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Detection and serotyping of African Horse Sickness virus circulating in Kenyan horses

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Abstract

African horse sickness (AHS) is an infectious and non-contagious disease of equidae transmitted mainly by Culicoides species. The disease is caused by AHS virus (AHSV), a linear dsRNA virus, within the genus Orbivirus of the Reoviridae family, having 9 different serotypes. Kenya has experience death of horses due to AHS, despite vaccination. The vaccine used has two parts, administered 2-3 weeks apart. Part 1 is trivalent, while part 2 is tetravalent, however, it lacks serotypes 5 and 9. The objective of this study was to determine AHSV serotypes circulating in Kenya to inform the best vaccine and control strategies for AHS. Samples were collected from 37 horses (24 dead, 13 live) in different regions in Kenya including Nanyuki (n=14), Nairobi (n=6), Nakuru/Naivasha (n=14) and Kisumu (n=3). Majority of horses showed different clinical signs characteristic of the acute pulmonary form, however, some showed the cardiac form. Whole blood samples were taken from the live animals, while heparinized blood, spleen, lung and lymph node samples were taken from dead animals (preserved in 10% buffered glycerin) and shipped in dry ice to the Central Veterinary Research Laboratory (CVRL) in Dubai for serotyping. All serotypes were detected from our samples except AHSV-6. Serotypes 2 and 4 were detected in all the regions with AHSV-4 having higher frequency (32.4%) than AHSV-2 (24.3%). All serotypes except AHSV-1 was detected in Nakuru/Naivasha region, with higher frequency of AHSV-7 (13.5%) followed by AHSV-4 (8.1%). AHSV-4 was the most frequent in Nanyuki while AHSV-2 was the most frequent in Nairobi. From these results we recommended that the horses in Kenya should be vaccinated with a vaccine containing all the 9 serotypes since all serotypes are circulating in Kenya.

Keywords: African Horse Sickness; Horses; Serotypes; Kenya

Introduction

African horse sickness (AHS) is an infectious and non-contagious disease of *Equidae* transmitted mainly by *Culicoides species (biting midges)* especially *C. imicola* and *C. bolitinos*, although other arthropod vectors have been implicated in the transmission of this disease [1]. AHS disease is caused by nine distinct serotypes of African horse sickness virus (AHSV) [2], a linear double stranded RNA (dsRNA) virus belonging to the *Orbivirus* genus in family *Reoviridae* should be [3]. AHSV genome consists of 10 segments of linear dsRNA, encoding seven structural (VP1 – VP7) and five non-structural (NS1-3, NS3a and NS4) proteins [3,4]. The NS proteins are involved in virus replication, assembly and transport from infected cells. The VP2 and VP5 are involved in virus attachment and cell entry, hence these proteins contain the antigenic determinants which elicit serotype-specific neutralizing antibodies and therefore, they are the most variable proteins and are used to determine the virus serotypes [5-7].

Upon the bite by the infected vector, the AHSV at first replicates in nearby lymph node before spreading throughout the entire body via the circulatory system, causing primary viraemia leading to lungs and lymphoid tissues infection. The virus predilection site is vascular endothelial cells where it causes extensive damage leading to pervasive hemorrhages [8]. In horses, the viraemia usually lasts between 4–8 days, whereas in donkeys, it may last for up to 4 weeks. In zebra, the viremia may be extended to approximately 40 days post-infection [7,9]. AHSV infection manifest in four forms: cardiac form, pulmonary form, mixed form, and horse sickness fever [8,10]. The cardiac form is characterized by a fever, edema of the head, neck, chest, petechial hemorrhages in the eyes, ecchymotic hemorrhages on the tongue, and colic. This form of disease may cause mortality of upto 50%. The pulmonary form (the most serious with >95% mortality) is associated with a rapid onset of symptoms including fever, depression, severe respiratory distress, severe dyspnea, coughing, and sweating.

Mixed form is a combination of the cardiac and the pulmonary form and is the most common leading to more than 70% mortality within 3-6 days. Horse sickness fever form is the mildest (with no reported mortality) where the animal just develops a moderate fever and some edema of the supraorbital fossae [8].

Studies have shown that mules and donkeys are less susceptible to AHS than horses, however, zebra has been shown to be resistant to the disease [11]. Besides *Equidae*, AHS has also been reported in ruminants such as camels, goats, and buffalo [12]. The first AHS case was recorded in Yemen following an epidemic that occurred in 1352 [13]. Although AHS is believed to have originated in Africa, the 1st case was only recorded in 1569 by Father Monclaro [14]. The most severe outbreak caused by AHSV serotype 9 (AHSV-9) occurred in Asia between 1959–1961 causing over a quarter million equid deaths. This was followed by epizootic of AHSV-9 in 1965 which spread throughout Northern Africa and Spain [14]. After these outbreaks, AHS was confined to sub-Saharan Africa for about 20 years until 1987, when AHSV-4 was reported again in Spain [15]. Senegal reported AHS outbreak in 2007 which was found to be caused by AHSV-2 [16]. Recently, The AHS has been reported in Chad (2019) and Thailand (2020) [17,18]. AHSV is listed as a notifiable equine disease by the World Organization for Animal Health (OIE) [19]. Vaccination is the main control strategy for AHS in both endemic and epidemic scenarios [20,21]. Live attenuated vaccine is the most common, however, a wide range of other vaccine candidates have been tested, including some that may provide cross-serotype protection against AHSV, but none have been used in the field to date, due to a lack of commercial viability [22]. The currently used live attenuated vaccine (LAV) comes in two vials containing 3 (AHSV-1, -3 and -4) and 4 (AHSV-2, -6, -7 and -8) AHSV serotypes each, hence, it lacks AHSV-5 and AHSV-9 [23]. AHSV-5 was withdrawn in 1990 from the vaccine after documentation of residual virulence [24]. However, AHSV-9 has never been included due to its low incidence especially in southern Africa where vaccine is produced. Additionally, cross protection between serotypes 1 & 2, 3 & 7, 5 & 8 and 6 & 9 has been documented [20,23], therefore, protection against AHSV-9 is expected to be provided by AHSV-6. AHS outbreak caused by both AHSV-5 and -9 was reported in South Africa in 2006, leading to questions of competency of the LAV for sufficient protection against the two serotypes. Mellor and Hamblin reported that gene segment re-assortment between wildtype and vaccine strains may result in new genetic variants or reversion to virulence of attenuated vaccine strains [14]. For example, recently whole genome sequences comparison of AHSV isolates which caused 2004 and 2014 outbreaks in Western Cape, South Africa, with AHSV vaccine and reference strains showed convincing evidence of re-assortment between and reversion to virulence of viruses within the vaccine itself [25]. In Kenya, AHS is an endemic disease affecting many horses and donkeys [26]. Recently, in Kenya, horse owners have been losing horses to AHS, despite vaccinating with commercially available AHS vaccine, mostly with LAV from Onderstepoorst Biological Products. We hypothesize that there could be reversion to virulence of some vaccine strains or there could be gene segment reassortment between wildtype and vaccine strains as has been reported elsewhere. Therefore, the aim of this study was to determine AHS serotypes circulating in Kenyan horses during the study period. This information is imperative for developing effective control strategies for AHS in the country including safe efficacious vaccines.

In this study we collected from 37 horses (24 dead, 13 live), in different geographical areas mainly in Kenya including Kisumu (n=3), Nanyuki (n=14), Nairobi (n=6) and Naivasha/Nakuru (n=14). The horses in the study areas are mainly kept in paddock grazing system and at night they are kept in stables or in open fields near owner's homestead, except in Nairobi where they are kept and fed entirely in stables. Horse owners use insect repellants and acaricide for insects and tick control, respectively. The affected horses showed different clinical signs of AHS mainly characteristics of per-acute or acute pulmonary form including high fever, severe pulmonary oedema, dyspnea and a frothy nasal discharge. However, some horses showed the cardiac form of AHS characterized by fever, edema of head, petechiae on the ventral surface of the tongue, tachypnoea and tachycardia. Paired whole blood samples taken from 13 live horses for processing serum. While samples from 24 horses that succumb to disease including heparinized blood, spleen, lung and lymph node were taken during postmortem (tissues were preserved in 10% buffered glycerine). The samples were store at -20°C before shipped on dry ice to the Central Veterinary Research Laboratory (CVRL), Dubai United Arabs Emirates for serotyping using the Virus neutralization test and real time RT-PCR, after screening them for AHSV antibody using competitive enzyme-linked immunosorbent assay (c-ELISA). The results were presented as descriptive statistics using GraphPad Prism 5 (GraphPad Software, Inc., CA, USA).

Overall, all nine serotypes of AHSV were detected in the samples collected except serotype 6 (AHSV-6). Our results showed that AHSV-2 and AHSV-4 occurred most frequently in the Kenyan horses with AHSV-4 (32.4%) occurring in higher frequency than AHSV-2 (24.3%) (Figure 1a). AHSV-7 (13.5%) was third most frequent followed closely by AHSV-9 (10.8%) and AHSV-5 (8.1%), respectively. Serotypes 1, 3 and 8 were less frequent. The dominance of AHSV-4 and AHSV-2 in the study area, raises the question of the efficacy of the multivalent vaccine (LAV) currently being used in Kenya and possibility of reversion to virulence of these serotypes. In terms of regional distribution (Figure 1b), AHSV-2 and AHSV-4 was detected in samples from all the regions, with high frequencies of the two serotypes (18.9% and 10.8%, respectively) recorded in Nanyuki region. AHSV-1 was detected in the Kisumu and Nanyuki regions, while AHSV-3 and AHSV-8 was detected only in Nairobi and Nanyuki regions, respectively. AHSV-5 was detected in Nanyuki and Nakuru/Naivasha regions, while AHSV-9 was detected in Nairobi and Nanyuki with higher frequency recorded in Nanyuki (Figure 1b). AHSV-7 was exclusively detected in Nakuru/Naivasha region and in high frequency than any other serotype in that region.

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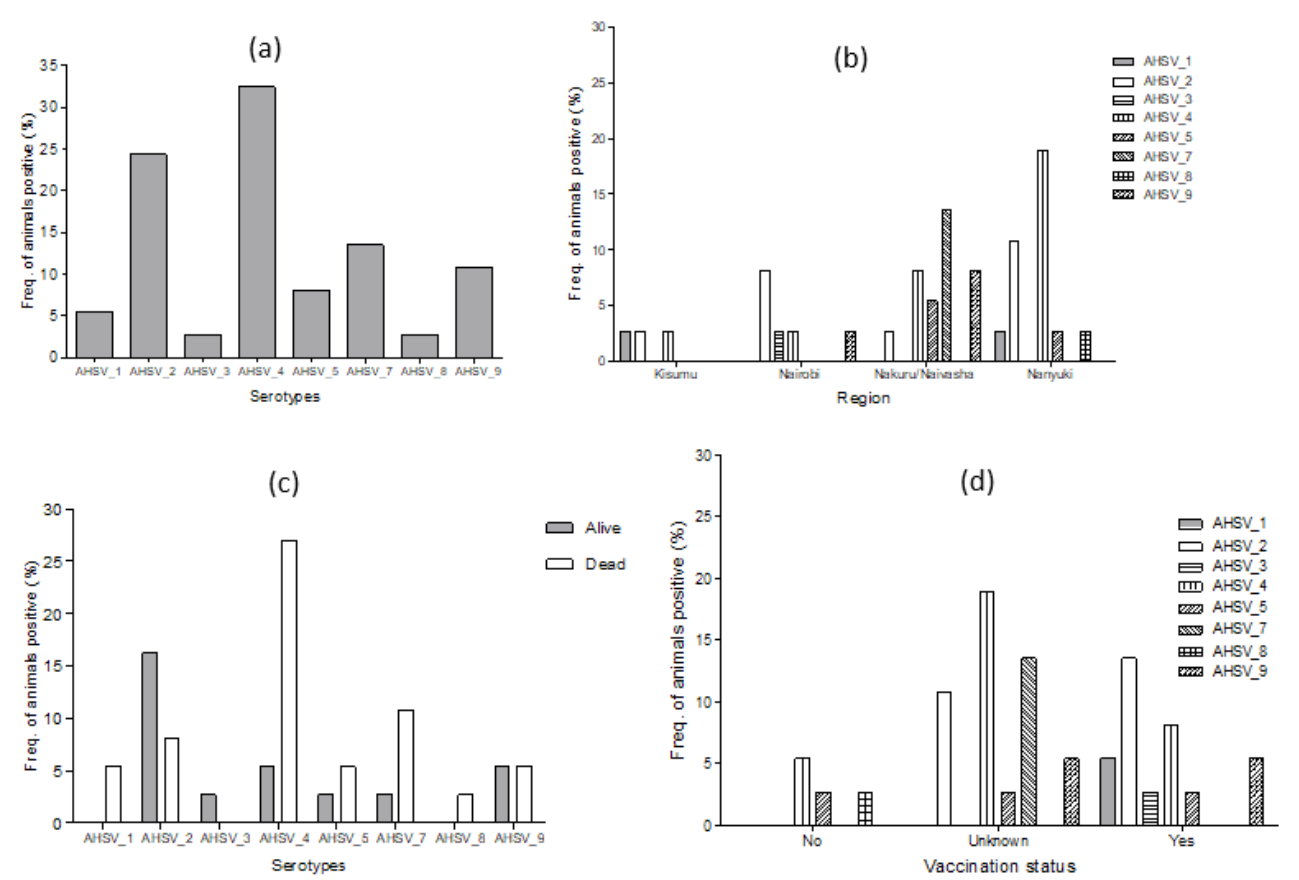

Figure 1: Summary of the frequency of AHSV serotypes detected in Kenya, overall (a), by regions of origin of samples (b); by dead or alive (c) and by vaccination status (d).

We also analyzed the serotype distribution between the samples from the sick horses that were a live and those that succumb to the disease (Figure 1c). In the live horses, serotypes 2, 3, 4, 5, 7 and 9 were detected with AHSV-2 occurring in higher frequency (16.2%) than the rest. On the other hand, all the serotypes, except AHSV-8, were detected from the samples of the dead horses, with higher frequency of AHSV-4 (27%) followed by AHSV-7 at 10.8% and AHSV-2 (8.1%). Although many horse owners did not avail to us the information about vaccination status of the affected horses, we examine the distribution of the serotypes based on known vaccination status of the horses. Our results showed that all serotypes were detected in vaccinated horses except AHSV-7 and 8, with AHSV-2 and AHSV-4 occurring in slightly higher frequency (Figure 1d). Serotype 8 was only detected in the unvaccinated dead horse in Nanyuki region. On the other hand, AHSV-3 was detected in one vaccinated horse which was alive in Nairobi area.

The detection of AHSV-5 and AHSV-9 that is not present in the vaccine (LAV) that is mostly used in Kenya indicates that the vaccine may not be protective against these serotypes. Besides, AHSV-5 and AHSV-9 is reported to be antigenically closely related to AHSV-8 and AHSV-6, respectively [24,27], and since AHSV-6 was not detected at all in our samples one would assume that the vaccine is offering total protection against AHSV-6 and by extension should offer protection against AHSV-9, which was not the case.

African Horse sickness virus was previously reported in Kenya, where Davies and colleagues showed that all the 9 serotypes of AHSV were circulating in the country [28]. Most recently, Gichure and colleagues reported AHS in donkeys in Kenyan highlands with prevalence of between 27 – 35% depending on the seasons [26]. One of our study sites was in the same area where Gichure et al conducted their studies, hence we hypothesize the possibility of AHSV circulating between horses and donkeys. Furthermore, all our study sites also have a sizeable number of zebras and donkeys, thus we also hypothesize the possibility of zebras, as reservoir host, since zebra are resistant to AHS disease [11]. AHS has been detected in Equidae in other countries in Africa including Namibia [29,30], Ethiopia [31,32], Sudan [33], Zimbabwe [34], Senegal [16], South Africa [25] among others. Currently, information about the prevalence of AHSV or its potential risk factors among equids in Kenya is limited. Since horses play an important role in horse

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races and companion, and donkeys play a role transport in remote areas, the control of AHSV and other emerging diseases in these equids is urgently needed in the country.

Conclusion

AHS outbreak in in Kenya has significant consequences for horse owners especially effect on sporting activities, indicating the pressing need to develop new, safe, efficacious and cost-effective vaccines for AHS. From our pilot results we concluded that the horses in Kenya should get vaccine with all the 9 serotypes since all serotypes are circulating in Kenya. The development of cross-protective AHS vaccines with long shelf life, rapid protection and could be differentiated from natural infections should be a foremost priority for research. From this pilot study, we recommend further investigation to evaluate the immune responses of horse population and other equids, in endemic areas such as Kenya, which have long-term exposure to AHSV and resistance/tolerance to AHS. Furthermore, the ecology of endemic maintenance of the AHSV in other equids such as zebra and donkey populations is unexplored, therefore, genetic and environmental drivers of AHSV vector competence need urgent research attention in Kenya. Finally, it is worth noting that, using our results, CVRL in United Arab Emirates has developed an inactivated vaccine containing all 9 serotypes which produces high ELISA and neutralizing antibodies, which need further evaluation in the field to establish its efficacy [18,35].

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