland it is thought to be impractical to apply insecticides or undertake habitat manipulation on sufficient scale to effectively control midges [137]. Certainly it appears unlikely that the large-scale coordinated effort required to manipulate the habitat could take place in time to help control an outbreak and environmental regulations prohibit the use of many insecticides. The covering of muck heaps on farms, which has been suggested as a smaller scale method of habitat manipulation, has been shown not to affect *Culicoides* abundance and is therefore unlikely to be an effective method of controlling arboviral disease [138].

CONCLUSIONS

In summary, climate change and globalisation have resulted in a myriad of factors that increase the risk of AHS to many parts of the world. There is extensive evidence that many AHS-free regions now have the conditions required to allow an AHS epizootic to occur and the introduction of AHSV-infected equines or *Culicoides* could produce extensive and persistent epidemics [16]. An outbreak of AHS in any disease-free region would have catastrophic effects on equine welfare and industry. The OIE regulations for disease-free countries are extensive and major stakeholders adhere stringently to these requirements, making the risk of AHS entry via a legally transported horse very low. Indeed, AHS is listed amongst six diseases for which the OIE requires additional mitigation measures in high health high performance (HHP) horses, despite these animals already being managed within systems that prioritise horse health, biosecurity and disease control. It is essential that international equid transport remains closely

monitored and illegal movement is prevented. Veterinary surgeons attending cases with clinical findings consistent with AHS, in particular in any equids that have travelled or are housed with equids that have travelled, must remain vigilant to the possibility of the disease occurring in areas currently considered disease-free.

Extensive research is required if the equine industry is to avoid or effectively contain an AHS epizootic in disease-free regions. This research should focus on four key areas: Firstly, investigating the AHSV vector competence of certain *Culicoides* species; secondly, improving the accuracy of disease modelling by increasing our knowledge of *Culicoides* distribution and the development of standardised recording of equid movement; thirdly, the development of more effective and practical methods to prevent blood-feeding by *Culicoides* on horses; and finally, the establishment of vaccination banks available for use by OIE disease-free regions that can be used in the event of an outbreak, preferably based on recombinant vaccine formulas.

576 **REFERENCES**
577

578

579

580

581

582

583

584

585

586

587 **TABLES AND FIGURE LEGENDS**

SPECIES	AHSV VECTOR ROLE	BTV VECTOR ROLE	REGIONS OF MOST IMPORTANCE
C. imicola	Primary Importance	Primary Importance	Africa, Southern Europe, Asia
C. bolitinos	Primary Importance	Primary Importance	Africa
C. brevitarsis	Unknown	Primary Importance	Australia
C. obsoletus	Suspected	Primary Importance	Europe
C. scoticus	Unknown	Primary Importance	Europe
C. chiopterus	Unknown	Primary Importance	Europe
C. dewulfi	Unknown	Primary Importance	Europe
C. pulicaris	Suspected	Primary Importance	Europe
C. punctatus	Unknown	Primary Importance	Europe
C. magnus	Unknown	Lesser Importance	Africa
C. sonorensis	Lab vector	Primary Importance	North and Central America
C. insignis	Unknown	Primary Importance	South and Central America
C. pusillus	Unknown	Primary Importance	South and Central America
C. actoni	Unknown	Lesser Importance	-
C. brevipalpis	Unknown	Lesser Importance	-
C. dumdumi	Unknown	Lesser Importance	-
C. filarifer	Unknown	Lesser Importance	-
C. fulvus	Unknown	Lesser Importance	-
C. furens	Unknown	Lesser Importance	-
C. gulbenkiani	Unknown	Lesser Importance	-
C. milnei	Unknown	Lesser Importance	-
C. nevilli	Unknown	Lesser Importance	-
C. nubeculosus	Unknown	Lesser Importance	-

C. orientalis	Unknown	Lesser Importance	-
C. oxystoma	Unknown	Lesser Importance	-
C. peregrinus	Unknown	Lesser Importance	-
C. puncticollis	Unknown	Lesser Importance	-
C. stellifer	Unknown	Lesser Importance	-
C. tilineatus	Unknown	Lesser Importance	-
C. tororoensis	Unknown	Lesser Importance	-
C. wadai	Unknown	Lesser Importance	-

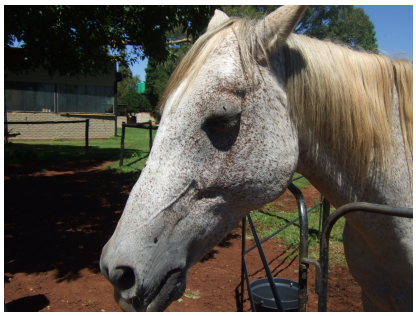
588

589 *Table 1:* The 31 species of *Culicoides* known to play a role in the transmission of bluetongue disease and their known or
590 suspected roles in African horse sickness virus transmission. Those in bold are more clearly implicated in field
591 transmission of bluetongue virus and therefore of more importance when considering African horse sickness virus.
592 Expanded and revised from Meiswinkel *et al*, 2004 [139].

593 *Figure 1: Sudden death associated with peracute form of AHS. Frothy fluid visible draining from nostrils (photo credit: Rudy*
594 *Meiswinkel)*

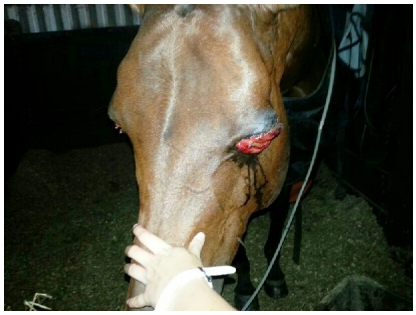
595

596 *Figure 2: A case of the cardiac form of AHS demonstrating oedema of the supraorbital space and head.*



597

598 *Figure 3: A case of the cardiac form of AHS showing chemosis and supraorbital oedema (photo credit: Maygan Jennings).*



599

600 *Figure 4: Flow chart summarising the response to AHSV infection based on the AHS control strategy for Great Britain* [21].

[1] Henning, M.M. (1956) Animal diseases in South Africa, 3 edn., Central news Agency. pp 785-808.

[2] Coetzer, J.A.W. and Erasmus, B.J. (1994) African horsesickness. In: *Infectious diseases of livestock with special reference to Southern Africa*, Eds: J.A.W. Coetzer, G.R. Thomson and R.C. Tustin, Oxford University Press. pp 460-475.

[3] Mellor, P.S. and Hamblin, C. (2004) African horse sickness. *Veterinary research* **35**, 445-466.

[4] Long, M.T. and Guthrie, A.J. (2014) African Horse Sickness. In: *Equine infectious diseases*, 2nd edn., Eds: D.C. Sellon and M.T. Long, Elsevier Health Sciences. pp 181-188.

[5] (OIE), W.O.f.A.H. (2015) Terrestrial Animal Code.

[6] Calisher, C.H. and Mertens, P.P. (1998) Taxonomy of African horse sickness viruses. *Archives of virology. Supplementum* **14**, 3-11.

[7] Gohre, D.S., Khot, J.B., Paranjpe, V.L. and Manjrekar, S.L. (1965) Observations on the outbreak of South African horse sickness in India during 1960–1961. *Bombay Veterinary College Magazine*, 5-15.

[8] Howell, P. (1960) The 1960 epizootic of African horsesickness in the Middle East and SW Asia. *Journal of the South African Veterinary Medical Association* **31**, 329-334.

[9] Diaz Montilla, R. and Panos Marti, P. (1967) Epizootologia de la peste equina en Espana. *Bull. Off. Int. Epizoot* **86**, 705-714.

[10] Rodriguez, M., Hooghuis, H. and Castano, M. (1992) African horse sickness in Spain. *Veterinary microbiology* **33**, 129-142.

[11] Aklilu, N., Batten, C., Gelaye, E., Jenberie, S., Ayelet, G., Wilson, A., Belay, A., Asfaw, Y., Oura, C., Maan, S., Bachanek-Bankowska, K. and Mertens, P.P.C. (2014) African horse sickness outbreaks caused by multiple virus types in Ethiopia. *Transboundary and emerging diseases* **61**, 185-192.

[12] Mellor, P.S. and Boorman, J. (1995) The transmission and geographical spread of African horse sickness and bluetongue viruses. *Annals of tropical medicine and parasitology* **89**, 1-15.

[13] Du Toit, R. (1944) The transmission of bluetongue and horsesickness by Culicoides. *Onderstepoort Journal of veterinary Science and animal Industry* **19**, 7-16.

[14] Mellor, P.S., Boorman, J. and Baylis, M. (2000) Culicoides biting midges: their role as arbovirus vectors. *Annual review of entomology* **45**, 307-340.

[15] Mellor, P.S. (1994) Epizootiology and vectors of African horse sickness virus. *Comparative immunology, microbiology and infectious diseases* **17**, 287-296.

[16] Purse, B.V., Mellor, P.S., Rogers, D.J., Samuel, A.R., Mertens, P.P. and Baylis, M. (2005) Climate change and the recent emergence of bluetongue in Europe. *Nature reviews. Microbiology* **3**, 171-181.

[17] Mintiens, K., Méroc, E., Mellor, P.S., Staubach, C., Gerbier, G., Elbers, A.R.W., Hendrickx, G. and De Clercq, K. (2008) Possible routes of introduction of bluetongue virus serotype 8 into the epicentre of the 2006 epidemic in north-western Europe. *Preventive veterinary medicine* **87**, 131-144.

[18] Maclachlan, N.J. and Guthrie, A.J. (2010) Re-emergence of bluetongue, African horse sickness, and other orbivirus diseases. *Veterinary research* **41**, 35.

[19] Guis, H., Caminade, C., Calvete, C., Morse, A.P., Tran, A. and Baylis, M. (2012) Modelling the effects of past and future climate on the risk of bluetongue emergence in Europe. *Journal of the Royal Society Interface* **9**, 339-350.

[20] Theiler, S.A. (1921) *African Horse-sickness:(pestis Equorum)*, Cape Times Limited, Government Printers.

[21] DEFRA (2012) The African Horse Sickness (England) Regulations 2012, Ed: F.a.R.A. Department for Environment, UK Government, London.

[22] Swanepoel, R., Erasmus, B.J., Williams, R. and Taylor, M.B. (1992) Encephalitis and chorioretinitis associated with neurotropic African horsesickness virus infection in laboratory workers. Part III. Virological and serological investigations. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde* **81**, 458-461.

[23] Van Rensberg, I.B., De Clerk, J., Groenewald, H.B. and Botha, W.S. (1981) An outbreak of African horsesickness in dogs. *Journal of the South African Veterinary Association* **52**, 323-325.

[24] van Sittert, S.J., Drew, T.M., Kotze, J.L., Strydom, T., Weyer, C.T. and Guthrie, A.J. (2013) Occurrence of African horse sickness in a domestic dog without apparent ingestion of horse meat. *Journal of the South African Veterinary Association* **84**.

[25] Gomez-Villamandos, J., Sanchez, C., Carrasco, L., Laviada, M., Bautista, M., Martınez-Torrecuadrada, J., Sánchez-Vizcaıno, J. and Sierra, M. (1999) Pathogenesis of African horse sickness: ultrastructural study of the capillaries in experimental infection. *Journal of comparative pathology* **121**, 101-116.

[26] Barnard, B.J. (1998) Epidemiology of African horse sickness and the role of the zebra in South Africa. *Archives of virology. Supplementum* **14**, 13-19.

[27] Health, W.O.f.A. (2014) Terrestrial Animal Code, OIE World Organisation for Animal Health Paris. http://www.oie.int. p Chapter 12.

[28] Hamblin, C., Graham, S.D., Anderson, E.C. and Crowther, J.R. (1990) A competitive ELISA for the detection of group-specific antibodies to African horse sickness virus. *Epidemiology and infection* **104**, 303-312.

[29] Hamblin, C., Mertens, P.P.C., Mellor, P.S., Burroughs, J.N. and Crowther, J.R. (1991) A serogroup specific enzyme-linked immunosorbent assay for the detection and identification of African horse sickness viruses. *Journal of Virological Methods* **31**, 285-292.

[30] Kweon, C.H., Kwon, B.J., Ko, Y.J. and Kenichi, S. (2003) Development of competitive ELISA for serodiagnosis on African horsesickness virus using baculovirus expressed VP7 and monoclonal antibody. *Journal of Virological Methods* **113**, 13-18.

[31] Laviada, M.D., Babín, M., Dominguez, J. and Sánchez-Vizcaíno, J.M. (1992) Detection of African horsesickness virus in infected spleens by a sandwich ELISA using two monoclonal antibodies specific for VP7. *Journal of Virological Methods* **38**, 229-242.

[32] McIntosh, B.M. (1956) Complement fixation with horsesickness viruses. *Onderstepoort J. Vet. Res.* **27**, 165-169.

[33] Agüero, M., Gómez-Tejedor, C., Cubillo, Á.M., Rubio, C., Romero, E. and Jiménez-Clavero, M.A. (2008) Real-time fluorogenic reverse transcription polymerase chain reaction assay for detection of African horse sickness virus. *Journal of veterinary diagnostic investigation* **20**, 325-328.

[34] Guthrie, A.J., MacLachlan, N.J., Joone, C., Lourens, C.W., Weyer, C.T., Quan, M., Monyai, M.S. and Gardner, I.A. (2013) Diagnostic accuracy of a duplex real-time reverse transcription quantitative PCR assay for detection of African horse sickness virus. *Journal of virological methods* **189**, 30-35.

[35] Weyer, C.T., Joone, C., Lourens, C.W., Monyai, M.S., Koekemoer, O., Grewar, J.D., van Schalkwyk, A., Majiwa, P.O.A., MacLachlan, N.J. and Guthrie, A.J. (2015) Development of three triplex real-time reverse transcription PCR assays for the qualitative molecular typing of the nine serotypes of African horse sickness virus. *Journal of Virological Methods* **223**, 69-74.

[36] Bachanek-Bankowska, K., Maan, S., Castillo-Olivares, J., Manning, N.M., Maan, N.S., Potgieter, A.C., Di Nardo, A., Sutton, G., Batten, C. and Mertens, P.P.C. (2014) Real Time RT-PCR Assays for Detection and Typing of African Horse Sickness Virus. *PloS one* **9**.

[37] Hamblin, C., Salt, J.S., Mellor, P.S., Graham, S.D., Smith, P.R. and Wohlsein, P. (1998) Donkeys as reservoirs of African horse sickness virus. *Archives of virology. Supplementum* **14**, 37-47.

[38] Mellor, P.S. (1993) African horse sickness: transmission and epidemiology. *Veterinary research* **24**, 199-212.

[39] Hopley, R. (2009) Partial Impact Assessment of African Horse Sickness legislation through the implementation of Directive 92/35, Ed: DEFRA, UK. pp 1-23.

[40] Braverman, Y., Ungar-Waron, H., Frith, K., Adler, H., Danieli, Y., Baker, K. and Quinn, P. (1983) Epidemiological and immunological studies of sweet itch in horses in Israel. *The Veterinary record* **112**, 521-524.

760 [41] Wilson, A., Mellor, P.S., Szmaragd, C. and Mertens, P.P. (2009) Adaptive strategies
761 of African horse sickness virus to facilitate vector transmission. *Veterinary*
762 *research* **40**, 16.
763
764 [42] Borkent, A. and Wirth, W.W. (1997) *World species of biting midges (Diptera:*
765 *Ceratopogonidae)*, American Museum of Natural History.
766
767 [43] Ortega, M.D., Mellor, P.S., Rawlings, P. and Pro, M.J. (1998) The seasonal and
768 geographical distribution of Culicoides imicola, C. pulicaris group and C.
769 obsoletus group biting midges in central and southern Spain. *Archives of*
770 *virology. Supplementum* **14**, 85-91.
771
772 [44] Kettle, D. and Lawson, J. (1952) The early stages of British biting midges
773 Culicoides Latreille (Diptera: Ceratopogonidae) and allied genera. *Bulletin of*
774 *entomological research* **43**, 421-467.
775
776 [45] Baylis, M., El Hasnaoui, H., Bouayoune, H., Touti, J. and Mellor, P.S. (1997) The
777 spatial and seasonal distribution of African horse sickness and its potential
778 Culicoides vectors in Morocco. *Medical and veterinary entomology* **11**, 203-212.
779
780 [46] Capela, R., Purse, B.V., Pena, I., Wittman, E.J., Margarita, Y., Capela, M., Romao, L.,
781 Mellor, P.S. and Baylis, M. (2003) Spatial distribution of Culicoides species in
782 Portugal in relation to the transmission of African horse sickness and bluetongue
783 viruses. *Medical and veterinary entomology* **17**, 165-177.
784
785 [47] Meiswinkel, R. (1998) The 1996 outbreak of African horse sickness in South
786 Africa - The entomological perspective. *Archives of Virology, Supplement* **1998**,
787 69-83.
788
789 [48] Rawlings, P., Pro, M.J., Pena, I., Ortega, M.D. and Capela, R. (1997) Spatial and
790 seasonal distribution of Culicoides imicola in Iberia in relation to the
791 transmission of African horse sickness virus. *Medical and veterinary entomology*
792 **11**, 49-57.
793
794 [49] Carpenter, S., Szmaragd, C., Barber, J., Labuschagne, K., Gubbins, S. and Mellor, P.
795 (2008) An assessment of Culicoides surveillance techniques in north-ern
796 Europe: have we underestimated a potential bluetongue virus vector. *J. Appl.*
797 *Ecol.* **45**, 1237–1245.
798
799 [50] Viennet, E., Garros, C., Lancelot, R., Allene, X., Gardes, L., Rakotoarivony, I.,
800 Crochet, D., Delecolle, J.C., Moulia, C., Baldet, T. and Balenghien, T. (2011)
801 Assessment of vector/host contact: comparison of animal-baited traps and UV-
802 light/suction trap for collecting Culicoides biting midges (Diptera:
803 Ceratopogonidae), vectors of Orbiviruses. *Parasites & vectors* **4**, 119.
804
805 [51] McDermott, E.G., Mayo, C.E., Gerry, A.C., Laudier, D., MacLachlan, N.J. and
806 Mullens, B.A. (2015) Bluetongue virus infection creates light averse Culicoides
807 vectors and serious errors in transmission risk estimates. *Parasites & vectors* **8**,
808 1-9.
809
810 [52] Elbers, A. and Meiswinkel, R. (2015) Limited attractant range of the black‐light
811 suction trap for the capture of Culicoides biting midges (Diptera:
812 Ceratopogonidae). *Journal of Applied Entomology*.
813

[53] Scheffer, E.G., Venter, G.J., Labuschagne, K., Page, P.C., Mullens, B.A., Maclachlan, N.J., Osterrieder, N. and Guthrie, A.J. (2011) Comparison of two trapping methods for Culicoides biting midges and determination of African horse sickness virus prevalence in midge populations at Onderstepoort, South Africa. *Veterinary parasitology*.

[54] Venter, G.J., Graham, S.D. and Hamblin, C. (2000) African horse sickness epidemiology: vector competence of south african Culicoides species for virus serotypes 3, 5 and 8. *Medical and veterinary entomology* **14**, 245-250.

[55] Boorman, J., Mellor, P.S., Penn, M. and Jennings, M. (1975) The growth of African horse-sickness virus in embryonated hen eggs and the transmission of virus by Culicoides variipennis Coquillett (Diptera, Ceratopogonidae). *Archives of virology* **47**, 343-349.

[56] Campbell, J.A. and Pelham-Clinton, E. (1960) X.—A Taxonomic Review of the British Species of Culicoides Latreille (Diptera, Ceratopogonidæ). *Proceedings of the Royal Society of Edinburgh. Section B. Biology* **67**, 181-302.

[57] Kremer, M. (1965) Contribution a l'étude du genre Culicoides Latreille, particulièrement en France.

[58] Mathieu, B., Cêtre-Sossah, C., Garros, C., Chavernac, D., Balenghien, T., Carpenter, S., Setier-Rio, M.L., Vignes-Lebbe, R., Ung, V., Candolfi, E. and Delécolle, J.C. (2012) Development and validation of IIKC: An interactive identification key for Culicoides (Diptera: Ceratopogonidae) females from the Western Palaearctic region. *Parasites and Vectors* **5**, 137.

[59] Nolan, D.V., Carpenter, S., Barber, J., Mellor, P.S., Dallas, J.F., Mordue, A.J. and Piertney, S.B. (2007) Rapid diagnostic PCR assays for members of the Culicoides obsoletus and Culicoides pulicaris species complexes, implicated vectors of bluetongue virus in Europe. *Veterinary microbiology* **124**, 82-94.

[60] Cêtre-Sossah, C., Baldet, T., Delécolle, J.-C., Mathieu, B., Perrin, A., Grillet, C. and Albina, E. (2004) Molecular detection of Culicoides spp. and Culicoides imicola, the principal vector of bluetongue (BT) and African horse sickness (AHS) in Africa and Europe. *Veterinary research* **35**, 325-337.

[61] Baylis, M., Mellor, P.S. and Meiswinkel, R. (1999) Horse sickness and ENSO in South Africa [8]. *Nature* **397**, 574.

[62] Guichard, S., Guis, H., Tran, A., Garros, C., Balenghien, T. and Kriticos, D.J. (2014) Worldwide Niche and Future Potential Distribution of Culicoides imicola, a Major Vector of Bluetongue and African Horse Sickness Viruses.

[63] Kluiters, G., Sugden, D., Guis, H., McIntyre, K.M., Labuschagne, K., Vilar, M.J. and Baylis, M. (2013) Modelling the spatial distribution of Culicoides biting midges at the local scale. *Journal of Applied Ecology* **50**, 232-242.

[64] Conte, A., Goffredo, M., Ippoliti, C. and Meiswinkel, R. (2007) Influence of biotic and abiotic factors on the distribution and abundance of Culicoides imicola and the Obsoletus Complex in Italy. *Veterinary parasitology* **150**, 333-344.

[65] Lord, C., Woolhouse, M., Rawlings, P. and Mellor, P. (1996) Simulation studies of African horse sickness and Culicoides imicola (Diptera: Ceratopogonidae). *Journal of medical entomology* **33**, 328-338.

[66] Meiswinkel, R. (1989) Afrotropical Culicoides: a redescription of C.(Avaritia) imicola Kieffer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied C.(A.) bolitinos sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *The Onderstepoort journal of veterinary research* **56**, 23-39.

[67] Medlock, J.M., Hansford, K.M., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H. and Bortel, W.V. (2012) A review of the invasive mosquitoes in Europe: Ecology, public health risks, and control options. *Vector-Borne and Zoonotic Diseases* **12**, 435-447.

[68] Rawlings, P. and Mellor, P. (1994) African horse sickness and the overwintering of Culicoides spp. in the Iberian peninsula. *Revue scientifique et technique (International Office of Epizootics)* **13**, 753-761.

[69] Mullens, B.A. (1992) Integrated management of Culicoides variipennis: a problem of applied ecology. In: *Bluetongue, African Horse Sickness, and Related Orbiviruses,*, Eds: T.E. Walton and B.I. Osburn, CRC press. pp 896-905.

[70] Mellor, P.S., Rawlings, P., Baylis, M. and Wellby, M.P. (1998) Effect of temperature on African horse sickness virus infection in Culicoides. *Archives of virology. Supplementum* **14**, 155-163.

[71] Wittman, E.J. (2000) *Temperature and the transmission of arboviruses by Culicoides biting midges*. PhD, Bristol, University of Bristol.

[72] Hulme, M. and Jenkins, G. (1998) Climate Change Scenarios for the United Kingdom, Summary Report, UK Climate Impacts Programme Technical Report No. 1., Climatic Research Unit: University of East Anglia, Norwich.

[73] Mellor, P.S. and Wittmann, E.J. (2002) Bluetongue virus in the Mediterranean Basin 1998-2001. *The Veterinary Journal* **164**, 20-37.

[74] Hoffmann, B., Bauer, B., Bauer, C., Bätza, H.J., Beer, M., Clausen, P.H., Geier, M., Gethmann, J.M., Kiel, E., Liebisch, G., Liebisch, A., Mehlhorn, H., Schaub, G.A., Werner, D. and Conraths, F.J. (2009) Monitoring of putative vectors of bluetongue virus serotype 8, Germany. *Emerging Infectious Diseases* **15**, 1481-1484.

[75] Mehlhorn, H., Walldorf, V., Klimpel, S., Jahn, B., Jaeger, F., Eschweiler, J., Hoffmann, B. and Beer, M. (2007) First occurrence of Culicoides obsoletus-transmitted Bluetongue virus epidemic in Central Europe. *Parasitology Research* **101**, 219-228.

[76] Mellor, P., Baylis, M. and Mertens, P. (2009) *Bluetongue*, 1st edn., Elsevier/Academic Press, London. pp xxi, 483 p., [420] p. of plates.

[77] Robin, M., Archer, D., Garros, C., Gardès, L. and Baylis, M. (2014) The threat of midge-borne equine disease: investigation of Culicoides species on UK equine premises. *Veterinary Record* **174**, 301.

[78] Mellor, P.S., Boned, J., Hamblin, C. and Graham, S. (1990) Isolations of African horse sickness virus from vector insects made during the 1988 epizootic in Spain. *Epidemiology and infection* **105**, 447-454.

[79] Mellor, P.S. (1990) The replication of bluetongue virus in Culicoides vectors. *Current Topics in Microbiology and Immunology* **162**, 143-161.

[80] Legisa, D.M., Gonzalez, F.N. and Dus Santos, M.J. (2014) Bluetongue virus in South America, Central America and the Caribbean. *Virus research* **182**, 87-94.

[81] Clavijo, A., Sepulveda, L., Riva, J., Pessoa-Silva, M., Tailor-Ruthes, A. and Lopez, J. (2002) Isolation of bluetongue virus serotype 12 from an outbreak of the disease in South America. *Veterinary record* **151**, 301-301.

[82] Carvalho, L.P.C. and Silva, F.S. (2014) Seasonal abundance of livestock-associated Culicoides species in northeastern Brazil. *Medical and veterinary entomology* **28**, 228-231.

[83] Dyce, A.L. (2001) *Biogeographic Origins of Species of the Genus Culicoids (Diptera: Ceratopogonidae) of the Australian Region*.

[84] FEI (2015) https://data.fei.org/Calendar/Search.aspx.

[85] Sabirovic, M., López, M., Patel, K., Kingston, A. and Hall, S. (2008) African Horse Sickness: Potential risk factors and the likelihood for the introduction of the disease to the United Kingdom., Ed: I.A.H. DEFRA, London. p 3.

[86] Sergeant, E.S., Grewar, J.D., Weyer, C.T. and Guthrie, A.J. (2016) Quantitative Risk Assessment for African Horse Sickness in Live Horses Exported from South Africa. *PloS one* **11**, e0151757.

[87] Sabirovic, M., López, M., Patel, K., Kingston, A. and Hall, S. (2008) African horse sickness: Potential risk factors and the likelihood for the introduction of the disease to the United Kingdom, Ed: DEFRA.

[88] Page, P.C., Labuschagne, K., Venter, G.J., Schoeman, J.P. and Guthrie, A.J. (2015) Efficacy of alphacypermethrin-treated high density polyethylene mesh applied to jet stalls housing horses against Culicoides biting midges in South Africa. *Veterinary parasitology* **210**, 84-90.

[89] Carpenter, S., Wilson, A. and Mellor, P.S. (2009) Culicoides and the emergence of bluetongue virus in northern Europe. *Trends in Microbiology* **17**, 172-178.

[90] Reiter, P. (2010) The standardised freight container: vector of vectors and vector-borne diseases. *Revue scientifique et technique (International Office of Epizootics)* **29**, 57-64.

[91] Gratz, N.G., Steffen, R. and Cocksedge, W. (2000) Why aircraft disinsection? *Bulletin of the World Health Organization* **78**, 995-1004.

[92] Napp, S., GARCÍA‐BOCANEGRA, I., Allepuz, A., Alba, A. and Casal, J. (2013) Assessment of the risk of a bluetongue outbreak in Europe caused by Culicoides

midges introduced through intracontinental transport and trade networks. *Medical and veterinary entomology* **27**, 19-28.

[93] Pedgley, D.E. and Tucker, M.R. (1977) Possible spread of African horse sickness on the wind. *The Journal of hygiene* **79**, 279-298.

[94] Gloster, J., Burgin, L., Witham, C., Athanassiadou, M. and Mellor, P.S. (2008) Bluetongue in the United Kingdom and northern Europe in 2007 and key issues for 2008. *The Veterinary record* **162**, 298-302.

[95] Oura, C.A., Ivens, P.A., Bachanek-Bankowska, K., Bin-Tarif, A., Jallow, D.B., Sailleau, C., Maan, S., Mertens, P.C. and Batten, C.A. (2012) African horse sickness in The Gambia: circulation of a live-attenuated vaccine-derived strain. *Epidemiology and infection* **140**, 462-465.

[96] Batten, C.A., van Rijn, P.A. and Oura, C.A.L. (2010) Detection of the European 'field' strain of bluetongue virus serotype 6 by real-time RT-PCR. *Veterinary microbiology* **141**, 186-188.

[97] Agüero, M., Arias, M., Romero, L.J., Zamora, M.J. and Sánchez-Vizcaíno, J.M. (2002) Molecular differentiation between NS1 gene of a field strain Bluetongue virus serotype 2 (BTV-2) and NS1 gene of an attenuated BTV-2 vaccine. *Veterinary microbiology* **86**, 337-341.

[98] Anwar, M. and Qureshi, M. (1972) Control and eradication of African horse sickenss in Pakistan. In: *Central Treaty Organization. CENTO Seminar on the Control and Eradication of Viral Diseases*. pp 110-112.

[99] Mourits, M.C.M. and Saatkamp, H.W. (2010) Kostenberekening van een uitbraak met Afrikaanse paardenpest in Nederland.

[100] Van Den Boom, R. and van Oldruitenborgh-Oosterbaan, M.S. (2013) Can Europe learn lessons from African horse sickness in Senegal? *Veterinary Record* **172**, 150-151.

[101] de Vos, C.J., Hoek, C.A. and Nodelijk, G. (2012) Risk of introducing African horse sickness virus into the Netherlands by international equine movements. *Preventative Veterinary Medicine*.

[102] Iacono, G.L., Robin, C.A., Newton, J.R., Gubbins, S. and Wood, J.L.N. (2013) Where are the horses? with the sheep or cows? Uncertain host location, vector-feeding preferences and the risk of African horse sickness transmission in Great Britain. *Journal of the Royal Society Interface* **10**.

[103] Robin, C.A., Lo Iacono, G., Gubbins, S., Wood, J.L.N. and Newton, J.R. (2013) The accuracy of the National Equine Database in relation to vector-borne disease risk modelling of horses in Great Britain. *Equine veterinary journal* **45**, 302-308.

[104] Hartig, W., Houe, H. and Andersen, P.H. (2013) Monitoring of equine health in Denmark: A survey of the attitudes and concerns of potential database participants. *Preventive veterinary medicine* **109**, 83-91.

[105] Vanderman, K.S., Dreschel, N.A., Swinker, A.M., Kniffen, D.M., Radhakrishna, R.B., Werner, J.R. and Jedrzejewski, E.A. (2009) Equine Veterinarians' and Health Care

Professionals' Concerns Related to the Implementation of the National Equine Identification System. *Journal of Equine Veterinary Science* **29**, 823-827.

[106] von Teichman, B.F., Dungu, B. and Smit, T.K. (2010) In vivo cross-protection to African horse sickness Serotypes 5 and 9 after vaccination with Serotypes 8 and 6. *Vaccine* **28**, 6505-6517.

[107] Weyer, C.T., Quan, M., Joone, C., Lourens, C.W., Maclachlan, N.J. and Guthrie, A.J. (2013) African horse sickness in naturally infected, immunised horses. *Equine veterinary journal* **45**, 117-119.

[108] Castillo-Olivares, J., Calvo-Pinilla, E., Casanova, I., Bachanek-Bankowska, K., Chiam, R., Maan, S., Nieto, J.M., Ortego, J. and Mertens, P.P.C. (2011) A modified vaccinia Ankara virus (MVA) vaccine expressing African horse sickness virus (AHSV) VP2 protects against AHSV challenge in an IFNAR−/− mouse model. *PloS one* **6**, e16503.

[109] Chiam, R., Sharp, E., Maan, S., Rao, S., Mertens, P., Blacklaws, B., Davis-Poynter, N., Wood, J. and Castillo-Olivares, J. (2009) Induction of antibody responses to African horse sickness virus (AHSV) in ponies after vaccination with recombinant modified vaccinia Ankara (MVA). *PloS one* **4**, e5997.

[110] Guthrie, A.J., Quan, M., Lourens, C.W., Audonnet, J.-C., Minke, J.M., Yao, J., He, L., Nordgren, R., Gardner, I.A. and MacLachlan, N.J. (2009) Protective immunization of horses with a recombinant canarypox virus vectored vaccine co-expressing genes encoding the outer capsid proteins of African horse sickness virus. *Vaccine* **27**, 4434-4438.

[111] House, J.A., Lombard, M., Dubourget, P., House, C. and Mebus, C.A. (1994) Further studies on the efficacy of an inactivated African horse sickness serotype 4 vaccine. *Vaccine* **12**, 142-144.

[112] Kanai, Y., van Rijn, P.A., Maris-Veldhuis, M., Kaname, Y., Athmaram, T.N. and Roy, P. (2014) Immunogenicity of recombinant VP2 proteins of all nine serotypes of African horse sickness virus. *Vaccine* **32**, 4932-4937.

[113] Papadopoulos, E., Rowlinson, M., Bartram, D., Carpenter, S., Mellor, P. and Wall, R. (2010) Treatment of horses with cypermethrin against the biting flies Culicoides nubeculosus, Aedes aegypti and Culex quinquefasciatus. *Veterinary parasitology* **169**, 165-171.

[114] Schaffartzik, A., Hamza, E., Janda, J., Crameri, R., Marti, E. and Rhyner, C. (2012) Equine insect bite hypersensitivity: What do we know? *Veterinary immunology and immunopathology* **147**, 113-126.

[115] Riek, R. (1953) Studies on allergic dermatitis of the horse II.–Treatment and control. *The Australian Veterinary Journal* **29**, 185-187.

[116] Paton, T. (1863) The ‘‘horse sickness’’of the Cape of Good Hope. *Veterinarian* **36**, 489-494.

[117] Carpenter, S., Mellor, P.S. and Torr, S.J. (2008) Control techniques for Culicoides biting midges and their application in the U.K. and northwestern Palaearctic. *Medical and veterinary entomology* **22**, 175-187.

[118] Meiswinkel, R., Baylis, M. and Labuschagne, K. (2000) Stabling and the protection of horses from Culicoides bolitinos (Diptera: Ceratopogonidae), a recently identified vector of African horse sickness. *Bulletin of entomological research* **90**, 509-515.

[119] Baker, T., Carpenter, S., Gubbins, S., Newton, R., Lo Iacono, G., Wood, J. and Harrup, L. (2015) Can insecticide-treated netting provide protection for Equids from Culicoides biting midges in the United Kingdom? *Parasites & vectors* **8**, 604.

[120] Lincoln, V.J., Page, P.C., Kopp, C., Mathis, A., von Niederhäusern, R., Burger, D. and Herholz, C. (2015) Protection of horses against Culicoides biting midges in different housing systems in Switzerland. *Veterinary parasitology* **210**, 206-214.

[121] Page, P.C., Labuschagne, K., Venter, G.J., Schoeman, J.P. and Guthrie, A.J. (2014) Field and in vitro insecticidal efficacy of alphacypermethrin-treated high density polyethylene mesh against Culicoides biting midges in South Africa. *Veterinary parasitology*.

[122] Venter, G.J., Labuschagne, K., Boikanyo, S.N., Morey, L. and Snyman, M.G. (2011) The repellent effect of organic fatty acids on Culicoides midges as determined with suction light traps in South Africa. *Veterinary parasitology* **181**, 365-369.

[123] de Jong, P., Wessels, M.J., Stoop, R.M.G.L.I., Jacobs, F., Nodelijk, G. and Sloet van Oldruitenborgh-Oosterbaan, M.M. (2012) The effect of insect blankets on the feeding rate of culicoides species in horses in The Netherlands. In: *5th Congress of the European College of Equine Internal Medicine*, Journal of Veterinary Internal Medicine, Edinburgh. pp 418-440.

[124] DEFRA (2009) African horse sickness - Guidance on protection from vector attack, Ed: DEFRA.

[125] Balenghien, T., Cêtre-Sossah, C., Grillet, C., Delécolle, J.C., Mathieu, B. and Baldet, T. (2008) Diurnal activity of potential bluetongue vectors in northern Europe. *Veterinary Record* **162**, 323-324.

[126] Stevens, D.P., Henderson, D., Vlaminck, K., Eley, J. and Kennedy, A.S. (1988) High-cis permethrin for the control of sweet itch on horses. *Veterinary Record* **122**, 308.

[127] De Raat, I., Van Den Boom, R., Van Poppel, M. and van Oldruitenborgh-Oosterbaan, M.M.S. (2008) The effect of a topical insecticide containing permethrin on the number of Culicoides midges caught near horses with and without insect bite hypersensitivity in the Netherlands. *Tijdschr Diergeneeskd* **838**, 842.

[128] Robin, M., Archer, D., McGowan, C., Garros, C., Gardès, L. and Baylis, M. (2015) Repellent effect of topical deltamethrin on blood feeding by Culicoides on horses. *The Veterinary record*.

[129] Mullens, B.A., Gerry, A.C. and Velten, R.K. (2001) Failure of a Permethrin Treatment Regime to Protect Cattle Against Bluetongue Virus. *Journal of medical entomology* **38**, 760-762.

[130] Trigg, J. (1996) Evaluation of a eucalyptus-based repellent against Culicoides impunctatus (Diptera: Ceratopogonidae) in Scotland. *Journal of the American Mosquito Control Association* **12**, 329.

[131] Page, P.C., Labuschagne, K., Nurton, J.P., Venter, G.J. and Guthrie, A.J. (2009) Duration of repellency of N,N-diethyl-3-methylbenzamide, citronella oil and cypermethrin against Culicoides species when applied to polyester mesh. *Veterinary parasitology* **163**, 105-109.

[132] Palmer, J. (1969) Toxicologic effects of aerosols of N, N-diethyl-m-toluamide (deet) applied on skin of horses. *American Journal of Veterinary Research* **30**, 1929.

[133] González, M., Venter, G.J., López, S., Iturrondobeitia, J.C. and Goldarazena, A. (2014) Laboratory and field evaluations of chemical and plant-derived potential repellents against Culicoides biting midges in northern Spain. *Medical and veterinary entomology* **28**, 421-431.

[134] Venter, G.J., Labuschagne, K., Boikanyo, S.N.B. and Morey, L. (2014) Assessment of the repellent effect of citronella and lemon eucalyptus oil against South African Culicoides species. *Journal of the South African Veterinary Association* **85**.

[135] Mands, V., Kline, D.L. and Blackwell, A. (2004) Culicoides midge trap enhancement with animal odour baits in Scotland. *Medical and veterinary entomology* **18**, 336-342.

[136] Harrup, L.E., Logan, J.G., Cook, J.I., Golding, N., Birkett, M.A., Pickett, J.A., Sanders, C., Barber, J., Rogers, D.J., Mellor, P.S., Purse, B.V. and Carpenter, S. (2012) Collection of Culicoides (Diptera: Ceratopogonidae) using CO2 and enantiomers of 1-octen-3-ol in the United Kingdom. *Journal of medical entomology* **49**, 112-121.

[137] Kettle, D. (1962) The bionomics and control of Culicoides and Leptoconops (Diptera, Ceratopogonidae= Heleidae). *Annual review of entomology* **7**, 401-418.

[138] Harrup, L.E., Gubbins, S., Barber, J., Denison, E., Mellor, P.S., Purse, B.V. and Carpenter, S. (2014) Does covering of farm-associated Culicoides larval habitat reduce adult populations in the United Kingdom? *Veterinary parasitology* **201**, 137-145.

[139] Meiswinkel, R., Gomulski, L., Delécolle, J., Goffredo, M. and Gasperi, G. (2004) The taxonomy of Culicoides vector complexes–unfinished business. *Veterinaria italiana* **40**, 151-159.