African horse sickness: The potential for an outbreak in disease-free regions and current disease control and elimination techniques

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Summary

African horse sickness (AHS) is an arboviral disease of equids transmitted by *Culicoides* biting midges. The virus is endemic in parts of sub-Saharan Africa and official AHS disease-free status can be obtained from the World Organization for Animal Health on fulfilment of a number of criteria. AHS is associated with case fatality rates of up to 95%, making an outbreak among naïve horses both a welfare and economic disaster. The worldwide distributions of similar vector-borne diseases (particularly bluetongue disease of ruminants) are changing rapidly, probably due to a combination of globalisation and climate change. There is extensive evidence that the requisite conditions for an AHS epizootic currently exist in disease-free countries. In particular, although the stringent regulations enforced upon competition horses make them extremely unlikely to redistribute the virus, there are great concerns over the effects of illegal equid movement. An outbreak of AHS in a disease free region would have catastrophic effects on equine welfare and industry, particularly for international events such as the Olympic Games. While many regions have contingency plans in place to manage an outbreak of AHS, further research is urgently required if the equine industry is to avoid or effectively contain an AHS epizootic in disease-free regions. This review describes the key aspects of AHS as a global issue and discusses the evidence supporting concerns that an epizootic may occur in AHS free countries, the planned government responses, and the roles and responsibilities of equine veterinarians.

Introduction

African horse sickness (AHS) is an infectious, noncontagious, vector-borne viral disease of equids. Possible references to AHS have been found from several centuries ago, although 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 and management measures are 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 Organization 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].

African horse sickness virus (AHSV) is a member of the genus Orbivirus (family Reoviridae) and consists of 9 different serotypes [6]. All 9 serotypes of AHSV are endemic in sub-Saharan Africa and outbreaks of 2 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 1987–1990, 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 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]. AHS is almost exclusively a disease of equids and is not considered zoonotic, although disease associated with the virus has been described in man 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 2–10 days, depending on viral load, viral virulence and host factors [4]. Four different clinical forms of AHS are recognised, each associated with different 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 soon as 30 min after the onset of clinical signs (Fig 1).

Figure 1. Sudden death associated with peracute form of African horse sickness. Frothy fluid visible draining from nostrils (photo credit: Rudy Meiswinkel).

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 (Fig 2), before extending to the conjunctiva (Fig 3) and then the remainder of the head and neck. The distal limbs and ventral abdomen are rarely affected. Dyspnoea, cyanosis, 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].

Figure 2. A case of the cardiac form of African horse sickness demonstrating oedema of the supraorbital space and head.

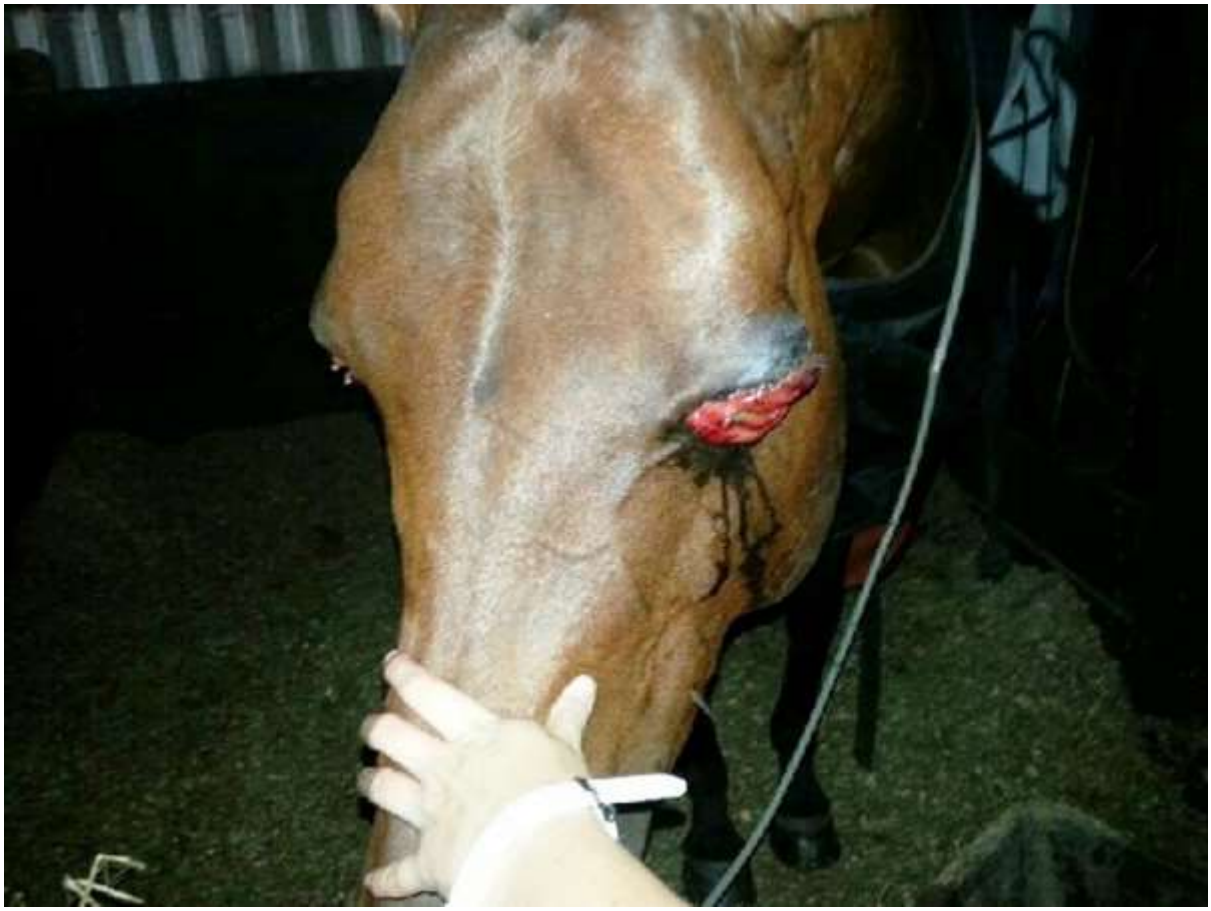

Figure 3. A case of the cardiac form of African horse sickness showing chemosis and supraorbital oedema (photo credit: Maygan Jennings).

Mixed form

Cases with this form are found to have a combination of pathologies at *post mortem*, although this is often not clinically apparent. 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

Any suspected cases of AHS in OIE disease–free countries must be reported to the state authorities and subject to laboratory confirmation [4]. Virus isolation is considered the gold standard for diagnosis, however the OIE accepts molecular evidence of viral presence by PCR and serological evidence of infection via enzyme-linked immunosorbent assays (ELISAs) [27]. Viral isolation is performed by inoculation of various cell cultures or mice cerebral

tissue and the process can be lengthy, which limits its use during initial control of a disease outbreak [4]. The use of serology for 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 immunosorbent 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]. PCR methods are currently under validation for recommendation 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 vaccines that allow differentiation of 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 certain 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 nonendemic, 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 <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.

Culicoides biting midges and their role in the epidemiology of AHS

Culicoides midges are among the world's smallest and most widespread insects. They are considered a biting nuisance to humans and livestock, transmit viral and parasitic diseases

and are the major cause of insect bite hypersensitivity (IBH) in horses [40]. There are currently over 1400 different species of *Culicoides* identified, with around 30 of these thought to be capable of virus transmission and over 50 different viruses isolated from midges worldwide [14, 41, 42]. Comparisons with the arboviral disease bluetongue are often made when considering AHS, as the viruses share vector *Culicoides* species within Africa and both have made incursions north into Europe [13, 16, 43]. The most relevant *Culicoides* species when considering AHSV and bluetongue virus (BTV) are shown in Table 1. The life-

Table 1. The 31 species of *Culicoides* known to play a role in the transmission of bluetongue disease and their known or suspected roles in AHS virus transmission. Expanded and revised from Meiswinkel *et al*. 2004 [139]

Species	AHSV vector role	BTV vector role	Regions of most importance
Culicoides imicola	Primary importance	Primary importance	Africa, Southern Europe, Asia
Culicoides bolitinos	Primary importance	Primary importance	Africa
Culicoides brevitarsis	Unknown	Primary importance	Australia
Culicoides obsoletus	Suspected	Primary importance	Europe
Culicoides scoticus	Unknown	Primary importance	Europe
Culicoides chiopterus	Unknown	Primary importance	Europe
Culicoides dewulfi	Unknown	Primary importance	Europe
Culicoides pulicaris	Suspected	Primary importance	Europe
Culicoides punctatus	Unknown	Primary importance	Europe
Culicoides magnus	Unknown	Lesser importance	Africa
Culicoides sonorensis	Lab vector	Primary importance	North and Central America
Culicoides insignis	Unknown	Primary importance	South and Central America
Culicoides pusillus	Unknown	Primary importance	South and Central America
Culicoides actoni	Unknown	Lesser importance	–
Culicoides brevipalpis	Unknown	Lesser importance	–
Culicoides dumdumi	Unknown	Lesser importance	–
Culicoides filarifer	Unknown	Lesser importance	–
Culicoides fulvus	Unknown	Lesser importance	–
Culicoides furens	Unknown	Lesser importance	–
Culicoides gulbenkiani	Unknown	Lesser importance	–
Culicoides milnei	Unknown	Lesser importance	–
Culicoides nevilli	Unknown	Lesser importance	–
Culicoides nubeculosus	Unknown	Lesser importance	–
Culicoides orientalis	Unknown	Lesser importance	–
Culicoides oxystoma	Unknown	Lesser importance	–
Culicoides peregrinus	Unknown	Lesser importance	–
Culicoides puncticollis	Unknown	Lesser importance	–
Culicoides stellifer	Unknown	Lesser importance	–
Culicoides tilineatus	Unknown	Lesser importance	–
Culicoides tororoensis	Unknown	Lesser importance	–
Culicoides wadai	Unknown	Lesser importance	–

AHSV = African horse sickness virus; BTV = bluetongue virus.

cycle of *Culicoides* includes the egg, 4 larval stages, the pupa and the adult [44]. As only female adults blood-feed, they are of primary importance when considering virus transmission.

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

In Africa, the most commonly implicated AHSV vectors are *Culicoides imicola*, which makes up over 90% of species caught using light traps in AHS endemic areas, and *Culicoides bolitinos*, which has more recently been recognised as an alternative vector in some regions [41, 54]. It is important to consider the evidence available to support the AHSV vector roles of these species. Biting insects have long been suspected to transmit AHSV and the disease was first induced in horses following inoculation with *Culicoides* extract in 1943 [13]. The ability of *Culicoides* to actually transmit AHSV was more convincingly demonstrated when the North American BTV vector, *Culicoides variipennis* (now *Culicoides sonorensis*), was shown to be an efficient laboratory vector for AHSV following oral inoculation [55]. Remarkably, transmission between live equid hosts has still not been demonstrated for any *Culicoides* species. Epidemiological studies have added some evidence to support this theory by demonstrating spatial and temporal associations between the abundance of *C. imicola* (as caught by light traps) and the incidence of AHS in Spain, Portugal, Morocco and South Africa [45-48].

Traditionally, *Culicoides* species are identified based on several morphological traits. The wing pattern in particular is very important, with variations in venation, colour, marking pattern and covering by short hairs used for differentiation. Other features, including thoracic colouring, antennae and abdominal spermathecae, are also used [56, 57]. Unfortunately, identification of many species requires a specialised knowledge of insect morphology that is no longer readily available [58, 59]. Given the importance of several of these species in arboviral transmission, PCR assays have recently been developed to provide rapid and accurate identification [59, 60].

The ability of *Culicoides* to cause outbreaks of AHS is dependent on the generation of large numbers of midges that can only occur when the appropriate weather conditions and biotic environment allow the development of large populations [14]. The 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, 2 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 5 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 south east 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

Culicoides species (Table 1) indigenous to disease-free countries might be able to transmit AHS 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]. The vectorial capacity of *Culicoides* has been shown to increase with ambient temperatures of 27–30°C and species traditionally considered nonvectors 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 straightforward, this is anticipated to increase the likelihood of competent AHSV vectors being present in this region [72].

Evidence for a potential role of indigenous *Culicoides* species is provided by comparisons with BTV epidemiology. AHSV and BTV share vector species (including *C. imicola*) and both have made incursions north into Europe [13, 16, 43]. During the recent bluetongue 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 *Culicoides pulicaris, Culicoides punctatus*, *Culicoides dewulfi, Culicoides obsoletus, Culicoides scoticus* and *Culicoides 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 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 considered 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].

Culicoides sonorensis is absent from much of Central America and all of South America [14]. BTV is endemic in Central America, where *Culicoides insignis* and *Culicoides pusillus* are the vectors of primary importance and it is suspected that the region acts as a source of BTV for both North and South America [80]. Although evidence is limited, BTV has been reported to be present in large parts of South America, where *C. insignis* and *C. pusillus* are again thought to be primary vectors [14, 81]. Brazil is of particular current importance, given the 2016 Olympic Games. Although very few *Culicoides* distribution data are available, a recent study showed that *C. insignis* accounted for 81% of livestock-associated catches in Brazil [82]. This species must therefore be considered of greater potential as an AHSV vector in the region.

In Australasia *Culicoides fulvus*, *Culicoides wadai*, *Culicoides actoni* and *Culicoides brevitarsis* are important vector species for BTV [14]. It has been suggested that *C. brevitarsis* and *C. imicola* may share a common ancestry and the competency of *C. brevitarsis* for AHSV should therefore be investigated [62, 83]. In Asia, BTV is transmitted by several vector species, although data are limited in many parts of the region. Of particular interest in this region is the presence of *C. imicola* in China [14].

When considering the current *Culicoides* species of global importance relevant to transmission of BTV and AHSV (as summarised in Table 1) it is clear that there is a dearth of basic research on the vector competence of many *Culicoides* species for AHSV. This has led to a reliance on BTV vector knowledge as a reference for AHSV and greater research effort is thus urgently required. In summary, it is possible that the appropriate *Culicoides* species and climatic conditions to support an outbreak of AHS are currently present in many AHS-free countries, although more research is urgently required.

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 [86].

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 6 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 under this scenario. The illegal transport of a reservoir equid (for example a donkey moved from northern Africa into Europe) represents a definite risk that cannot be quantified [87]. The likelihood of the introduction of AHSV to Great Britain via the legal trade of equine semen, ova and embryos, meat, and other specified biological products is considered to be negligible [87].

4 – Viral introduction within infected Culicoides

There are 2 possible ways that a virus-infected *Culicoides* midge could reach a previously unaffected geographical area. The first is within a plane or freight container in transit, especially those containing vegetative materials such as packaged flowers [89, 90]. While this is well documented for other vector insects, there is no suitable information available for estimating the risk of AHSV introduction via inadvertent transportation of *Culicoides* [89, 91]. An assessment of the risk of a European bluetongue outbreak caused by *Culicoides* movement via intracontinental transport and trade concluded that large numbers of vectors would have to be transported to pose a significant risk [92]. An even greater number of *Culicoides* would likely have to be transported for an extensive AHS outbreak, as the number of resident equid hosts is generally fewer compared to livestock affecting BTV transmission.

The second potential method of virus introduction via *Culicoides* is wind dispersal. Although adult *Culicoides* rarely fly further than a few hundred metres from their breeding grounds,

they can be passively dispersed over much greater distances if wind patterns are appropriate [14]. The wind dispersal of infected *Culicoides* has been implicated as the cause of the overseas spread of AHSV from Morocco to Spain in 1966 and BTV from mainland Europe to the UK in 2007 [93, 94].

5 – Reversion to virulence of vaccine strains

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 thus 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

An AHS epizootic would have severe consequences for equine welfare and industry in affected regions. During a 3-year outbreak in Asia between 1959 and 1961 over 300,000 equids died and in Spain 110 horses died as a direct result of AHS from 1987 to 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]. AHS 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 *AHS control strategy for Great Britain*, which is freely available online [21]. A summary of the measures is provided in Figure 4.

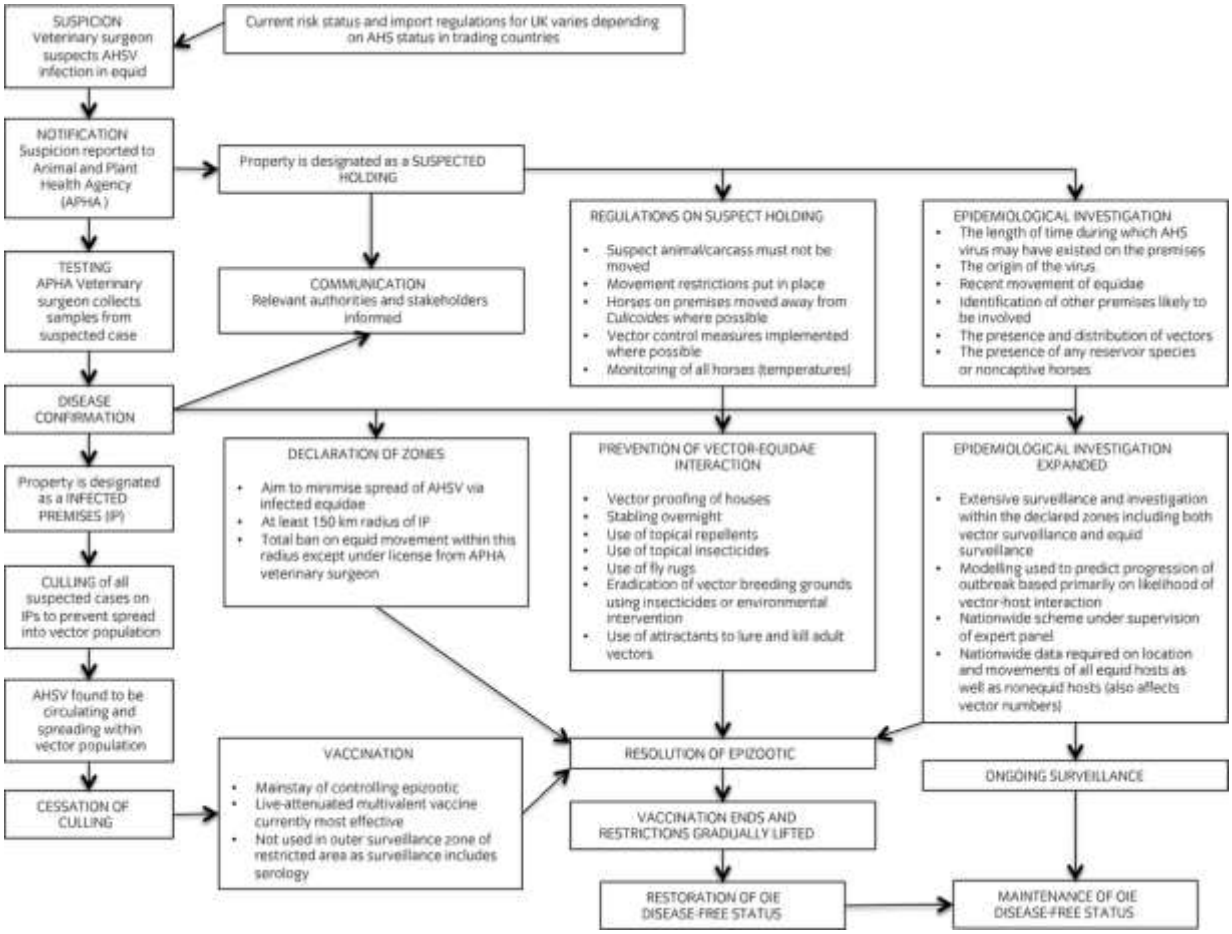

Figure 4. Flow chart summarising the response to African horse sickness virus (AHSV) infection based on the AHS control strategy for Great Britain [21].

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 later found to be infected. 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 south east 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 (EU) 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, 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 National Animal Identification Scheme (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 7 of the 9 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 protect all horses fully [4]. A recent study showed that 16% of immunised horses in an AHS endemic area were infected with AHSV over a 2-year period [107]. As half of these cases were subclinically 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 subclinically 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 EU 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 nonendemic countries, and could allow for differentiation of infected from vaccinated animals.

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 by bite prevention [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 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 reduce further 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, 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 neither licensed in the horse nor specifically against midges in any species [21]. The application of permethrin to horses with IBH significantly improved clinical signs in 86% of 43 horses [126]. Other studies in horses do not support the use of topical deltamethrin or permethrin solution as a repellent to prevent *Culicoides* from biting horses [127, 128]. However, these studies did not investigate the possible insecticidal effects of deltamethrin in reducing onward transmission of disease from viraemic horses or the numbers of adult *Culicoides* within an area. This emphasises the important and often poorly defined distinction between insecticides and repellents [127]. Possibly the most direct indication of the effects of the permethrins on the transmission of arboviral disease is a field study conducted in cattle. This study demonstrated that 2-weekly application of topical permethrin did not reduce exposure to BTV as measured by serology [129]. Injectable avermectins are used to control ectoparasites in many species, including

the horse. Unfortunately, their efficacy against different *Culicoides* species varies significantly, with near toxic doses required in some cases and there are no data available on their efficacy against European *Culicoides* species [117].

N,N-diethyl-3-methylbenzamide (DEET) has been shown to reduce the biting rate of *C. impunctatus* in man [130]. The application of 15% DEET-impregnated mesh to suction light traps has been shown to significantly reduce *Culicoides* catches when compared to untreated mesh [131]. However, there is in vivo evidence of adverse effects (including hypersteatosis and dermatosis) occurring in horses when DEET is repeatedly applied topically at concentrations >15%, although many were only mild reactions [132]. Recent work has demonstrated that a combination of DEET and plant-derived organic fatty acids may provide an effective and long-lasting repellent effect against *Culicoides* [133]. Citronella oil, while known to be an effective mosquito repellent, has been repeatedly shown to have either no repellent effect or potentially an attractant effect on *Culicoides* [131, 134].

Other control methods, such as the use of chemoattractants with 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 control midges effectively [137]. It appears certainly 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 6 diseases for which the OIE requires additional mitigation measures in high health, high performance 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 further 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 4 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 vaccine 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.

Authors' declaration of interests

No competing interests have been declared.

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