

AFRICAN HORSE SICKNESS CHARACTERISTIC FEATURES OF THE PATHOGEN, EPIZOOTIOLOGY, CLINICAL SIGNS, DIAGNOSIS, AND MONITORING MEASURES (REVIEW ARTICLE)

O. M. Chechet (ORCID ID 0000-0001-5099-5577), **L. Ye. Konienko** (ORCID ID 0000-0001-6832-0789), **V. V. Ukhovskiy*** (ORCID ID 0000-0002-7532-3942), **M. S. Karpulenko** (ORCID ID 0000-0001-8982-9031), **H. V. Kyivska** (ORCID ID 0000-0002-2390-8498), **O. A. Moroz** (ORCID ID 0000-0003-4853-4573)

The State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise Kyiv (Ukraine); e-mail: uhovskiy@ukr.net*

Abstract *The paper presents a review of scientific literature about the epizootic situation with the African horse sickness. It gives current information on characteristic features, ways of transmission, and disease processes. The paper outlines the following key avenues of pathogen transmission: by vectors, by wild or domestic host animals transporting, and by sick (infected) animals in the early stages of sickness. The paper gives special attention to clinical signs, course, and pathological changes caused by the disease, and serological and molecular-genetic diagnostic methods. It reviews global experience in the localization and prevention of this disease and gives the reasons for implementing an active system of epidemiological surveillance of horses to find manifestations of the disease which is crucial for transition and spread prevention and early outbreak detection in Ukraine.*

Keywords: African horse sickness, epizootic situation, pathogen spread factors, diagnosis, risk assessment, prevention measures.

Introduction. African horse sickness (AHS) (Lat. *Pestis africana equorum*; Engl: *African horse sickness* (AHS); syn.: *Peste Equina Africana* – a viral disease that has peracute, acute, or subacute clinical forms of manifestation, characterized by fever, subcutaneous edema, and hemorrhages in internal organs.

African horse sickness is a natural focal viral disease that transmits over significant territories in a short time. The disease has epizootics manifestation. Young animals are more affected. The virus is transmitted by hosts and vectors. The disease affects all ungulates, but equidae are the most susceptible. It affects mules, donkeys, and zebras less, but they have longer viremia. Other known susceptible species include dogs, wild carnivores (hyenas and jackals), elephants, and camels (Zientara et al., 2015; Assefa et al., 2022). The disease is included in the WOAHP list of terrestrial animal diseases that require mandatory reporting to the World Organization for Animal Health. Due to the severity and potential risk of the disease, WOAHP eliminates the possibility of its rapid global spread (Susan et al., 2019). AHS remains the most economically significant global disease of equidae.

The paper's goal was to study and systematize information from the scientific literature regarding outbreaks of African horse sickness in the world. The information analysis deliverables were a description of the disease etiology, determined epizootiological characteristics, a character of clinical signs and pathological-anatomical changes, and an outline of modern diagnostic methods and measures for monitoring and prevention.

Methods and materials. The review was based on the study and analysis of the scientific literature on African horse sickness. The material for the review was the statistical data from WOAHP (WAHIS), ProMED, and OIE Publications.

Results. The earliest known recorded information on AHS was found in an Arabic document called "Le Kitab El-Akoual El-Kafiah Wa El Chafiah" which probably described an outbreak that occurred in Yemen in 1327. However, it is believed that the virus originated in Africa, and the first recorded evidence of the disease on the continent was made by Father Monclaro in his narrative of Francisco Barréto's journey to East Africa in 1569. Unlike zebras, which are endemic to the region, horses are not native to southern Africa. The first mention of AHS in South Africa was approximately fifty years after horses and donkeys were

introduced to the Cape of Good Hope by Dutch settlers in 1657. The significant outbreak was in 1719, when almost 1700 animals died (Dennis et al., 2019). Until 1953, outbreaks regularly occurred at intervals of approximately 20–30 years, the most significant outbreak was in South Africa in 1854–1855, when nearly 70,000 horses died (Vandenbergh, 2010; Carpenter et al., 2017). African horse sickness (AHS) is still periodically recorded in South African countries, but geography cannot hold the virus and it spreads further: to the countries of North Africa, the Middle East, the Arabian Peninsula, Southwestern Asia, and the Mediterranean region (Carpenter et al., 2017; Dennis et al., 2019). From 1959 to 1963 a significant epizootic occurred in the Middle East and Southwestern Asia, during which more than 300,000 equines perished (Carpenter et al., 2017). Researchers note that countries with milder climatic conditions are increasingly at risk of this disease outbreaks due to the vectors' (midges) migration northward as a result of global warming and climate change (De Vos et al., 2012; Hopley and Toth, 2013). Such an AHS outbreak in Europe would have significant economic and emotional consequences for horse owners on the continent, indicating the pressing need to develop new, safe, efficacious and cost-effective vaccines which would additionally allow differentiation between vaccinated and infected animals (DIVA).

Pathogen characteristics. African horse sickness virus (AHSV) is a virus of the *Orbivirus* genus belonging to the *Reoviridae* family. Nine different serotypes have been described (from AHSV-1 to AHSV-9) (Mohamed et al., 2022). There are 9 antigenically distinct serotypes of AHS virus (AHSV) identified by virus neutralization (VN) with crossreaction of homological antiserum, (some crossreaction has been observed between 1 and 2, 3 and 7, 5 and 8, and 6 and 9). Such cross-protection was observed in the field conditions common in South Africa, when multivalent vaccines do not contain certain serotypes, due to cross-protection of other types (e.g. cross-protection of African horse sickness serotype 5 and 9 after vaccination with serotypes 8 and 6) (Ngoveni et al., 2019).

The genome of AHSV consists of 10 double-stranded RNA segments, encoding seven structural (four major and three minor) and five non-structural (NS1, NS2, NS3/NS3A, and NS4) proteins. Segments encode the NS1 and NS2 proteins, which are highly conserved among the 9 AHSV serotypes. However, the RNA encoding NS3 and NS3A, and the two segments encoding the outer capsid proteins, are more variable between the AHSV serotypes (Zwart et al., 2015; Zientara et al., 2015; Dennis et al., 2019; Wall et al., 2021). Two major structural proteins, VP5 and VP2, form the viral coat, while the other two major structural proteins, VP3 and VP7, as well as three minor structural proteins, VP1, VP4, and VP6, make up the short particle of AHSV virion. The virions of the pathogen are non-enveloped, with a diameter of approximately 80 nm, structured as a two-layered icosahedral capsid composed of 32 capsomers. The virion core is formed by two major proteins, VP3 and VP7, which are highly conserved among the nine AHSV serotypes and are responsible for serogroup (Faber et al., 2016). The VP2 and VP5 proteins form the outer shell. Dominant antigenic sites inducing serotype specific neutralizing antibodies (nAbs) are mainly located on VP2 (Kanai et al., 2014).

The virus is sensitive to low pH and is easily inactivated at pH below 6.0. However, it is relatively stable at alkaline pH (7.0–8.5). The pathogen is sensitive to disinfectants at regular concentrations, including formalin, phenol, as well as UV rays.

After lyophilization the pathogen can be stored for several years; in blood, it can live for a few weeks, and in soil at 37°C for 11 days. At refrigerator temperature (4°C), strains maintain virulence for up to 90 days. The virus becomes inactive at a temperature of 45°C after 6 days, at 55°C – in 10 minutes, and at 60°C - in (5–15) minutes. Under the UV rays, the pathogen is inactivated in 1 minute (Zientara et al., 2015).

AHSV is morphologically almost identical to the bluetongue virus (BTV) (Mayo et al., 2021). The pathogen (in significant quantities) is detected in the blood during viremia, internal organs, exudate and tissue fluids, urine, and milk of infected animals. The virus is cultivated on various lines of primary and continuous cell cultures and in chicken embryos. Assays are conducted on suckling mice.

Epizootological features. African horse sickness is a natural focal viral disease that can be transmitted over significant territories in a short time.

African horse sickness is endemic in tropical and subtropical regions of Africa, south of the Sahara. The pathogen is common in South Africa but occasionally can be found in North African countries. Several outbreaks have been recorded outside of Africa, in the Middle East, and the Pyrenean Peninsula. Outbreaks of AHS were documented in almost entire the Republic of South Africa. During the last decades of the 20th century, the frequency of AHS outbreaks significantly dropped especially in the southern regions of South Africa, coinciding with a decline in the population of plain zebras (*Equus burchellii*), which are considered the natural reservoirs for the virus. (Porphyre and

Grewa, 2019). In the period 1959 to 1961, serotype 9 affected Saudi Arabia, Lebanon, Syria, Jordan, Iraq, Turkey, Cyprus, Iran, Afghanistan, Pakistan, and India. In late 1961, after a mass vaccination campaign and the loss of more than 300,000 horses, the registration of the disease in Asia ceased. In 1965 serotype 9 spread beyond the Sahara and appeared in Morocco, after spreading to Algeria and Tunisia. In October 1966, the virus appeared in Spain, in the Gibraltar area, in total 637 horses died or were slaughtered. Spain achieved viral eradication in three weeks after the application of intense vaccination and slaughter campaigns (Zientara et al., 2015).

In September 1987, serotype 4 was reported in the safari Community near Madrid. This outbreak was probably caused by the importation of subclinical infected zebras from Namibia. The disease spread through the Alberche and Perales rivers basins, contaminating a strip of land 100 kilometers in length and 50 kilometers in width. Before December 1987, when epizootic was over, one hundred forty-six (146) equines died or were slaughtered. Meantime 38 000 equids were vaccinated with multivalent attenuated vaccine (Zientara et al., 2015). In early October 1988, at a distance of nearly 600 kilometers from the previous outbreak, AHS appeared in the province of Cadiz. The disease affected various municipalities in the southern part of the Cadiz and Malaga provinces and caused the death of 156 horses. The last AHS outbreak was reported in December 1988. Around 18,000 animals were vaccinated with an attenuated multivalent vaccine and then with a monovalent vaccine of type 4. The epidemic resumed in July 1989, in the provinces of Badajoz, Cadiz, Huelva, Cordoba, and Seville. AHS cases were reported in September 1988 in the Algarve (Portugal), and Morocco declared the outbreak in the northern part of the country, near the Gibraltar Strait, in October 1989. In Spain, 110 animals died of the disease, and over 900 animals were slaughtered. Before January 1990, approximately a hundred outbreaks had been registered in the five affected provinces. 242,000 susceptible animals in 12 provinces were vaccinated with a monovalent, modified live serotype 4 vaccine to create a buffer zone that, in many cases, took more than 250 kilometers from the most peripheral identified outbreaks (Carpenter and Mellor, 2017). Portugal implemented monitoring and mitigation measures, which were based on mass vaccination of all susceptible animals, slaughtering animals on infected farms, and strict control of animal movements. The disease was eradicated within three months. 206 cases of AHS were registered in Portugal, in 16 districts (Verwoerd, 2012). In early September 1990, in Malaga province, a new case of AHS was reported. The disease appeared in 12 municipalities of this province, including Torremolinos and Mijas, and killed 66 horses. The latest case was in November 1990. Mandatory vaccination of the entire horse population in Andalusia was carried out. During this time, new cases of African horse sickness were recorded in the northern part of Morocco, caused by the AHSV 4 (Carpenter and Mellor, 2017). In 1989, the outbreak was in Morocco, in 3 provinces (Larache, Tanger, and Tetuan) 512 cases were recorded. In 1990 the disease spread to 4 other provinces (Chéfchaouen, Kénitra, Sidi Kacem, and Taounate) with a total 555 of cases (Zientara et al., 2015). After the outbreaks in 1989–1991 that affected the Iberian Peninsula and Morocco, the disease was silent outside the endemic territory in the south of Sahara, except for Yemen, where it was sporadically recorded. Most African countries, implemented vaccination, movement restrictions, surveillance, border controls, and stamping-out. However, countries in the northern part of the continent, such as Egypt, Morocco, and Algeria, controlled their outbreaks without vaccination. Eritrea, Ethiopia, Namibia, Senegal, and South Africa carried out vaccination campaigns. Despite efforts aimed at disease control on the continent, Ethiopia, Senegal, and South Africa periodically report outbreaks of this disease.

The virus is transmitted by hosts and vectors. *Culicoides* midges, *Aedes*, *Culex*, and *Anopheles* mosquitoes are the vectors, which can transmit and replicate the virus for up to five weeks. *Culex pipiens*, *Anopheles stephensi*, and *Aedes aegypti* insects can also transmit the virus. During the interepizootic period, the virus is maintained in the wild animal population. Zebras and African donkeys are considered to be reservoirs (Zientara et al., 2015).

All odd-toed ungulates are susceptible to the virus, but horses are the most. Mules, donkeys, and zebras are less susceptible but have longer viremia. Other known susceptible species include dogs, wild carnivores (hyenas and jackals), elephants, and camels (Zientara et al., 2015; Assefa et al., 2022). Dogs and other carnivorous animals can become infected by consuming the meat of infected animals that died from the disease (Hanekom et al., 2023). However, the role of dogs in the spread of the virus is considered insignificant since they do not attract vectors (Riddin et al., 2019). Serological studies indicate that infection with African horse sickness is widely spread among various African wildlife carnivore species (van Vuuren and Penzhorn, 2015). During a sero study of camels (*Hyalomma dromedarii*) in Nigeria (panel of 104 camels), 10 animals were classified as seropositive (Carpenter et al., 2017). Levels and time of viremia were unknown. In general, it is unlikely that camels can play any significant role in the spread of African horse sickness (Zientara et al., 2015). African elephants

(*Loxodonta africana*) also demonstrate low levels of antibodies to the pathogen and are considered to be poorly susceptible and unlikely reservoirs for the virus (dead-end hosts) (Carpenter et al., 2017). During serological study sheep and goats showed no antibodies to the virus, indicating that under natural conditions, these species are not susceptible. Livestock can be infected experimentally, and the disease will have a fever sign.

There are three ways of pathogen transmission:

- 1) via vectors;
- 2) via transporting wild or domestic host animals to other areas;
- 3) via sick (infected) animals in the early stages of sickness.

The *Culicoides* midge is the primary vector of the AHSV, which gets the virus while feeding on infected horses (Riddin et al., 2019). The most important vector is *Culicoides imicola*, but other species like *C. variipennis* (widespread in many parts of the United States) and *C. bolitinos* (present in Africa) are also potential vectors of the virus (Fall et al., 2015; Assefa et al., 2022). Infection primarily lasts during the warm, rainy season when midges are abundant and almost ceases after frost when midges die. Most animals become infected during the daytime when midges are most active. It's important to note that infected animals and host animals also play a significant role in the persistence of the virus. In Africa, the virus circulates freely in areas populated by zebras. In cases where the zebra population decreases, African horse sickness cases also tend to decline (Zientara et al., 2015). For the past decade, the globe has faced climate change. This results in the extension of seasons for *Culicoides* midges. Thus, the winter period has shortened or even disappeared, contributing to the vector's capacity and its spread to other regions (Riddin et al., 2019).

In general, in Europe and the Mediterranean basin, vectors can disperse several kilometers from breeding sites or via longer-distance with wind dispersion (Durr et al., 2017). This has been studied in the Gibraltar Strait (Southern Spain), where it was concluded that sandstorms facilitated the movement of *Culicoides* from northern Morocco to southern Spain, and these storms were consistently associated with outbreaks (EFSA, 2021). Analysis of field observations regarding the outbreak dynamics indicates that windborne dispersal of midges may contribute to the spread of the virus over short distances, but long-distance "leaps" of infection are consistently caused by the movement of virus reservoirs (equines). The transportation of infected animals or hosts (both wild and domestic) can cause outbreaks in areas located thousands of kilometers away from the source of infection. It happened during the outbreak in Spain and Portugal, from 1987 to 1990, caused by the importing of infected zebras from Namibia (Carpenter et al., 2017). Zebras were transported to Spain through Lisbon. One group of zebras was left in Madrid and another was transported to Alicante on the western coast of Spain. There was no explanation for why the virus was not recorded on the western coast. Subsequent studies showed that the low density of *Culicoides* on the western coast was insufficient for the spread of the virus. However, the zebras from the first group in the province of Madrid contacted *Culicoides* midges in the area, which caused the outbreak in three provinces. There were 27 cases reported and 250 horses killed (EFSA, 2021). The transportation of the infected animals caused the outbreak 7000 km away from their habitat.

The presence of the vector is connected with climate factors such as temperature, rain, and relative humidity (Riddin et al., 2019). The wind is the most important factor for *Culicoides* midge's dispersal (Durr et al., 2017). Due to the small size of midges (1-3 mm), the wind can carry them for over 700 km under suitable temperatures and wind speed conditions (Riddin et al., 2019). Sandstorms can be monitored with satellite images. The example, of the fact that *Culicoides* midges were carried by a storm, was confirmed when BTV in southern Spain was sequenced and compared with BTV serotype 4 from northern Morocco, and the homology was 100%. No animal transportation between Spain and Morocco was recorded at that time.

In March 2020, Thailand veterinary authorities reported to the WOAHA about a confirmed outbreak of African horse sickness in Pak Chong, Nakhon Ratchasima. The outbreak was considered to start earlier, in February. Since then, despite the implementation of various control measures, (including animal transportation controls, the use of insect nets, and vaccination with a live attenuated vaccine), the disease spread to other regions of the country. 15 outbreaks were recorded, causing over 500 equids killed, with mortality levels of more than 90 %. This was the first outbreak of African horse sickness in Thailand and the first case in Asia for approximately 60 years (King et al., 2020; Lu et al., 2020; Castillo-Olivares, 2021). Eventually, another outbreak of African horse sickness was recorded in Malaysia, near the Thailand border (OIE, 2020).

Pathogenesis. African horse sickness involves generalized processes in the blood and lymphatic vessels. Clinical signs and lesions are associated with damage to the vessel endothelium and increased permeability, which can vary in severity depending on the strain, serotype, and susceptibility of the host (Aklilu et al., 2014). After an infected (*Culicoides*) midge bite, the virus replicates in the endothelium of lymphatic capillaries and regional lymph nodes, primary viremia is initiated. Then, the virus spreads to the capillary vessels of multiple organs, primarily the lungs, large intestine, and lymphoid organs, causing secondary viremia. In susceptible horses, viremia can last from 4 to 8 days, but rarely longer than 21 days. The virus titer in tissues can reach up to $10^{5.0}$ TCID₅₀/cm³. The viremia is characterized by the fever which lasts until its end (Zientara et al., 2015).

Virions are adsorbed on erythrocytes and monocytes (an insignificant amount of the virus is present in the plasma) and are transported by the blood to endothelial cells of the lungs, spleen, and other tissues, which are the main sites of secondary replication. Despite the relatively low level of replication in these organs, the virus seriously damages endothelial cells and shows signs of edema and pleural effusion, which characterize the severe form of AHS believed to be the result of increased vascular permeability and impaired circulatory and respiratory function (Scacchia et al., 2015; Schliewert et al., 2022).

Even though AHSV can be detected in many organs, it has been found that the cell tropism is limited to endothelial cells, some macrophage cell lines, and reticular cells in lymphoid organs. No antigen of the pathogen has been detected in myocardial muscle cells and lymphocytes (Scacchia et al., 2015). Viruses also replicated in intravascular lung macrophages, interstitial macrophages, and fibroblasts (Hopley and Toth, 2013). Replication in endothelial cells resulted in cell damage with changes in intercellular connections, endothelial loss, increased capillary permeability, and subendothelial deposition of cell debris and fibrin. In many organs, especially in the myocardium and lungs, swelling, hemorrhage, and microthrombi can be observed (Scacchia et al., 2015; Schliewert et al., 2022).

Several studies have contributed to understanding the pathogenesis of AHS in donkeys and zebras (Hopley and Toth, 2013). The species, which are considered reservoirs of the pathogen, have predominantly subclinical infection. Experimentally infected with AHSV-4 donkeys develop and persist viremia for at least 12 days (confirmed by virus isolation, although at a lower titer than in ponies infected similarly). During another study, viral RNA was detected continuously in donkeys for up to 47 days (Scacchia et al., 2015).

Zebras commonly have subclinical infection. Experimentally infected zebras have much longer viremia than horses. After infecting, the virus could be isolated in 40 days from the blood and in 48 days from the spleen. Because of this subclinical viremic condition, zebras can be hosts to the pathogen and introduce it into new territories (Hopley and Toth, 2013).

Clinical signs and forms of the disease. The incubation period can be up to 2 months. There are peracute, acute, or subacute forms of the disease. The clinical signs of the peracute form are a rapid increase in the body temperature to 41°C and higher, which is observed on the (2nd-3rd day) after infection. The high temperature stays for 1–2 days and then becomes normal. Animals have conjunctivitis, rapid breathing, and an increased pulse rate. Animals die of acute heart failure on the 5th-7th day of the sickness.

The acute form of the disease is characterized by the predominant damage of lung tissue (with a mortality rate in animals that can be up to 95%). There is a sudden increase in body temperature (fever), breathing becomes difficult, the neck extends, and symptoms like shortness of breath, dry cough, and yellowish nasal discharge can appear. The conjunctiva becomes brownish-red with a yellowish tint. Tearing and photophobia develop. Within (24–48) hours before the death of the animal, there is a rapid development of lung edema, with foamy fluid discharge from the nostrils and blueness of visible mucous membranes. The duration of the disease in such form lasts for (10–15) days.

The subacute form particularly the cardiac or edematous, is characterized by swelling of the head, and neck, and constant cardiac disturbances. Subcutaneous edema of the head and neck are the most typical clinical signs. Supraorbital edema and conjunctival hyperemia are observed. Hemorrhages in the conjunctiva may also be observed. The mortality rate for this form can be approximately 50%. Sometimes, the swelling spreads to the abdominal area of the animal. On the (10th-12th) day, swellings also appear in both temporal fossae. The visible mucous membranes are swollen, and the pulse is weak, rapid, and sometimes not traceable. Infected animals predominately die.

In addition, there is a *pulmonary* form of the disease, characterized by fever, depression, sweating, spasmodic cough, and in the terminal stages, frothy nasal discharge. This form mortality rate is 95 %. The *cardiac* form, with a mortality rate of approximately 50%, is characterized by fever, swelling

of the head, neck, and supraorbital pits, and petechial hemorrhages in the eyes may sometimes be observed. A *fever* form is also recorded. This is the mildest form of the disease, typically not fatal, and is characterized by a sub-febrile fever, anorexia, depression, and hyperemia of the mucous membranes. In most cases, a *mixed* form of the disease is recorded, which is a combination of the cardiac and pulmonary forms of the disease. This form also has a high mortality rate, approximately 70%, with death usually occurring within (3–6) days after the onset of fever (Spence et al, 2019). The mortality rate among horses can be up to 70–95%, among mules up to 90%, and among donkeys up to 10%.

Post-mortem ***pathological and anatomical changes*** depend on the clinical form of the disease. For the pulmonary form, the main changes include alveolar and interstitial lung edema and hydrothorax. This form also often brings swellings around the connective tissue of the trachea and aorta, as well as hyperemia of the fundic part of the stomach. The petechial hemorrhages can be observed in the serous membranes of the peritoneum, pleura, and pericardium, as well as hydropericardium. Many horses may have foamy, whitish, or pinkish fluid coming from their nostrils.

In the cardiac form, the most characteristic changes include edematous infiltration of the muscles of the head and neck with subcutaneous swelling of the head. Pericardial effusion along with hemorrhages in the epicardium and endocardium are constantly observed. Pale areas of myocardial degeneration have been described. Pulmonary edema may also be observed.

The mixed form may have a mixture of these lesions. Actually, after necropsy, most lethal cases are confirmed as mixed forms with lesions of the heart or lungs (Hopley and Toth, 2013).

Lethal cases in dogs typically look like the pulmonary form in horses, with pulmonary edema and congestion as pathognomonic manifestations (O'Dell et al., 2018).

Diagnostic techniques. African horse sickness diagnosis is based on clinical signs, laboratory tests specific to AHS, and epizootiological data.

Laboratory tests are typically conducted in authorized laboratories. When there is a suspicion of African horse sickness the samples – blood from sick animals and small pieces of spleen, lungs, and lymph nodes of dead animals are sent to authorized laboratories for testing. Samples should be collected no later than 4–6 hours after the animal's death. Samples should be kept at 4°C during transportation and short-term storage before processing in an authorized laboratory. In the case of samples from non-vaccinated animals, to detect antibodies to AHSV, an authorized laboratory conducts serological tests. In the case of samples from vaccinated animals and non-vaccinated animals with detected antibodies to the AHSV after serological tests, laboratory studies are conducted to detect the antigen or RNA of this virus.

Serotyping and identification of the virus are conducted according to the methods (methodologies) of laboratory research outlined in the international standards, instructions, and/or recommendations.

Serotyping by a type-specific serological test such as virus neutralization (VN) is in general the gold standard (Zientara et al., 2015). Reliable tests are based on complement fixation test, indirect ELISA, immunoblotting) and PCR (Mayo et al., 2021) these tests allow for the specific detection of the agent or its components. New testing based on real-time RT-PCR allows to identify certain serotypes (Hemida et al., 2017).

The positive cut-off for the African horse sickness diagnosis is:

- African horse sickness virus is isolated and identified;
- antigen of the virus or RNA of the African horse sickness virus, specific to one or more serotypes of this virus, have been identified in samples from one or more animals exhibiting clinical signs of African horse sickness or epizootically connected with a confirmed case or a suspected case of African horse sickness.

Differential diagnostics. African horse sickness should be differentiated from anthrax (through bacteriological testing), piroplasmiasis, and trypanosomiasis (using blood samples microscopy and parasitological tests).

Immunity and prevention. The host's genetics is the key factor influencing the immune response and susceptibility of the animal. Both horses and zebras are susceptible to this agent, but horses are exposed to the sickness (show signs and severe course of the disease). If an animal has recovered from a previous infection, it is fully protected against new infection. However, in the case of infection with a different serotype of the agent, fever or manifestations of the cardiac form of the disease may still occur.

The live attenuated vaccine that has been used in South Africa to protect horses from the infection for approximately 60 years (Castillo-Olivares, 2021). Although, this vaccine hasn't been licensed for use outside the African subcontinent. Therefore, several new-generation vaccines have been developed and tested by biotechnology laboratories (RNA vaccines, subunit vaccines, poxvirus vector vaccines, etc.) (Dennis et al., 2019).

Prevention and measures of control. Research on disease prevention conducted in South Africa has demonstrated that keeping horses indoors at night provides significant protection against AHSV infection. It has been explained that the most important vector of the disease, *Culicoides imicola*, is exophilic. Another important vector of AHSV in South Africa, *C. bolitinos*, has demonstrated more endophilic behavior. However, closed doors and windows resulted in a 14-fold reduction in the number of *C. bolitinos* and *C. imicola* in the stables (Robin et al., 2016). Possible methods for controlling *Culicoides* populations include:

- treating livestock with insecticides, repellents, or systemic antiparasitic agents (e.g., avermectins);

- treating breeding sites for larvae or resting places for imagoes with insecticides;

- treating areas where animals are kept and/or transported with insecticides;

- removing or reducing breeding sites for larvae on farms (drinking bowls etc.).

However, the EU does not allow insecticide products against *Culicoides*. Information regarding the effectiveness of pyrethroid-based products is questionable (Benelli et al., 2017).

AHSV control and eradication were achieved in Portugal, Morocco, and Spain at some point (Carpenter et al., 2017). The key to success in controlling the disease in Spain was the use of a multivalent attenuated vaccine produced by Onderstepoort and an attenuated monovalent type 4 vaccine from the same source. It also involved establishing protective areas, controlling animal transportation, slaughtering infected animals, controlling *Culicoides*, and conducting serological surveillance.

Current European legislation stipulates that AHS must be controlled through the slaughtering of infected animals, disposal of carcasses, and the setting of a protection area with a radius of at least 100 kilometers around infected premises. The next area of surveillance is located approximately 50 kilometers beyond the protection area. Such procedure shall be in force for at least 12 months. The size of these areas is explained by the assumption that the virus can be transported over long distances by infected *Culicoides* vectors, carried by the wind (EFSA, 2021).

AHSV prevention procedure in Ukraine is described in the "Instruction on the Prevention and Control of African horse sickness" (Order No. 124, as of January 22, 2021, by the Ministry of Economic Development, Trade, and Agriculture of Ukraine) (hereinafter referred to as the Instruction).

To prevent the disease importing animals to the territory of Ukraine from countries/zones that are free from African horse sickness is only possible when the following conditions are observed:

- on the day of departure, the animals must not exhibit any relevant clinical signs;

- the animals must not have been vaccinated for at least 40 days before departure;

- the animals must have been kept in a country/zone that is free from African horse sickness for at least 40 days before departure;

- the animals should not have been transited through countries/zones that are affected by African horse sickness.

If there is a confirmed case of African horse sickness during the quarantine of animals, the owners and keepers of the animals have to immediately report it to the head of the independent structural unit of the territorial authority acting on relevant administrative territory and responsible for veterinary medicine.

The serological test is carried out in areas that are free from African horse sickness and is applied in locations with the highest risk of infection based on the results of earlier monitoring of animal health regarding African horse sickness and risk assessment.

During serological diagnosis tests, the following requirements shall be observed:

- various factors that could hinder the spread of African horse sickness, including geographical, climatic, administrative, ecological, historical, and epizootiological;

- tests are carried out at a distance of at least 100 kilometers from the border of an area/country that is affected by African horse sickness, however, the distance may be reduced if there are natural or geographical barriers that could hinder the spread of the disease.

Virological diagnosis tests are used for the following purposes:

- to confirm the case of African horse sickness;

- to confirm positive results of serological diagnosis tests:

- to identify AHSV serotypes present in the area/country.

Virological diagnosis tests are conducted to identify AHSV serotypes in cases where the serological tests were positive.

Monitoring of animal health regarding African horse sickness-positive/negative diagnosis involves both passive clinical observation and active laboratory diagnosis (serological and virological tests).

Monitoring of animal health regarding African horse sickness, to determine vector-free territories (countries/areas), includes observing the vectors.

Measures in case of suspicion of African horse sickness. In case of suspicion of African horse sickness, veterinarians, animal owners, and other individuals with reasonable grounds for suspicion must immediately report such information to the head of the relevant independent territorial unit of the competent authority.

Reports of African horse sickness suspicion can be submitted in any form, including email.

In case of African horse sickness suspicion, owners and caretakers of animals under suspicion have to take measures to prevent the spread of the disease before the arrival of representatives from the competent authority. These measures may include:

- animals of susceptible species isolated in areas protected from the vector;
- cessation of the transportation of susceptible animals between farms, their slaughter, and the sale of animal products, following the requirements of the Instruction;
- application of disinfection measures allowed in Ukraine indoors and around the premises where animals are kept
- conducting a thorough accounting of all susceptible animals and determine the number of deceased animals and those under suspicion.

Meat, meat products, skin, and fur obtained from animals suspected of African horse sickness can be transported between farms after processing or treatment using methods that ensure the eradication of AHSV.

The head of the structural unit of the territorial competent authority, who receives a report of AHSV suspicion, shall perform the following measures on the husbandry:

- continuous government-regulated veterinary and sanitary control, monitoring of animal health, conducted to identify cases of this disease;
- making a list of susceptible animal species with the number of dead, sick animals, and those under suspicion.

The head of the structural unit of the territorial competent authority shall arrange measures for other husbandries if their location, geographic proximity, or contacts may give reasons to suspect AHSV in these husbandries. He/she shall also ensure that measures are taken for the laboratory-confirmed results of AHSV presence before the outbreak eradication plan is commenced.

Measures to eradicate the outbreak. After the AHS positive diagnosis, the relevant State Extraordinary Epizootic Commission (SEEC) should make decisions to set quarantine measures and establish the boundaries of the affected unit, affected area, protected area, and surveillance area where epizootic measures shall be taken. When determining the boundaries of the protected area, several factors shall be taken into account, including geographical, climate, epizootic, vector presence, and measures of state control to limit the spread of the disease.

The relevant SEEC is responsible for approving a plan of measures to prevent the spread and eradicate African horse sickness.

Plan of measures to eradicate AHS shall include: the following actions are typically taken as part of the plan to control African horse sickness in *the affected unit* – slaughter of the AHSV positive and with clinical signs animals; disposal of corpses (carcasses) of dead animals by incinerating, ground disposal or otherwise, not to allow possible spread of AHSV; *in the affected area*: the actions to eradicate the disease are taken. Vaccination of all susceptible animals against African horse sickness may also be conducted. The decision to vaccinate shall take into account epidemiological, meteorological, geographical, and climatic factors. Any measures to control the disease (as outlined in the Instruction) can be applied in the affected area on the relevant SEEC decision; measures may include actions to eliminate vector habitats; as well as conducting epizootic investigation to get reliable information regarding the origin of the agent.

The epizootic investigation shall provide the following information:
the time of AHSV remains in the affected unit;

the AHSV source and a list of other husbandries where animals are kept, and there is suspicion of the disease, from the same source;

the presence of the disease vector;

information about the transportation of animals and/or their corpses (carcasses) to or from the affected husbandry, and other husbandries under AHSV suspicion.

AHSV eradication action plan in the protected area shall include:

- making a list of susceptible animals kept in the husbandries within the protection area;
- conducting continuous clinical examination of susceptible animals, if there is a suspicion of AHSV in any of these animals, blood or biological samples should be collected and sent to an authorized laboratory for testing to identify this disease.

The actions mentioned above shall be documented in compliance with the effective laws and regulations.

In the protected area, the transportation of animals out of the premises where they are kept is only possible to a designated slaughterhouse. The animals' transportation in the protected areas should be conducted under the supervision of the officials from the territorial competent body. Vaccination of all susceptible animals in the protected area may be conducted with vaccine against AHS, with all due requirements.

Vaccination against AHSV in the surveillance area is prohibited.

In the case of AHSV vaccination, the action plan for AHSV eradication shall be extended for at least 12 months.

The animals' transportation within areas with the same veterinary-sanitary status can be carried out with the decision of the head of the structural unit of the competent territorial authority, provided the following requirements are met: animals do not show AHS clinical signs;

all animals are listed and have veterinary documents containing information about their health status, place of origin, route, and purpose of transportation;

animals have not been AHSV vaccinated for at least 60 days before the transportation.

The transportation of animals, not intended for immediate slaughter, and/or their reproductive material from farms located within the surveillance area to farms beyond can be carried out with the decision of the head of the structural unit of the competent territorial authority, provided that the veterinary-sanitary requirements are met.

Lifting restrictions. The farm changes its status to "not-affected" at least 60 days after the last slaughtering of AHSV-positive or animals under suspicion, in case of conducting all AHS eradication measures in compliance with the plan of action.

A country/area is considered to eradicate AHS, when there is no systematic AHSV vaccination and when at least one of the following requirements is met:

- there have been no AHS outbreaks recorded for two years. The country/area does not border the AHSV-affected countries/areas;

- the absence of African horse sickness outbreaks in a country/area has been confirmed for at least two years;

- the absence of African horse sickness outbreaks in a country/area has been confirmed for 40 days and the absence of the vector has been confirmed for two years based on the results of monitoring the animals' health.

Quarantine restrictions in the affected unit, affected area, protected and surveillance areas are lifted by the decision of the SEEC based on the results of verifying the implementation of the actions specified in the African horse sickness eradication action plan, and if the following requirements are met:

- 12 months have passed since the last case of animal death from AHSV;

- the owners and caretakers have ensured the mechanical cleaning and disinfection of all premises, the area of paddocks, equipment, vehicles, as well as other places and objects related to the AHSV-positive livestock.

Conclusion

The review of current scientific literature resulted in the following: the AHSV has spread beyond Africa to Saudi Arabia, Lebanon, Syria, Jordan, Iraq, Turkey, Cyprus, Iran, Afghanistan, Pakistan, India, and Spain. The agent is transmitted by vectors – *Culicoides* midges, *Aedes*, *Culex*, and *Anopheles* mosquitoes, which can transmit and replicate the disease for up to five weeks in their organisms. African horse sickness should be differentiated from anthrax, equine piroplasmiasis, and equine trypanosomiasis. In this connection, there is a common requirement to establish an active system of

epidemiological surveillance of horses for AHSV which is very important for prevention of entry and spread of the infection in the territory of Ukraine.

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ХАРАКТЕРИСТИКА ЗБУДНИКА, ЕПІЗООТОЛОГІЧНІ ОСОБЛИВОСТІ, КЛІНІЧНІ ОЗНАКИ, ДІАГНОСТИКА ТА ЗАХОДИ БОРОТЬБИ З АФРИКАНСЬКОЮ ЧУМОЮ КОНЕЙ (ОГЛЯДОВА СТАТТЯ)

О. М. Чечет, Л. Є. Корнієнко, В. В. Уховський, М. С. Карпуленко, Г. В. Київська, О. А. Мороз

Державний науково-дослідний інститут з лабораторної діагностики та ветеринарно-санітарної експертизи, Київ (Україна); e-mail: uhovskiy@ukr.net*

Резюме. У статті описано результати аналізу наукових літературних джерел щодо епізоотичної ситуації з африканської чуми коней. Наведені актуальні відомості про характеристику, шляхи розповсюдження і патогенез збудника. Описано ключові шляхи розповсюдження збудника захворювання, серед яких виділяють: розповсюдження інфікованими векторами, транспортуванням диких або домашніх тварин-носіїв на інші території, хворими (інфікованими) тваринами на ранніх стадіях захворювання. Особливу увагу присвячено клінічним ознакам, перебігу та патолого-анатомічним змінам, викликаним захворюванням, серологічним і молекулярно-генетичним методам діагностики. Із урахуванням світового досвіду обґрунтовано основні складові заходів боротьби з цим захворюванням і його профілактика, аргументовано необхідність проведення активної системи епіднадзора серед коней з метою виявлення випадків хвороби, що, у свою чергу, матиме рішуче значення для попередження занесення і розповсюдження хвороби на територію України та раннього виявлення спалахів хвороби.

Ключові слова: африканська чума коней, епізоотична ситуація, фактори розповсюдження збудника, діагностика, оцінка ризику, заходи боротьби.

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