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Epidemiological Study of African Horse Sickness in Sudan

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Abstract- A cross-sectional study was conducted from September 2016 to October 2017 to determine the seroprevalence and to investigate the risk factors for African horse sickness (AHS) in Sudan. A total of 920equines (590 horses and 330 donkeys) were randomly selected and sampled. Competitive Enzyme Linked Immuno-Sorbent Assay (c-ELISA) was employed to detect antibodies to AHS virus. The overall seroprevalence was 72.2 %, while it was 80% in horses and 58.2% in donkeys. The univariate analysis of associations of potential risk factors with seroprevalence of AHS showed statistically significant ($p \leq 0.05$) results with state ($\chi2 = 47.434$, $p<0.001$), species ($\chi2 = 50.163$, $p<0.001$), sex ($\chi2 = 26.206$, $p<0.001$), housing ($\chi2 = 26.477$, $p<0.001$), vaccination ($\chi2 = 44.466$, $p<0.001$), breed ($\chi2 = 57.256$, $p<0.001$), water bodies ($\chi2 = 26.271$, $p<0.001$), Cullicoides ($\chi2 = 42.658$, $p<0.001$), ticks ($\chi2 = 23.608$, $p<0.001$), activity of animal ($\chi2 = 41.435$, $p<0.001$), awareness of owner ($\chi2 = 25.639$, $p<0.001$),age ($\chi2 = 20.186$,$p<0.001$), health score($\chi2 = 12.038$, $p<0.001$), pregnancy ($\chi2 = 3.249$,$p = 0.0355$),and infection with other disease($\chi2 = 14.637$,$p<0.001$). However, the risk factors of presence of other animals and pervious infection with AHS did not show statistically significant ($p>0.05$) associations.

Keywords: african horse sickness, AHS, c-ELISA, seroprevalence, risk factors, equines, horses, donkeys, sudan.

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Keywords: african horse sickness, AHS, c-ELISA, seroprevalence, risk factors, equines, horses, donkeys, sudan.

I. Introduction

African Horse Sickness (AHS) is a vector-borne viral disease of horses, mules, and donkeys. The clinical signs and lesions occur as a result of increased vascular permeability and are characterized by an impairment of the respiratory and circulatory system (Radostits et al., 2007). AHS was first recognized in southern Africa, with the first outbreak recorded in 1719, when more than 1700 animals died. Although endemic in equines in Sub-Saharan Africa, outbreaks have also been recorded in North Africa, the Middle and Near East and southern Europe (MacLachlan and Guthrie 2010). AHS virusis transmitted by the biting midges Culicoides. *Culicoidesimicola*is the field vector of AHS virus although *Culicoidesbolitinos* has been shown to play an important role in the transmission of AHS virus in the cooler upper highlands of South Africa (Meiswinkel and Paweska 2003). The highest incidence of the disease usually occurs in the late summer and early autumn in years when the climatic conditions favor an abundance of *Culicoides*midges (Coetzer and Guthrie 2004).

Clinically, the disease is characterized by anacute pulmonary form, a cardiac form or sub-acute form, mixed form and a mild form known as horse sickness fever(Upadhyaya, 2011). AHS is one of the viral diseases characterized by up to 95, 50and 10% mortality rates in horses, mules, and donkeys, respectively (OIE, 2008).

In a study performed in horses and donkeys from southern Darfur and Khartoum states, the sera were analyzed by passive haemagglutination test and serum neutralization test, the overall seroprevalence was 42.64% and 27.75%, respectively (Ihsan 2004).In different regions of Ethiopia, Tesfaye et al.(2012), Kassa (2006), Demissie (2013),Ende et al.(2013), Molalegne et al. (2010),and Yeshitila and Bekele(2017)found that the apparent seroprevalence of AHS was 24.60%, 23%, 33.04%, 46.2%, 25%and 23.47%,respectively. There were no significant variations (P>0.05) among age groups and sex for seroprevalence of the disease (Ende et al. 2013,Kassa 2006,Tesfaye et al.2012). In the Khartoum state of Sudan,Abu Elzein et al. (1989) found that the seroprevalence in donkeys was 98% for AHS by using the micro AGID test. Also, in the same area antibodies to AHS virus were detected in horses, donkeys, goats, cattle and Dorcas gazelle in a rate of 78.9%, 76.7%, 20%, 15% and 11.1%, respectively (Elghazali and Ali 2013). In Zimbabwe, results indicated a higher seroprevalence of the disease in the late rainy season (68.9%) compared to other seasons. Age and

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vaccination of horses were found to be significant factors (Gordon et al., 2013).

Sudan livestock population for the year 2009 was 41.65 million cattle, 51.55 million sheep, 43.27 million goats, 4.52 million camels, 7.51 million donkeys and 784 thousand horses (Statistical Bulletin for Animal Resources 2009).There were previous studies conducted to investigate AHS in Sudan (Eisa 1974; Abu Elzein et al. 1989; Ihsan 2004; Elghazali and Ali 2013). However, these studies were conducted in few states, and small sample sizes were investigated. Hence, there was a gap of research information and published studies which point out the seroprevalence of AHS and investigate its association with potential risk factors. Therefore, this study was conducted to fill the information gap regarding the seroprevalence and potential risk factors associated with the disease in Sudan.

II. Materials and Methods

a) Study design

The study was designed as a cross-sectional observational study with a multistage sampling technique. Four states; Northern, River Nile, Khartoum, and Darfur were randomly selected from the whole country. Then from each state, four localities were selected. Finally, animals were investigated by visiting markets, farms and villages.

b) Collection of blood samples

The blood was taken aseptically from the jugular vein into sterile vacutainers without anti coagulants, allowed to clot for 1-2 hours at room temperature, stored vertically overnight at 4°C and then centrifuged at 4000 rpm for 5 minutes. The sera were taken in sterile bijou bottles and inactivated in the water bath at 56°C for 30 minutes, allowed to cool and then stored at –20°C until assayed in the laboratory.

c) Data collection

A pre-tested structured questionnaire with the primary objective of elucidating the multi-factorial background of AHS was conducted in an interactive manner with every individual owner of horses and donkeys. The format was designed to investigate individual animal characteristics, management, and environmental risk factors.

d) Laboratory procedure

Blood samples were examined at The Central Laboratory, Ministry of Higher Education and Scientific Research, Khartoum. Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) was used to detect the presence of specific antibodies against the AHS virus in the collected sera samples following manufacturer's protocol.

e) ELISA protocol

i. *Preparation of reagents*

Washing solution: one part of the concentrate washing solution which provided in the kit was diluted in 24 parts of distilled or deionized water (40 ml of concentrate solution and 960 ml of water) and remained stable at +4°C.

Controls (+ve) and (-ve): were ready to use and didn't need any preparation.

Conjugate and substrate were ready to use and didn't need any preparation.

1. All reagents were brought to room temperature before use.
2. 100 µl of the positive control was dispensed into two wells and 100 µl of the negative control into two wells. Sera samples were diluted 1/5 in provided diluents. This step was made directly in the wells of the plate by adding 80 µl of the diluents and 20 µl of serum samples and the plate was shaken carefully for homogenization. The plate was covered and incubated for 1 hour at 37°C.
3. Washing steps:

The content of the plate was thrown out by a brusque turnover of the plate to avoid the possible mixture of the content from one well to another.

A volume of 300 µl of washing solution was dispensed on each well.

The plate was shaken delicately, avoiding the contamination between wells.

The plate was turned over to empty the wells.

The process was repeated five times as indicated on the instructions of the kit.

Before empty the content of the last washing step, the next reagent was ready to use. The plate was not maintained dry more than strictly needed.

After the last step of washing the plate was shaken and turned over on the absorbent filter paper.

4. The plate was washed five times as described in step 3.
5. 100 µ / well of the conjugate was added. Incubated for 30 minutes at 37°C.
6. The plate was washed five times as described in step 3.
7. 100 µl/well of the substrate solution was dispensed by using a multi-channel pipette. Incubated for 10 minutes at room temperature.
8. 100 µl well of the stop solution was dispensed and dispensing of bubbles was avoided.
9. The plate was read at 405 nm using spectrophotometer.
10. Validation criteria:

The ELISA test validation was checked for each plate based on two criteria set by the manufacturer for the mean optical density (OD) of the positive and negative control. The OD of the positive control was less

than 0.2 and the OD of the negative control was higher than 1.0.

11. Interpretation

Blocking percentage (BP) of each sample was calculated based on OD value applying the following formula:

$$BP = \frac{OD\ (-Control\ ) - OD\ (Sample\ )}{OD\ (-Control\ ) - OD\ (+Control\ )} x100$$

Samples showed BP value lower than 45% were considered to be negative for antibodies of AHS virus. Samples showed BP value higher than 50% were considered as positive for antibodies of AHS virus.

Samples with BP value between 45% and 50% were considered doubtful, and they were retested. If the result is the same, another extraction was made and tested 2 weeks later.

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ii. *Statistical analysis*

All data collected were entered into Microsoft excel spreadsheet. For analysis of the data, SPSS version 21 software was used. Data were analyzed descriptively in the first step, using the frequency table and cross tabulation. Then the association of the potential risk factors with the seroprevalence of AHS at the individual level was analyzed using the Chi-square test. The level of significance was set at $P<0.05$. For the investigation of the association between the seroprevalence in response to individual animal characteristics, management, and environmental potential risk factors, a multivariate analysis was performed in which logistic regression was used. The strength of association between the risk factors and the sero-prevalence of AHS was quantified using the odds ratio (OR) and the level of significance was set at $p \leq 0.05$.

III. Results

a) Prevalence and associated risk factors

In a total of 920 equines (590 horses and330 donkeys) sampled and examined, the overall seroprevalence of AHS was72.2 %. Within states, the highest seroprevalence of AHS was reported in Khartoum and Darfur states (81%) and (78.8%), respectively. Table 1shows the univariate analysis of the association of potential risk factors with the seroprevalence of AHS. The table shows the risk factors which have been investigated, number of animals tested, number of animals positive with their percent (%), the value of Chi-square (χ^2) and the P value.

The seroprevalence of AHS showed statistically significant association with state (χ^2 = 47.434, $p<0.001$), species (χ^2 = 50.163, $p<0.001$), sex (χ^2 = 26.206, $p<0.001$), housing (χ^2 = 26.477, $p<0.001$), vaccination (χ^2 = 44.466, $p<0.001$), breed (χ^2 = 57.256, $p<0.001$), the presence of water bodies (χ^2 = 26.271, $p<0.001$), presence of cullicoides (χ^2 = 42.658, $p<0.001$), presence of ticks (χ^2 = 23.608, $p<0.001$), activity of animals (χ^2 = 41.435, $p<0.001$), awareness of owners (χ^2 = 25.639, $p<0.001$), age (χ^2 = 20.186, $p<0.001$), health score(χ^2 = 12.038, $p<0.001$),pregnancy status(χ^2 = 3.249, p = 0.0355), and infection with other diseases(χ^2 = 14.637, $p<0.001$). However, risk factors of presence of other animals and pervious infection with AHS did not show statistically significant associations ($p>0.05$).

The final multivariate model revealed that equines in Khartoum state were almost five times more likely to be sero-positive compared with equines in Northern state (OR = 4.909, p = 0.017), local equines were two times and half more likely to be sero-positive compared with cross equines(OR = 2.532, p = 0.004), horses were 3.8times more likely to be sero-positive compared with donkeys (OR = 3.776, p = 0.017), equines raised in areas with water bodies were two times more likely to be sero-positive compared with those in dry areas (OR = 2.172, p = 0.033), and non-vaccinated equines were 17 times more likely to be sero-positive compared with vaccinated ones (OR =17.298, $p<0.001$).

IV. Discussion

The current study indicatedan overall seroprevalence of AHS in equines at the study states of 72.2 %. This result was higher than those reported in Khartoum and South Darfur states (Elghazali and Ali 2013, Ihsan 2004), different regions of Ethiopia (Tesfaye et al. 2012, Ende et al. 2013, Kassa 2006) and Zimbabwe (Gordon et al. 2013). In contrast, our findings of the seroprevalence of AHS in donkeys was lower than the previous of Abu Elzein et al. (1989) in Khartoum state. The difference in seroprevalence of AHS in the present study and other previous studies could be probably due to differences in season of sampling, geographic location, study methods and diagnostic techniques employed by the investigators.

Out of 15 risk factors that showed significant statistical association ($p \leq 0.05$) with seroprevalence of AHS in the univariate analysis and entered in the multivariate analysis, only five risk factors (state, species, breed, water bodies and vaccination) showed significant statistical associations ($p \leq 0.05$) with seroprevalence of AHS. The results indicated higher seroprevalence in Khartoum state and South Darfur state which characterized by high rainfall, the presence of water bodies and good vegetation with over-abundance of midges, while River Nile state and Northern state were located in semi-desert and desert climate, respectively. The variations in seroprevalence of

AHS at different states in our study were significant ($p \leq 0.05$), and this result was in close agreement with the results reported by Ende et al. (2013) and Tesfaye et al. (2012) in different regions in Ethiopia.

Furthermore, there was a significant variation of seroprevalence between the two species of equines (horses and donkeys).A higher seroprevalence was observed in horses as compared with donkeys, and the difference was statistically significant ($p \leq 0.05$). This finding is in agreement with a couple of previous studies (Alemayehu and Benti, 2009; Yeshitila and Bekele, 2017). Also, by the OIE (2008) reports which stated that among equines horses were the most susceptible to AHS with a mortality rate of 50-95% followed by mules with a mortality rate around 50% and donkeys with a mortality rate of 5-10%. However, our result differed with the findings of other previous studies conducted by Tesfaye et al. (2012) and Ende et al. (2013) in Ethiopia.

The current study further revealed that there isa statistically significant variation ($p \leq 0.05$) of seroprevalence between the local and cross breed. A higher seroprevalence was observed in the local compared with the cross breed. However, this significant difference could be attributed to the fact that the cross horses receive high care by raising them in safe stables that protect them from the infestation of vectors and vaccinated annually against the disease.

Our findings indicated a statistically significant variation between areas with water bodies and dry ($p \leq 0.05$). A higher seroprevalence of AHS was observed in equines raised in areas with water bodies, and this result is in close agreement with the results reported by Coetzer and Guthrie (2004), Demissie (2013) and Radostitis et al. (2007) who explained that AHS endemic areas are more likely to be low lying, flooded by rain water, warm and marsh regions that create favorable environment for multiplication of *Culicoides* and mechanical vectors.

Furthermore, our final multivariate model revealed a statistically significant ($p \leq 0.05$) variation between non-vaccinated and vaccinated equines. A higher seroprevalence of AHS was observed in non-vaccinated equines. Lack of vaccination is the most strong risk factor (OR =17.298, P <0.001) among the risk factors investigated and found statistically significantly associated with AHS. However, the observed association could be confounded with poor management and lack of knowledge of equines owners about the disease and the importance of vaccination as a protective tool against AHS. Unfortunately, both risk factors were not investigated.

Regarding sex, our findings showed significant variation ($p \leq 0.05$) in the se roprevalence between sexes in the univariate analysis, with a higher seroprevalence in males than in females. This result is in close agreement with the result reported in Ethiopia by Yeshitila and Bekele (2017). However, the risk factor of sex did not remain statistically significant in the final multivariate model.

In the current study, we have used the odds ratio to quantify the strength of association between potential risk factors and the seroprevalence of AHS. To our best knowledge, this is the first study to quantify the strength of association between potential risk factors and AHS in Sudan. However, although the discussed risk factors were statistically significantly associated with AHS in the final multivariate model, it is difficult to consider them as necessarily causally related and should be interpreted in light of the causal criteria that have been proposed by Thrusfield (2005).

V. Conclusion

The present study indicated that AHS seroprevalence was highly prevalent in equines in the study states of Sudan. Furthermore, the seroprevalence of AHS was statistically significantly associated with the vaccination status of equines, geographic location of state, species, breed, and presence of water bodies. These identified risk factors should be carefully considered when control strategies for AHS are implemented.

Table 1: Univariate analysis of potential risk factors associated with sero-prevalence of AHS in equines in four states of Sudan

Risk factors	No. tested	No. +ve (%)	χ^2	p-value
State:			47.434	<0.001
Northern	263	150 (57%)		
River Nile	92	63 (68.5%)		
Khartoum	253	209 (81%)		
Southern Darfur	312	242 (78.8%)		
Species:			50.163	<0.001
Horses	590	472 (80%)		
Donkeys	330	198 (58.2%)		
Sex:			26.206	<0.001
Male	587	455 (77.9%)		
Female	333	209 (62.2%)		
Vaccination:			44.466	<0.001
Yes	197	105 (53.3%)		

No	723	559 (77.3%)		
Health Score:			12.038	<0.001
Good	776	523 (69.7%)		
Bad	164	141 (82.9%)		
Housing:			26.477	<0.001
Barn	170	99 (58.6%)		
Backyard	622	482 (77.2%)		
Farm	128	83 (65.4%)		
Age (yrs):			20.186	<0.001
1 – 3 years	319	285 (80.3%)		
4 – 8 years	547	333 (67.8%)		
9– 12 years	54	34 (60.7%)		
>12 years	18	12 (66.7%)		
Breed:			57.256	<0.001
Local	836	633(75.7%)		
Cross	84	31 (36.9%)		
Pregnancy Status:			3.249	0.0355
Yes	28	16 (57.1%)		
No	892	648 (72.6%)		

Table 1: Continued

Risk factors	No. tested	No. +ve (%)	χ^2	p-value
Presence of water bodies:			26.271	<0.001
Yes	293	369 (79.7%)		
No	627	295 (64.6%)		
Presence of cullicoides:			42.658	<0.001
Yes	383	451 (79.8%)		
No	537	213 (60%)		
Presence of ticks:			23.608	<0.001
Yes	189	167 (86.1%)		
No	731	497(68.5%)		
Infection with other diseases:			14.637	<0.001
Yes	77	74 (90.2%)		
No	843	590 (70.4%)		
Presence of other animals:			0.944	0.165
Yes	8	7 (87.5%)		
No	912	657 (72%)		
Pervious infection with AHS:			0.095	0.379
Yes	16	11 (68.8%)		
No	904	653(72.2%)		
Activity of equines:			41.435	<0.001
Racing	131	63 (49.2%)		
Back	352	286 (78.6%)		
Cart	437	315 (73.6%)		
Awareness of owners:			25.639	<0.001
Yes	521	408 (78.8%)		
No	399	256 (63.7%)		

Table 2: Multivariate analysis of potential risk factors associated with sero-prevalence of AHS in equines in four states of Sudan

Risk factors	No. tested	No. +ve (%)	OR	95% CI for OR	p-value
State:					
Northern	263	150 (57%)	Ref.		
River Nile	92	63 (68.5%)	1.637	0.986 – 2.719	0.057
Khartoum	253	209 (81%)	4.909	1.329 – 18.133	0.017
Darfur	312	242 (78.8%)	1.349	0.359 – 5.070	0.658
Breed:					
Local	836	633(75.7%)	2.532	1.339 –4.790	0.004
Cross	84	31 (36.9%)	Ref.		
Species:					
Horses	590	472 (80%)	3.776	1.264 – 11.281	0.017
Donkeys	330	198 (58.2%)	Ref.		
Presence of water bodies:					
Yes	463	369 (79.7%)	2.172	1.063 – 4.437	0.033
No	457	295 (64.6%)	Ref.		
Vaccination:					
Yes	197	105 (53.3%)	Ref.		
No	723	559 (77.3%)	17.298	8.673 – 34.501	<0.001

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