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Review Article

Review on the Biology of African Horse Sickness Virus and Its Vector

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Abstract: There are many infection and non infection disease those can decrease productivity of equine and even destroy their life. From those African horse sickness which caused by African horse sickness virus is the most characterized by high morbidity and mortality rates on equine. According to the International Committee on Taxonomy of Viruses, the virus was Family: *Reoviridae*, Subfamily: *Sedoreovirinae* and Genus: *Orbivirus* which shares many morphological and structural characteristics with the other members of this genus, Bluetongue virus and Equine encephalosis virus. This virus double stranded RNA virus which contains two different proteins with nine serotypes. Transmission of this virus is based on presence of culicoidmidget arthropod mainly *Cullicoides* specious (*c. imucola*). This blood feeding arthropod transmit virus mechanically and transovarially, however entrance of the virus through different mechanism that virus used based on protein contains. Replication is done by replicate enzyme of virus in host cytoplasm & virus protein translation by host machinery. From all sero-type AHS types 1-8 are considered to be highly pathogenic for horses and disease results in high mortality (90-95%). This virus resistant to different chemical, temperature range and PH value due to presence of some proteins. *Cullicoides* have different specious those are small insect, obligate blood sucker. In general, after surveillance was done for both virus and insect, control and prevention of African horse sickness is overcome by flow control strategy of virus and insect. Even though this review have best contribution during develop control strategy.

Keywords: African Horse Sickness Virus, Bluetongue Virus, Cullicoides, Equines, Orbivirus, Protein, Serotype

1. Introduction

The equines have core role in farmer life through transportation and for enjoyment [33]. Those performances of equine are decreased by factor like non infection and infection disease. From infection factor, African horse sickness disease is the one that characterized by high morbidity and mortality rates [63]. Among the equidae, horses are the most susceptible to AHS with a mortality rate of 50-95%, followed by Mules with mortality around 50%. Zebras are thought to be the natural reservoir hosts and play important role, donkeys can also develop viraemia sufficient to infect *cullicoides* while, dogs do not play a significant role in the maintenance or spread of AHS [64].

In tropical and sub-tropical areas of sub-Saharan Central and East Africa AHSV is endemic disease. This disease possesses different forms: cardiac form, pulmonary form, mixed form, and horse sickness fever. The cardiac, pulmonary, and mixed forms are regularly fatal [57] and occur during late summer/autumn season and cyclical incidence. Listed by World Organization for Animal Health (OIE) under endemic disease in sub-Saharan Africa [64]. This disease is noncontiguous, vector born orbivirus that is transmitted primarily by culicoidmidget (*Cullicoides imicola and Cullicoides bolitinos*) during blood meal, which they require for their reproduction [90].

African horse sickness virus is a non-enveloped virus with a segmented, dsRNA genome. The 10 segments of the genome are enclosed within a two layer icosahedral (having 20 identical equilateral triangular faces) capsid. The 10 segments code for 7 structural proteins (VP1–VP7) and 5 non-structural proteins (NS1–NS4 & NS3a) [72, 98]. Two structural proteins, VP3 and VP7, which consist of the inner layer of the capsid,

are conserved across all serotypes. VP2 and VP5 make up the outer layer; VP2 varies antigenically among the serotypes [73]. AHSV is subdivided into nine serotypes (AHSV-1 to AHSV-9) [63].

There is partial antigenic relationship between each serotypes, with cross-reactivity of homologous antiserum to other subtypes in VN assay (cross-neutralization between types 1 and 2, 3 and 7, 5 and 8, and 6 and 9) [84]. Epidemiological survey in Ethiopia indicate that majority of outbreak occurred previously is serotype (9, 8, 6, 4, 2) which is characterized at laboratory level by viral isolation technique [14]. To control this circulating serotype attenuated monovalent freeze dried AHS vaccine produced and given ever year. Even though vaccination is given yearly many outbreaks occur in the country, this lead serious consequences for international trade of animals for the country [65]. Such highly mortality and morbidity virus require strong strategy for control and prevention however, well understanding of biology of Agent and vector is recommendable for developing the strategy.

Therefore the objective of this review is:
1) To review the biology of African horse sickness virus
2) To review biology of *cullicoides* responsible for transmission of virus.

2. Literature Review

2.1. History

African horse sickness is ancient equine disease which most like originate from Africa where circulate in natural zebra population and described for first time in Arabic document entitled 'Kitâb el-Akouâl el Kafiah wa elFoucoûl ef Charfiah' dating back to the early 1300s [99]. Epizootics with horses suffering from an AHS-like disease apparently occurred in 1327 in the Yemen [58]. The degree and severity of the disease are illustrated the truth that a purchaser and a salesman are discussing the price of a horse when, suddenly, the horse falls on the floor and dies. But recognition of the virus is after 60 years it affecting African horse in 1657 and the first outbreak occur in 1719 which 1700 animals are dead over [35]. Then large outbreak was followed which kill out large population of equine in different part of the world (Table 1).

Table 1. AHS outbreak in different country at different time.

Country	Year of occurrence	Reference
South Africa	1854-1855	[15, 4]
Afghanistan, Cyprus, India, Iraq, Jordan, Lebanon, Pakistan, Saudi Arabia, and Syria	1959-1961	[6, 36, 61]
Spain, and Portugal	1988-1991	[57]
Southern Morocco	1965	[57]
Ethiopia	1977-1983	[44]

2.2. African Horse Sickness Virus Biology

Characterization of AHSV is according to the International Committee on Taxonomy of Viruses, Family: *Reoviridae*, Subfamily: *Sedoreovirinae* and Genus: *Orbivirus* which shares many morphological and structural characteristics with other members of this genus, Bluetongue virus (BTV) and Equine Encephalosis virus (EEV) [37]. The contrast between amino acid sequences of AHSV serotype 4 (AHSV-4) and BTV-10 suggested that these, two viruses contribute to a common ancestor and have diverged under the selective pressure of the immune systems of their respective and dissimilar hosts [19]. It is double stranded, non-enveloped, 55-70nm in diameter composed of 10 segments with the core RNA particles having characteristic ring shaped and structured as a two layered icosahedral capsid composed of 32 capsomeres. The all segments are designated by their size and increasing electrophoretic mobility in 1% agarose gels and by molecular weight as L1-L3 (large segments), M4-M6 (medium segments), and S7-S10 (small segments). These segments code for the seven structural proteins (VP1 to 7) and five non-structural proteins (NS1, NS2, NS3, NS3a and NS4). The non-structural proteins are coded by the segments M5, S8, S9 and S10 [59].

2.2.1. Major External Protein VP2 and VP5

The external capsid poised of VP2 and VP5 is mostly dependable for serotype antigenic uniqueness; neutralizing epitopes are regularly located in VP2. VP2 and VP5 are involved in cell attachment and membrane penetration [91]. VP2, coded by segment 2, is the serotype specific protein and the major target for neutralizing antibodies and determines the virus serotype [40]. Mainly based on its genetic variability, nine serotypes of AHSV have been recognized. AHSV neutralizing epitopes, in addition to the virus receptor-binding site, are positioned in VP2, as confirmed by the binding of neutralizing monoclonal antibodies [19]. But VP2 of AHSV has no activity in viral replication as in vitro which is approved by deletion of VP2 during replication [83].

The VP2 protein of orbiviruses also has haemagglutinating activity and involved in virus entry and exit of the cell via its interaction with NS3 [56]. The second protein VP5 is coded by segment 6 dsRNA which is postulated to be a type 1 fusion protein based on its high structural similarity to BTV-1 VP5 [51]. The studies on BTV VP5 have indicated that it has pH-dependent fusion activity [91] and activated in the low pH environment of the late endosome allowing the virus core to enter the cytosol [69].

2.2.2. Core Proteins: VP3 and VP7

The internal of the virion is composed two of major proteins, VP3 and VP7, highly conserved near with the nine AHSV [56]. The core particle is a highly stable structure that does not disassociate in the cytoplasm of the infected cell. They prevent

the dsRNA genome from exposure to pattern recognition (PAMPs) by the host cell. They encoded by segment 3 and 7 dsRNAs respectively. Additionally they are the main components of the inner core and contain group-specific antigenic determinants [19].

The VP7 proteins of AHSV are similar to serotypes 4 and 9 with 99.7% identity at the amino-acid level. VP7 proteins are also highly conserved between AHSV, BTV and epizootic haemorrhagic disease of deer virus and, consequently, these proteins are used for the serological diagnosis of AHSV sero group-infected animals [20]. VP7 trimer structure does not change the distribution of the two domains in the particle; the upper interacts with VP2 and the lower with VP3. The β sheet structure of the upper domain also contains an Arg-Gly-Asp (RGD) tripeptide motif. It is postulated that the VP7 RGD motif assists entry of orbiviruses into insect host cells [78]. The VP3 is critical for the creation of the core and viral particles as VP7 does not have the ability to form the core without VP3 and cannot associate with dsRNA or the transcription complex protein [38].
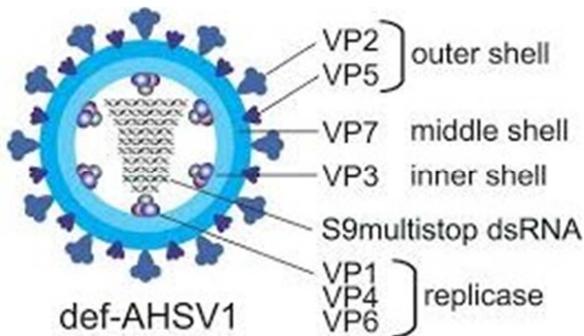
Source: [48]

Figure 1. *Structure of African horse sickness virus.*

The outer layer of AHSV is composed of the VP2 and VP5 proteins. The core of the particle packages the viral dsRNA genomic segments and the minor structural proteins VP1, VP4 and VP6. The layer of the core is formed by the VP3 and VP7 proteins. VP3 forms the scaffold of the layer on which VP7 is arranged and interacts with the outer layer.

2.2.3. Transcriptional Complex Proteins: VP1, VP4 and VP6

Three of the minor core proteins, VP1, VP4 and VP6 form the replicase complexes that locate in the core of the AHSV viral particle [90]. VP1, VP4 and VP6 are AHSV proteins, very little functional work has been undertaken and thus assignment of function is based on BTV [48]. AHSV, the minor structural proteins VP1 (RNA-dependent RNA polymerase, 150kD), VP4 (capping enzyme, 78kD) and VP6 (helicase and ATPase, 36kD) form 12 flower-shaped transcription complexes (TC) attached to the VP3 layer directly under each of the fivefold vertices [51]. AHSV VP1 is a putative RNA dependant RNA polymerase that is encoded by segment 1 of AHSV several motifs required for RNA-dependent RNA polymerase activity have been identified in AHSV VP1 [19]. AHSV VP6 is able to bind specifically dsRNA [21].

2.2.4. Non-structural Proteins

There are 5 non-structural proteins (NS1, NS2, NS3 NS3a and NS4) which play great roles in the viral life cycle [72]. NS1 is mostly expressed in the cytoplasm of infected cells, while a small amount is found in viral inclusion bodies. Removing of NS1 decrease virus induced CPE and virus budding via the plasma membrane which indicates it is viral virulence factor [67]. The tubular structures commonly observed in the cytoplasm of infected cells are composed of NS1, which has been shown to play a role in the preferential up-regulation of viral Protein synthesis [10]. de la Poza *et al* report in 2013 AHSV NS1 is a key protein in vaccine composition for protection of IFNAR (-/-) mice [19]. They involved in virus replication due to it translated early during virus replication [38].

Non structural protein 2 is main part of viral inclusion bodies (VIBs) which has ssRNA binding ability and able to take viral ssRNA to the VIBs. The capability to attach ssRNA was found to be through the N-terminus of the protein in VIBs, this allows NS2 to form dimers [42]. The expression of recombinant NS3 proteins or in infected cells is lower than the other non-structural proteins NS1 and NS2. Viral particle release is mediated by NS3/NS3a, the only AHSV glycosylated membrane protein NS3 localises to sites of virus release, it is proposed to have a role in membrane destabilization to allow the virus to exit via budding [14]. NS3 has a role in virus release, reverse genetic-engineered viruses that did not express NS3 could replicate efficiently in BSR cells but not in the insect vector cell line [81].

The non-structural protein 4 (NS4) was initially identified by bioinformatics analysis in several different orbiviruses (BTV, GIV, etc.) and its expression has been confirmed in AHSV infected samples [81]. The NS4 is encoded in the +1 reading frame of segment 9, which also encodes VP6 in the +3 reading frame [72; 98]. Localized in the nucleoli of infected cells, the NS4 is assumed to 10 participate in virus-host interaction by acting against antiviral responses of the host [72; 98]. NS4 has a clear role in antagonizing the IFN response and a diagnostic indicator of the clinical outcome of disease [38].

2.2.5. African Horse Sickness Virus Genome

African horse sickness virus genome composed of 10 dsRNA segments which measure 19.5kb [38]. As discussed before there is segment variation base on contents of their base pair to small (765 bp) and large (3965 bp). AHSV segment contains 5' and 3' untranslated regions at both sides of the open reading frame. At 5' end, it ranges from 12 bp (segment 2) to 25 bp (segment 5), while at the 3' end range from 29 bp (segment 6) to 100 bp (segment 7). The six terminal nucleotides are conserved and present on the 5' and 3' end of each segment of the AHSV genome [38]. The consensus sequence of this hexanucleotide sequence is found on all genome segments and is unique for each member e.g., AHSV (5′-GUU(A/U)A(A/U)……AC(A/U)UAC-3) and BTV (5′-GUUAAA…..(A/G)CUUAC-3′), which is very important for viral packaging [11] and specific translation of viral proteins in the infected host [14]. The segments are mostly

monocistronic, although second functional, in-frame initiation codons or additional protein-coding ORFs have been identified in the AHSV genome as well as in BTV. Additional option ORF was identified in segment 10 of AHSV and BTV [77].

2.2.6. Mechanism of Viral Entry to Host Cell

The entry of the virus to the cell is initiated by VP2 which is the host cellular membrane fusion proteins due to it have putative sialic acid (SA), which is binding domain surface receptor [96]. For attachment antigenic tip domains bind to certain surface sulphate as one possible candidate [54], but due to the high sequence variability in the AHSV outer cell receptors which have yet to be identified. Different receptors and entry mechanisms used by different serotypes to enter different cell types [76]. However, bond was stabilized by inter linkage between surface glycoprotein (glycophorin A) and SA-binding domain which is a heavily glycosylated sialoglycoprotein abundantly exist on the surface of equine erythrocytes [20].

The VP2 is sensitivity to serum proteases and it is a central domain at the top of the AHSV VP2 triskelion hub which is target site for such a horse serum protease [51]. This domain is situated directly above the BTV putative SA-binding site and cleavage of AHSV VP2 in this region would thus increase accessibility to this potential binding site [20]. The process is initiated by proteolytic cleavage of VP2 either in infected insect saliva or in the host serum, after which the virion attaches to the host cell membrane and the viral cell entry was able by clathrin-mediated endocytosis [27]. However, more recently evidence has been presented which describes a macropinocytosis-like entry route dependant on actin and dynamin [76]. In both case, the low pH (6.0–6.5) within the early endosome disturbs the interactions between VP2 and VP7, facilitating detachment of the VP2 trimers and disrupting the zinc finger motif situated at the interface between the VP2 hub and body domains, which is believed to play a role in controlling conformational changes [97].

The removal of VP2 causes a re-folding of VP5, which in turn leads to the outward protrusion of barb-like structures from the particle surface with the VP5 protein trimers remaining tethered to the particle by their anchoring domains [97]. These barb-like fusion peptides insert themselves into the endosomal membrane, causing release of the viral core particle into the cytoplasm. But as Van *et al*, in 2017 experimentally improved VP2 is not essential for viral replication in contrast viral releasment from insect cell was completely abolished without VP2 expression [83]. But newly reverse genetic experiments including truncations of AHSV VP2 demonstrated that it is not required for entry into BSR, Vero or Kc cells [83] show that there are multiple entry strategies used by AHSV to infect cells. The removal of the outer capsid proteins and the release of viral cores into the cellular environment containing the necessary host substrates and transcription factors cause the core to become transcriptionally active [97].

2.2.7. Viral Replication

Entrance of the of the virion was followed by simultaneously and repeatedly transcribed by VP2 to produce ssRNAs [97], which are modified by the capping and methylation activity of enzyme VP4 within the core before being released into the cytoplasm [20]. When cells are infected simultaneously by at least two viruses, the segmentation of orbivirus genomes allows for the exchange of genome segments between orbiviruses of the same serogroup but either belong to different serotypes or originate from different geographical regions. Genome re-assortment is responsible for the rapid genetic evolution of orbiviruses. The frequency of genome re-assortment is variable, some genes being more frequently exchanged than others [97]. AHSV re-assortments have been obtained *in vitro* by co-infection of cells by serotypes 2, 3 and 4 [19]. According Von Teichman *et al in 2008* suggest that *in vivo* re-assortment occurs more frequently in the insect than in the vertebrate host and that re-assortment in vertebrate hosts is less common for AHSV than for BTV [88].

Viral inclusion body's act as the sites of viral assembly and protein NS2 directly and specificaly sequestering the 10 ssRNAs, together with the three enzymatic proteins VP1, VP4 and VP6. During mRNA transcription, 10 single-stranded RNA (ssRNA) molecules are synthesized inside the viral core, corresponding to each of the genome segments. These ssRNA transcripts encode for the production of viral proteins translated by host cell machinery [68]. Inner core protein VP3, for encapsidation and the formation of new sub-core particles [68]. The deposition of VP7 trimers serves to stabilize the particles, and phosphorylation of NS2 then regulates their exit from the VIBs in order to acquire the two outer capsid proteins VP5 and VP2 for the formation of mature progeny virions [62].

Then virions release from infected cells by cell lysis or budding mechanism for viral egress earlier on in the infection cycle. The latter is mediated by utilising the host exocytosis pathway and the membrane destabilizing action of non-structural glycoprotein NS3, which functions as a viroporin and also interacts with calpactin to function as a bridging molecule between the new virions and the host cell export machinery [62]. In vector, no viral infection and CPE, while viral release is mediated exclusively through vesicle formation at the cytoplasmic membrane [68]. Cytopathic effect of AHSV is mainly effective by NS3, which is caused more CPE by AHSV than in BTV [86].
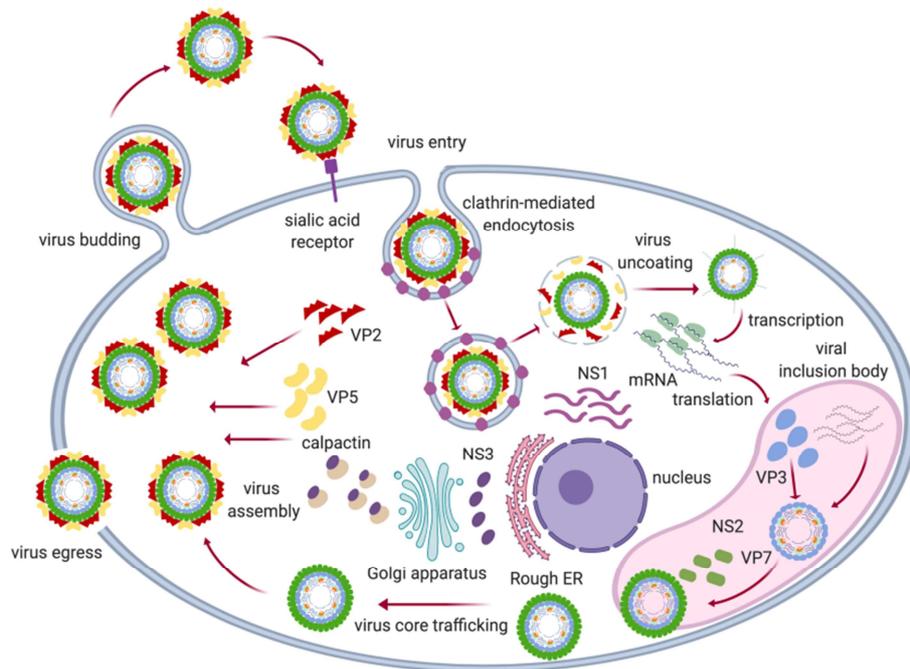
Source: [20].

Figure 2. Diagrammatic representation of the replication cycle of BTV/AHSV.

Additionally the primary route of BTV and AHSV host infection is thought to be initiated by the outer capsid proteins, there is facts to propose that BTV core-like particles (CLPs), mean particles that have lost the external capsid proteins, are also able to infect both insect and, to a lesser extent, mammalian cells [20]. The upper domains of both BTV and AHSV is VP7 trimers posses characteristic of Arg-Gly-Asp (RGD) motifs, albeit in slightly different position [62].

RGD domains in biological systems are linked with integrin-ligand recognition and fusion of molecules to cell membranes [20]. In fact there is holes on the surface of the outer capsid layer of both BTV and AHSV particles makes it tempting to speculate that RGD sites on VP7 may play a role in the ability of viral CLPs to infect cells. Transcription and translation of viral proteins occurs, utilizing the host cell machinery and the VIBs act as sites of assembly for the progeny virions as indicate on figure 2.

2.2.8. Host Phatogene Interaction

African horse sickness virus types 1-8 are considered to be highly pathogenic for horses and disease results in high mortality (90-95%). But, type 9 AHS virus is less pathogenic and disease may result in lower mortality (70%) [66]. Colonization preferable lymph node and initially replicates of the virus occur after exposed to bite infected vector which followed by disseminating of the virus throughout the entire body via the circulatory system [32]. Infection of lung and lymphoid organ are considered as primary viremia while, further replication in target organ (endothelial Cells and Mononuclear cells of the lung, spleen, and Lymphoid tissue) is which in turn give rise to secondary viremia. The virus damage endothelial cell which is followed by activation of infected microphage with subsequent cytokine production

(IL-1, TNFa) that lead to increase permeability of the cell, effusions into body cavities and tissues, and widespread haemorrhages which named as edema [80].

The association of intravascular coagulation with infection virulent AHSV and its absence in horses infected with a virulent suggest that virulence is associated with a strain's ability to cause endothelial injury. Virulence of AHSV associated with thrombocytopenia, prolongation of coagulation times and the presence of fibrin degradation products as Clinic pathological studies. As John reported in 2019 AHSV-4 infection is correlated with oedema and pneumonia in the lungs, inflammation in the liver and meningitis plus perivascular cuffing in the cerebrum. Other data shows that different strains of AHSV differ in terms of their pathogenicity and tropism [39].

Host interact the damage due to pathogen induce different immune mechanism. There are different types of innate immunity including intrinsic innate immunity where existing cellular factors directly restricts virus replication [50]. Another arm of the innate immune response is the indirect restriction of viral infection by inducing interferons and up-regulating the expression of other antiviral molecules. Apoptosis, pyroptosis and necroptosis are also mechanisms used by the cell after sensing virus infection in order to restrict replication and virus spread [38]. But African horse sickness virus cause apoptosis during entry of the virus into the cytosol [87].

2.2.9. Physio-chemical Characteristics of AHSV

This characteristic is typical for AHSV as other genus of Orbivirus. The virus is acid sensitive, stable at alkaline resistant to lipid solvents and relatively heat resistant. It's infectivity is remarkably stable at 4°C, particularly in the

presence of stabilizers such as serum and OCG [100].

Table 2. Physical and Chemical Resistance Characteristics of AHSV.

Physical and Chemical Resistance Characteristics of AHSV	
Action	**Resistance**
Temperature	Relatively heat stable, especially in presence of protein. AHSV in citrated plasma still infective after heating at 55–75°C (131–167°F) for 10 minutes. Minimal loss of titer when lyophilized or frozen at -70°C (158°F) with Parker Davis Medium. Infectivity is remarkably stable at 4°C (39°F), particularly in the presence of stabilizers such as serum and OCG: blood in OCG can remain infective >20 years. Can be stored >6 months at 4°C in saline with 10% serum. Fairly labile between -20°C (-4°F) and -30°C (-22°F).
pH	Survives pH 6.0 – 12.0. Readily inactivated below 6.0. Optimal pH (for survival) is 7.0 to 8.5.
Chemicals/Disinfectants	Inactivated by formalin (0.1%) for 48 hours, β-propiolactone (0.4%) and binary ethyleneimine. Resistant to lipid solvents Inactivated by acetic acid (2%), Potassium peroxymonsulfate/ Sodium chloride -Virkon® S (1%), and sodium hypochlorite (3%).
Survival	Putrefaction does not destroy the virus: putrid blood may remain infective for >2years, but virus is rapidly destroyed in meat by rigor mortis (lowering pH). Vaccine strains survive well in lyophilized state at 4°C (39°F).

Source: OIE. 2013. "African Horse Sickness." Technical Disease Card. www.oie.int (OIE, 2013) [100].

2.2.10. Clinical Sign and Incubation Period of the Virus

The incubation period of the virus is from 3 day to 2 weeks based on virus strain, viral load, viral virulence and sensitivity of equid host [47]. The infective period, or when animals are a source of infection for *Culicoides* midges, is 40 days for domestic horses [100]. AHSV have four clinical forms of the disease: cardiac form, pulmonary form, mixed form, and horse sickness fever [23]. An intermittent temperature of 40-41°C is characteristics of all forms [71].

Table 1. Incubation period of the four disease form of AHS.

Disease form	Incubationperiod
Per acute (pulmonary)	3-5 days
Sub-acute (edematous or cardiac)	7-14days
Acute (mixed) form	5-7 days
Horse sickness fever	5-14 days

Source [63].

There are not known viral determinants or host-associated factors that determine the severity of the clinical manifestations. As reported in 2015 mixed form is the most frequently occurring clinical outcome [97]. However, a retrospective study on data from the 2009–2010 outbreaks in Ethiopia reported all forms of the disease with cardiac the most prevalent form (54.8%), followed by horse sickness fever, pulmonary (7.1%) and 2.4% with the mixed form [1].

2.3. Vector Biology

2.3.1. Morphology and Ecology

Culicoides is a genus of biting midges in the order Diptera, family Ceratopogonidae [9]. In worldwide there are approximately 1400 recognized species of *Culicoides*, of which 96% are obligate bloodsuckers [7]. According to Tesfaye and Abdisa reported in 2019 there are six Culicoid species in the western parts of Ethiopia identified as: *C. imicola, C. milnei C. neavei, C. zuluensis, C. fulvithorax and C. isioloensis* [79].

Culicoides spp is the smallest insect which measure 1-3mm and their mouthparts form a proboscis well adapted for wounding skin and sucking blood. Wings are well developed, and biting midges are commonly identified to complex or species level based on the wing maculation [52]. Due to this method is base on professional experience, there is an alternate method like (PCR) with subsequent sequencing and phylogenetic comparison of DNA marker regions [101] and DNA microarray was developed to identify *Culicoides* species of the obsoletus group [18]. The life cycle of Culicoides includes three immature stages, egg, larva and pupa and a mature or imago stage, each characterized by species-specific environmental demands [22].

They are found in all continents (except Antarctica) from sea level up to around 4, 200 m [25]. Ecologically they favor enriched moist soil like clay soil to complete their vector's life cycle which stays from seven to ten day. The *Culicoides* are largely dependent on the level of rainfall, and heavy rains can significantly increase the loads of adult *Culicoides*, in some cases up to 200-fold [52]. The environment is a key determinant limiting distribution, abundance and seasonal occurrence [12]. Subtropical areas, after the rainy season and temperate regions, from the beginning of spring until autumn are greatest in hot and humid climates which directly related with periods of vector activity [43].

During climatic variables outbreaks occurrence of AHS are interactive and affect by two phenomena either directly, through temperature, or indirectly through their influence on developmental habitat formation. As temperature increases, infection rates to increase, virogenesis is more rapid and transmission can occur earlier [31].

2.3.2. Veterinary and Public Health Importance

This *Culicoides* biting midges can cause painful lesions and the saliva even induces acute allergic reactions such as the "common summer eczema (insect hypersensitivity)" in horses [82]. Due to this anthropoid is obligate blood feeder they feed blood from the animal 3-5 day based on availability of animal [95].

Above 50 viruses are isolated from Culicoides species [86; 13]. There are many viruses belong to the families *Reoviridae* those can transmitted by *culicoides:* (e.g., African horse sickness virus (AHSV), bluetongue virus (BTV), or epizootic hemorrhagic disease virus, bovine ephemeral fever virus (BEFV), Akabane virus (AKAV), Schmallenberg virus (SBV), or Oropouche virus (OROV) but, Rift Valley fever virus

(RVFV), is essentially transmitted by mosquitoes [13]. Out of all, African horse sickness (AHS) and bluetongue (BT) are the two major important animal diseases transmitted by Culicoides and they have international significance. *Cullicoides spp C. imicola* and *C. bolitinos* are known to transmit AHSV while *C. imicola* which is particular species has been recorded in Africa, Asia and Europe [55]. The female midge of the genus *culicoides* are principal vector for AHSV and other viral disease during blood sucking to provide a protein source for egg production [26].

Extrinsic incubation period (EIP), pried between ingestion and the insect being able to transmit the virus based on temperature adapted by vector it may stay approximately eight days [90]. The salivary of the *culicoides* inhibit phagocytic activity of host cell like macrophage, which is termed as saliva activated transmission this lead to animal caught with virus by single bite of insect [5]. Midges are not merely mechanical vectors, they slightly allow the replication of the virus in themselves before transmission but, there is no report of transovarial transmission so far [45].

The minimum dose needed to experimentally infect *C. imicola* is 104–104.5 TCID50/0.02 ml blood. A high viraemia in the host is therefore necessary for vector contamination [97]. In other way direct transmission is described in dog contaminated to oral root after ingestion of infected meat [45], but recent report has shown the direct transmission of AHSV to dogs without Ingesting contaminated meat [85]. Even though human being are not host for African horse sickness virus no more adverse effect, but neutropic vaccine strain, adapted to mice can cause cephalitis and retinitis in human being [75].

2.4. Epidemiology

African horse sickness is endemic in sub-Saharan Africa. Serotype 9 is widespread in the endemic region, while serotypes 1 to 8 occur in limited areas. The greatest virus diversity has been reported in southern and eastern Africa. Some serotypes have recently caused outbreaks in countries where they were not previously found. In particular, serotypes such as 2, 4, 6, 7 and 8 have been detected in regions where only serotype 9 was once common. African horse sickness outbreaks have occurred outside Africa in the Middle East, the Mediterranean region of Europe and parts of Asia (e.g., the Indian subcontinent). Although all outbreaks, to date, were eventually eradicated, AHSV was able to persist for years in some areas [75].

Survey conducted between 1977 and 1981 in Ethiopia indicated that the majority of African horse sickness disease caused by serotype 9 [44]. Serotype 7 and 2 are identified by Leforban in (1983) in Oromiya region and by Gebreegziabhe in SNNPR and west Oromiya at 2008 respectively [29]. As Aschalew reported in 2005 two serotypes of AHSV in Ethiopia (serotypes 6 and 9) from isolated, serotype 9 perversely isolated while serotype 6 was first reported by Aschalew. Epidemiological survey examined by ELISA out pout result in 2006 in different ecological area of the country indicate prevalence rate of AHS in Ethiopia 10.4%, 29.7% and

10.3% in horses, donkeys and mules respectively [41]. According Aklilu and colleague report that from outbreak of 2009-2010, out of 42 sample five serotype of AHSV like AHSV-2, 4, 6, 8 and 9 identified [1]. The difference in seroprevalence of AHSV at different sites of the study area was statistically significant. The study by Kassa (2006) indicated that seropositivity increases as one goes from high land to lowland [41]. AHS seroposetivity was also assessed in relation to agro ecology and the prevalence of midland was 52.72% while it was 14.28% in the highland due to formation of favor climatic condition in low land for running life cycle of *Culicoides.* [30].
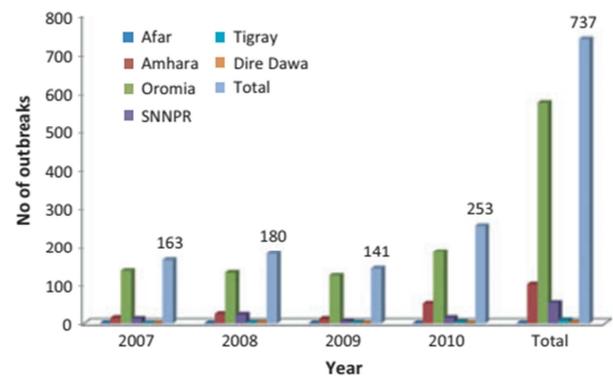
Source [1].

Figure 3. Outbreak of AHS in different region of Ethiopia (2007-2010).

2.5. Diagnosis

The clinical signs and lesions in association with epidemiological information may be sufficient for clinical diagnoses but, conformation is through isolation and identification of the virus. For characterization sample like from whole blood collected in anticoagulant during the febrile stage of infection and from tissues like spleen, lung, lymph nodes and salivary glands [45]. For isolation and characterization it's possible to inoculate embryeonate hens eggs, several mammalian derived cell lines (BHK Vero, monkey kidney (MS),) for isolation and appreciation of CPE within seven days propagated in to insect cell cultures such as mosquito (Aedes albopictus) C6/36 cells and Culicoides (KC) cells but, they don't show CPE [17].

Additionally intra cerebral inoculation of newborn mice can be done. Isolate virus from brain sample of new mice is serotype by virus neutralization to select strain for vaccine purpose. Sandwich ELISA is used for rapid identification [65]. AHSV is directly identified from whole blood and tissue sample by using probe and primer at molecular level by RT-PCR technique [89].

2.5.1. Differential Diagnosis

The differential diagnosisof the AHSV are equine viral arteritis, equine infectious anemia, Hendra virus infection, purpura haemorrhagica and equine piroplasmosis. In Africa, equine encephalitis virus, another Orbivirus transmitted by *cullicoides*, causes a syndrome resembling horse sickness fever [80]. Those causes of sudden death like toxins, anthrax

as well as disease that result in severe respiratory distress, should also be considered [66].

2.5.2. Post Mortem Diagnosis

Post Mortem diagnosis based on gross lesion findings in acute cases includes severe hydrothorax and pulmonary edema and moderate ascites. The congestion of liver, edema formation on the alimentary canal in prorolonged time and accumulation of fluid in chest cavity which can measure up to 3-5 litter are evenly appreciated [45]. Some lymph node exist in thoracic cavity and abdominal part are enlarged. The lungs are filled with frothy fluid that make distended and typically mottled red, even accumulate in trachea, bronchi and bronchioles. These frothy exudates may expelled through the nostrils [49]. In cardiac form horse sickness there is marked hydro pericardium, endocardial hemorrhage, myocardial degeneration and anasarca, yellow gelatinous infiltrate can be seen in the subcutaneous and intramuscular fascia of the head, neck and shoulders, and occasionally the brisket, ventral abdomen and rump especially of the supra orbital fossa [49].

2.6. Control and Prevention

The virus has no treatment at all rather than treating secondary complication during animal on recovery period and non-steroidal anti-inflammatory drugs are used for alleviating pain and reducing fever, antimicrobials to fight secondary bacterial infection or corticosteroids to help stabilize cell membranes and preserve vascular membrane integrity, have been employed [45]. To control and prevent African horse sickness virus three things; Vaccination against the agents, vector control and animal quarantine the majors [74].

2.6.1. Vaccination Against the Agent

Vaccination is the most model for control of the virus [58]. Live attenuated vaccines are routinely used to control in endemic regions. Monovalent or polyvalent vaccines may be in use, based on the viruses circulating in the area [88]. Reactivity to some vaccine strains is reported to be better than to others, and protection may be incomplete in some cases: clinical cases and mild or subclinical infections have been reported in some horses that had received as many as 5 vaccine doses in Africa. The currently available vaccines are teratogenic in pregnant mares, and vaccine strains may be transmitted by *Culicoides* vectors [97].

2.6.2. Controlling of Vector

There are four major vector controlling technique like mechanical, chemical, biological, and genetic. Culicoid habitat modification is mechanical technique to reduce or eradicate larve of midgate which straightforward within relatively dry climates [70]. More recent studies have manipulated these habitats via removal of lagoons suitable for *C. sonorensis* development and examined the impact on adult populations [53]. However, this study demonstrated that the process of habitat elimination had a negligible impact on adult populations and subsequent on BTV & AHSV transmission [53]. Di-ethyl toluamide (DEET) commercially available repellent that has a significant effect against Culicoides up to four hours [46]. Since C. imicola suck blood during the first four hours of the night, if the repellent applied nightly to susceptible animals, DEET may have a significant even though some effect by reducing the biting rate of this species [58].

With biological control method, using larvicidal effects of insect pathogenic fungi which can kill colony-reared [2] and reduce adult survival in laboratory [2].

2.6.3. Restricting of Animal Movement

The Terrestrial Animal Health Code of the OIE makes the following recommendations about the conditions that must be met by infected exporting the country. 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. 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 or 40 days, with two serological tests no less than 21 days apart to show lack of seroconversion. At 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 result have shown to be circulating in the area [100].

3. Conclusion and Recommendation

African horse sickness is the disease characterized by high mortality and morbidity rate on equine specious which is caused by African horse sickness virus. The virus is organized by different proteins those are use for virus entrance, replication, transcription and translation. All mechanism are also occur in obligate blood feeding anthropoid named as *culicoides* (transmitter of virus among equines), however the mechanism was not clearly described. The severity of the disease was determined by those mechanisms. Resistivity and sensitivity of AHSV for different chemicals, pH value and temperature is due to existence of different protein and different stabilizer. In general, transmission of African horse sickness virus based on presence of *culicoides* between equines. But there is some assumption that virus transmit from equine to dogs by *culicoides*. More over in endemic country like Ethiopia equine vaccinated yearly even though out break occur. Whit my review I access research gab on area of *culicoides* surveillance. Which indicate control strategy based on only virus is not successfully over came. The reason behind that understanding a biology and surveillance of the vector is best supporter for virus controlling method.

4. Recommendation

1) Transmission of African horse sickness virus by *culicoides* among only equine, but assumption state transmitted to dog by *culicoides* is the one that need researcher delegation.
2) Vaccinating equine is not only solution to control African horse sickness virus in endemic country like

Ethiopia rather survey and control *culicoides*.

3) Mode of viral replication in vector is the other point need researcher delegation.

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