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Seroprevance of African Horse Sickness in Khartoum State, Sudan

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ABSTRACT

A cross sectional study was conducted on equine to determine the seroprevalence of African Horse Sickness Virus (AHSV) antibodies and identification of potential risk factors in equine population in the three Khartoum localities in Khartoum State namely Khartoum, Khartoum north and Omdurman. A total of 187 serum samples were collected for competitive ELISA test to determine the presence of African Horse Sickness (AHS) antibodies. The seroprevalence of 87, 84 and 100% were found in the Khartoum, Khartoum north and Omdurman localities, respectively. The apparent seroprevalence was found to be 94% in donkey and 84% in horses. The overall seroprevalence of AHS virus was found to be 84%. There was no significant variation between the horse and donkey in the seropositivity ($p>0.05$). Significant variation was not observed in seroprevalence among age groups and sex of equine. All age groups as well as male and female of equine population were equally affected. Knowledge base of equine owner about AHS, *Clucoides* vector and mode of transmission of the disease in the study areas were assessed through structured questionnaire. The survey indicated that almost all equine owners did not know about *Clucoides* vector and mode of transmission. Therefore, there should be awareness reaction about AHS and Clucoides vector among the people through an organized extension package to the present study areas.

INTRODUCTION:

African horse sickness (AHS) is a non-contagious, insect-borne disease of equids caused by a double stranded RNA virus – namely, AHS virus (AHSV) – which belongs to the genus *Orbivirus* (family *Reoviridae*) and shares some morphological features with bluetongue and equine encephalosis viruses. The biting midge *Culicoides imicola* is considered the most important vector of AHSV in Africa (Mellor and Hamblin 2004).

There are nine distinct serotypes of African horse sickness virus (AHSV-1 to AHSV-9) that can be distinguished in serum neutralization tests by the specificity of their reactions with neutralizing antibodies. These different serotypes are all

classified within the species of African horse sickness virus, genus Orbivirus, family Reoviridae (Katarzyna *etal*., 2014); of the nine serotypes, types 1 to 8 are typically found only in restricted areas of sub-Saharan Africa while type 9 is more widespread and has been responsible for virtually all epidemics outside Africa, the only exception being the 1987–1990 Spanish-Portuguese outbreaks which were due to AHSV-4 (Philip and Christopher, 2004). African Horse Sickness outbreaks have been occasionally reported outside the African continent: Middle East (1959-63), Spain (1966, 1987-90), Portugal (1989), Saudi Arabia, Yemen (1997), and Cape Verde Islands (1999) (Anwar and Qureshi 1972, Coetzer *et al.* 2004, Howell 1960, Howell 1963, Mellor and Hamblin 2004). Such epidemics mainly resulted from the movement of infected animals (Mellor and Hamblin 2004), although the propagation of infected vectors by wind over long distances cannot be ruled out, as it is possible given the case concerning the bluetongue virus (Alba *et al.* 2004, Mellor and Hamblin 2004, Sellers *et al.* 1978). Such epidemics mainly resulted from the movement of infected animals (de Sá *et al.* 1994, Mellor and Hamblin 2004), although the propagation of infected vectors by wind over long distances cannot be ruled out, as it is possible given the case concerning the bluetongue virus (Alba *et al.* 2004, Mellor and Hamblin 2004, Sellers *et al.* 1978). .African horse sickness virus affects all equine species including horses, donkeys, mules, hinnies and zebras. Morbidity and mortality rates vary between species, horses being most susceptible to the virus. Mortality in horses can reach 95%, while infections in zebras are mostly subclinical (Coetzer and Guthrie, 2004). In addition to equids, camels, goats, and buffalo can become infected. Additionally, some carnivores such as dogs can become infected via ingestion of contaminated meat. However, there have been no documented cases of transmission of AHSV in carnivores in the wild animal, and it is considered that they area 'dead-end' host, rather than a reservoir of infection (Geoffrey *et al*., 2012).Orbiviruses are transmitted to animals mainly by arthropod vectors. The distribution of these viruses is very similar to the distribution of their specific vector and it is therefore influenced by climatic conditions. AHSV is transmitted by midges of the genus *Culicoides* which are also the vectors involved in Bluetongue (BT) transmission (MacLachlan and Guthrie, 2010). In horses, AHS is characterized by clinical signs, which develop as a result of damage to the circulatory and respiratory systems giving rise to serous effusion and hemorrhage in various organs and tissues. The extent and severity of the clinico-pathological findings have been used to classify the disease into four forms. In ascending order of severity these are horse sickness fever (which usually affects only mules, donkeys and partially immune horses), the sub-acute or cardiac form, the cardio-pulmonary or mixed form and the per acute or pulmonary form. All forms of disease can occur in any one outbreak but in susceptible populations of horses the mixed and pulmonary forms tend to predominate, so mortality rates in these animals will be very high (Philip and Christopher, 2004) .Two types of vaccines have been described for AHS. Attenuated live vaccines (monovalent and polyvalent) and inactivated vaccine. New vaccines, including a subunit vaccine, have been evaluated experimentally (Sánchez-Vizcaíno, 2004). The aim of this study was to determine the prevalence rate of the disease in Khartoum state, Sudan.

MATERIALS AND METHODS:

Study area: A cross sectional study was conducted on equine to determine the seroprevalence of African Horse Sickness Virus (AHSV) antibodies and identification of potential risk factors in equine population in selected areas of Khartoum state, three localities namely Khartoum, Omdurman and Khartoum North.

Study design: Determination of the seroprevalence of AHSV by using competitive ELISA. Data on the potential risk factors associated with the occurrence of AHS were collected during sampling through recording and questionnaire administration.

Sampling method and sample size determination: Systematic random sampling was applied to select the study population; for an infinite population with 95% confidence level, 5% desired absolute precision and 50% expected prevalence, since there was no previous information on the prevalence of AHS antibodies in the study areas. The sample size was determined according to Thrusfield (2005). Even if 384 samples were the minimum sample size required, to increase the precision, 480 equine serums sample from two zones were collected.

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Collection of Samples: A total of 187 serum samples of horses and 50 of donkeys were collected. Whole blood of 10 mL was collected by vein puncture using sterile venoject needles and plain vacutainer tubes including needle holder under aseptic conditions. Each sample was labeled with identification number. The blood was allowed to clot over night at room temperature. The recovered serum was decanted into another tube and labeled with similar identity. The sample was transported at +4ºC then kept at -20ºC until evaluated with competitive ELISA. Data were collected in each area of the study and these were, age, sex, type of equidae (horse and donkey), and vacutainer identification number was recorded at the time of sampling. The serological test was performed using Indirect – ELISA to identify AHSV antibodies. The indirect ELISA was performed according to protocol provided by the ELISA kits manufacturing company (INGENASA, MADRID).

Questionnaire survey: A questionnaire for 67 owners was used to collect information about the knowledge of disease and the control of the vectors , nutrition, presence of other animal in the stable and the source of animals, that was aimed at assessing the potential risk factors such as management practice (stabling condition and vaccination history). Also questionnaire included presence of equine biting insects and availability of water bodies near the study areas and the knowledge base of equine owners about AHS was prepared to interview individual owners of horse and donkeys.

Statistical analysis: Data recorded during sampling, laboratory findings were entered and stored in Microsoft excel spread sheet. The data were thoroughly screened for errors and properly coded before subjected to statistical analysis. The data were brought from the Microsoft excel spread sheet and analyzed using SPSS version 16 software to establish associassion (χ^2 test) between

serological test results and risk factor. Tables are used to present the results and the overall positive seroprevalence are calculated by dividing total number of positive sample over the total sample and multiplying with hundred.

RESULTS:

Seroprevalence survey: One hundred and eighty seven serum samples from Khartoum, Omdurman and Khartoum North were examined for AHSV using indirect ELISA the seroprevalence of AHS was found to be 80%, 100% and 87%, respectively. Of the 187 sampled equidae from three localities the overall seroprevalence of 84% was found as indicated in Table 1.

Table 1: Sero prevalence of African horse sickness virus in different localities in Khartoum state

locality	Number of tested	Number of positive	Number of negative	Percentage of prevalence
Khartoum	123	99	24	80%
Omdurman	17	17	0	100%
Khartoum north	47	41	6	87%
Total	187	157	30	84%

As shown in table (2) there was no statistical significant between risk factors in this study.

Table 2: Descriptive statistics of risk factors associated with AHS in Khartoum state

The Factor		% Within ELISA		
		Positive	Negative	Total
Gender	Male	39 (67.2)	5 (55.6)	44 (65.7)
	Female	19 (32.8)	4 (44.4)	23 (34.3)
Stable	Khartoum	31 (53.4)	5 (55.6)	36 (53.7)
	East Nile	27 (46.6)	4 (44.4)	31 (46.3)
Mixing with others animals	Yes	15 (25.9)	2 (22.2)	17 (25.4)
	No	43 (74.1)	7 (77.8)	50 (74.6)
Age (Years)	< 5	8 (13.8)	2 (22.2)	10 (14.9)
	5-10	27 (46.6)	4 (44.4)	31 (46.3)
	> 10	23 (39.7)	3 (33.3)	26 (38.8)
Animal Origin	Khartoum	32 (55.2)	5 (55.6)	37 (55.2)
	West Sudan	25 (43.1)	3 (33.3)	28 (41.8)
	Foreign	1 (1.7)	1 (11.1)	2 (3)
Total		58 (100)	9 (100)	67 (100)

DISCUSSION:

African horse sickness represents a major health concern and negatively impacts the equine industry, mainly in those countries, such as Sudan, where high quality horses are bred and vaccination is not compulsory. A freeze-dried, polyvalent, live attenuated vaccine against AHS (Onderstepoort Biological Products, OBP) is currently used in Africa. Horses should be inoculated 3 times – at 6, 9, and 12 months of age – and then annually re-vaccinated, before the rainy season, to become immune to all the serotypes in the vaccine. As a result, the immune status of Sudanese horses is likely to be variable, such speculation reasonably explaining the occurrence of severe, sometimes fatal AHS, in a number of immunized horses. Furthermore, a growing body

of evidence indicates that prophylactic immunization against AHS is useful to prevent serious losses, but cannot fully protect horses from infection and disease under natural condition (Coetzer *et al.* 2004, Crafford *et al.* 2013, Weyer *et al.* 2013, Molini *et al.*2015, forthcoming).The prevalence of AHSV antibodies detected by ELISA in the horse stables in Khartoum state, Sudan, was estimated at 84%. This high prevalence is in harmony with previous serological studies in Sudan Ihsan (2012) showed that the percentage of the antibodies were 74% and 79.4 % by using SNT and ELISA techniques respectively , and that may be an indication of continual exposure of the horses regardless of age, sex and breed. The study area Khartoum state extends in three localities, with thick vegetation and hot and dry climatic conditions which favor vector propagation and virus transmission. In Nigeria, no preventive vaccination is routinely applied against AHSV, particularly in indigenous and local crossbred horses. There appears to be a form of innate resistance to infection by AHSV as corroborated. It could explain the probable absence of reported outbreak of the disease in the region, despite the high prevalence of antibodies. In contrast, newly imported horses, particularly from AHS-free areas, are susceptible to infection and are therefore usually vaccinated before importation. But once established in the country (the animals of exotic breeds examined during the study had been introduced more than ten years ago or were born in the country), they are seldom revaccinated (Adeyefa and Hamblin, 1995) and therefore vulnerable to AHSV infection (Ehizibolo *et al.*, 2014). From the study it can be conclude that, the seroprevalence of AHSV was 87%, 84 and 100% in Khartoum, Khartoum north and Omdurman localities, respectively. From the total sample tested the seroprevalence of 84% in donkeys and 94% horses was obtained, the overall prevalence with 84%. These findings showed that the African horse sickness affects equally almost all age groups and sexes, hence there is no significant variation in the seropositivity among the equine. The questionnaire survey result indicates that the knowledge base of equine owners about AHS, mode of transmission and clucoides vector was not satisfying. They are unaware of culicoides vectors and mode of transmission of AHS. Generally AHSV exists in all localities and affect the two types of equidae in the study areas. Based on the above conclusion following recommendation are forwarded: There should be awareness reaction about AHS and Clucoides vector among the people through an organized extension package to the present study areas. Appropriate equine enclosure system to avoid insect bite by stabling them some hours before sunset and letting them out a few hours after sun rise as Clucoides are nocturnal in nature and are not inclined to enter equine stables and also control of insect using chemical or biological method. All equine greater than 6 month in the study area should be vaccinated with polyvalent vaccine in the study areas. There is a need of deeper understanding of the epidemiology of African horse sickness in the study areas by an integrated approach of serotyping and identification of the clucoides vectors as well as other potential vectors.

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