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Full Length Research Paper

Seroprevalence and risk factors of African horse sickness in mules and donkeys in selected sites of West Amhara Region, Ethiopia

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The study was conducted with the aims of determining the seroprevalence and risk factors of African Horse Sickness (AHS) in mules and donkeys in selected sites of West Amhara region. A total of 390 (191 mules and 199 donkeys) serum samples were collected from November, 2009 to February, 2010. Blocking ELISA was employed to determine the presence of African Horse Sickness Virus (AHSV) antibodies. The overall seroprevalence of AHS in this study was found to be 57.4% (95% CI = 52.35 to 62.39%). The prevalence of AHS was found to be lower in mules (55.5%) than donkeys (59.3%). Statistical analysis of the data showed that there were no significant variation (P>0.05) in the prevalence of AHS between the two species of animals examined in this study. The seroprevalence of the disease was higher in mules and donkeys >5 years old when compared to those <5 years old. But there was no significant variation (P> 0.05). Males were found to be affected by AHS more than female animals do but there were no significant difference (P>0.05) between the two sexes of mules and donkeys examined. The result showed higher seroprevalence of AHS in lowland than the midland agro ecological zones of the study area. Analysis of the data showed statistically significant (P<0.05) variation of AHS between the two agro ecological zones. Of the 100 equine owners interviewed about AHS 25 (25%) were familiar and 75 individuals (75%) of the respondents know nothing regarding the disease AHS. Hence, the knowledge of equine owners about AHS was assessed to be at infancy. This suggests the need to implement strict awareness creation among equine owners on vaccination of their animals and methods of insect control in the study area in order to decrease the prevalence of AHS.

Key words: African horse sickness, mules and donkeys, West Amhara Region, seroprevalence.

INTRODUCTION

Ethiopia has the largest equine population, probably with the highest density per square kilo-meter in the world and it has a total of 6.9% of the World's and 42.4% of Africa's equine population. Moreover it has 65% of all African mules, almost 50% of horses and 30% of donkeys (Alemayehu, 2004). Horses, mules and donkeys represent a significant share of the working animal population of a number of countries in Africa (Feseha, 1998). According to the Central Statistical Authority of Ethiopia there are about 5.42 million donkeys, 1.78 million horses and 373 519 mules in Ethiopia (CSA, 2009).

Equines play an important role in the transport of farm produce, fodder, firewood, agricultural inputs and construction materials. They provide the best alternative in places where the road network is poor or the terrain is rugged and mountainous, and in cities where narrow streets prevent easy delivery of merchandise. However, many factors contribute to the poor performance of

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Table 1. Geo reference data base of the study areas.

Study areas	Zone	Elevation (m)	Latitude (N)	Longitude (E)
Bahir Dar	West Gojjam	1770	12.775	36.414
Metema	North Gondar	790	11.59	37.388
Dangla	Awi	2116	11.434	36.979

Table 2. Number of sampled mules and donkeys in different age categories.

Category	Age in years	Sampled equidae		
		Mules	Donkeys	Total
1	< 5	10	32	42
2	≥ 5	181	167	348

equines, among which the viral disease, African Horse Sickness (AHS) is incriminated as the leading cause of morbidity and mortality in these animals (Williams and Masaga, 1998).

African horse sickness is a serious, often fatal, arthropod-borne viral disease of horses, donkeys, zebras and mules. The mortality rate can be as high as 95% in some forms of this disease. Asymptomatic or mild infections can occur in horses, as well as zebras and donkeys, previously infected with a different serotype of the virus. Infected animals or vectors may carry the virus into AHS-free regions. The hosts in order of decreasing severity of AHS are horses, mules, donkeys and zebras. Horses and mules have the highest mortality, donkeys have a lower mortality, and African donkeys have a subclinical infection. The horse is an amplifier of African horse sickness virus (AHSV) and source of virus for arthropods (Mellor et al., 1998).

African horse sickness results from infection with the AHSV, a member of the genus *Orbivirus* in the family Reoviridae. African Horse Sickness Virus is a double stranded RNA virus, which causes a non contagious, but infectious arthropod borne disease of equines and occasionally dogs. There are 9 serotypes of AHSV. No cross-reactions with other known Orbiviruses have been observed (Radostitis et al., 2007).

All serotypes of AHSV occur in eastern and southern Africa; this distribution reflects that zebra cycles the virus asymptomatically. At present AHSV is endemic in tropical and subtropical areas of Africa, South of the Sahara occupying a broad band stretching from Senegal in the West to Ethiopia, Kenya and Somalia in the East, and extending to South Africa (Mellor et al., 1998). In countries such as Ethiopia where large population of equines are raised, the presence of multiple serotypes of such a devastating virus poses a serious hindrance to the national development. Therefore, this study was aimed to determine the seroprevalence of AHS in mules and donkeys and identify the risk factors associated with AHS.

MATERIALS AND METHODS

Description of the study areas

The study was conducted in three districts of Western part of Amhara national Regional State. Specific study sites encompassed include Bahir Dar town, Dangilla and Metema districts. The altitude of the study areas ranges from 790 m.a.s.l. in Metema to 2116 m.a.s.l. at Dangilla. The rain fall in the study areas is bimodal in distribution and falls in the range of 1288.7 mm in Bahir Dar to 1909 mm in Dangilla per annum. There are long and short rainy seasons extending from June to September and February to March, respectively. The minimum and maximum air temperatures of the study areas are 9 to 23.5℃, 12.4 to 26.8℃ and 19.6 to 35.7℃ for Dangilla, Bahir Dar, and Metema, respectively. About 70% of the total land of Bahir Dar town and its surrounding is featured by plain plateaus with some patches of sloppiness, drained by Abay River and marked at some portions of the land by Lake Tana. Metema even though looks like desert; has a water reservoir around the study area. The geo-reference and equine population of the study areas are indicated as follows (Table 1).

Study population

The study population were the 1816 mules and 39 500 donkeys which were found in the study areas. Horses were excluded from the study due to the reason that they were available only in one of the study areas (Dangilla) and they were already vaccinated at the time of sampling. Both sexes of mules and donkeys above six months of age with no previous history of vaccination against AHS were sampled (Table 2).

Study design

The study was a cross-sectional study. Data on the potential risk factors associated with the occurrence of AHS were collected during sampling through recording and questionnaire. Serum and data collection was conducted from November, 2009 to February, 2010.

Sampling method

Equines were taken as the primary units of the sampling. 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.

Sample size determination

To calculate the total sample size, the following parameters were used: 95% level of confidence (CL), 5% desired level of precision and with the assumption of 50% expected prevalence of AHS, the sample sizes were determined using the formula given in Thrustfield (2005).

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

n = required sample size, Pexp = expected prevalence, d = desired absolute precision

Using the above formula the sample size was calculated to be 384 but a total of 390 serum sample comprising 191 mules and 199 donkeys were collected. Sample size greater than determined by the formula was used to improve precision estimates of the study.

Study methodology

Seroprevalence survey

For the seroprevalence survey, serum was collected from 390 equines (191 mules and 199 donkeys). To get serum whole blood of 7 to 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 in to another tube and labeled. The samples were kept at -20℃ until tes ted with Blocking ELISA. Area of sampling, age sex, type of equidae (mule, donkey), date of collection, agro ecology (lowland or midland) and identification number was recorded at the time of sampling.

Blocking ELISA test was conducted at the immunology laboratory of National Veterinary Institute (NVI), Debre Zeit, Ethiopia (1983). The antigen was fixed in a solid support (polystyrene plate). After incubation with sera samples, an AHSV specific monoclonal antibody (Mab peroxidase conjugated) was added. If the sample contains antibodies specific of the virus, they will not allow the binding of the labeled Mab to the antigen whereas if it does not contain specific antibodies, the Mab will bind to the antigen coating the plate.

After washing the plate to eliminate all non-fixed material, presence or absence of labeled Mab can be detected by adding the substrate which, in presence of the peroxidase, will develop a colorimetric reaction. If there has been color development, the conjugate has bound to the antigen, being the sample negative. On the other hand, if there are antibodies specific (Abs) of AHSV in the sample, they will block the binding of conjugate and there will not be color development. The antigen used in this kit was VP7 recombinant protein from the AHSV (serotype 4) obtained using the baculovirus expression system. The use of serotype 4 confers many advantages to the assay. From the safety point of view, it is due to the total absence of infectivity and from the accuracy point of view, VP7 is one of the major proteins from AHSV, as well as the most antigenic one and the most conserved with in the 9 different serotypes. Besides to this it has not been found any infected or vaccinated animal without antibodies to this protein.

Test procedure used to identify AHSV antibodies in our study was as shown below:

1. All reagents were brought to room temperature before use.
2. 100 µl of diluted sample was dispensed in to appropriate wells (dilution1/5). 100 ml of positive control was dispensed into two wells and 100 ml of negative control was dispensed into two wells. The plates were covered and incubated for 1 h at 37℃
3. Washed 5 times
4. 100 µl of conjugate per well was added and incubated for 30 min at 37℃.
5. Washed 5 times
6. 100 µl of substrate solution in each well was dispensed using a multichannel pipette and incubated for 10 min at room temperature.
7. 100 µl of stop solution was dispensed to each well.
8. Read at 405 nm.

Validation criteria

The optical density (OD) of the positive control must be lower than 0.2 and the OD of the Negative control must be higher than1.0. Samples showing Blocking percentage (BP) value lower than 45% were considered negative for antibodies to AHSV where as samples showing BP value higher than 50% were considered as positive to antibodies to AHSV. Samples with BP value between 45% and 50% were considered doubtful and were retested.

Interpretation:

Blocking percentage of each sample was determined using the following formula;

$$BP = \frac{Abs\ (control\text{-})\text{-}Abs\ (sample)}{Abs\ (control\text{-})\text{-}Abs\ (control+)} \times 100$$

Example: Average Abs Positive control=0.110; Average Abs Negative control=1.859; Abs sample=0.865.

$$BP = \frac{1.859-0.865}{1.859-0.110} \times 100 = 56.8 \text{ sample is positive}$$

Questionnaire survey

A questionnaire format that was aimed at assessing the potential risk factors such as management practices (stabling condition and vaccination history), presence of biting insects, availability of water bodies near the study areas and the knowledge of equine owners about AHS was prepared to interview individual owners of mules and donkeys. A total of 40 equine owners, 20 from Bahir Dar 15 from Dangla and 5 from Metema were interviewed.

Data management and statistical analysis

Data recorded during sampling and laboratory findings were entered and stored in MS-Excel. The data were thoroughly screened for errors and properly coded before subjected to statistical analysis. The data was statistically analyzed using Pearson chi-square to establish association between serological results and different risk factors considered in the study, Intercooled Stata 7.0 (Stata corporation 1985 to 2001) was used for the statistical analysis.

Table 3. Seroprevalence of AHS in different study areas.

Study areas	Samples tested	Samples positive	Seroprevalence (%)
Metema	60	42	70
Bahir Dar	230	130	56.52
Dangla	100	52	52
Total	390	224	57.4

Table 4. Seroprevalence of AHS in donkeys and mules.

Species	Examined	Positive	Seroprevalence (%)	χ^2- value	p-value
Donkey	199	118	59.3	0.5754	0.448
Mules	191	106	55.5		
Total	390	224	57.4		

Table 5. Seroprevalence of AHSV in two age categories of equidae.

Age (years)	Examined	Positive	Seroprevalence (%)	χ^2- value	p-value
< 5	42	22	52.40	0.4920	0.483
>5	348	202	62.4		
Total	390	224	57.40		

Table 6. Seroprevalence of AHS between sexes of equidae

Sex	Examined	Positive	Seroprevalence (%)	χ^2- value	p-value
Male	293	169	57.67	0.0285	0.866
Female	97	55	56.70		
Total	390	224	57.40		

RESULTS

Seroprevalence survey

Out of 390 serum samples collected and tested for AHSV antibodies by Blocking ELISA, 224 were found positive giving a seroprevalence of 57.4% (95% CI = 52.35% to 62.39%) Table 3.

The seroprevalence of AHS was found to be lower in mules (55.5%) than donkeys (59.3%). Statistical analysis of the data showed that there were no significant variation (P>0.05) in the prevalence of AHS between the two species of animals examined in this study (Table 4).

The seroprevalence of the disease was higher in mules and donkeys >5 years old when compared to those <5 years old. But there was no significant variation (P> 0.05) in the seroprevalence of AHS between age groups (Table 5).

The seroprevalence of AHS was found to be higher in male mules and donkeys than female mules and donkeys. Comparision of seroprevalence between sexes showed no significant difference (P>0.05) between the two sexes of mules and donkeys examined (Table 6).

The result proved higher seroprevalence of AHS in lowland than the midland agro ecological zones of the study area. Analysis of the data showed statistically significant (P<0.05) variation of AHS between the two agro ecological zones (Table 7).

Questionnaire survey

A total of 100 equine owners were interviewed regarding the disease AHS. 25 (25%) of them replied that they knew the disease; 75 individuals (75%) of the respondents know nothing regarding the disease. Respondents which were familiar with AHS put the order of equines susceptibility in the way that horses are the

Table 1. Seroprevalence of AHS in different agro ecological zones of the study area.

Agro ecology	Examined	Positive	Seroprevalence (%)	χ^2- value	p-value
Lowland	60	42	70	4.5785	0.032
Midland	330	182	55		
Total	390	224	57.40		

first to be affected followed by mules while donkeys are relatively resistant. They also suggested that both sexes are equally affected and most of them said that adults are more affected. Furthermore, some owners replied that the disease is common in areas of low elevations than in high elevated areas. Respondents failed to explain the mechanism of transmission of the disease, except some, who proposed transmission could be mainly by close contact among equines and by biting flies.

Many of the owners said that there are insects in their localities called by different local names challenging their mules and donkeys. They said that flies were abundant in the months of September to November. All of the respondents were however, unaware of *culicoides* and their role as vectors and even some owners said that it is due to hotness of the environment that animals become diseased.

In relation to housing management practices of equines, most owners replied that stabling their equines is a common practice during the night so as to protect them from predators wild animals. In Bahir Dar town and Dangilla, most equines are found stabled in round fenced compound without roof and some mules are found tethered. All Donkeys of Metema unlike to the above areas are tethered during night time in the absence of stable.

In regard to environmental influences, respondents in Bahir Dar reported that there were permanent water bodies such as Lake Tana and river Abay surrounding them making their surrounding environment swampy. There were water points and water reservoirs in Dangilla and Metema. Dwellers of Metema informed that there is continuous immigration of donkeys from different neighboring districts for trading purpose.

DISCUSSION

Seroprevalence survey

The study conducted in selected sites of western part of Amhara national regional state indicated an overall seroprevalence of African Horse Sickness to be 57.4%. The seroprevalence of the disease was 55.5% in mules and 59.3% in donkeys. The high prevalence seen in the study is an indication of active circulation of the virus in the study area. Similarly high seropervalences were recorded by other studies done in other parts of Ethiopia (Demssie, 2006).

A relatively higher seroprevalence was observed in donkeys as compared to mules but the difference was not statistically insignificant (P>0.05). Previous studies in Ethiopia including in Amhara region reported a higher prevalence in donkeys as compared to mules (Keith, 2005). According to OIE (2004) and Radostits et al. (2007) among equidae, horses are the most susceptible to AHS with a mortality rate of 50 to 95% followed by mules with mortality around 50% and donkeys are affected by AHS with the mildest form, which is frequently overlooked in natural outbreaks. From these facts, it can be inferred that, there is high chance of recovery in donkeys from infection due to AHS unlike that of horses and mules. Demissie (2006) during active disease search and sero-survey indicated that, getting cases of seropositive recovered mules after being exposed to natural challenges is rare. The higher seroprevalence observed in donkeys in this study is in line with the above argument that seroprevalence in donkeys is higher due to less fatality by the infection.

In our study there was no significant variation (P>0.05) in seropositivity between the age groups of equidae. Seroprevalence of 52.4% in young (< 5 years) and 58.04% in older age groups ($\geq$5 years) was found. This finding is supported by Keith (2005) who reported that, all foals that had lost their maternal antibody by six month of age and different age groups of equidae that are above six month of age had equally seroconverted. From this study and Keith's survey results, it can be inferred that all age groups of mules and donkeys seem likely to be equally affected by AHS provided that the equines were not previously exposed and recovered or vaccinated. Demissie (2006) also found that there was no significant variation in seropositivity among different age categories.

A seroprevalence of 56.7% in female and 57.7% in male was found but this difference was statistically not significant (P=.0.866). Field experience conducted by Demssie (2006) also proved that both sex groups are equally likely to be affected by African Horse Sickness.

Statistically significant higher seropositivity of AHS was obtained in the lowland as compared to midland (P = 0.032). According to NVI (1983), the distribution of the disease seems to have positive correlation with the ecology of its vectors. Furthermore, the disease is considered to be endemic to the lowlands and midlands

of Ethiopia. However, some cases of the disease are known to appear in surveyed highlands of Ethiopia. According to Radostits et al. (2007) the breeding status and movement of vectors is governed by climatic conditions. They further described that *culicoides* have almost worldwide distribution that makes spread of AHS universal. Mellor et al. (1998) described that increased use of irrigation, water lakes, manure, urine, dung pats, tree holes, rotten vegetation, stagnant surface water are ideal larval habitats for the multiplication of *culicoides*. In the present study, the prevalence of AHS in increasing order was found in Dangla, Bahir Dar and Metema. This indicates that the prevalence was higher in lowland (Metema) as compared to the midland (Bahir Dar and Dangla). Within the midland, seropositivity is higher in Bahir Dar than Dangla. This could be because of the environmental variation in which Lake Tana and river Abay which are surrounding Bahir Dar create conducive environment to the multiplication of the vector as compared to that of Dangla.

Questionnaire survey

From the questionnaire survey, the majority of the owners were found to have no experience of the disease. However, those owners which have closer relation with equines (cart mules and pack donkeys) were able to recognize the disease more than those with less contact to equines. They described horses to be most affected, mules were the second most susceptible and donkeys were seen rarely with the disease.

It was determined that both sexes of equines were equally affected by AHS. The disease was quite frequently seen in lowlands than higher elevations. These responses from owners are consistent with the results of seroprevalence survey. None of the respondents were able to describe the mechanism of transmission of the disease among animals.

Equine biting flies are very common mainly in the lowland than midland. Equine biting flies are called by different names in the study areas. “Ewir zinb”, “tingn” and “yemeskerem zinb” in Bahir Dar and Dangla while in Metema “bowda” was the name for the midge. In the assessment made to know the knowledge base of equine owners about *culicoides* vectors, none of the respondents were able to know these vectors and the role they play as well rather, they are totally unaware of them. It was conclusively described that flies are abundant immediately after the rainy season, because the climate is conducive for their multiplication. Equines in the study areas are either stabled but allowing entrance of insects or they are simply tethered without stabling.

Bahir Dar is located close to permanent water bodies such as Lake Tana and river Abay; even in Metema and Dangla water points and water reservoirs supporting insect breeding were not uncommon.

In general, the poor knowledge of equine owners regarding their management and environmental influences creating conducive environment to the disease could contribute to the high prevalence of the disease observed in the present sero-survey.

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