Vet. Res. 35 (2004) 445–466

DOI: 10.1051/vetres:2004021

Review article

African horse sickness

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(Received 30 December 2003; accepted 1 March 2004)

Abstract – African horse sickness virus (AHSV) causes a non-contagious, infectious insect-borne disease of equids and is endemic in many areas of sub-Saharan Africa and possibly Yemen in the Arabian Peninsula. However, periodically the virus makes excursions beyond its endemic areas and has at times extended as far as India and Pakistan in the east and Spain and Portugal in the west. The vectors are certain species of *Culicoides* biting midge the most important of which is the Afro-Asiatic species *C. imicola*. This paper describes the effects that AHSV has on its equid hosts, aspects of its epidemiology, and present and future prospects for control. The distribution of AHSV seems to be governed by a number of factors including the efficiency of control measures, the presence or absence of a long term vertebrate reservoir and, most importantly, the prevalence and seasonal incidence of the major vector which is controlled by climate. However, with the advent of climate-change the major vector, *C. imicola,* has now significantly extended its range northwards to include much of Portugal, Spain, Italy and Greece and has even been recorded from southern Switzerland. Furthermore, in many of these new locations the insect is present and active throughout the entire year. With the related bluetongue virus, which utilises the same vector species of *Culicoides* this has, since 1998, precipitated the worst outbreaks of bluetongue disease ever recorded with the virus extending further north in Europe than ever before and apparently becoming endemic in that continent. The prospects for similar changes in the epidemiology and distribution of AHSV are discussed.

Culicoides **/ vectors / epidemiology / climate / disease prediction**

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1. INTRODUCTION

African horse sickness (AHS) is a non-contagious, infectious, insect-borne disease of equids caused by a virus of the same name (AHSV). Although zebra and donkeys rarely exhibit clinical signs, the effects of the disease, particularly in susceptible populations of horses can be devastating and mortality rates for this species may exceed 90%. As a consequence of its severity and because it is able to expand rapidly and without apparent warning out of its endemic areas AHS has been allocated OIE (Office International des Epizooties) List A status.

In horses, AHS is characterised by clinical signs, which develop as a result of damage to the circulatory and respiratory systems giving rise to serous effusion and haemorrhage in various organs and tissues. The extent and severity of the clinico-pathological findings have been used to classify the disease into four forms. In ascending order of severity these are horse sickness fever (which usually affects only mules, donkeys and partially immune horses), the subacute or cardiac form, the cardio-pulmonary or mixed form and the peracute or pulmonary form. All forms of disease can occur in any one outbreak but in susceptible populations of horses the mixed and pulmonary forms tend to predominate so mortality rates in these animals will be very high. The clinical signs occurring in naturally and experimentally infected horses have been described in detail by various workers [7, 25, 70, 73, 98, 122].

2. HISTORY

AHS is an ancient disease and the first reference to it concerns an epidemic that apparently occurred in 1327 in the Yemen [97]. However, the virus almost certainly originated in Africa and was first recognised there subsequent to the introduction of horses during the exploration of central and east Africa, the first observations being

made by a certain Father Monclaro in 1569 [121]. In southern Africa the virus has probably been present from distant times but disease was not recognised until some 60 years after the first introduction of horses in 1657, the first major outbreak occurring in 1719 when over 1 700 animals died [50]. Subsequently and over the next 217 years at least 10 major and several lesser outbreaks of AHS have been recorded in southern Africa the largest being in South Africa in 1854–1855 when over 70 000 horses died [9, 25]. The frequency, extent and severity of outbreaks in southern Africa has declined significantly over the last century, coinciding with major decrease in the horse and zebra populations and the introduction of AHS vaccines.

3. AETIOLOGY

African horse sickness virus (AHSV) is a member of the genus *Orbivirus* in the family Reoviridae and as such is morphologically similar to other orbiviruses such as bluetongue virus (BTV) of ruminants and equine encephalosis virus [117, 119, 129]. The virion is an unenveloped particle about 70 nm in diameter and is made up of a two-layered icosahedral capsid, which is composed of 32 capsomeres [25]. The genome comprises 10 double stranded RNA segments, each of which encodes at least one polypeptide [43]. The genome is enclosed within the core particle, which comprises two major proteins, VP3 and 7 which are highly conserved among the nine AHSV serotypes, and three minor proteins, VP 1, 4 and 6 [20, 100, 108]. Together they make up the group specific epitopes [42, 52, 105]. The core particle is surrounded by the outer capsid composed of two proteins VP2 and 5. VP2 is the protein that has most responsibility for antigenic variation [21, 72, 130]. At least three non-structural proteins have been identified in infected cells (NS1, 2 and 3/3a) [28, 60, 69].

To date, nine antigenically distinct serotypes have been identified the last being in 1960 [58]. There is considered to be some cross relatedness between the serotypes, typically between AHSV types 1 and 2; 3 and 7; 5 and 8; and 6 and 9, although there is no evidence from the field that there is any intratypic variation [25]. Of the nine serotypes, types 1 to 8 are typically found only in restricted areas of sub-Saharan Africa while type 9 is more widespread and has been responsible for virtually all epidemics outside Africa, the only exception being the 1987–1990 Spanish-Portuguese outbreaks which were due to AHSV-4.

The physio-chemical characteristics of AHSV are fairly typical for the genus *Orbivirus*. The virus is acid sensitive, being readily inactivated at pH values below 6.0 but remains relatively stable at more alkaline pH values (7.0–8.5). It is resistant to lipid solvents and relatively heat resistant. Infectivity is remarkably stable at 4 °C, particularly in the presence of stabilisers such as serum and OCG (sodium oxalate, carbolic acid and glycerine). Minimal loss of titre occurs with viruses that have been lyophilised or frozen at –70 °C with Parker Davis Medium but the viruses are fairly labile between –20 °C and –30 °C [25, 91].

4. HOST RANGE

Zebra have long been considered the natural vertebrate host and reservoir of AHSV, and are believed to play a vital role in the persistence of the virus in Africa. This species rarely exhibits clinical signs of infection. All other equid species and crossbreeds are also susceptible to infection but because of the high mortality rate frequently recorded in horses and occasionally mules these should be regarded as accidental or indicator hosts. Indeed, the failure of AHSV to become established outside tropical and sub-tropical regions of Africa, where zebra principally reside, indicates that horses, mules and donkeys generally speaking are not long-term reservoirs of AHSV and are not involved in the permanent persistence of the virus. The

sole exception may be AHSV-9, which survives perfectly well in West Africa where zebra no longer occur.

Dogs are susceptible to experimental infection with AHSV and may die from the effects of the virus. Infection also occurs readily following ingestion of infected horsemeat [123]. However, most authorities believe that this species does not play a role in the spread or maintenance of AHSV as viraemia is thought to be low level and transient and as vector insects are rarely attracted to them.

Infection in camels is rare and inapparent and no details are available on the level and duration of viraemia, if any. It is likely that like the dog this species has no significant role in the epidemiology of AHS [8].

5. PATHOGENESIS

On entry into the vertebrate host initial multiplication of AHSV occurs in the regional lymph nodes followed by dissemination throughout the body via the blood (primary viraemia) and subsequent infection of target organs and cells, namely the lungs, spleen and other lymphoid tissues, and certain endothelial cells [25]. Virus multiplication in these tissues and organs gives rise to a secondary viraemia, which is of variable duration and titre dependent upon a number of factors including host species. Under natural conditions the incubation period to the commencement of secondary viraemia is less than nine days although experimentally it has been shown to vary between 2 and 21 days [81]. In horses a titre of up to $10^{5.0}$ $TCID_{50}$ of virus/mL may be recorded but viraemia usually lasts for only four to eight days and has not been detected beyond 21 days [25]. In donkeys and zebra the levels of viraemia are lower ($< 10^{3.0}$ $TCID_{50}$/mL) but may extend for up to four weeks [25, 47].

In experimentally infected horses, high concentrations of AHSV rapidly accumulate in the spleen, lungs, caecum, pharynx, choroid plexus and most lymph nodes. Subsequently virus is found in most organs, probably due to their blood content. In the blood, virus is associated with the cellular fraction both red blood cells [50, 122] and the buffy coat, while very little is present in the plasma [65]. This may be similar to the situation that occurs with the related BTV of ruminants where virus is sequestered in the cell membrane of infected red blood cells and is thereby protected from the effects of humoral antibody leading to both circulating in the system together. In ruminants this leads to extended viraemia, a situation that seems not to occur with AHSV in horses although viraemia in the presence of circulating antibody has been reported in zebra [25, 122]. In AHS the onset of viraemia usually corresponds with the appearance of fever and persists until it disappears [66].

Wohlsein et al. [136] have shown that in experimentally infected horses with the peracute form of disease antigen is found primarily in the cardio-vascular and lymphatic systems, and to lesser extent throughout the body. In animals with horse sickness fever antigen is concentrated in the spleen, with lesser amounts elsewhere. The main locations of antigen were endothelial cells suggesting them to be a primary target of the virus and large cells of the red pulp of the spleen. The presence of antigen in large mononuclear cells, resembling phagocytic cells, and surrounding lymphoid follicles suggests that these cells might also be involved in virus replication and in the transport of viral protein to the lymphoid follicles [136].

5.1. Clinical signs

AHSV can cause four forms of disease: horse sickness fever, cardiac form, mixed form and the pulmonary form [37].

Horse sickness fever is invariably mild usually involving only mild to moderate fever and oedema of the supraorbital fossae

– there is no mortality. It frequently occurs following infection with less virulent strains of virus or when some degree of immunity exists and is the only form of disease exhibited by the African donkey and zebra [59].

The cardiac or sub-acute form of the disease is characterised by fever and a time course of several weeks. The main clinical finding is subcutaneous oedema, particularly of the head, neck and chest but also of the supraorbital fossae. Oedema is never seen in the lower limbs. The conjunctivae may be congested, petechial haemorrhages may be seen in the eyes and ecchymotic haemorrhages may be seen on the ventral surface of the tongue. Colic is often a feature and mortality rates may exceed 50% [25].

The mixed form is often the most common form of AHS and is a combination of the cardiac and pulmonary forms of disease. The mortality rate is approximately 70% and death usually occurs within 3 to 6 days after onset of fever [25].

The pulmonary form is peracute and may develop so rapidly that an animal can die without previous indication of illness. Usually there will be marked depression and fever (39–41 °C) followed by onset of respiratory distress and severe dyspnoea. Coughing spasms may occur, the head and neck are extended and severe sweating develops. There may be periods of recumbency and terminally quantities of frothy fluid may be discharged from the nares. Anorexia is not a feature. The prognosis for horses suffering from this form of AHS is extremely grave and mortality rates commonly exceed 95% [25, 81]. The pulmonary form of AHS is also the form most usually seen in dogs [25].

5.2. Pathology

5.2.1. Macrolesions

These vary in accordance with the disease. With the pulmonary from the most conspicuous lesions are interlobular oedema of the lungs and hydrothorax. The sub-pleural and interlobular tissues are infiltrated with a yellowish gelatinous exudate and the entire bronchial tree may be filled with a surfactant, stabilised froth. Ascites can occur in abdominal and thoracic cavities and the mucosa of the stomach may be hyperaemic and oedematous.

In the cardiac form the most prominent lesions are gelatinous exudate in the subcutaneous, sub-fascial and intramuscular tissues and lymph nodes. Hydropericardium is seen and haemorrhages are found on the epicardial and/or endocardial surfaces. Petechial haemorrhages and/or cyanosis may also occur on the serosal surfaces of the caecum and colon. In these instances a distinct demarcation can often be seen between affected and unaffected parts. This may be due to a selective involvement of endothelial cells. As in the pulmonary form ascites may occur but oedema of the lungs is either slight or absent.

In the mixed form of AHS, lesions common to both the pulmonary and cardiac forms of the disease occur.

5.2.2. Microlesions

The histopathological changes are a result of increased permeability of the capillary walls and consequent impairment in circulation. The lungs exhibit serous infiltration of the interlobular tissues with distension of the alveoli and capillary congestion. The central veins of the liver are distended and interstitial tissue contains erythrocytes and blood pigments while the parenchymous cells show fatty degeneration. Cellular infiltration can be seen in the cortex of the kidneys while the spleen is heavily congested. Congestion may also be seen in the intestinal and gastric mucosae, and cloudy swelling in the myocardial and skeletal muscles [51, 73, 91].

6. EPIDEMIOLOGY

6.1. Distribution

At present AHSV is endemic in tropical and sub-tropical areas of Africa south of the Sahara occupying a broad band stretching from Senegal in the west to Ethiopia and Somalia in the east, and extending as far south as northern South Africa [59, 84]. The Sahara desert however, seems to provide an effective geographical barrier which has prevented the virus from establishing itself permanently in northern Africa or beyond. The virus may also be endemic in one place outside Africa, in Yemen in the Arabian Peninsula [112], although its long-term status in this area is so far uncertain.

6.1.1. AHSV in South Africa

Interestingly, into recent historic times AHSV has been endemic across virtually the whole of South Africa and outbreaks of AHS in horses are recorded on an almost countrywide basis throughout the whole of the 19th Century and into the first few decades of the 20th Century [9]. However, the decline in the annual number of AHS outbreaks over this period, particularly in the southern areas of South Africa, seems to have coincided with the elimination, through hunting, of the large free-ranging populations of zebra (considered to be the reservoir) from almost all of that country. For most of the 20th Century large populations of zebra have been restricted to game parks in the northeastern parts of South Africa, where AHSV is still endemic [9]. In the northeast of South Africa seroconversion to AHSV occurs in zebra in every month of the year and virtually all adult animals have specific antibodies to all nine serotypes of the virus. At present populations of zebra elsewhere in South Africa are small, comprising isolated herds of less than 100 individuals each which is presumably insufficient to maintain AHSV throughout the year as the virus only occurs seasonally or occasionally in these areas. However, as restocking of zebra is reported to be taking place in many parts of South Africa it may well be that at some ill-defined population density, conditions for the re-establishment of permanent foci of AHSV across the country may be generated [9].

6.1.2. AHSV outside sub-Saharan Africa

Until relatively recently AHSV was believed to be confined to sub-Saharan Africa except for occasional excursions into North Africa or the Arabian Peninsula [91, 106]. However, in the period 1959 to 1961 AHSV-9 expanded out of Africa and spread in a broad swathe across Saudi Arabia, Syria, Lebanon, Jordan, Iraq, Turkey, Cyprus, Iran, Afghanistan, Pakistan and India [41, 57, 59, 91, 106]. Nevertheless, by the end of 1961 in the face of a massive vaccination campaign and the death of over 300 000 equids the disease in Asia ended [6]. The inability of the virus to persist in these regions was probably due to a combination of factors including vaccination, vector control campaigns and adverse climatic conditions that reduced or prevented adult vector activity during the winter periods [80]. It was also the case that the mortality rate from AHS was so high that much of the area was virtually depopulated of susceptible equids.

During 1965 AHSV-9 again spread beyond its sub-Saharan endemic zones and appeared first in Morocco then spreading into Algeria and Tunisia before crossing the Straits of Gibraltar into Cadiz Province, Spain, in October 1966 [29, 30, 63, 95]. The virus was eliminated from Spain within three weeks, following the application of a vigorous vaccination and slaughter policy but overall it persisted in North Africa for almost two years [30]. The appearance of AHSV in North Africa was thought to be due to the movement of nomads and their animals, particularly donkeys, across the Sahara from west Africa where AHSV-9 is endemic [74, 103].

Subsequent to 1966 AHSV was reported only from sub-Saharan Africa for over 20 years. However, in July 1987 an outbreak of AHS due to serotype 4 of the virus was reported in central Spain, in the Provinces of Madrid, Toledo and Avila [70]. The outbreak was apparently caused by the importation of a number of sub-clinically infected zebra from Namibia into a safari park at Aldea del Fresno some 45 km SW of Madrid [70]. This park subsequently became the site of the first 27 cases of AHS in Spanish horses in 1987. The epidemic continued for 3 to 4 months in central Spain and then apparently ended during October [3, 31]. Although the 1987 Spanish outbreak caused some alarm because AHS is a notifiable disease and this was the furthest north that it had ever been recorded, concern was allayed because all previous information suggested that the virus was incapable of overwintering in Europe. Consequently, the apparent end of the epidemic in October 1987 was in line with expectations and was assumed to be the finish of the matter. However, this was not the case and further more severe outbreaks occurred in Spain during 1988, 1989 and 1990, in Portugal in 1989 and in Morocco in 1989, 1990 and 1991. All of these outbreaks were due to AHSV-4 a serotype that had never previously been seen outside southern Africa [80]. During the course of the outbreaks there was no other evidence of AHSV of any serotype within 2000 km of Spain or northern Morocco so it seems certain that the virus had persisted in the area for at least five years overwintering four times in the process. At the time this situation was unprecedented and led to much speculation on how and why the epidemiological situation had changed.

It is now known that AHSV is able to overwinter in southern Spain and Portugal, and in Morocco because an efficient vector species of *Culicoides* is present in these regions and because the climate is sufficiently mild for the adults of this vector to be active throughout the year [16, 22, 90].

6.2. Vectors, transmission and climate

AHSV has long been thought to be transmitted between its equid hosts by biting arthropods and during the last century suspicion fell, at one time or another, on a wide variety of species and genera. However, in more recent times it has become apparent that certain species of *Culicoides* are by far the most important vectors of AHSV. Du Toit [34] first showed that wild-caught *Culicoides* species (mixed pools) were infected with AHSV and later in 1945 Wetzel et al. [133] demonstrated the transmission of the virus by *Culicoides* bite from an infected to a susceptible horse 12 days after the insects' infecting blood meal. However, subsequent to that finding it was not until 1975 that Mellor et al. [86], and Boorman et al. [15] using *C. variipennis* (= *sonorensis*) a N. American species as a model finally confirmed Du Toit's original work. These authors demonstrated for the first time that AHSV is able to replicate in a species of *Culicoides* subsequent to ingestion by a factor of up to 10 000-fold and that transmission was also possible after 7 to 10 days incubation at 26 °C.

6.2.1. Culicoides imicola

In Africa, the work of several workers has shown that the major vector of AHSV in that continent is *C. imicola,* and indeed until recently this species was considered to be the only *Culicoides* involved in AHSV transmission in the field [16, 77, 78, 124, 125].

Culicoides imicola is an Afro-Asiatic species that is common throughout Africa and much of SE Asia stretching as far east as Laos [56, 131, 135]. This makes *C. imicola* the most widely distributed of all *Culicoides* species though it was only in 1982 that it was first recorded in Europe – in parts of southern Spain [87]. However, it is now known to be widespread across virtually the whole southern part of that continent having been recorded throughout most of Portugal, much of Spain and Italy, large areas

of mainland Greece, many Mediterranean islands including Corsica, the Balearics, Sardinia, Sicily, Malta and much of the Greek archipelago [40, 85]. Indeed, the most recent finding in southern Switzerland is, at 46° N, the furthest north that this species has so far been found anywhere in the world (Cagienard and Griot, 2003 – personal communication). The presence of *C. imicola* in Spain and Portugal and in Morocco [10, 11, 16] is clearly the major reason why AHSV could be transmitted in those areas. Equally importantly, the all-year-round presence of adult *C. imicola* in parts of Spain, Portugal, and Morocco is the main reason why AHSV was able to overwinter four times in the region until it was finally eliminated by a concerted vaccination and control campaign [11, 22, 90].

Whether the more recent findings of *C. imicola* across southern Europe represent a real and general movement northwards of this species, perhaps in response to climate-change, or whether they are merely a reflection of more intensive sampling due to the 1998–2003 bluetongue epizootic in the area is difficult to say although in some regions at least the former seems to be the more likely option. For example, in mainland Greece, a vector insect survey in 1983 included 16 collections of *Culicoides* comprising 19 species taken from locations where there was intense BTV transmission in 1999 but not a single *C. imicola* was recorded [88]. However, more recent surveys in 1999–2002 from locations in the same regions have found *C. imicola* to be widespread and abundant [5, 102]. Be that as it may, the newly discovered presence of populations of *C. imicola* in eastern Spain, Menorca, Mallorca, Ibiza, Corsica, Sardinia, mainland Italy, mainland Greece and Tunisia means that each of these areas is vulnerable to AHSV incursion. Indeed the recent devastating incursions of the related BTV, which is transmitted by the same vector species of *Culicoides*, into virtually all of these areas between 1998 and 2003 provides a graphic illustration of what could happen with AHSV.

6.2.2. Prediction of AHS risk by modelling the distribution of the major vector

As the major field vector of AHSV is *C. imicola* the presence of populations of this midge clearly confers a risk of AHS (cf. the outbreaks of AHS in Spain during 1987–1990). In this context, recent work by Baylis et al. [10, 12] has shown that the distribution and abundance of *C. imicola* can be modelled with some accuracy using a series of satellite-derived proxy climatic variables. The most important of these are the normalised vegetation index (NDVI) which provides a measure of photosynthetic activity and is therefore also correlated with soil moisture, and land surface temperature (LST). NDVI is presumably important because *C. imicola* breeds in damp or wet soil and, therefore, a high NDVI potentially correlates with the presence of breeding sites. The best model, which combined the minimum LST and minimum NDVI as predictors of *C. imicola* abundance, accounted for nearly 67% of variance. In the most recent predictive models of *C. imicola* abundance and distribution which have been developed in relation to BTV rather than AHSV, altitude, NDVI, middle infra-red reflectance, LST and air temperature have all been included [120]. These authors then used discriminant analysis to identify the best models from 40 temporally Fourier-processed 1 km spatial resolution remotely-sensed variables based on the above. The best models correctly predicted *C. imicola* presence and absence at 83 of 87 sites (95%) and abundance at 76 sites (87%).

6.2.3. Other potential Culicoides *vectors of AHSV*

As long ago as 1975 it was shown that *C. sonorensis* (= *variipennis*) the North American vector of BTV is also an efficient laboratory vector of AHSV [15, 86]. This suggests that should viraemic equids gain entry to those parts of North America where

C. sonorensis occurs (most of the southern and western United States) transmission of the virus would be likely [82].

In the Old World, *C. imicola* has long been considered to be the only confirmed field vector of AHSV. However, recent work by Venter et al. [127] has now implicated a second African species, *C. bolitinos,* as a potential field vector of this virus. *Culicoides bolitinos,* which has also been implicated as a BTV vector, has a wide distribution in southern Africa and is particularly common in cooler highland areas where *C. imicola* is rare [127].

During the 1987–1990 outbreaks of AHS in Spain and Portugal most isolations of AHSV, as expected, were made from *C. imicola* the known major field vector [89]. However, surprisingly isolations were also made from mixed pools of *Culicoides* consisting almost entirely of *C. obsoletus* and *C. pulicaris* but excluding *C. imicola* suggesting that one or both of these species might also be involved in the transmission AHSV in Europe [89]. More than 10 years after this suggestion, *C. obsoletus* and *C. pulicaris* group midges have both been implicated as vectors of BTV during the 1998–2003 incursions of that virus into Europe [23, 85, 114]. Indeed, the presence of these novel field vectors has been cited as the main reason why BTV has been able to penetrate into wide areas of Europe where *C. imicola* is absent [83, 85]. Consequently, as BTV and AHSV utilise the same species of *Culicoides* as vectors it is probable that in regions where *C. obsoletus* and *C. pulicaris* are abundant future incursions of the equid virus could also extend well beyond the distribution of *C. imicola.* In this context, it should be noted that the *obsoletus* and *pulicaris* groups of midges are probably the commonest *Culicoides* species across the whole of central and northern Europe [83], a fact that veterinary authorities and those concerned with control would do well to take on board. But does this mean that any future incursion of AHSV into Europe could extend across virtually the whole of the continent? The answer is, not necessarily so. As we shall see from the next section, in addition to susceptible hosts, competent vectors and the virus, ambient temperature is critically important and is often the controlling variable governing transmission.

6.2.4. Temperature and AHSV infection of vector Culicoides

AHSV infection rates of vector *Culicoides* and rates of virogenesis within them are temperature dependent [132]. As temperature increases infection rates also tend to increase, virogenesis is faster and transmission can occur sooner. However, midge survival rates decrease. Conversely, as temperature is reduced the reverse is true for each of these variables. The likelihood of transmission is therefore a function of the interaction of these two opposing sets of trends.

Furthermore, replication of AHSV does not seem to occur below 15 °C and at temperatures below this level the apparent infection rate rapidly falls to zero [132]. However, when midges are maintained for extended periods at these cooler temperatures and then transferred to temperatures within the virus permissive range, "latent" virus that has presumably persisted at very low levels in some individuals, commences replication and rapidly reaches sufficiently high titres for transmission to occur [132].

In related studies other workers have shown that adults of *C. imicola*, the major vector of AHSV, are active at temperatures as much as 3 °C lower than the minimum required for AHSV replication [116]. This finding suggests that AHSV transmission may be possible only over the warmer parts of a vector's range while in the cooler areas, even if the virus were to be introduced, transmission would be impossible or only possible at certain times of the year (e.g. summer) or in climatically sheltered localities. At face value this work suggests that even if *C. imicola* were to continue its

expansion northwards its ability to transmit AHSV will progressively decrease with increasing latitude unless accompanied by climate moderation. Nevertheless, the fact that midge survival at these low temperatures is dramatically extended – to as long as 90 days in some cases, must also be taken into account as must the possibility that "latent" virus may be present in some of these surviving midges that will commence replication should temperatures rise to permissive levels e.g. during spring. These phenomena could constitute a virus overwintering mechanism in the absence of vertebrates and also in the apparent absence of infected vectors (Tab. I).

6.2.5. AHS and the El Niño/Southern Oscillation

In South Africa major epidemics of AHS occur every 10–15 years but the cause of this pattern has been uncertain. However, recently a strong link between the timing of these epidemics and the warm (El Niño) phase of the El Niño/Southern Oscillation (ENSO) has been discovered. Baylis et al. [13] have suggested that the link is due to the combination of heavy rain and drought that ENSO brings to South Africa, and *C. imicola,* the vector of AHSV in southern Africa. *Culicoides imicola* breeds in wet soil and in years of heavy rain its populations can increase by over 200-fold [78]. Baylis et al. [13] have also shown that since 1803, 13 of the 14 major epidemics of AHS in South Africa have coincided with a warm phase ENSO. However, since 1803, 42 ENSOs have occurred which were not accompanied by AHS epidemics. This apparent anomaly is explained by showing that in South Africa, warm phase ENSOs typically bring heavy rain followed by drought – no AHS epidemics occurred during such years. However, a sub-set of ENSO years exist where the pattern is reversed – i.e. drought followed by heavy rain. These are the years when the 13 AHS epidemics occurred. The reasons why this pattern of drought followed by heavy rain is conducive to AHS are unclear though these authors [13] suggest that it may have to do with the congregation of zebras (the AHSV reservoir) near remaining waterholes during the drought period where they come into contact with and infect more vectors which then disperse rapidly once rain provides more breeding sites. Whatever the reason, recognition of this pattern means that prediction of AHS epidemics in South Africa should now be significantly easier.

7. DIAGNOSIS

Clinical signs and lesions in association with previous epidemiologial information may be sufficient for clinical diagnoses, however because most of the clinical signs and macroscopic lesions are not pathognomic AHS must be confirmed by isolation

Table I. A postulated African horse sickness virus overwintering mechanism.

1. AHSV seems to require temperatures ≥ 15 °C to replicate in and be transmitted by vector *Culicoides*.
2. Adults of the vector *C. imicola* are active at temperatures as low as 12 °C and can survive even lower temperatures in an inactive state.
3. Since AHSV requires a higher minimum temperature than the vector this suggests that transmission may be possible over only part of the range of the vector.
4. However, during cold periods virus can survive at very low levels in adult vectors, whose life span is extended significantly at these temperatures.
5. When temperature rises to permissive levels (≥ 15 °C), e.g. in spring, replication of this surviving virus commences and transmission becomes possible.
6. This sequence of events could constitute an AHSV overwintering mechanism in the absence of vertebrate involvement.

and identification of the virus. Traditionally this has depended on the isolation of the infectious virus from whole blood collected in anticoagulant (preferably EDTA) during the febrile stage of infection [49]. Because susceptible horses usually die following an acute infection virus may also be readily recovered and identified after death directly from tissues such as spleen, lung, lymph nodes and salivary glands [37, 46]. Whole blood should be washed and lysed as soon as possible after collection to remove the anticoagulant and any antibody that might be present in the serum component. Toxic effects can be significantly reduced if not eliminated by diluting the lysed blood 1 in 10 before inoculation for virus isolation. A 10–20% suspension of each tissue sample in diluent should be prepared by grinding the sample in a mortar with sterile sand.

Intracerebral inoculation of 2 to 4 day-old suckling mice is the preferred method for primary isolation of AHSV although the virus will adapt and grow in embryonated hens eggs following intravenous inoculation. AHSV can also be propagated in insect cell cultures such as mosquito (*Aedes albopictus*) C6/36 cells [94] and *Culicoides* (KC) cells [71]. However, since they do not show cytopathic effects (CPE) such cell assay systems are usually used only as a sensitive intermediary to amplify virus as a prelude to virus isolation in mammalian cells. Several mammalian derived cell lines including baby hamster kidney (BHK), African green monkey (Vero) and monkey kidney (MS) cells are available for AHSV isolation all of which usually show CPE within seven days [35, 36, 91].

7.1. Antigen identification

AHSV can be identified directly in whole blood and other tissues using molecular probes [96, 126] and RT-PCR using group specific primers for segment 3 [113] and VP7 [137]. The indirect sandwich ELISA is also extremely useful for the rapid identification of AHSV antigen in solid tissues taken from animals that have died following an acute infection [33, 45, 67]. In addition, isolated viruses can be identified by group specific tests such as complement fixation [75] and direct and indirect fluorescence [27].

Until recently serotyping AHSV has relied largely upon virus neutralisation (VN) tests using type specific antisera [58, 129]. These tests depend upon the presence of live replicating virus and can be performed using mammalian cells [48] or suckling mice [76] as the indicator of VN. They tend to be onerous to perform and take five or more days before results are obtained. The recent development of a type-specific RT-PCR by Sailleau et al. [112] has now provided a method of confirming the identity of AHSV in tissue samples within 24 hours. A significant advantage of this method is that it can be used to type very low levels of AHSV antigen in samples that do not contain live virus, and the results are available within a few hours.

7.2. Antibody identification

Group specific antibody against AHSV can be detected using a several diagnostic assays that are directed primarily toward the VP7, including complement fixation [14], agar gel immunodiffusion [4], immunofluorescence [27] and ELISA [44, 46, 68]. An indirect ELISA based on the detection of antibody against segment 10 (NS3) has also been described for AHSV serotype 4 [69]. This assay can be used to distinguish between naturally infected animals and those vaccinated with purified, inactivated vaccine.

Serotype specific antibody can be detected using serum neutralisation tests (SNT) [14, 54]. The SNT is not often used for primary diagnosis but may have value in epidemiological surveillance and transmission. In endemic regions where multiple AHSV serotypes are likely to be present, it is essential that paired sera collected at a minimum of two week intervals be assayed serologically to confirm a diagnosis.

8. DIFFERENTIAL DIAGNOSIS

The clinical signs and lesions reported for AHS can be confused with those caused by a closely related *Orbivirus*, equine encephalosis virus (EEV). Many aspects of the epidemiology of the diseases caused by these two viruses are also similar. They have a similar geographical distribution and vertebrate host range and the same vector species of *Culicoides* [26, 128]. As a result both can occur simultaneously in the same locations and even in the same animal. Fortunately, rapid, sensitive and specific ELISA's are available to enable the detection of the antigen and antibody of both AHSV and EEV [24, 44–46, 134], and if used in conjunction with each other can provide a rapid and efficient differential diagnosis.

Several other diseases may also be confused with one or other of the forms of AHS. The haemorrhages and oedema reported in cases of purpura haemorrhagica and equine viral artritis may be similar to those seen in the pulmonary form of AHS, although with AHS the oedema tends to be less extensive and the haemorrhages are less numerous and widespread [25]. The early stages of babesiosis (*Babesia equi* and *B. caballi*) can be confused with AHS, particularly when the parasites are difficult to demonstrate in blood smears [25].

9. PREVENTION AND CONTROL

There is no specific treatment for animals suffering from AHS apart from rest and good husbandry. Complicating and secondary infections should be treated appropriately during the recovery period.

AHSV is non-contagious and can only be spread via the bites of infected vector species of *Culicoides*. Control may therefore be effected by:

(a) Introducing animal movement restrictions to prevent infected animals initiating new foci of infection,
(b) Slaughter of viraemic animals, in certain circumstances (e.g. for welfare reasons or very early in an epidemic) to prevent them acting as a source of virus for vector insects,
(c) Husbandry modification,
(d) Vector control,
(e) Vaccination.

While the first two measures are largely self-explanatory, the part played by husbandry modification, vector control and vaccination in AHS control may require additional comment.

9.1. Husbandry modification

This measure is aimed at denying or reducing vector access to susceptible animals. Most vector species of *Culicoides* including *C. imicola* are exophilic [79] so stabling susceptible equids during times of maximum vector activity (i.e. the crepuscular periods and during the night) will significantly reduce biting rates and hence the likelihood of infection. In addition, if obvious portals of access to such housing such as windows and doors are screened with material of fine mesh (e.g. sand-fly netting) or with coarser material impregnated with insecticide (e.g. a synthetic pyrethroid) this will further reduce biting rates [17].

9.2. Vector control

It is rarely possible to completely eliminate populations of vector *Culicoides*. The main aim, is to reduce the number of potentially infecting bites that susceptible animals receive, to levels where maintenance of an epidemic becomes unsustainable (i.e. to where the basic reproductive rate $R_o = < 1$), [2]. Vector control can be tackled in a number of ways but it is important to remember that a combination of approaches is likely to yield the best results.

9.2.1. Habitat alteration

This control method is dependent upon an ability to recognise and destroy the

breeding sites of vector species of *Culicoides*. *Culicoides imicola* usually breeds in organically enriched moist but not waterlogged soils. Such areas may be bare or covered with short grass (e.g. irrigated pastures) and need to remain moist for sufficient time to complete the developmental part of the vector's life cycle (i.e. at least 7 to 10 days). Consequently, slow draining or clay soils are better for *C. imicola* than free-draining, nutrient-poor, sandy soils. High populations of *C. imicola* usually occur only on livestock farms and seem to be a phenomenon of this type of activity. In such locations humans inadvertently provide an ideal *C. imicola* habitat by providing water, soil contaminated with animal dung and a sedentary "blood bank" of domestic animals. *Culicoides imicola* breeding sites can vary in size from a few metres in diameter (irrigation pipe leaks, horse trough overflows, leaking taps) to whole pastures. When potential breeding sites are few in number and are small their elimination may be achieved easily through habitat modification (e.g. by turning off taps, mending leaks and filling in or draining damp areas) but this may be impossible or not economically feasible in other situations [17].

9.2.2. Adulticiding

Recommendations for broad-scale, aerial application of insecticides with all of the attendant risks to non-target organisms are not likely to find favour in these more environmentally conscious times. However, targeted application of insecticides of known low mammalian toxicity (e.g. the synthetic pyrethroids) in and around stables, and directly to equids themselves can be efficacious against *Culicoides* species and are more likely to be environmentally acceptable [17, 19]. Application of systemic insecticides such as Ivermectin may also be effective at killing biting *Culicoides* [118]. An additional advantage with this system, and with such insecticidal food additives as tetrachlorvinphos, is that these drugs are eliminated in the faeces which, should they be deposited on breeding sites, are toxic to the immature stages of *Culicoides* [62, 118].

9.2.3. Larviciding

Application of a larvicide such as "Abate" (American Cyanamid) (5% temephos granulated with gypsum) to *Culicoides* breeding sites, provides a slow but sustained release of the insecticide and may be effective for periods as long as 30 days [53]. Such preparations are effective even when used on breeding sites that are rich in organic matter which makes them particularly suitable in *Culicoides* control [17]. Biological control of larval *Culicoides* by agents such as *Bacillus thuringensis* has apparently not proved successful [64].

9.2.4. Repellents

There are several candidate and established repellents that have been tested on *Culicoides*. However, none are completely effective and the deterrent effect, even of the best, rarely persists for more than a few hours [18, 115]. Di-ethyl toluamide (DEET) seems to be the only commercially available repellent that has been shown to have a significant deterrent effect against *Culicoides* for periods of up to four hours [18]. Since *C. imicola* attacks apparently peak during the first four hours of the night, if applied nightly to susceptible animals, DEET may have a significant but temporary effect in reducing the biting rate of this species.

9.3. Vaccination

9.3.1. Attenuated vaccines

Polyvalent, attenuated vaccines are commercially available from Onderstepoort Biological Products (OPB), Onderstepoort, South Africa. The early vaccines were based on virus strains, attenuated by multiple suckling mouse brain passage [1]. They gave

solid immunity but occasionally resulted in serious side effects, including fatal cases of encephalitis in horses and donkeys, particularly after primary vaccination [99, 101]. These problems were minimised by further attenuation of the vaccine virus strains through passage in cell culture [92]. These cell culture adapted viruses still form the basis of the currently available OPB vaccines.

The OPB, AHS vaccines currently used in southern Africa are supplied in two polyvalent vials containing AHSV types 1, 3 and 4, and 2, 6, 7 and 8, respectively. AHSV-5 is currently not included having been withdrawn in October 1993 because of reports of severe reactions and deaths in some vaccinated animals [39]. AHSV-9 is also not included because type 6 is strongly cross protective and because type 9 is rarely present in southern Africa and is considered to be of low virulence.

In South Africa most susceptible animals in areas considered to be at risk to AHS are vaccinated, routinely, twice in the first and second years of life, annually thereafter. The simultaneous inoculation of several vaccine virus types usually results in the production of protective antibody to each virus serotype. However, in some individuals interference between the virus serotypes may result in incomplete protection after the first course of vaccination [25], and some strains of vaccine virus may be only weakly immunogenic [38, 65, 76]. Consequently, several courses of vaccination may be required to achieve full immunity.

Monovalent attenuated AHSV 9 vaccine (National Laboratory, Senegal) is used extensively in West Africa where this is the only serotype known to be circulating regularly. Outside sub-Saharan Africa monovalent vaccination has been used successfully, in combination with other control measures, to eradicate the virus from the incursive areas e.g. the 1959–1961 outbreaks in the Middle East, Arabia, India and Pakistan [41, 57, 59, 91, 106], the 1955–1956 outbreaks in North Africa and Spain [30], and the 1987–1991 outbreaks in Spain, Portugal and Morocco [80, 104]. It is noteworthy that in the latter outbreak, initially, only a polyvalent vaccine was available for use in Spain. Despite this, the decision was taken to ring vaccinate equids in and around Madrid using this polyvalent vaccine. Subsequent to its use AHSV types other than the outbreak type were isolated from horse blood samples in the area (H. Hooghuis, 1987 – personal communication) suggesting that the vaccine viruses were inducing a viraemia in vaccinated animals raising the possibility of insect transmission. The significance of such transmission should it occur has yet to be evaluated.

Despite the evident success of these live attenuated vaccines in endemic situations there are still concerns about their use in epidemic situations. These can be summarised as:

(1) The only widely available commercial AHSV vaccines are manufactured in South Africa, and no AHSV vaccines are licensed for use in the EU.

(2) These vaccines are all live attenuated formulations and the South African vaccines are offered only as two standard polyvalent preparations. Manufacture of monovalent or other polyvalent formulations appropriate to specific outbreaks involves a delivery delay of several weeks or months, therefore, emergency vaccination in response to incursions may not be possible.

(3) The live virus vaccines may cause teratogenic effects and are therefore not recommended for use in pregnant mares.

(4) The seed viruses for the South African vaccines originated in South Africa. Use of these vaccines elsewhere, therefore, involves introducing virus topotypes from other ecosystems.

(5) AHSV has a 10-segmented genome. Consequently, reassortment (exchange of genome segments) could occur between live vaccine viruses and wild-type viruses, in either the vertebrate or invertebrate

hosts. This could result in "new" viruses with different or even enhanced virulence characteristics or that express novel antigenic properties.

(6) The South African vaccines are likely to stimulate a vaccine virus viraemia in some vaccinated equids. However, the proportion of vaccinated animals developing a viraemia, the duration of viraemia and the titre of viraemia are all unknown.

(7) It is not known whether vector *Culicoides* can ingest vaccine virus from vaccinated equids facilitating reassortment, transmission and/or reversion to virulence.

In the light of these important concerns it is likely that some authorities will refuse to use these vaccines and consequently a coherent, effective region-wide vaccination policy may be difficult to develop. In a situation where the major vector (*C. imicola*) is expanding its range, where novel vectors may be involved and where future incursions of AHSV are likely to involve new geographical areas, the prospects for control or prevention in the absence of such a policy is poor. It is therefore of the utmost urgency that inherently safe (i.e. inactivated), efficacious, vaccines be developed that can be used with confidence by all parties. An additional advantage to the use of inactivated vaccines is that tests based upon one or other of the non-structural proteins of the virus can be developed that will enable differentiation between naturally infected and vaccinated animals [61, 69].

9.3.2. Inactivated vaccines

Inactivated vaccines have the advantage that they do not contain a live and potentially dangerous agent. However, they may be expensive to produce and multiple inoculations may be required to elicit and maintain high levels of protective immunity. It may also be difficult to ensure complete vaccine inactivation [65].

There are no inactivated AHS vaccines available on the market at the time of writing although such vaccines have previously been developed [32, 55, 93]. During the 1987–1991 AHSV epidemic in Spain, Portugal and Morocco, one of these, a formalin-inactivated vaccine "Equipest®" was developed commercially [32]. The initial clinical trials were carried out in France, and were followed by two field trials in Spain and Morocco. Although this vaccine proved to be reasonably efficacious, it was withdrawn shortly after the eradication of AHSV from Europe and is no longer available.

9.3.3. Sub-unit vaccines

Sub-unit vaccines against AHS have not so far been developed commercially although a considerable amount of research has been undertaken on the subject dealing mainly with the related BTV [109–111]. In relation to AHSV, Roy and Sutton [107] have described the development of baculovirus expression systems for the preparation of the synthetic, outer capsid proteins, VP2 and VP5, of AHSV-4. These were used together or VP2 was used alone, to form the basis of experimental AHS vaccines for use in horses. With both vaccines animals developed neutralising antibodies that apparently conferred protection against challenge with virulent homologous virus six months after vaccination.

With BTV, work has progressed further and recombinant baculovirus expression systems have been developed and used to produce a series of virus like and core like particles containing either the five major structural proteins (VP2, 3, 5, and 7 – VLPs) or the three major core proteins (VP3 and 7–CLPs). These synthetic particles are apparently highly immunogenic, presumably, because although they lack genetic material they accurately mimic the spatial configuration of authentic virions. VLP-based experimental vaccines when used in sheep conferred complete protection against homologous virulent virus challenge for up

to 15 months post vaccination [107, 109–111]. CLP-based experimental vaccines conferred only partial protection [107] but presumably have the potential for development as a BT-group specific rather than type specific vaccine as they are composed only of VP3 and 7 which are common across all 24 serotypes of BTV. Although the technology for sub-unit vaccine development has been available for several years, to date the techniques have not been commercialised, which may be a reflection of cost and/or difficulties with large-scale production.

10. CONCLUSION

AHS is one of the most lethal of equid diseases. At present its endemic zones are restricted to sub-Saharan Africa and possibly Yemen but the virus has a history of rapid expansion, without warning, into countries far beyond these areas. The distribution of the disease is controlled largely by the abundance, prevalence and seasonal incidence of its insect vectors, which are certain species of *Culicoides* biting midge. Climate-change has recently resulted in the major vector species, *C. imicola*, expanding northwards into many areas of Europe previously considered to AHS-risk free. The related BTV, which is transmitted by the same vector species of *Culicoides* has already entered many of these locations and is causing unprecedented outbreaks of disease in ruminants. This strongly suggests that at some stage in the future AHSV could do the same. Current studies are improving our understanding of the requirements for AHSV transmission by its vectors and of the relationship between disease transmission and climate, and in the future such investigation may enable AHS epidemics to be predicted. At present, *Culicoides* vector control measures are poorly developed and the only vaccines available are live attenuated preparations that are not registered for use in Europe and whose deployment would be viewed with concern by some veterinary authorities. There is therefore an urgent need to enhance our predictive ability, improve vector control measures and develop efficacious, inherently safe, inactivated, vaccines.

ACKNOWLEDGEMENTS

This paper was written during the course of two EC-funded and two DEFRA-funded projects on *Culicoides*-transmitted arboviral diseases (Contracts QLK2-CT-2000-00611, QLK2-2001-01722, SE 2610 and "Contract for Supply of Exotic Disease Reference Laboratory Facilities"). The authors are grateful for the financial support.

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