African horse sickness

S. Zientara [1]*, C.T. Weyer [2] & S. Lecollinet [1]

(1) Université Paris Est, Unité mixte de recherche 1161, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, Institut national de la recherche agronomique, École nationale vétérinaire d'Alfort, 23 avenue du Général de Gaulle, 94706 Maisons-Alfort, France
(2) Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa
*Corresponding author: szientara@vet-alfort.fr

Summary

African horse sickness (AHS) is a devastating disease of equids caused by an arthropod-borne virus belonging to the *Reoviridae* family, genus *Orbivirus*. It is considered a major health threat for horses in endemic areas in sub-Saharan Africa. African horse sickness virus (AHSV) repeatedly caused large epizootics in the Mediterranean region (North Africa and southern Europe in particular) as a result of trade in infected equids. The unexpected emergence of a closely related virus, the bluetongue virus, in northern Europe in 2006 has raised fears about AHSV introduction into Europe, and more specifically into AHSV-free regions that have reported the presence of AHSV vectors, e.g. *Culicoides* midges. North African and European countries should be prepared to face AHSV incursions in the future, especially since two AHSV serotypes (serotypes 2 and 7) have recently spread northwards to western (e.g. Senegal, Nigeria, Gambia) and eastern Africa (Ethiopia), where historically only serotype 9 had been isolated. The authors review key elements of AHS epidemiology, surveillance and prophylaxis.

Keywords

Africa – African horse sickness – Arbovirus – *Culicoides* – Equids – Midges – *Orbivirus.*

Introduction

African horse sickness (AHS) is a devastating disease of horses which is caused by an arbovirus belonging to the *Reoviridae* family, genus *Orbivirus*, and is transmitted by haematophagous *Culicoides* midges. Owing to its vectorial transmission pattern, AHS appears seasonally when vectors are the most active (after the rainy season in the tropics, in the summer and autumn in temperate regions). African horse sickness virus (AHSV) principally infects horses and other equids (mules, donkeys and zebras) and causes a fatal, acute or subacute disease characterised by respiratory and/or circulatory impairments.

Historical background

The pathology was first described in Africa following the introduction of European horses during the exploration of central and eastern Africa (1) and the disease was initially recognised during the 1719 epizootics in South Africa (2). Since then, severe epizootics have been reported in various African and Mediterranean countries. This has raised concern among veterinary authorities, as AHS represents a serious threat for horse populations owing to its ability to rapidly and insidiously spread out of its endemic areas.

African horse sickness disease was first described in an Arabic document entitled 'Kitâb el-Akouâl el Kafiah wa el Foucoûl ef Charfiah' dating back to the early 1300s (2). Epizootics with horses suffering from an AHS-like disease apparently occurred in 1327 in the Yemen. The extent and severity of the disease are illustrated by the following description: 'a purchaser and a salesman are discussing the price of a horse when, suddenly, the horse falls on the floor and dies'.

African horse sickness virus was first identified in South Africa during the first major AHS outbreak in the Cape region in 1719, during which over 1,700 animals died; however, the virus was most likely circulating in the wildlife population of the area before this (2). The disease is considered endemic to most areas of southern Africa and at least ten other major outbreaks of AHS have been recorded in the region since 1719, the largest being in 1854–1855, with more than 70,000 horse fatalities (3).

316

Rev. Sci. Tech. Off. Int. Epiz., **34** (2)

Geographic distribution and economic consequences

African horse sickness virus is endemic in tropical and subtropical areas of Africa, south of the Sahara, from Senegal in the west to Ethiopia and Somalia in the east and extending as far south as South Africa (Fig. 1a) (4). The virus repeatedly spread out of its African basin during the last century and caused severe outbreaks in the newly infected territories: in 1943–1944 there were outbreaks in Egypt, Syria, Jordan, Lebanon and Palestine, and in 1959–1960 outbreaks which caused the death of over 300,000 equids occurred in the Middle East and South-West Asia (Cyprus, Turkey, Lebanon, Iran, Iraq, Syria, Jordan, Palestine, Pakistan and India). In 1965, AHS was first reported in Morocco before reaching Algeria and Tunisia and crossing the Strait of Gibraltar into Spain in 1966 (5). These latter outbreaks were all caused by serotype 9 viruses.

Europe faced a second AHS epizootic, this time due to serotype 4 virus, in 1987 (3). It began in the province of Madrid, but although extensive control measures were taken (38,000 equids were vaccinated), AHSV successfully spread to southern Spain in 1988 and to Portugal and Morocco in 1989 (6). The high mortality rate and the control measures implemented to limit AHSV circulation had a major impact on the international trade of horses and on the horse industry in infected countries and caused major economic losses. Over 2,000 horses died in 1989, and the costs associated with AHS control were approximately US$30 million (7). Spain and Portugal were finally cleared of AHSV in 1991 (the last clinical cases being reported in 1990).

Since 2007, AHSV serotypes 2 and/or 7 have unexpectedly spread to several countries in West and East Africa (e.g. Nigeria, Senegal, Mali, Gambia, Ethiopia) where historically only serotype 9 had been isolated (Fig. 1b). They caused numerous outbreaks with mild clinical outcomes (232 outbreaks declared in Senegal in 2007, with 1,137 deaths of local and exotic horses) (11). This recent expansion of AHSV is of great concern, in light of the recent experiences gained with the closely related bluetongue virus (BTV), which have shown that BTV can easily and quickly spread in the Mediterranean Basin once it has reached North Africa.

In South Africa, outbreaks of AHS occur every summer throughout the country, with the exception of the region of the southern Cape. Historically, the area around the Cape of Good Hope has been free of AHS, with sporadic outbreaks caused by movement of AHS-infected horses into the area. This led to the establishment of an AHS-free zone in the region in order to facilitate the export of horses to Europe, which was accepted by the European Union in 1997. In the same year, the South African authorities established an AHS-controlled area, which contained a surveillance zone and a protection zone in addition to the free zone (12). Since the implementation of this regionalisation, outbreaks within the surveillance zone have occurred in 1999, 2004, 2011 (13) and more recently in 2013 and 2014 (14). The increase in the frequency of outbreaks in the AHS-controlled area has had a negative impact on the equine export industry of the country. It has also raised serious concerns relating to the increase in the spread of the disease and the possibility of an increase in the frequency of outbreaks in historically free areas.

Epidemiology

Affected species

The disease principally affects equids under natural conditions. Horses are highly susceptible and generally develop acute and subacute forms with elevated mortality rates, while mules and donkeys develop a curable form of the disease. In zebras, the infection is asymptomatic.

Apart from horses, the only other species shown to be highly susceptible to AHS is dogs, which can develop fatal pulmonary forms (4). Generally, infections in dogs have been linked to ingestion of infected horse meat, but recent reports have shown that transmission of the virus from midges to dogs is possible (15). However, it is believed that canines play only a minor role (if any role at all) in the transmission cycle of AHS and they are currently regarded as epidemiological bottlenecks.

Pathogen

The ultra-filterability of the AHS-causing agent and its antigenic plurality were demonstrated in 1910 (16). Nine AHSV serotypes (numbered 1 to 9) have been reported. The virus was classified in the arbovirus group because of its mode of transmission and it was identified as a member of the *Orbivirus* genus in 1976 (17).

### Viruses of the genus *Orbivirus*: biology and pathogenesis

Orbiviruses are responsible for major animal diseases, such as bluetongue and AHS, whereas they are minor pathogens for humans. Members of the *Orbivirus* genus share morphological, physicochemical and genomic characteristics. They are non-enveloped viruses, surrounded by a ring-shaped capsid composed of 32 capsomers. Orbivirus particles enclose a segmented and double-stranded (ds) RNA genome.

The *Orbivirus* genus is divided into serogroups, further subdivided into serotypes on the basis of virus neutralisation

Rev. Sci. Tech. Off. Int. Epiz., **34** (2)

317
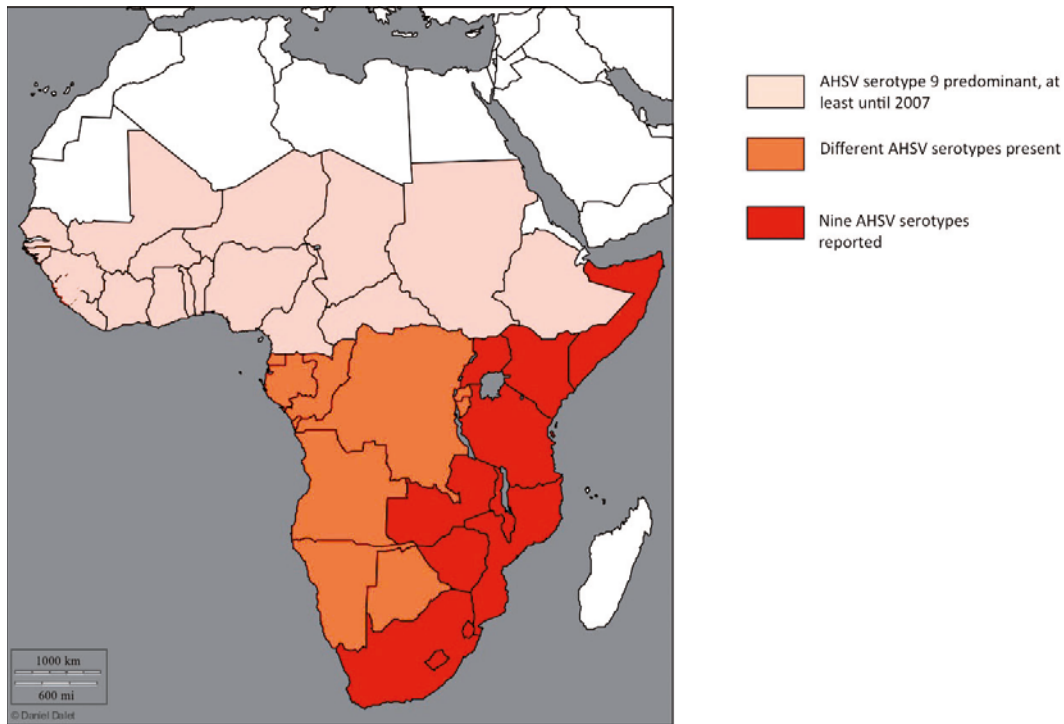
a) Areas of endemic African horse sickness virus

The genetic diversity of African horse sickness virus follows an increasing gradient from north to south (serotype 9 has historically been the sole serotype identified in central Africa while the nine AHSV serotypes have regularly been described in southern and eastern Africa)
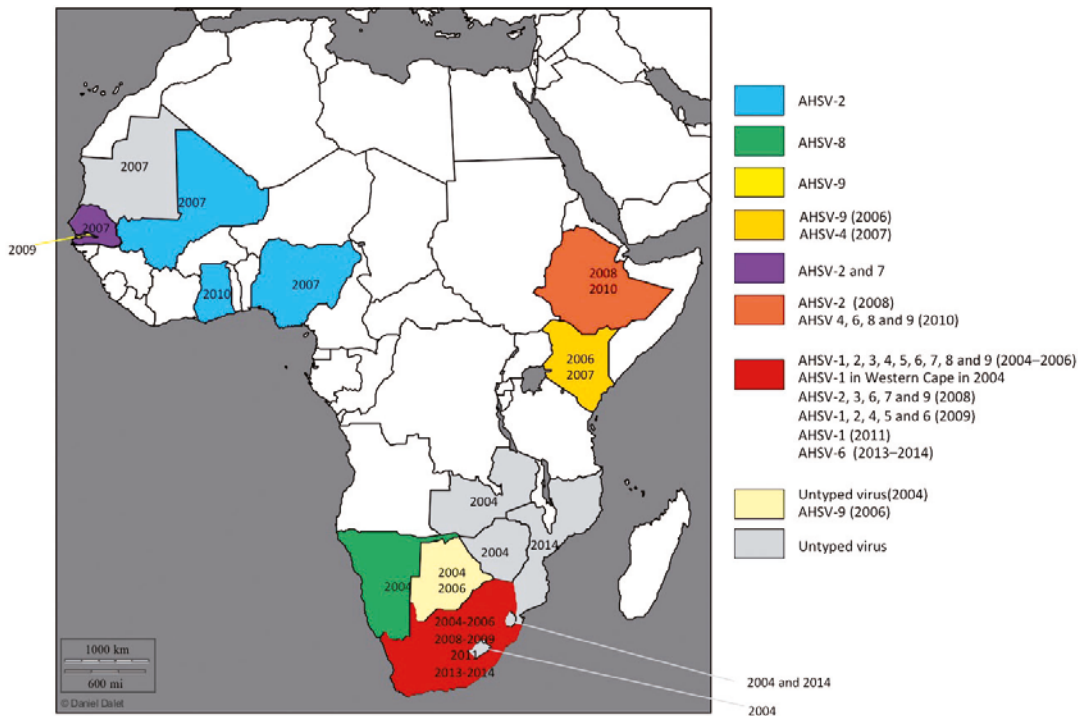
b) Recent outbreaks of African horse sickness (2004–2014)

Based on data from the OIE World Animal Health Information Database, Promed alerts and a review of the literature (8, 9, 10)

Fig. 1
Geographic distribution of African horse sickness

tests. Orbiviruses are transmitted by insects or other arthropods such as *Culicoides* biting midges, mosquitoes, sandflies and ticks. They can infect a variety of vertebrate hosts, e.g. humans, equids, monkeys, rabbits, dogs, cattle, deer and suckling mice.

Serogroup members share a common antigen, VP7, localised in the inner capsid (Fig. 2), that is strongly immunogenic and is widely used in rapid diagnostic tests (e.g. enzyme-linked immunosorbent assay [ELISA], complement fixation tests and quantitative reverse-transcription polymerase chain reaction [RT-qPCR] assays). Serotype-specific antigens are localised on the outer capsid and induce neutralising antibodies.

African horse sickness virus

The first molecular characterisation of AHSV was performed in the late 1960s by Verwoerd and Huismans (19). Since then, over the past 25 years, detailed molecular and genetic analyses have been undertaken.

Structure

Purified AHSV particles contain seven structural proteins (VP1–VP7) that are similar to those of BTV (Fig. 2). The outer capsid shell of mature virions consists of two proteins, VP2 and VP5, while the intermediate layer is composed of two major proteins, VP3 and VP7, and the inner capsid contains three minor proteins, VP1, VP4 and VP6 (20). Five non-structural proteins, NS1, NS2, NS3, NS3A and NS4, are also observed in AHSV-infected cells (21). The AHSV genome is composed of ten dsRNA segments of different lengths, which can be distinguished by polyacrylamide gel

electrophoresis and are numbered 1 to 10 according to their migration order (22). The AHSV genome has been entirely sequenced (with sequences mainly representing serotype 4).

Major structural proteins

VP2 protein, encoded by segment 2, is the principal component of the outer capsid and is the main serotype-specific antigen (23). Based on its genetic variability, nine serotypes of AHSV have been identified. AHSV-neutralising epitopes, as well as the virus receptor-binding site, are located in VP2, as demonstrated by the binding of neutralising monoclonal antibodies (24). A comparison between amino acid sequences of AHSV serotype 4 (AHSV-4) and BTV-10 suggests that these two viruses share a common ancestor and have diverged under the selective pressure of the immune systems of their respective and different hosts (ungulates for BTV and equids for AHSV) (22).

VP5 protein is coded by segment 6 dsRNA. The 505 aa-long protein, although located on the outer capsid, is apparently less exposed at the surface of the virion than the VP2 protein. VP5 does not seem to be involved in AHSV antibody-mediated neutralisation (22).

VP3 and VP7, encoded by segment 3 and 7 dsRNAs respectively, are the major components of the inner core and contain group-specific antigenic determinants (22, 25). The VP7 proteins of AHSV serotypes 4 and 9 exhibit 99.7% identity at the amino-acid level. VP7 proteins are also highly conserved between AHSV, BTV and epizootic haemorrhagic disease of deer virus (EHDV) and, consequently, it is these
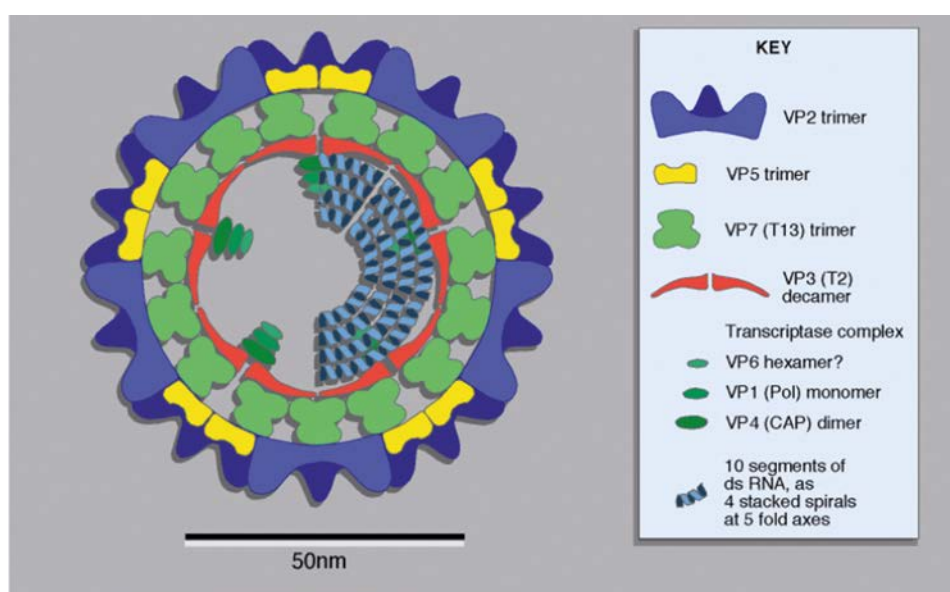

Fig. 2
African horse sickness virus structure (adapted from 18)

Rev. Sci. Tech. Off. Int. Epiz., **34** (2)

319

proteins that are used for the serological diagnosis of AHSV-serogroup-infected animals.

Although little information is available on AHSV transcription, replication or morphogenesis processes, there is no doubt that these processes and the proteins involved are the same as those of BTV. When cells are infected simultaneously by at least two viruses, the segmentation of orbivirus genomes allows for the exchange of genome segments between orbiviruses that are of the same serogroup but either belong to different serotypes or originate from different geographical regions. Genome reassortment is responsible for the rapid genetic evolution of orbiviruses. The frequency of genome reassortment is variable, some genes being more frequently exchanged than others (26). AHSV reassortants have been obtained *in vitro* by co-infection of cells by serotypes 2, 3 and 4 (22). Data suggest that *in vivo* reassortment occurs more frequently in the insect than in the vertebrate host and that reassortment in vertebrate hosts is less common for AHSV than for BTV (27).

Virus transmission

African horse sickness virus is indirectly transmitted to equids by haematophagous arthropods. Direct transmission has only been seen in dogs after oral contamination with infected meat. Many vectors have been found infected with AHSV, in particular, mosquitoes of the *Aedes*, *Culex* and *Anopheles* genera, or ticks of the *Hyalomma* or *Rhipicephalus* genera (6). However, the main biological vector is a biting midge of the *Culicoides* genus: *C. imicola* (28). The minimum dose needed to experimentally infect *C. imicola* is 10^4–$10^{4.5}$ $TCID_{50}$/0.02 ml blood. A high viraemia in the host is therefore necessary for vector contamination. In the Old World, *C. imicola* has long been considered to be the only confirmed field AHSV vector. However, recent work by Venter *et al.* has shown that a second African species, *C. bolitinos*, can efficiently transmit AHSV (29). *Culicoides bolitinos*, which has also been implicated as a BTV vector, is widely distributed across southern Africa and is common in cooler highland areas where *C. imicola* is rare. A transovarial transmission mechanism in *Culicoides* midges has never been substantiated by experimental or epidemiological studies.

During the 1987–1990 outbreaks of AHS in Spain and Portugal, AHSV was mostly isolated from *C. imicola,* as expected (4). However, surprisingly, isolates were also obtained from mixed pools of *Culicoides* that did not include *C. imicola* and consisted almost entirely of *C. obsoletus* and *C. pulicaris*, suggesting that one or both of these species might also be involved in AHSV transmission in Europe. More than ten years after this observation, *C. obsoletus* and *C. pulicaris* midges were both implicated as BTV vectors during the 1998–2003 BTV incursions into Europe (4).

The distribution of AHS is largely controlled by the abundance, prevalence and seasonal incidence of its insect vectors. Climate change has recently resulted in the expansion northwards of its major vector species, *C. imicola*, into many areas of Europe. The related bluetongue virus, which is transmitted by the same vector species of *Culicoides*, has already been reported in many of these locations and has caused unprecedented outbreaks in ruminants over the last decade. This strongly suggests that at some stage in the future AHSV could do the same.

Virus reservoir

Occasionally, multiple cases of AHS occur suddenly over a short period of time. This explosive emergence can only occur when there are large numbers of contaminated insects, which suggests the existence of a virus reservoir with a long-lasting, reactivateable viraemia or (more likely) a continuous circulation of the virus within a reservoir herd. For this to occur the herd needs to be large enough and have non-seasonal breeding so that naive young animals are perpetually exposed. This situation is likely to occur in an area such as the Kruger National Park (30). Sometimes, it is only when large numbers of horses are affected that it becomes clear that one or more of the surrounding populations of animals (zebras and African donkeys) is serving as a reservoir host.

The persistence of AHS in Spain for four years could, according to Mellor, be explained by the survival of infected *C. imicola* during winter, especially in the south temperate region of Spain (6). An alternative hypothesis would be that European donkeys (*Equus asinus*) or mules acted as virus reservoirs. The virus could have been maintained during the cold season through low-level transmission between reduced insect populations and infected donkeys or mules. In these animals, the viraemia has been shown to last longer (10 to 27 days) than in horses (intense viraemia for 4 to 8 days, maximum 18 days) (5, 6, 31).

Evolution

The spatial evolution of AHS outbreaks depends on the vector biotope. The biotopes with conditions favourable for larval development and adult survival are wet areas (swamps, edges of rivers) and areas near waterholes where the canopy maintains humidity.

The time course of AHS outbreaks is directly linked to periods of vector activity, which is greatest in hot and humid climates, i.e.:

– subtropical areas, after the rainy season

– temperate regions, from the beginning of spring until late autumn.

The rates of AHSV infection of *Culicoides* vectors and of virogenesis are temperature dependent. As temperature increases, infection rates tend to increase, virogenesis is more rapid and transmission can occur earlier. However, midge survival rates decrease. Conversely, as temperature is reduced, the reverse is true for each of these variables. The transmission likelihood is the result of the interaction of these two opposite data sets.

Furthermore, AHSV replication does not seem to occur below 15°C (32). 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 (32). In related studies, Sellers *et al.* have shown that adult *C. imicola*, the major AHSV vector, are active at temperatures as much as 3°C lower than the minimum required for AHSV replication (33). Together, the dramatically extended midge survival rate at low temperatures (to as long as 90 days in some cases) and the replication of 'latent' virus in warmer temperatures (e.g. during spring) could constitute a virus overwintering mechanism in the absence of infected vertebrates.

Pathogenesis

After an animal is bitten by *Culicoides* midges, AHSV initially replicates in the regional lymph nodes. The virus subsequently spreads via the bloodstream, resulting in an intense viraemia, with virus particles tightly bound to erythrocytes (24). Once in the blood, the virus replicates secondarily in endothelial and mononuclear cells and enters target organs (lung, heart, spleen and lymphoid tissue) (34). Virus replication in the target organs results in a second viraemia phase, with damage to endothelial cells and macrophage activation, which is responsible for the release of inflammatory cytokines (such as interleukin-1 and tumour necrosis factor alpha). Oedema and effusion, typical of the more severe forms of AHS, are almost certainly the result of increased vascular permeability caused by direct viral injury to endothelial cells, although indirect effects mediated by inflammatory cells or soluble factors cannot be ruled out. The variable AHSV tropism for cardiac or pulmonary endothelial cells can account for the various clinical forms of AHS disease. Recent work suggests the existence of a correlation between strain virulence *in vitro* and the severity of necropsy lesions *in vivo* (34, 35). The association of intravascular coagulation with infection by virulent AHSV isolates and its absence in horses infected with an avirulent isolate suggest that virulence is associated with a strain's ability to cause endothelial injury.

Clinicopathological studies with virulent AHSV variants have shown that virulence is associated with thrombocytopenia, prolongation of coagulation times and the presence of fibrin degradation products (36).

Clinical signs

The incubation period of AHS varies from 3 to 15 days according to the virus strain and the sensitivity of the equid host. AHSV can cause four forms of AHS disease: horse sickness fever, a cardiac form, a mixed form and a pulmonary form, in ascending order of severity. The nature of the clinical signs and the prognosis of the infection are determined by virus-dependent factors (strain virulence, infectious dose, etc.) and host-dependent factors (breeding conditions, immune status, etc.).

Horse sickness fever is invariably mild and not lethal, usually involving only mild to moderate fever and oedema of the supraorbital fossae. It is more frequently encountered in infected African donkeys and zebras, or in more susceptible equids that are infected with less virulent strains or are partially immune (vaccinated).

The cardiac or subacute form of the disease is characterised by fever (39–40°C) lasting several weeks. The main clinical finding is subcutaneous oedema, particularly of the head (including the supraorbital fossae), neck and chest (Fig. 3a). Equids suffering from this form of AHS are said to have the *dikkop* form of the disease ('thick head' in the South African language Afrikaans), referring to the pronounced supraorbital swelling that is commonly observed. The conjunctivae may be congested (Fig. 3b), petechiae may be observed at the level of the conjunctiva and ecchymotic haemorrhages may be seen on the ventral surface of the tongue. Colic can also be reported and mortality rates may exceed 50%.

The pulmonary form, known as *dunkop* ('thin head' in Afrikaans), is peracute and may develop so rapidly that an animal can die without previous signs of illness. Usually, there will be marked depression and fever (39–41°C), followed by the onset of respiratory distress and severe dyspnoea. Coughing spasms may occur, the head and neck are extended and severe sweating develops (Fig. 4). There may be periods of recumbency and, in the terminal stages, large quantities of frothy fluid may be discharged from the nostrils. The prognosis for horses suffering from this AHS form is extremely poor and mortality rates commonly exceed 95%.

The mixed form is the most common form of AHS and is a combination of the cardiac and pulmonary forms of the disease. The mortality rate is approximately 70% and death usually occurs within three to six days of fever onset.

Rev. Sci. Tech. Off. Int. Epiz., **34** (2)

321

a) Oedema of the supra orbital fossae
Courtesy of J.-P. Ganière, Nantes Atlantic College of Veterinary Medicine, Food Science and Engineering (ONIRIS)
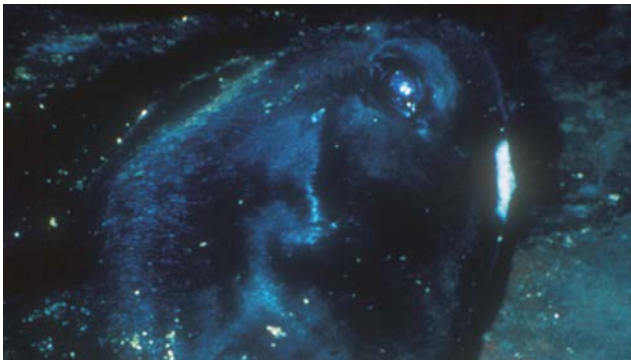
b) Severe oedema of the conjunctiva, supraorbital fossae, eyelids and neck
Courtesy of J.-P. Ganière, ONIRIS

Fig. 3
Cardiac form of African horse sickness

Fig. 4
Late phase of the pulmonary form of African horse sickness
Abundant frothy discharge flowing from the nostrils
Courtesy of J.-P. Ganière, ONIRIS

It has been known for some time that donkeys and zebras can have subclinical infections, but, recently, it has been shown that subclinical infections can occur in horses as well (13, 37). This has important implications, as movement control of horses in the infected areas of endemic countries is generally limited to a clinical examination of the horse prior to transportation, on the premise that infected horses would show clinical signs of some sort.

Diagnosis

The differential diagnosis of AHS includes equine infectious anaemia, equine arteritis, infection with equine encephalosis virus (another orbivirus), equine pneumonia caused by Hendra virus infection, anaplasmosis, babesiosis or theileriosis, anthrax, and *purpura haemorrhagica*. Clinical evolution, post-mortem examination, epidemiological factors (seasonal abundance of competent vectors) and laboratory diagnosis will allow for refinement of diagnostic hypotheses.

Post-mortem diagnosis

The clinicopathological presentation of AHS can be pathognomonic; for example, the supraorbital swellings which are often present in horses with subacute AHS or the presence of secretions in the trachea, bronchial tubes and larynx can be sufficient for a tentative diagnosis (36).

Laboratory diagnosis

Samples

Blood is the ante-mortem sample of choice for virus identification; blood should be collected on EDTA. Post-mortem samples are usually taken from the spleen, lungs, lymph nodes and heart. Samples must be sent rapidly to a specialised laboratory at 4°C.

Virus detection

Virus isolation

Virus isolation and serotyping are of utmost importance whenever outbreaks occur outside endemic regions, since the choice of prophylactic measures depends on the infecting serotype. Highest viraemia is found during the early febrile period and spleen, lungs, and lymph nodes have the highest viral load. Virus isolation can be performed by:

– inoculation of cell cultures, such as the Vero or BHK-21 cell line (cell lysis and cytopathogenic effects generally appear three to seven days after primary inoculation or blind passages, and virus isolation consequently requires at least 7 to 14 days)

322

Rev. Sci. Tech. Off. Int. Epiz., **34** (2)

– intracerebral inoculation of newborn suckling mice (16).

Virus identification

The traditional methods of virus identification are serological techniques that use the isolated virus as antigen, e.g. the virus neutralisation test. Recently, however, the use of real-time or classical RT-PCR assays, which allow rapid virus identification and typing (in a few hours), has replaced serological techniques in most laboratories. RT-PCR assays amplify conserved genomic sequences (1, 3, 5, 7 or 8 dsRNA segments) for group diagnosis, or highly variable segments (segment 2 encoding VP2 in particular) for typing (8, 9, 38, 39, 40, 41, 42, 43, 44).

Confirmatory diagnosis of AHS is better achieved through virus detection techniques than through serology, because many horses will die before the induction of AHSV antibodies, antibodies being detectable 10 to 14 days post infection. Furthermore, in areas where vaccination is routinely carried out, serological results can be difficult to interpret. However, serological surveys can provide confirmation of virus circulation in a specific region or country. Moreover, several serological techniques are prescribed by the World Organisation for Animal Health (OIE) for the purposes of animal trade (45). They include:

a) Complement fixation test. The presence of complement-fixing antibodies in animal sera indicates recent infection. However, the anti-complementarity observed with sera of donkeys as well as some horses precludes its wider use. These tests are group specific but lack sensitivity (46).

b) Serum neutralisation test. Neutralising antibodies can be detected as early as three weeks post infection by the serum neutralisation test and can persist for many years. Their identification allows for the typing of circulating viruses. Some cross-neutralisation between AHSV serotypes has been observed, in particular between serotypes 9 and 6, and also, to a lesser extent, between serotypes 1 and 2, 3 and 7, and 5 and 8 (47).

c) Enzyme-linked immunosorbent assays. Several indirect or competitive ELISAs have been developed for the detection of AHSV group antigens over the last twenty years. They appear to have satisfying sensitivity (48, 49, 50) and are now widely implemented in diagnostic laboratories, some being commercially available. These tests use polyclonal or monoclonal antibodies as well as virus purified antigens or recombinant proteins (mainly VP7) expressed in prokaryotic or eukaryotic systems (51, 52, 53, 54) and detect group-specific antibodies. Laviada *et al.* have developed a DIVA assay (i.e. one that differentiates infected from vaccinated animals), based on

recombinant NS3 from serotype 4 AHSV, for the detection of anti-NS3 antibodies; such antibodies are usually only induced after infection with AHSV or vaccination with a live vaccine (55).

Control

In order to prevent the import of infected equids from AHSV at-risk areas to AHSV-free countries, the *Terrestrial Animal Health Code* of the OIE makes the following recommendations about the conditions that must be met by infected exporting countries (56):

a) The horse should show no clinical signs of illness on the day of shipment, and should not have been vaccinated against AHS within the last 40 days.

b) The horse should have been isolated in a vector-protected establishment for either:

– 28 days, with serological testing after this period to show a negative serological result

– 40 days, with two serological tests no less than 21 days apart to show lack of seroconversion

– 14 days, with an agent identification test carried out on day 14 to show absence of the virus (e.g. RT-qPCR), or

– 40 days, after having been vaccinated against all serotypes that surveillance programmes have shown to be circulating in the area.

If AHSV should emerge outside AHS endemic areas, the control programme implemented should contain three components: quarantine, vector control and vaccination. Suspect cases of AHS should be quarantined in insect-proof enclosures. Vector control mainly consists of shielding infected stables and treating them with authorised pesticides, such as alphacypermethrin, to reduce adult populations (57). Because of the severity of AHS and due to the contagion risk from infected animals, slaughter of confirmed cases and of in-contact animals may be required by national regulations. Movement restrictions for equids in infected regions must also be enforced by Veterinary Services.

Vaccination is central to AHS control. The early vaccines were based on virus strains attenuated by multiple passage in suckling mouse brain (58). They induced solid immunity but occasionally resulted in serious side effects, including fatal cases of encephalitis in horses and donkeys. These problems were minimised by further attenuation of the vaccine strains through passage in cell culture. These cell-culture-adapted viruses still form the basis of the currently

available Ondersteepoort Biological Products (OBP) vaccines (4). The OBP AHS vaccines currently used in South Africa are supplied in two polyvalent vials: the first contains AHSV types 1, 3 and 4, and the second contains types 2, 6, 7 and 8. AHSV-5 was withdrawn in October 1993 because of severe reactions and deaths in some vaccinated animals. AHSV-9 is also not included because type 6 is strongly cross-protective and because type 9 has rarely been reported in the country and is considered of low virulence. In South Africa, most susceptible equids in at-risk areas are vaccinated routinely, twice in the first year of life, and annually thereafter. Simultaneous inoculation with several vaccine types usually results in the production of protective antibody to included serotypes. However, in some individuals, interference between virus serotypes may result in incomplete protection after the first course of vaccination (36). 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, because, before the appearance of serotypes 2 and 7 in 2007, AHSV-9 was the only serotype known to be circulating in this region. Monovalent vaccination (against serotype 9 or 4) has also been used successfully in combination with other control measures to eradicate the virus during outbreaks in the incursive areas, e.g. the 1959–1961 outbreaks in the Middle East, the 1955–1956 outbreaks in North Africa and Spain, and the 1987–1991 outbreaks in Spain, Portugal and Morocco (4).

Despite the evident success of these live attenuated vaccines in controlling enzootic transmissions, there are still concerns about their use in epizootic situations, namely:

– the seed virus strains used in OBP vaccines originate from South Africa and the use of these vaccines elsewhere involves introducing virus topotypes from other ecosystems

– live virus vaccines may cause teratogenic effects and are therefore not recommended for use in pregnant mares

– live virus vaccines are likely to stimulate viraemia in some vaccinated equids and to infect biting *Culicoides*

– reassortment could occur between live vaccine viruses and wild-type viruses, in either the vertebrate or invertebrate hosts (27); this could result in 'new' viruses that have different or even enhanced virulence characteristics or that express novel antigenic properties.

As a consequence, efforts should be made to develop safer vaccines, such as inactivated, sub-unit or recombinant vaccines. An inactivated vaccine was used to successfully combat the 1987–1991 AHSV epizootic in Europe (59, 60) after clinical trials in France and field trials in Spain and Morocco showed that it was effective at blocking AHSV transmission. However, once the epizootic had ended, the vaccine was withdrawn, and there are currently no inactivated vaccines on the market. Although they are effective, they can be expensive to produce and sometimes multiple inoculations are needed to elicit high levels of protective immunity; consequently, efforts are underway to develop improved and less expensive inactivated vaccines. In addition, other promising vaccine candidates are under development. They are based on the administration of a poxvirus vector expressing VP2 protein, alone or in combination with VP5 (61, 62), or of recombinant capsid proteins, e.g. VP2 alone, or in association with VP5 and VP7, that self-assemble into immunogenic virus-like particles (47, 63). They have yet to be tested in horses, but the immunogenicity and safety of these two vaccines have been shown to be satisfactory in mammal models (e.g. guinea pigs).

Conclusion

African horse sickness virus has already caused large epizootics in the Mediterranean region and the Middle East on three occasions. The introduction in 2006 into northern Europe of BTV, an orbivirus close to AHSV, and the recent occurrence of two new AHSV serotypes in central Africa have revived concerns about the possible resurgence of AHS in the Mediterranean region. Enhanced awareness among veterinarians and increased preparedness of Veterinary Services in AHSV-free countries should be strongly encouraged in the coming years.

Peste équine

S. Zientara, C.T. Weyer & S. Lecollinet

Résumé

La peste équine est une maladie extrêmement grave des équidés causée par un *Orbivirus* appartenant à la famille des *Reoviridae*. Le virus est transmis par des arthropodes. La maladie constitue une menace sanitaire majeure pour les équidés des régions endémiques de l'Afrique subsaharienne. Le virus de la peste équine est à l'origine de vastes épizooties récurrentes dans la région méditerranéenne (particulièrement au nord de l'Afrique et au sud de l'Europe), associées aux échanges internationaux d'équidés infectés. L'émergence inattendue du virus de la fièvre catarrhale ovine dans le nord de l'Europe en 2006, virus étroitement apparenté à celui de la peste équine, a suscité de grandes inquiétudes quant au risque d'introduction du virus de la peste équine en Europe et plus particulièrement dans les régions indemnes de cette maladie mais ayant rapporté la présence des vecteurs compétents pour le virus, notamment les moucherons du genre *Culicoides*. Les pays d'Afrique du Nord et d'Europe devraient se préparer à faire face à des incursions du virus de la peste équine à l'avenir, en particulier depuis la récente propagation de deux sérotypes du virus (les sérotypes 2 et 7) en direction du nord, aussi bien en Afrique occidentale (Sénégal, Nigeria, Gambie...) qu'en Afrique orientale (Éthiopie), régions où par le passé seul le sérotype 9 avait été isolé. Les auteurs font le point sur les principaux éléments de l'épidémiologie, la surveillance et la prophylaxie de la peste équine.

Mots-clés

Afrique – Arbovirus – *Culicoides* – Équidé – Moucheron piqueur – *Orbivirus* – Peste équine.

Peste equina

S. Zientara, C.T. Weyer & S. Lecollinet

Resumen

La peste equina es una devastadora enfermedad de los équidos cuyo agente etiológico es un virus transmitido por artrópodos del género *Orbivirus*, familia *Reoviridae*. Esá considerada una importante amenaza sanitaria para los caballos de las zonas del África subsahariana en las que es endémica. En repetidas ocasiones, las operaciones comerciales con équidos infectados por el virus han causado grandes epizootias en la región del Mediterráneo (norte de África y sur de Europa en particular). Desde 2006, cuando en el norte de Europa apareció inesperadamente el virus de la lengua azul, estrechamente emparentado con el de la peste equina, existe el temor de que este último penetre en Europa, y más concretamente en regiones hasta ahora exentas de él donde está descrita la presencia de vectores como los jejenes *Culicoides*. Los países norteafricanos y europeos deben estar preparados para responder en el futuro a incursiones del

Rev. Sci. Tech. Off. Int. Epiz., **34** (2)

325

virus de la peste equina, máxime cuando dos de sus serotipos (el 2 y el 7) se han propagado en fechas recientes hacia el norte hasta alcanzar el África Occidental (Senegal, Nigeria, Gambia...) y Oriental (Etiopía), zonas donde hasta entonces solo se había aislado el serotipo 9. Los autores pasan revista a los principales aspectos de la epidemiología, vigilancia y profilaxis de la enfermedad.

Palabras clave

África – Arbovirus – *Culicoides* – Équidos – Jejenes – *Orbivirus* – Peste equina.

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327

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