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Research Article

Seroprevalence of African Horse Sickness at Central Highland of Ethiopia

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ABSTRACT

A cross-sectional study was undertaken to determine the seroprevalence of African Horse sickness virus (AHSV) antibodies and identify potential risk factors in equine population at selected areas of central high land of Ethiopia from November 2011 to April 2012. A total of 546 sera (506 horses, 18 mules and 22 donkeys) were collected randomly. Competitive Enzyme Linked Immuno Sorbent Assay (c-ELISA) configuration was employed to determine the presence of AHSV antibodies. The apparent prevalence of AHSV was found to be 46 % in horses, 61.1 % in mules and 36.4 % in donkeys. The overall apparent seroprevalence of AHSV in three species of equine was found to be 46.2 %. Statistically significant ($p<0.05$) difference in seroprevalence was observed at the different study areas confirming the existence of agro-ecology based variation in the occurrence of AHS. The highest seroprevalence of AHSV was documented at the mid highland followed by highland areas. There were no significant variations ($P> 0.05$) among age groups and sexes for seroprevalence of AHSV. In this study, all age groups as well as male and female populations were equally affected by African horse sickness disease. Questionnaire survey also indicated the presence of African horse sickness disease in the study areas. Therefore, control strategies as annual vaccination and appropriate housing system should be targeted at all ages and in both sex.

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INTRODUCTION

Ethiopia has the largest livestock population in Africa. The overall livestock population was about 43.1 million cattle, 23.6 million sheep, 6.5 million equine (5.42 million donkeys, 1.78 million horses and 373,519 mules) (CSA, 2009). The country has the largest equine population with 6.9 % of the world and 42.4 % of the African equine population. Moreover, 65% of all African mules, 50% horses, and 80% of donkeys are found in Ethiopia (Alemayehu and Benti, 2009).

Equine play important role in Ethiopia in the transportation of farm products, fodder, firewood, agriculture inputs, construction, and waste materials in both rural and urban transport system which is low cost and viable which provides the best alternatives in places where the infrastructure is insufficiently developed (Alemayehu and Benti, 2009). On the other hand, many factors contribute to the poor performance and health of equidae. Among the most important ones are nutritional disorders, bacterial, fungal, protozoal and viral diseases. A viral disease like African horse sickness (AHS) characterized by high morbidity and mortality rates is worth enough to be mentioned. Hence, it is the viral diseases characterized by up to 95%, 50%, and 10 % mortality rate in horse, donkey and mule respectively (Fiseha, 1998; OIE, 2008; Maclachlan and Gutherie, 2010).

African Horse Sickness is an infectious, non contagious arthropod borne disease of eqiudae, caused by a double stranded RNA orbivirus belonging to the family Reoviridae (Mellor and Hamblin, 2004). The spread of diseases is influenced by climatic conditions which favor the survival of vector insects including warm moist weather and high rainfall, as well as spread by wind dispersal. Virus replication within the vector requires a sustained minimum temperature. This explains why the disease does not persist in countries with colder climates (Mellor *et al*., 1998).

African Horse Sickness occurs endemically in all part of Africa south of the Sahara with periodic spread further north. At present AHS virus is endemic in tropical and subtropical area of Africa, south of Sahara occupying a broad band stretching from Senegal in the west to Ethiopia and Somalia in the east, and extending as far south as north south Africa (Mellor and Hamblin, 2004).

There were previous works done on the seroprevalence of the disease in southern Ethiopia by Tesfaye *et al.* 2012) and western Amhara by Mulualem *et al.*(2012). However, there is gap of research information and published studies which point out the prevalence of African horse sickness at central highland of Ethiopia. Therefore, the present study was conducted to fill the information gap regarding the disease in the central highlands of Ethiopia and rollout the potential risk factor associated with the disease occurrence.

MATERIAL AND METHODS

Study Area

The study was carried out from November 2011 to April 2012 at central highland of Ethiopia at selected district of South West and West Shewa zone which are found in Oromia regional state. Woliso and Wonchi districts were selected from

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Southwest Shewa zone while Ejere and Jeldu woreda were designated from West Shewa zone.

Study Population

The equine population was the main target of the study. Horse, mules and donkeys above six months of age, both sexes and with no pervious history of vaccination against African horse sickness were sampled. Age of the horses was determined according to Mair *et al.*(2002) using dentitions and wearing patterns of incisor teeth.

Study Design

Cross-sectional study was carried out to assess the seroprevalence of AHS from sera of equine and data on the potential risk factors associated with the occurrence of AHS were collected during sampling through recording and questionnaire.

Sampling Method

Purposive sampling method was employed to select four woreda from the total woreda of both Southern and Western Showa zone. All equids according to their age, sex, agro-ecology and history of non-vaccination were sampled. Stratification of equines in to mules and donkeys was made at peasant association level. Discussion was made with owners of mules and donkeys to know their respective areas and avoid repetition during sampling.

For sample size determination the total equine population in southern and western showa zone were used by taking the expected prevalence of 32.5 % which was reported by Molalegne *et al.*(2010) and the absolute precision of 5% with 95% confidence level had been used, according to the formula given by Thrusfield (2005) which is placed as follow;

$$n = \frac{1.96^2(Pexp(1-Pexp))}{d^2}$$

Where; n= required sample size, Pexp= expected prevalence of AHS, d= desired absolute precision, 1.96= the value of "z" at 95% confidence interval.

Hence, a total of 546 equines were sampled from the study areas. But, expected sample to be collected were 337 equines.

Study Methodology

Whole blood of 10 ml was collected by vein puncture using sterile venoject needles and plain vacutainer tubes including needle holder were used under aseptic precautions. 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 vacutainer tube and labeled with similar identity. The samples were kept at – 20^0C until evaluated with c-ELISA. The test was done after serum collection using ELISA kit. Standardization and validation of the kit was conducted before the test sera are subjected to c-ELISA test procedures.

Questionnaire survey

A structured questionnaire format was prepared to interview individual owners of horses, donkeys and mules. The format was used to assess the predisposing factors: seasons, presence of equine biting insects and result after vaccinations at study areas and the knowledge base of equine owners about AHS. A total of 215 equine owners were interviewed.

Data management and analysis

The data collected from the study areas, which were processed in the laboratory was entered into Microsoft-excel spread sheets and was analyzed by using statistical software (SPSS version 16.0). Chi-square test was applied to test statistical association between the potential risk factors (age, sex, and species) with prevalence of the disease. For all the analysis P< 0.05 was considered statistically significant. Tables were 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 of African Horse Sickness

From total of 546 equids (506 horse, 22 donkeys and 18 mules) sampled and examined, 252 were found serologically positive for African horse sickness virus with overall prevalence of 46.2 % using c-ELISA. Out of 546 equids the prevalence of the disease in horses, mules and donkeys was 233 (46 %), 11 (61.1 %) and 8 (36.4 %), respectively
(Table 1)

Table 1: Seroprevalence of African Horse sickness

Species	c-ELISA			
	Positive	Negative	Total	X^2 (p-value)
Horse	233 (46.0%)	273 (54.0%)	506 (100%)	
Donkey	8 (36.4%)	14 (63.6%)	22 (100%)	2.47 (0.291)
Mule	11 (61.1 %)	7 (38.9%)	18 (100%)	
Total	252 (46.2%)	294 (53.8%)	546 (100%)	

The highest prevalence of the disease was encountered in Jeldu (59.9%) followed by Ejere (49%) and Woliso (36.6%), while the lowest one was observed at Wonchi (35.7%) district (Table 2).

Table 2: Seroprevalence of African Horse sickness in relation to study areas

Address	c- ELISA			
	Positive	Negative	Total	X^2 (*P*- value)
Woliso	41 (36.3%)	72 (63.7%)	113 (100%)	
Wonchi	50 (35.7%)	90 (64.3%)	140 (100%)	22.77 (0.000)
Jeldu	94 (59.9%)	63 (40.1%)	157 (100%)	
Ejere	47 (49.0%)	49 (51.0%)	96 (100%)	

The study revealed that higher prevalence of AHS was more in female (51.5%) than male (43.9%) horses (Table 3).

Table 3: Seroprevalence of African Horse sickness in relation to sex

Sex	c-ELISA			
	Positive	Negative	Total	X^2 (P value)
Female	67 (51.5%)	63 (48.5%)	130 (100%)	22.77 (0.131)
Male	165 (43.9%)	211 (56.1%)	376 (100%)	

The result indicated that equids (horses) between 7 and 13 years were serologically positive in the study areas with the highest prevalence 50.2% followed by ages greater than 13 years (48.4 %) and 1-6 years (39.6 %) (Table 4).

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Table 4: Seroprevalence of African Horse sickness in relation to age groups

Age	c-ELISA			
	Positive	Negative	Total	X^2 (Pvalue)
1-6	78 (39.6%)	119 (60.4%)	197 (100%)	
7-13	124 (50.2%)	123 (49.8%)	247 (100%)	5.15 (0.076)
>13	30 (48.4%)	32 (51.6%)	62 (100%)	
Total	232 (45.8%)	274 (54.2%)	506 (100%)	

Questionnaire Survey

The questionnaire survey was conducted at four sites of the study areas regarding the type of equine diseases, predisposing factors and the fate of the diseases after vaccination.

According to the questionnaire survey, about 37.3% of respondents replied that rainy season is the first predisposing factor followed by high number of insect (36.6%) and movement of horses (34.5%) for the occurrence of AHS .

According to the respondents, AHS more commonly occurred during the month of September to November (31 %), March to May(40.6 %) and December to February (45.7 %). Most of the animal owners got 60 % of their equine vaccinated; however, 40% of them did not get their equines (horses) vaccinated against major equine diseases. From these, 37.5% (48) had their animals vaccinated for AHS. From the questionnaire survey, 48.8% respondents said that vaccinated animals died shortly after vaccinations. Conversely, 26% respondents revealed that death was occurring two months after vaccination. Among participants 25% said that no death appeared after vaccination of the equine.

DISCUSSION

The overall prevalence of AHSV in equines at the study areas was found to be 46.2 %. The seroprevalence of AHS in horse, donkey and mules was 46 %, 36.4 % and 61.1 %, respectively. There was no significant variation ($p > 0.05$) of seropositivity between the three type of equidae (horse, mule, donkey). This is well supported by the report of Tesfaye *et al.*(2012) However, according to OIE(2008) among equidae horse were the most susceptible to AHSV with a mortality rate of 50-95% followed by mules with mortality around 50%, donkey with mortality rate 5-10%.

The result indicated that the highest seroprevalence was recorded in Jeldu (59.9%) followed by Ejere (49 %), Woliso (36.6 %) and Wonchi district (35.7 %). The variations in seroprevalence of AHS at different site of studies were significant ($p < 0.05$). Jeldu, Ejere and Woliso are mid highlands and Wonchi district is relatively more highland with respect to the other study areas. The strength of association between seropositivity and potential risk factors indicated that types of agro-ecology contributed for the occurrence of AHS. This coincides with the report of (Kassa 2006, Personal communication) who said that seropositivity increases as one goes from highland to midland and lowland areas. The present study areas consisted of permanent water bodies (like lake), river irrigation, wide spread tree holes which are favorable for insect survival. As per Radostitis *et al.* (2007) and Demissie (2013) endemic areas are more likely to be low lying, warm and marsh regions that create favorable environment for multiplication of *Culicoids* and mechanical vectors.

In the present study the seroprevalence of AHSV was determined in the two sex groups of equidae. A seroprevalence of 51.5 % in female and 43.9 % in male was found. There was no significant difference ($p > 0.05$) in the occurrence of AHS between male and female. This is well supported by the finding of (Kassa 2006, Personal communication) who found no significant variation in seropositivity in the two sex groups; rather they are equally affected by the AHS.

In the present study, the seroprevalence of AHSV in different age groups of equids were assessed. The finding showed no significant variation ($p > 0.05$) in seropositivity among the different age groups of equids. This might be due to the fact that feeding system of insects those transmit AHSV is not selective feeding among the age of equids. This finding is supported by Molalegne *et al.* (2010) and Keith (2005) in that all foals that have lost their maternal antibody by six months of age would be protected by vaccination. Keith (2005) further described that different age groups of equids that are above six months of age had equally seroconverted and were protected after they were being vaccinated. From the findings of Keith (2005) one can infer that all age groups of equidae seem to be equally affected by AHS provided that the equines were not previously exposed, recovered or vaccinated.

The present finding revealed that AHS occurred following long and short rainy season with the increment of number of insects and movement of horses from one agro- ecology to the other based on retrospective study or questionnaires survey about this disease. The reason could be that rainy season facilitates multiplication of insects. This agrees with Mulualem *et al.* (2012) claimed that multiplications of insects is influenced by temperatures and weather conditions of the environment.

As majority of respondents expressed equine (horse) which took vaccine died shortly after vaccination or after two months of vaccination. From these ideas it can be inferred that there was vaccine failure which could be due to poor administration, variation in serotype and relapse of diseases after vaccination of equine (horses) with specific serotype of AHSV. The above finding clearly indicates that there is lack of knowledge of equine owners. This knowledge scarcity created conducive environment to the disease and contributed to the high prevalence of the disease observed in the present sero-survey. This conforms to the observation of Mulualem *et al.* (2012).

Therefore, from the finding of the present study and contextual comparison of the present findings with previous survey results of other authors in different parts of Ethiopia, it can be inferred that AHS exists in all agro ecological zones of the present study areas (midland and highland) even though lowland was not included in the study.

CONCLUSION

The present study found that African Horse sickness was highly prevalent in equines at central highland of Ethiopia. The greatest risk of this disease was also associated with weather condition and agro-ecology of the environments. The disease is equally likely to affect all age groups of equids; hence there was no significant variation in seropositivity among the different age categories of equids. Moreover, both sexes are equally affected by African horse sickness. The highest seroprevalence was observed in the midland followed by highland areas. Generally, the disease has been observed in all agro-ecological zones of the study area and affected horses, donkeys and mules in the study areas. It was found that equine owners of the study area had poor knowledge about the disease. It is therefore recommended to intensively create awareness among the community about the disease and design appropriate control measures suitable for the agro-ecology. Further studies should be made by an integrated approach of serotyping and identification of the clucoides vectors as well as other potential vectors.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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