

Serological survey of African horse sickness in selected districts of Jimma zone, Southwestern Ethiopia

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Abstract A cross-sectional serological survey was undertaken in selected districts of different agro-ecology of Jimma zone (Dedo, Yebu, Seka, Serbo, and Jimma town) from November 2009 to February 2010 to determine the seroprevalence of African horse sickness virus and associated risk factors of the disease. Two hundred seventy-four equids (189 horses, 43 mules, and 47 donkeys) with a history of non-vaccination for at least 2 years were selected randomly from the above areas. Sera samples were collected and assayed for the presence of specific antibody against African horse sickness virus using blocking ELISA. An overall seroprevalence of 89 (32.5%) was found and it was 24 (51.1%) for donkeys, 13 (30.2%) for mules, and 52(28.3%) for horses. Seroprevalence was significantly ($X^2=11.05$, $P<0.05$) different among the different species of equids. Seroprevalence was also significantly ($X^2=11.43$, $P<0.05$) different among the different agro-ecological areas being higher in highlands 47 (40.5%) followed by midland 30 (34.5%) and lowland 12 (16.9%). Age and sex were not significantly ($X^2=3.15$, $P>0.05$ and $X^2=3.38$, $P>0.05$, respectively) associated with seroprevalence of AHSV. The present study showed that African horse sickness (AHS) is highly prevalent disease for the horses followed by mules and then donkeys in Jimma zone explained by lower seroconversion rate.

Therefore, control strategy against AHS should target at high risk species of all age and sex in their locality in the initial stage for better containment of the disease.

Keywords African horse sickness · B-ELISA · Equines · Seroprevalence · Southwestern Ethiopia

Introduction

According to FAO (2003), there are 115.2 million domestic equidae (comprising horses, donkeys, and mules) in the world of which 44.3 million are donkeys; 57.6 million are horses and 13.3 million mules. Ethiopia has 5.2 million donkeys, 2.8 millions horses, and 0.6 million mules (FAO 2003). Ethiopia has the largest equine population probably with the highest density per square kilometer in world (Alemayehu and Benti 2009), and it has a total of 6.9% of the world's and 42.4% of the African equine population. Moreover, it has 65% of all African mules, 50% of horse, and 30% of donkeys. Horse, donkeys, and mules represent a significant share of working animal population of a number of countries in Africa (Alemayehu and Benti 2009). According to the Central Statistical Authority of Ethiopia (CSA) (2009), there are 5.42 million donkeys, 1.78 million horse, and 373,519 mules in Ethiopia. An important but often overlooked aspect is that in most cases, donkeys and mules reared by landless and marginal farmers are the means of subsistence for millions in the least privileged parts of the world. These beasts of burden receive little care and are subjected to intensive work throughout their lives. Their life expectancy is short, their work output is low, and the reproductive performance is poor (Alemayehu and Benti 2009).

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Equidae play an important role in the transportation of farm products, fodder, firewood, agricultural inputs, construction, and waste materials. Equine power is used both in rural and urban transport system which is cheap and viable (Alemayehu and Benti 2009). However, many factors contribute to the poor performance of equidae. Among the most important ones are nutritional disorders, bacterial, fungal, and viral diseases could be listed. Viral disease like African horse sickness (AHS) characterized by high morbidity and mortality rates is worth enough to be mentioned. Hence, it is one of the viral diseases characterized by up to 95%, 50%, and 10% mortality rate in horse, mule, and donkey, respectively (Office International Des Epizootics OIE 2008; Maclachlan and Guthrie 2010).

AHS is an infectious but non-contagious viral disease affecting all species of equidae caused by an *Orbivirus* of the family *Reoviridae* and characterized by alterations in the respiratory and circulatory functions (Office International Des Epizootics OIE 2008; Maclachlan and Guthrie 2010). AHS is transmitted by at least two species of *Culicoides*. Nine different serotypes have been described. Although the clinical signs and lesions are characteristic, it can be confused with those of other equine diseases. As a viral disease, detection of AHS virus is based on the identification of infectious virus, virus nucleic acid, viral antigens, or specific antibodies (Radostits et al. 2007; Office International Des Epizootics OIE 2008; Wilson et al. 2009; Maclachlan and Guthrie 2010).

It is necessary to examine the status and impact of the disease and existing control measures with a view to improve them and attain better control strategies (Venter et al. 2006). The study conducted by Keith (2005) and Kassa (2006) in some parts of Ethiopia indicated that AHS exists in all age group, both sexes and all agro-ecology zones of the surveyed areas. However, little is known about the status of the disease in the present survey area, south west of Ethiopia. This was the rationale that initiated this study.

Therefore, the objectives of the present study were to determine the seroprevalence of African horse sickness in different agro-ecological zones of the survey area in horse, mule and donkey and to identify the potential risk factors associated with the disease.

Materials and methods

The study area

The study was conducted at selected district of Jimma zone which is located at south western part of Ethiopia. Seka, Yebu, Serbo, Jimma town, and Dedo were districts of Jimma zone selected for this study due to their variation in

altitude. Areas with elevation of 1,500 m above sea level (masl) or less were taken as lowland, between 1,500 and 2,500 masl as midland and over 2,500 masl as high land (Keith 2005).

The altitude in the study areas ranges from 800 to 2881 masl. The lowest altitude measure in Dedo while the highest is from Seka. The rainfall in the study areas is bimodal in distribution and within the in range of 1,400–1,700 mm per annum. There are long and short rainy seasons extending from June to September and from February to March respectively. The minimum, maximum, and average air temperature of the study areas are 14°C, 28°C, and 21°C respectively.

Target population

The equine population of Jimma zone is 74,574 horses, 28,371 mules, and 49,489 donkeys (Central Statistical Authority CSA 2009). Domestic equids above 6 months of age and both sexes with a history of non-vaccination were sampled. The age of equine was determined according to Mair et al. (2002) using dentition and wearing patterns of incisor teeth and by asking the animal owners.

Sampling technique

Purposive sampling method was employed to select five districts from the existing 17 districts of Jimma zone. Then individual animals were selected randomly from the nominated districts.

Sample size determination

The sample size was determined by assuming the expected prevalence of 41.4% from the previous study by Hyera and Baipoledi (2008) in Botswana since there is no published report about the seroprevalence of African horse sickness virus (AHSV) in Ethiopia. Accordingly, the sample size of equine sampled was 274 based on the formula given by Thrusfield (2005); of these, 67.1% were horses, 17.1% were donkeys, and 15.6% were mules.

Study design

Cross-sectional study design was carried out to determine the prevalence of AHS antibody and its associated risk factors in selected districts of Jimma zone, south west of Ethiopia.

Study methodology

Four to 8 ml of blood was collected by venipuncture using sterile venoject needles and plain vacutainer tubes. Each

sample was labeled with identification number. The blood was allowed to clot overnight at +4°C. Then serum was harvested and kept at -20°C until tested. The blocking ELISA (B-ELISA) kit (Ingezim AHSV Compac plus, Spain) which is African horse sickness virus serotype non-specific was used to test the serum collected. The test plate was coated with an antigen of VP7 (viral protein 7) which is the dominant and highly conserved sequence region among the nine serotypes of the virus. This antigen was recombinant protein from the African horse sickness virus. Area of sampling, identification number, date of collection, and risk factors such as age, sex, species of equid (donkey, horse, and mule) and agro-ecology were recorded at the time of sampling (Office International Des Epizootics OIE 2008).

Data management and analysis

Data collected during sampling and laboratory analysis were entered and stored in MS Excel spreadsheet. Data were thoroughly scrutinized for errors and proper coding before subjected to statistical analysis and analyzed using SPSS soft ware version 16.0.

Result

Out of 274 equids examined, 89 (32.5%) were found serologically positive for AHSV using B-ELISA. Seroprevalence was significantly ($P<0.05$) higher in donkeys than in mules and horses. There is statistical variation between species of equids ($P<0.05$; Table 1).

The seroprevalence of AHSV was variable with the variation in the study area. It was found that the highest prevalence of AHSV was in Dedo followed by Jimma town and Yebu. The difference in seroprevalence of AHSV with the variation in the study area was found to be statistically significant ($P<0.05$; Table 2)

The study indicated that 8–14 years of age equids were seropositive in the selected study areas. However, this difference in the seroprevalence of AHSV due to age variation was not statistically significant ($P>0.05$; Table 3).

Table 1 Seroprevalence of AHSV in three species of equids

Species	Total tested	Number of positive (%)	(χ^2) (P value)
Horse	184	52 (28.3)	8.949 (0.011)*
Mule	43	13 (30.2)	
Donkey	47	24 (51.1)	
Total	274	89 (32.5)	

*Significant ($P<0.05$)

Table 2 Seroprevalence of AHSV in equids at selected districts of Jimma zone

Study area	Total tested	Positive (%)	χ^2 (P value)
Jimma town	32	13 (40.6)	11.05 (0.026)*
Serbo	60	15 (25.0)	
Seka	45	8 (17.8)	
Yebu	84	29 (34.5)	
Dedo	53	24 (45.3)	
Total	274	89 (32.5)	

*Significant ($P<0.05$)

The study also examined the impact of agro-ecology and sex as risk factors for the seroprevalence of AHSV and the result revealed that highest prevalence was recorded in highland areas than midland and lowland and the difference was statistically significant ($P<0.05$). On the same fashion, the seroprevalence of AHSV was higher in males than in females. However, the variation was not statistically significant ($P>0.05$; Table 4).

Discussion

The overall seroprevalence of AHSV-specific antibodies in equids in the study area was found to be 32.5%. Species-related seroprevalence has significant difference. It measured 51.1% in donkeys, 30.2% in mules, and 28.3% in horses. Donkeys have high seroprevalence followed by mules and horses, and this might be due to low mortality rate of donkeys as compared to the deadly effect of this disease to the horses and mules. Office International Des Epizootics (OIE) (2008) and Maclachlan and Guthrie (2010) described that donkeys are affected by horse sickness fever, the mildest form, which is frequently overlooked in natural outbreaks. This finding is in line with the cohort study conducted among the donkey and mule population of Amhara and Tigray regions by Keith (2005). He confirmed that AHS was not a significant cause of mortality in donkeys, but it was a significant cause of mortality in mules and horses. It is also in agreement with the report of Venter et al. (2006) and Office International

Table 3 Seroprevalence of AHSV in equids of different age group

Age in years	Total tested	Positives (%)	χ^2 (P value)
1–7	134	38 (28.4%)	3.15 (0.207)
8–14	134	50 (37.3%)	
>15	6	1 (16.5%)	
Total	274	89 (32.5%)	

Table 4 Seroprevalence of AHSV in different agro-ecological zone and sex

Risk factor	Total tested	Positives (%)	χ^2 (<i>P</i> value)
Agro-ecological zone			
High land	116	47 (40.5)	11.43 (0.003) *
Midland	87	30 (34.5)	
Lowland	71	12 (16.9%)	
Total	274	89 (32.5%)	
Sex			
Female	117	31 (26.5%)	3.38 (0.068)
Male	157	58 (36.9%)	
Total	274	89 (32.5)	

*Significant ($P < 0.05$)

Des Epizootics (OIE) (2008) of which AHSV causes disease in horse, mule, and donkey with up to 95% mortality in susceptible horse population. Office International Des Epizootics (OIE) (2008) and Wilson et al. (2009) also reported that among equidae horses are the most susceptible to AHSV with a mortality rate of 50–95%.

The highest seropositivity was obtained in high land followed by midland and lowland in case of agro-ecology. The difference in seroprevalence of AHSV at different sites of the study area was statistically significant. The study by Kassa (2006) indicated that seropositivity increases as one goes from high land to lowland. This contradicts to the present findings, but the study area of Kassa (2006) was in the lowlands near permanent water bodies such as lakes, river irrigations, canals, watering points, and water reservoir that support insect breeding and multiplications. Nevertheless, the present study area is in the high land which has wide spread tree holes, swampy, and rotting vegetations which is more favorable for insect breeding and multiplications. The study conducted by Kassa (2006) indicated that AHSV infects equines in different agro-ecological area if and only if the area is favorable to multiplication and survival of vectors. The report by Radostits et al. (2007) and Wilson et al. (2009) also indicates that endemic areas are more likely to be in low-lying, warm, and marsh regions that create favorable environment for multiplication of culicoides and mechanical vectors.

Radostits et al. (2007) and Wilson et al. (2009) revealed the breeding status and movement of vectors is governed by climatic condition. These authors further described that culicoides have almost worldwide distribution, so that spread of AHS is universal. Mellor et al. (1998) describes that increase use of irrigation, water lakes, manure, urine, dung pats, tree holes, rotting vegetations, and stagnant water surface are ideal larval habitats for the multiplication of culicoids.

Keith (2005) explained that it is environment rather than species or husbandry that is relevant to seroconversion. According to Kassa (2006), AHSV exists in all agro-ecological zones and it is consistent to the present findings.

In this study, the seroprevalence of AHSV in different age groups and sexes of equids were assessed. No significant variation in seropositivity among the different age groups and sexes of equine were found. This finding is consistent with Keith (2005), Messeret (2007), and Tsegabirhan (2007, unpublished thesis) in that all foals (both sexes) that have lost their maternal antibodies by 6 months of age would be protected by vaccination. It was further described that different age group of equids that are above 6 months of age had equally seroconverted and protected after they were being vaccinated. From the finding of Keith (2005) and Kassa (2006), it can be concluded that all age group and both sexes of equids seem to be equally affected by AHSV provided that the equids were not previously exposed and recovered as well as vaccinated.

Conclusions and recommendations

The present study showed that there was high seroprevalence of AHS in the survey area during the study period. It also showed that agro-ecology and species could be taken as risk factors in the seroprevalence of AHS. The highest seropositivity was obtained in high land followed by midland and lowland in case of agro-ecology. Donkeys showed the highest seroprevalence followed by mules. However, the horses were less seropositive that could be due to the seropositive horses were dying as the result of the virulent nature of the virus in the survey area. Nevertheless, age and sex categories were not a significant factor to precipitate AHS. From the result obtained in the present survey and considering the reality of Jimma zone the following relevant points are recommended to alleviate the problem.

- Studies should be undertaken to differentiate which virus serotype is prevailing in the survey area.
- There should be annual vaccination programs for horses, mules, and donkeys in their order of importance at different agro-ecological zone with appropriate serotype vaccine, unless it will have devastating impact in the horses of the survey area.
- The epidemiology and biology of culicoids and other potential vectors should be studied in the survey area.

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