Review on the Epidemiology of African Horse Sickness

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Abstract: African horse sickness (AHS) virus causes a non-contagious, infectious, arthropod-borne disease of equines and occasionally of dogs. The virus is widely distributed across sub-Saharan African where it is transmitted between susceptible vertebrate hosts by the vectors. These are usually considered to be species of *Culicoides* biting midges but mosquitoes and/or ticks may also be involved to a greater or lesser extent. Periodically the virus makes excursions beyond its sub-Saharan enzootic zones but until recently does not appear to have been able to maintain itself outside these areas for more than 2-3 consecutive years at most. This is probably due to a number of factors including the apparent absence of a long term vertebrate reservoir, the prevalence and seasonal incidence of the vectors and the efficiency of control measures (vaccination and vector abatement). Natural infection occurs in Equidae, the most severe disease occurring in horses, with mules, donkeys and zebras showing lesser degrees of susceptibility in that order. AHS has both a seasonal and an epizootic cyclical incidence. The disease most commonly occurs in late summer to early autumn, and after periods of drought followed by heavy rains because the warm, moist conditions are optimal for vector breeding. There are a number of factors that decide the outcome in the horse that is bitten by a midge infected by AHS virus, including the virulence of the individual virus serotype and the immune status of the horse, however when the disease first appears in an area, affected equines should be eliminated immediately, and the non-infected equines vaccinated with polyvalent vaccine and rested for a week.
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Introduction

African horse sickness (AHS) (equine plaque) is an infectious, non-contagious arthropod borne viral disease of equidae (28). It is caused by a double-stranded RNA Orbivirus belonging to the family Reoviridae, characterized by alterations in the respiratory and circulatory functions (OIE, 2008). AHS virus affects all species of equidae family (horses, mules, donkeys and zebras) and transmitted by a biting midge belonging to the genus *cullicoides* (23). This devastating disease was listed by the Office of International des Epizootics (OIE) as endemic in sub-Saharan Africa (28). In the 1980s, AHS spreads outside its endemic region, reaching Spain and Portugal (21; 22; 28).

The disease has both a seasonal (late summer/autumn) and a cyclical incidence with major epizootics in Southern Africa during warm-phase. Mortality due to AHS is related to the species of equidae affected and to the strain or serotype of the virus (5). At least two field vectors are involved: *Cullicoides imicola* and *Cullicoides bolitinos*. Among the equidae, horses are the most susceptible to AHS with a mortality rate of 50-95%, followed by mules with mortality around 50%. In enzootic regions of Africa, donkeys are very resistant to AHS and experience only sub-clinical infections (39). In European and Asian countries, however, donkeys are moderately susceptible and have a mortality rate of 10%. Zebras are also markedly resistant with no clinical signs, except fever, and may have extended viraemia (up to 40 days) (28; 33; 39).

The disease is confined to sub-saharan Africa, although periodic epizootics have caused severe outbreaks of the disease outside enzootic regions, i.e., North Africa, the Middle East, and Southern Europe (39). The nine known virus serotypes of AHSV have been isolated from clinical cases of the disease in Kenya. The most important factor in the epizootology of AHS is the reservoir host. The existence of the reservoir of the infection is suggested by the fact that the disease passes from one season to another in a particular area. An outbreak mostly occurs during rainy seasons and quickly disappears during dry and cool periods before reappearing when wet and warm weather returns (16; 34).

There are 5.42 million donkeys, 1.78 million horses and 373,519 mules in Ethiopia (8). It has the largest equine population, probably with the highest density per square kilometer in the world (16). Many factors can contribute to the poor performance of equines, among which viral diseases characterized by high morbidity and mortality rates are to be the first one, and African horse sickness is one of the viral diseases. Although, it is the principal killing disease at the area of existence, there is little yet research of

economic significance on horse and mules. Due to its nature and consequence capacity of rapid spread, it was regarded as list A disease (28). Therefore the major objectives of this review paper are:

- ❖ To have an overview, on current status of the disease from global and Ethiopian perspective
- ❖ To highlight the transmissions of African horse sickness
- ❖ To aware the risk factor of African horse sickness virus

Literature Review

Etiology

African horse sickness virus (AHSV) belongs to the family Reoviridae, genus Orbivirus. Like other Orbiviruses, AHSV virions are double layered, with their genomes composed of 10 double-stranded RNA (dsRNA) segments. The virion is non enveloped particle of a size around 70 nm. There are nine serologically distinct AHS virus serotypes (AHSV-1 to AHSV-9) have been identified with no evidence of cross-neutralization among themselves (5; 27). African horse sickness (AHS) is associated with a viscerotropic orbivirus of which nine antigenic strains (serotypes) are recognized.

Epidemiology

Broadly, any occurrence of a disease is subject to specific interactions between multiple factors (determinants) which include a susceptible host, pathogen, environment and sustainable management to address these factors and interactions (38). This may also include biological vectors, if applicable. In addition, other determinants such as genetic constitution, nutrition and toxic agents may also contribute and impact on animal health and production (38).

Epidemiology of AHS is highly dependent on the interaction between the infected host, a competent vector and susceptible non-infected equidae under favourable environmental conditions. Once the disease has been introduced, cyclic outbreaks (usually during the late summer through late autumn) would coincide with the availability and abundance of the competent vector (s) (10).

History and Geographical Distribution

Probably the first historical reference to AHS concerns an epizootic in the Yemen which occurred in 1327 (25). However despite this early record the virus group appears to have originated in Africa and was first recognised as a distinct disease entity there subsequent to the introduction of highly susceptible breeds of equine during the exploration of Central Africa (25). In southern Africa the disease has been recognized since the occupation of the Cape of Good Hope by the Dutch East India Company at the beginning of the 18th century when large numbers of deaths occurred in imported horses (17).

However, it was not until 1900 that M'Fadyean using samples of infected horse blood showed that the agent of horse sickness fever was able to pass through bacterial filters and concluded that it was an ultravisible virus. In a series of experiments covering several years (1908, 1915 and 1921) then recognized that AHS virus existed as a number of antigenically distinct strains but not until 1962 were the 2 most recent internationally accepted serotypes (8 and 9) identified and characterised (18). The isolation of an additional strain of AHS virus in Kenya (G-75), that apparently is not neutralized by antisera to any of the 9 recognised serotypes, suggests that a further serotype should now be added to the internationally recognised list (12;13).

World distribution and its occurrence

AHS virus is widely distributed across sub-Saharan Africa and is enzootic in a band stretching from Senegal and Gambia in the west to Ethiopia and Somalia in the east (18). It also occurs as far south as South Africa and may extend at times to Egypt in the north. The Sahara desert, however, provides an effective geographical barrier which usually, though not invariably, prevents incursions into North and NW Africa from the infected areas further south.

Figure 1: Geographic distribution of African horse sickness in endemic areas
Source: (29)

Until relatively recently AHS virus was believed to be confined to Africa with the exception of occasional excursions across the Red Sea into South West Arabia. However, in the summer of 1959 the situation changed. Horse sickness fever appeared first

in Saudi Arabia and the southern regions of Iran, and then spread northwards, eastwards and westwards to involve Afghanistan and Pakistan as well as in Spain, Portugal, Morocco, Pakistan and India (34).

All serotypes of AHS occur in Eastern and Southern Africa. Only AHS serotype 9 and 4 have been found in West Africa, from where they occasionally spread into countries surrounding the Mediterranean. Spread also occasionally occurs from North Africa to the Iberian Peninsula. This distribution is primarily dictated by the presence of the principal insect vector, C*ullicoides imicola* (Sinclair, 2006). The virus could have been introduced into Ethiopian equines by wind-borne infected midges (*Cullicoides*) from endemic regions of Africa because the vectors were known to be wind-driven and migrate, carrying the virus over 700 km (32).

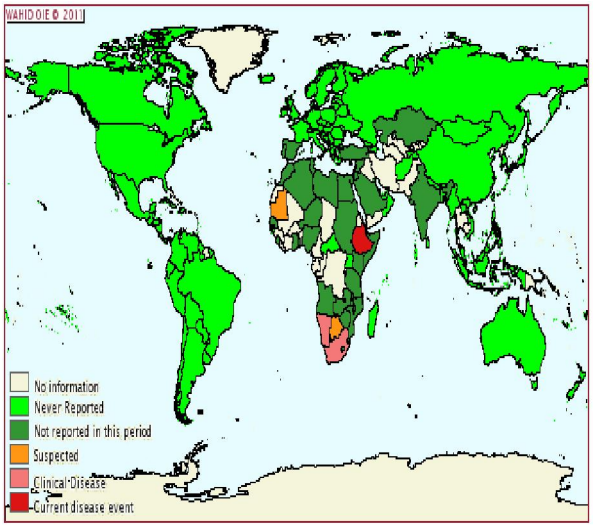

Figure 2: Outbreaks of African horse sickness for July-December 2010.
Source: (30)

African Horse Sickness in Ethiopia

Of the nine serotypes identified, type 9 is predominantly found through out the African continent, and it is the only serotype previously identified in Ethiopia. the distribution of the disease seems to have positive correlation with the ecology of its vectors. Furthermore, the disease is considered to be endemic to the lowlands and midlands of Ethiopa (26).

In 2002–2003 Ethiopia faced serious and repeated outbreaks of AHS in different regions, including southern, western, central, and northern Ethiopia. The outbreak affected horses vaccinated with monovalent vaccines containing type 9 AHSV (AHS Vaccine, National Veterinary Institute, Debre Zeit, Ethiopia). It is well documented that in spite of its wide distribution, serotype 9 of AHSV has a lower virulence than other serotypes, killing few horses in enzootic areas. The outbreak encountered in 2002–2003, however, resulted in high mortality (3). Donkeys, which were thought to be resistant to AHS, were observed to encounter clinical AHS in the Tgrai region of northern Ethiopia.

The most amenable means of control of AHS for countries such as Ethiopia is immunization of susceptible hosts with suitable vaccines. Considering the diversity of AHSV serotypes and the absence of cross-protection, it has been strongly recommended that polyvalent vaccines are used **(**14**)**.

Table 1: Seroprevalence (using ELISA test) of African Horse sickness in some places of Ethiopia

Study areas	Species	Prevalence (%)	Sources
Arsi & Bale	Horse	20.34	(36)
	Donkey	24.6	
	Mule	20	
Bahir Dar	Horse & Donkey	56.52	
Dangla	Horse & Donkey	52	(37)
Metema	Horse & Donkey	70	
Southern Ethiopia	Horse	36.2	(14)
	Donky	25.42	
	Mule	33.33	
Woliso	Horse, Donkey & Mule	23	(19)
Central Ethiopia	Horse, Donkey & Mule	36.3	(1)

Modes of transmission

AHS is not a contagious disease. It is transmitted between susceptible animals by the bite of hematophagous insects including midges (*Culicoides* spicies), ticks (*Hyalomma dromadarii* and the brown dog tick, *Rhipicephalus sanguineus*), and mosquitoes (various species in laboratory studies) (23). Midges are by far the most important vector in the spread of the spontaneous disease. The source of virus for midges is blood of infected horses, donkeys, mules and zebra. Horses and mules have clinical signs of disease while viremic, but donkeys are often and, most importantly, zebra are always, in apparently infected. Zebras may remain viremic for 6 weeks, donkeys for 12 day and horses for 18-21 day (4; 35).

Transmission of the virus to areas where it does not usually exist occurs both by movement of infected animals such as zebras and horses, and by transportation of midges by wind. Mechanical transmission of the virus on contaminated surgical instruments and needles should be considered a possibility. In areas in which the disease is enzootic the virus persists by cycling between the mammalian host, the zebra, and vectors year round (4; 35). Zebra in enzootic areas can seroconvert during any month of the year, indicating that persistence of the virus is associated with sequential infection of zebra within a herd or region. Persistence of the virus in a region is

attributable to the long period of viremia in zebra and the presence of a herd of sufficient size to support cycling of infection among animals (Barnard, 1998). Transmission by insects other than midges is thought to be a minor source of infection. Virus replication occurs mainly in the lungs, spleen and lymph nodes. Although virus is present in urine, milk and other body secretions of infected animals, no transmission of disease by contact, inhalation or ingestion of these materials is known (6; 33).

Vectors

Culicoides are not simply mechanical vectors; they rather allow the replication of the virus in themselves before transmission. In fact there is no report of transovarial transmission so far. *Cullicoides imicola* is the vector responsible for the transmission of AHSV within its enzootic area and during epizootics. *Cullicoides bolitinos* is also a vector of AHSV in southern Africa while the other *Cullicoide* species are unlikely to be important as they are unable to maintain the infection (22). However *Cullicoides varipenis, Cullicoides pulicaris* and *Cullicoides absoletus* are competent and likely important vectors because of their ability to maintain infection over winter, as demonstrated in Portugal (32).

The abundance of midges can be predicted from measure of soil moisture content and land surface temperature. Midges breed in dump soils that are rich in organic material, such as irrigated pastures that provide soil moisture adequate for completion of the life cycle (at least 7 to 10 days). Higher temperatures increase the rates of infection of midges, virogenesis within midges and transmission rate, but decrease midge longevity. Replication of AHSV in midges does not occur at temperatures less than 15°C, although midges continue to be active at 12°C. Midges can be transported by winds for up to 700 km (32). As the main means of spread of AHS virus is by biting midges, conditions that favor the presence of large populations of these insects are required for epidemics of the disease to occur. Favorable conditions are high temperatures and humidity after widespread rains (11).

Cullicoides midges generally feed in the twilight periods after sunrise and sunset, on fine clear nights. In overcast and cloudy conditions or in cooler weather, biting activity will occur in late afternoon, before sunset, and in the mornings after sunrise. Analyses of meteorological conditions suggest that windborne spread of infected vectors may have been responsible for a number of outbreaks of AHS in Spain, around the Mediterranean and in the Middle East and India. Distances involved varied from 40 - 700 kilometers (22; 27).

Susceptible species

All members of the horse family (Equidae: horses, mules, donkeys and zebras) are susceptible, with horses generally experiencing severest disease and highest mortality rates (11). The most serious infections occur in horses and mules, which appear to be accidental hosts. Dogs are also susceptible. The disease does not affect humans. Zebras and donkeys rarely develop serious clinical signs (4; 5; 20). Although zebras are thought to be the natural reservoir hosts, horses, mules and donkeys can also develop viraemia sufficient to infect *cullicoides*. Most sources suggest that dogs do not play a significant role in the maintenance or spread of AHS. Expermentaly dogs have been found to be susceptible to the AHS virus. Infection typically occurs following the consumption of virus infected horse meat. Canines are not thought to become naturally infected with AHS through vector bites, and are also not considered important in the spread or maintenance of the virus. It is generally accepted that dogs play no role in the spread or maintenance of AHS because they do not develop viraemia sufficient to infect vectors. Camels and zebras can be in apparently infected with AHS virus. In an Egyptian survey, antibodies were found in sheep, goats, and buffaloes (4; 28).

Risk factors

Environment factors

AHS has both a seasonal and an epizootic cyclical incidence. The disease most commonly occurs in late summer to early autumn, and after periods of drought followed by heavy rains. The warm, moist conditions are optimal for vector breeding. Some authors speculate that global warming could increase the risk for spread of arthropod born diseases such as AHS. Epizootics of AHS outside the enzootic sub Saharan zone do not appear to be maintained for more than 2 to 3 consecutive years. Outbreaks are abruptly curtailed by severe frost. During the harsh winter months; the virus must survive in an appropriate reservoir (35). The incidence of the disease is often seasonal because of the seasonal variations in the number of Culicoides species present. (35). Local factors, including topography, influence the distribution of midges within their overall range and therefore the disease has a geographical distribution: the areas most severely affected are low lying and swampy (5).

Animal factors

Natural infection occurs in Equidae, the most severe disease occurring in horses, with mules, donkeys and zebras showing lesser degrees of susceptibility in that order. The virus causes severe disease in dogs. Elephants seroconvert when exposed to infection, but are probably not an important reservoir (Barnard, 1998). The case fatality rate varies depending on the severity of disease but can be as high as 90% in susceptible horses, but is lower in mules and donkeys. After natural infection or vaccination

immunity to that strain, but not to heterogonous strains, is solid. Foals from immune dams derive passive immunity from the colostrums and are immune until 5-6 months of age (31).

Clinical signs

There are four different forms of African horse sickness. These are: the peracute or pulmonary form, the subacute or cardiac form, the acute or mixed form and the horse sickness fever (6; 11; and 34).

The pulmonary form of African horse sickness is characterized by an incubation period of 3-5 days. Acute fever of 40-42°C for 1-2 days is followed by the sudden onset of severs respiratory distress. Infected animals often stand with forelegs spread, head extended nostrils fully dilated, serofibrinious nasal discharge, and coughing (28; 32).

The cardiac form of AHS is a subacute disease with a longer incubation period (1 - 2 weeks) and a more protracted course than the acute respiratory form. The fever (39 - 41°C) last less than two week and is followed by swelling of the supraorbital fossa, which is pathognomonic. Swelling usually extends to the eyelids, facial tissues, neck, thorax, brisket and shoulders. Death (50 - 70%) usually occurs within one week from cardiac failure (11; 28; 34).

In the mixed form of African horse sickness, symptoms of both the pulmonary and cardiac forms are seen with incubation period of 5-7 days. In most cases, the cardiac form is subclinical and is followed by severe respiratory distress, occasionally; mild respiratory signs may be followed by edema and death from cardiac failure (11; 28; 32).

The fever form of AHS is a mild to subclinical infection; the incubation period varies from 4 to 14 days. The characteristic fever of 39- 40°C usually lasts for 3 to 8 days; morning remissions and afternoon exacerbations are often seen, and may be the only clinical sign observed. Other symptoms are generally mild and may include mild anorexia or depression, edema of the supraorbital fossae, congested mucous membranes and an increased heart rate. Almost all animals affected with this form recover (6; 11; 28; 34).

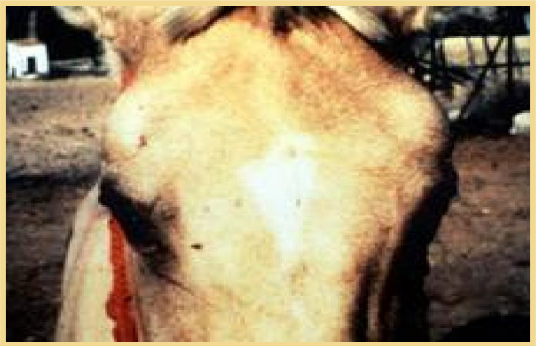

Figure 3: Swelling of the supraorbital fossa
Source: (34)

Pathogenesis

There are a number of factors that decide the outcome in the horse that is bitten by a midge infected by AHS virus, including the virulence of the individual virus serotype and the immune status of the horse (32). After the virus is inoculated into the body, it is carried to the regional lymph nodes where it finds conditions favorable to its multiplication. Virus is released into the blood whereby it finds itself infecting the target organs, namely the lungs and other lymphoid tissues of the body. The viraemia is associated with the red blood cells and lasts for about four to eight days. By the third day after inoculation, the virus may be found in organs such as the spleen, lungs and pharynx, as well as most lymph nodes. The heart is not primary site for virus replication (15; 22).

Diagnosis

In endemic areas, clinical signs and lesions may lead to a presumptive diagnosis. African horse sickness should be suspected in animals with typical symptoms of the cardiac, pulmonary or mixed forms of the disease. The supraorbital swellings are particularly characteristic of the disease. The horse sickness form can be difficult to diagnose (15).

In live animals, blood samples collected into anticoagulant tubes should be taken for virus isolation. Success is most likely if these samples are collected in heparinized tubes from acutely sick animals in the early febrile stage of the disease (27). From relatively fresh dead bodies or moribund animals, small pieces (5-10gram) of spleen, lung and lymph nodes will be collected, which are the samples of choice for AHS virus diagnosis. Samples will be kept at +4°C or in glycerol-saline solution till processed (2; 27). Cell culture that showed CPE will be assayed for identification of the responsible serotype involved in the infection using the standard nine serotypes of AHS virus and sera following the procedure of virus neutralization test (27).

Serological diagnosis of the acute disease may be difficult because many horses die before they mount a detectable antibody response. In horses that survive for at least 10 day, agar gel immunodiffusion (AGID), indirect fluorescent antibody (IFA), complement fixation test (CFT), virus neutralization and ELISA tests are all effective in detecting antibody to the virus (32).

Reverse-Transcription Polymerase Chain Reaction (RT-PCR) will be applied for the confirmation of virus and for the discrimination of the nine AHS virus serotype (9).

Treatment, prevention and controls

There is no specific treatment for African horse sickness. Supportive care and treatment of complications of the disease should be provided (32). Generally, control of AHS relies on effective

quarantine and movement controls to stop the spread of infection, animal management to limit vector exposure, establishing immunity by vaccination (6; 34).

Vector control

In the quest to prevent AHS transmission to horses, one has to consider the life cycle and typical habits of the midges (11). Protection of horses has to be focused on preventing the midges from entering stables (to be able to bite the horses) or by confusing them so that the midges cannot find the horses. A stable which has effective mosquito-proof mesh applied to all apertures should prove successful. Whenever possible, all equines should be stabled in insect-proof housing. At a minimum, stabling from dusk to dawn, the period when *cullicoides* are most active, is recommended (28).

Vector control measures such as modifications of *cullicoides* breeding areas, insect repellents, and targeted applications of insecticides or larvicides may also be useful (34). The *cullicoides* midges predominantly bite on the top of horses and therefore physical barriers like blankets or face masks, etc. will prevent midge's biting (7).

Vaccination

When the disease first appears in an area, affected equines should be eliminated immediately, and the non-infected equines vaccinated with polyvalent vaccine and rested for a week. Three types of vaccines can be considered: inactivated ('killed'), attenuated ('live') and recombinant virus vaccines (27).

Economic importance

In a fully susceptible horse population, the effect of AHS can be devastating, because up to 95% mortality can be expected. The serious nature of the disease for equines is compounded by tremendous problem of eradication: vaccination reduces the ravages of horse sickness, but even when practical on a wide scale it cannot eradicate the disease because the infection is insect borne, and uncontrolled hosts provide a reservoir of infection (32).

Conclusion And Recommendation

African horse sickness is an infectious but not contagious disease of Equidae. It is spread by the bite of blood-feeding insects. The disease is enzootic in sub-Saharan Africa, causing clinical disease in horses, donkeys, mules and dogs, and infecting zebras, elephants and perhaps other wildlife. The AHS virus is thought to be maintained in endemic areas as a result of a continuous cycle between insect vectors and infected wild or domestic equines or other wild reservoir hosts. The disease is currently an economic concern because of the costs associated with preventive measures in enzootic areas, monitoring for introduction of disease in neighboring unaffected areas, and restrictions on importation of horses from countries in which the disease is enzootic. In countries such as Ethiopia, where large populations of equines are raised, the presence of multiple serotypes of such a devastating virus poses a serious hindrance to national development.

Based on the above conclusion, the following points are recommended:

- Awareness should be created to those equine owners to look for timely vaccination.
- There should be further epidemiological study to come up with appropriate control measure.
- There should be development of protective vaccine that incorporates the strain in the area; for this, the virus circulating in the area should be identified and incorporated in the vaccine given.
- Whenever possible, all equines should be stabled in insect-proof housing. At a minimum, stabling from dusk to dawn, the period when *culicids* are most active.

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References

1. Alemayehu, L. (2004): Case studies on reproductive activity of equines in relation to environmental factors in central Ethiopia: Hand book on Animal Disease in Tropics: 3rd ed. London: British Veterinary Association, Pp 1- 3.
2. Alexander, K., Kat, P., House, J., House, C., Brien, S., Laurenson, M., MucNutt, J., and Osburn, B. (1995): African Horse Sickness and African Carnivores. *Veterinary microbiology*, 47: 133 - 140.
3. Aschalew, Z., Teshale, S., Keith, P. and Feseha, G. (2005): Isolation and Identification of circulating serotype of African Horse Sickness Virus in Ethiopia. *The Journal of Applied Research in Veterinary Medicine*. 3: 1 - 42.

4. Barnard, B.J.H. (1998): Epidemiology of African horse sickness: Zebra virus reservoir. *In Foot and mouth Disease, African Horse sickness and pleuropneumonia*, summaries and conclusions from the OIE scientific conference, April 1994, OIE, Paris, France.
5. Baylis, M., Mellor, P., and Meiswinkel, R. (1999): Horse sickness and ENSO in South Africa. *Natural*, Pp 397 - 574.
6. Binepal, V., Wariru, B., Davies, F., Soi, R. and Olubayo, R. (1992): An attempt to define the host range for African horse sickness virus (Orbivirus, Reoviridae) in East Africa, by a serological survey in some equidae, camalidae, loxo dentidae and carnivore. *Veterinary microbiology*, 31:19 - 23.
7. Bremer, C., Dungu-Kimbenga, B. and Viljeen, G. (1998): Detection of African horse sickness virus in Zebra by RT-PCR and the development of different methods for confirming AHSV specificity of RT-PCR products. Proceedings of the Eighth International conference on Equine infectious disease, Dubai, 23-26 March 1998. R and W publications (New market) Ltd, New market, UK.
8. Central statistical authority (2009): Federal Democratic Republic of Ethiopia. Central Statistical Authority (CSA), agricultural sample silvery 2008/2009 (2001 E.C), Report on livestock characteristics (private peasant holdings), Addis Ababa.
9. Cetre-Sossah, C., Mathieu, B., Setior-Rio, M., Grillet, C., Baldet, T., Dellocole, J. and Albina, E. (2008): Development and evaluation of a real-time PCR assay for *cullicoides imicola*, one of the main vectors of bluetongue (BT) and African Horse Sickness (AHS) in Africa and Europe. Research in veterinary science, in press.
10. Chevalier, V., de la Rocque, S., Baldet, T., Vial, L., Roger, F. (2004): Epidemiological process involved in the emergence of vector-borne diseases: West Nile fever, Rift Valley Fever, Japanese encephalitis and Crimean-Congo haemorrhagicfever. OIE Scientific and Technical Review, 23, 535 - 555.
11. Coetzer, J.A. and Guttrie, A.J. (2004): African horse sickness: In infectious diseases of Livestock, 2nd Ed., and Pp 1231 - 1246.
12. Davies, F. (1976): AHS vaccine. *Veterinary Research*, Pp 98 - 36.
13. Davies, F., and Lund, L. (1974): The application of fluorescent antibody techniques to the virus of African horse sickness. *Research Veterinary Science* 17,128 - 130.
14. Demssie, K. (2006): African Horse Sickness: Seroprevalence and identification of risk factors in Equidae at selected sites in Ethiopia. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, Pp. 42 - 45.
15. Ferhandey, J., Ferhandey – Pacheco, P., Rodriguey, B., Sotelo, E., Robles, A., Arias. M. and Sanchey-vichiano, J. (2007): New advances in the molecular diagnosis of African Horse sickness (AHS). Proceedings of the 13th international symposium for the world association of veterinary laboratory Diagnosticians, 11-14 November, Melbourne, Australia.
16. Feseha, G. (1998): Proceedings on equine infectious diseases. 8th international conference on equine infectious disease, Dubai April, Pp 318 - 319.
17. Henning, M. (1956): Animal Diseases in South Africa. Central News Agency Ltd, South Africa, 3rd Ed, Pp 785 - 808.
18. Howell, P. (1963): The isolation and identification of further antigenic types of African horse sickness virus. *Onderstepoort Journal Veterinary Research*, 29:139 - 149.
19. Kassa, D. (2006): African Horse Sickness: Seroprevalence and identification of risk factors in Equidae at selected sites in Ethiopia. Unpublished M.Sc. Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, Pp 39 - 40.
20. Koekemoer, M.J., Potcieter, A.C., Paweska, J.T. and Van Dijk, A.A. (2000): Development of probe for typing African horse sickness virus isolates using a complete set of clone Vp-2 genes. *Journal virology Methods*, 88:155 - 144.
21. Maclachlan, N.J., Balasuriya, U.B., Davis, N.L., Collier, M., Johnston, R.E., Ferraro, L.L. and Guthrie, A.J. (2007): Experiences with new generation vaccines against equine viral arteritis, West Nile disease and African horse sickness, vaccine, 25:5577 - 5502.
22. Meiswinkel, L. and paweska, J. (2003): Evidence for a new field Culicoides vector of African horse sickness in South Africa. *Preventive veterinary medicine*, 60:24-53.
23. Mellor, S. (1995): Epizootology and vector of African horse sickness virus. *Comparative Immunological Microbiological Infectious Disease*, 17: 287-296.
24. M'Fadyean, J. (1900): African horse sickness. *Journal Comparativ Pathology*, 13: 1-20.
25. Mouie, L. (1896): Histoire de la Medecine Vétérinary. Maulde, Paris, p. 38.
26. National Veterinary Institute (1983): The Pathomorphological Changes of African Horse Sickness, Annual Report, Debre Zeit, Pp 1-2.

27. Office Internationa lDes Epizootics (OIE) (2004): African horse sickness, In manual of diagnostic tests and vaccine for terrestrial animals, 5th Ed. Paris: Office International des Epizootics, Pp 1 - 21.
28. Office international Des epizootics (OIE) (2008): African horse sickness, in manual of diagnostic tests and vaccine for terrestrial animals. Paris: *Office International des Epizootics*, Pp 823 -838.
29. Office international Des epizootics (OIE) (2010): Geographic distribution of African horse sickness in endemic area, Pp 3 – 34.
30. Office international Des epizootics (OIE) (2011): Outbreaks of African horse sickness for July-December 2010, Pp 5 – 57.
31. Portas (1999): *Medical Epidemiological Infection*, Pp 123 - 337.
32. Radostitis, O.M., Gay, C.C., Hinchchiff, K.W., and Constable, P.D. (2007): veterinary medicine, A text book of the disease of cattle, sheep, pigs, Goats, and Horse, 10th Ed. London: W.B. Saunders company ltd, Pp 1179 - 1183.
33. Roy, P., Peter, P., Mertens, C. and Casal, I. (1994): Africa horse sickness virus structure. *Comparative Irnminology Microbiology Infectious Disease*, 17: 245-273.
34. Sinclair, M. (2006): The epidemiology of African horse sickness outbreak in the Western Cape province of South Africa in 2004.
35. Skowronek A. (1995): In African horse sickness. Wein, Austria: Springer-Verlag, *Veterinary Pathology*; 31:12 -. 54.
36. Tesfaye, M. (2012): Seroprevalence and Associated Risk Factors of African Horse Sickness in Arsi and Bale Zones, Southeastern Ethiopia. *International. Journal of. Animal health,* 4: 426 -432.
37. Teshome, M., Mekonnen, A., and Wudu, T. (2012): Seroprevalence and risk factors of African horse sickness in mules and donkeys in selected sites of West Amhara Region, Ethiopia. *African Journal of Microbiology Research*, 6: 4146 - 4151.
38. Thrusfield, M. (1995): sampling in veterinary epidemiology, 2nd Ed. London: Blackwell science Ltd, Pp 179-204.
39. Williams, C.F., Inoue, T., Lucus, A.M., Zanatto, P.M.A. and Roy, P. (1998): The complete sequence of four major structural proteins of African horse sickness virus serotype 6: evolutionary relationship within and between the Orbiviruses. *Virus research*, 53: 55 - 73.

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