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A cross-sectional sero-survey of some infectious diseases of working equids in Central Ethiopia

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Despite the large numbers of working equids and their significant contribution towards communities as well as the national economy, the attention given to study the health and welfare problems of working equids in Ethiopia is quite minimal. The main objective of this study was to investigate the sero-prevalence of some important infectious diseases infecting working equids and asses their spatial distribution in the different agro-ecological zones of Ethiopia. Sera collected from 1007 equids selected by simple random sampling technique were tested using the Office Internationale des Epizootic (OIE) approved serological tests. The overall sero-prevalence for all equids were 10.5% (n=288) for African horse sickness (AHS), 0.7% (n=997) for Dourine, 3.8% (n=982) for Glanders, 0.1% (n=1002) for equine infectious anaemia (EIA), 13.5% (n=208) for equine herpes virus 1 (EHV-1), 88% (n=208) for equine herpes virus 4 (EHV-4) and 65% (n=20) for Piroplasmosis. Significant interspecies (P=0.001) and spatial (P=0.01) variations were observed for AHS, Glanders, Dourine and EHV-1. However, the age and sex of the animals had no significant effect on the prevalence of the tested diseases. Infection of equids with more than one infectious disease was diagnosed. Out of the 208 equids tested for herpes, 11.1% were sero-positive for both EHV-1 and EHV-4. Co-infections of AHS and EHV-1 (1%), AHS and EHV-4 (4%), Glanders and EHV-1 (2%) and Glander and EHV-4 (7.7%) were also observed. This study has shown not only the high prevalence of some of the infectious diseases in the equine population but their wide distribution across the different agro-ecological zones of the central regions of Ethiopia.

Key words: Working equids, infectious diseases, epidemiology, sero-prevalence, Ethiopia.

INTRODUCTION

The world equine population is estimated at 44 million donkeys, 11 million mules and 59 million horses (FAOSTAT, 2012). More than 97% of the world's donkey and mule populations, and over 72% of the world's horse population is found in developing countries specifically kept for draft purpose. These vast numbers of working

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Table 1. Number of equids examined from the different agro-ecological zones in central regions of Ethiopia.

Agro-ecological zone	Altitude range*	No. of equids examined			Total
		Donkeys	Mules	Horses	
Highlands (Dega)	2400-2850	347	40	258	645
Midlands (Weyna Dega)	1500-2400	197	11	2	210
Lowlands (Kola)	≤1500	123	0	29	152
Total		667	51	289	1007

*Meters above seas level.

equids play crucial roles in both urban and rural areas, providing agricultural energy and transport, and in many cases, the sole means of generating income for their resource-limited owners (Fielding and Pearson, 1991; Swaan, 2006). Ethiopia has more than 6 million donkeys, the second largest donkey population in the world next to China, 1.9 million horses and over 350, 000 mules (FAOSTAT, 2012) specifically kept for work. Despite their huge numbers and significant contribution to the communities and the national economy (Behnke and Metaferia, 2011), the attention given to study the health aspects of working equids in Ethiopia is quite minimal. Among the multiple health and welfare problems affecting working equids, infectious diseases are one of the major constraints to their productivity and work performance; this often leads to high morbidity and mortality (El Idrissi and Lubroth, 2006; Knottenbelt, 2009). Apart from studies made on some parasitic infections (Getachew, et al., 2010; Hagos, et al., 2010; Getachew, et al., 2012; Gizachew, et al., 2013) and few studies on African horse sickness and Epizootic lymphangitis (Asfaw, et al., 2012; Ayelet et al., 2013) information on other equine infectious diseases is virtually unknown in Ethiopia. The present study was therefore conducted to investigate the prevalence of some infectious diseases in working equids and their spatial distribution in the central regions of Ethiopia.

MATERIALS AND METHODS

Study areas

The study covered areas of the central regions of Ethiopia and targeted the rural and sub-urban of Addis Ababa and Akaki, eastern and northern shewa districts of Ormia region, and southern Wollo zone of the Amhara region, representing different agro-ecological zones. Based on the traditional agro-climatic classification of Ethiopia, the animals were sampled from 'Dega', highland; 'Weyna Dega', midland and 'Kola', lowland areas (Table 1). Geographically the study areas covered latitude and longitude ranges of 08^0 09'472" to 11^0 09' 810" North and 38^0 05'248" to 39^0 61'740" East. Within the target districts one to two villages or peasant associations were purposively selected based on their road accessibility. Lists of equine owners were then made to help random selection of animals.

Study animals

A total of 1007 working equids including 667 donkeys, 289 horses and 51 mules were randomly selected using a simple random sampling technique (Table 1) from the list of owners generated. Only a maximum of two equids were sampled from each owner having more than three equids. Because of differences in the distribution of the equine species in the different agro-ecological zones and availability of animals during sampling periods, it was not possible to collect equal numbers of samples from each equine species. More than 85% of the equine population of the study areas were donkeys. This was particularly the case in mules. Mules are mostly found in highland areas and owned by limited number of people; they are very expensive. However, the total number of equids sampled was more than the required sample size calculated based on the assumption of 50% expected prevalence and 95% confidence interval with a 5% precision level (Thrusfield 2007). All equids were kept by resource-limited communities under extensive traditional management system. As per the information collected the sampled equids were not vaccinated or treated against any of the diseases under investigation. The study included both sexes (male and female) and all age groups (> 6 months) of equids. Age of the animals was estimated on the basis of their dentition according to Crane (1997) and Evans (2001). The study was conducted by the Donkey Sanctuary (DS) project based in Ethiopia in collaboration with CVRL from September 2005 to April 2006.

Blood sampling and storage

Five to ten ml of blood samples were collected from the jugular vein into 10ml plain test tubes and transported under cold-chain using cool-box according to OIE (2013b) to the diagnostic lab at the Addis Ababa University, College of Veterinary Medicine and Agriculture (CVMA). The samples were allowed to clot overnight at room temperature, and sera were separated and stored at -20 ^oC until processed. The sera were then exported under cold-chain protocol to the Central Veterinary Research laboratory (CVRL) to be analysed. CVRL is OIE accredited diagnostic laboratory in Dubai, United Arab Emirates (UAE).

Laboratory testing

Sera samples were investigated for African horse sickness (AHS), Dourine, equine infectious anaemia (EIA), Glanders, equine herpes viruses (EHV-1 and 4) and equine Piroplasmosis (*Theileria equi* and *Babesia caballi*) at CVRL using the OIE approved serological tests (OIE, 2013b). OIE approved serological tests used to analyse the different diseases are shown in Table 2. To reduce nonspecific reactions, donkey sera were inactivated at 62 ^oC for 30 min for complement fixation test (CFT). Because of the insufficient sera-samples due to variation in the amount of blood taken and the recovered sera during separation, it was not possible to test each serum for all diseases.

Data analysis

Twenty-four and fourteen sera-samples which showed doubtful or

Table 2. Serological tests used to investigate the different infectious diseases of equids in central regions of Ethiopia.

Disease[a]	Serological tests[a]
African horse sickness (AHS)	Competitive Enzyme-Linked Immunosorbent Assay (cELISA)
Glanders and Dourine	Complement Fixation Test (CFT)
Equine infectious anaemia (EIA)	Agar-gel immunodiffusion (AGID)
Equine herpes virus (EHV-1&4)	Indirec t-ELISA
Equine Piroplasmosis (*T. equi* and *B. caballi*) (IFAT)	Immunofluorescence antibody test

[a]OIE approved serological tests (OIE, 2013b. All diseases were diagnosed at CVRL, OIE accredited diagnostic laboratory in Dubai.

inconclusive results (OD = 0.1- 0.2) for EHV-1 and EHV-4, respectively, were excluded from the analysis. It was not possible to repeat the test due to shortage of sample. The association of sero-prevalence of the tested diseases and the different agro-ecological zones, equine species, age and sexes were evaluated by multivariable binary logistic regression analysis. Where appropriate, a pair-wise contrast analysis (R library Gremisc) was performed between these variables (risk factors) that were found to be significant. Descriptive statistics were performed using data analysis tools in Excel© (Microsoft, 2007) and Minitab© statistical software (Minitab release 14), whereas all statistical analyses were carried out in R (R Development Core Team 2009). The significance level for all statistical tests was set to $p<0.05$.

Results

The number of equids examined and percentage serologically positive for the tested diseases are shown in Table 3. Significant interspecies differences were observed for AHS, Glanders, and EHV-1 ($p = 0.001$). Mules showed a higher sero-prevalence of AHS when compared to donkeys and horses, while Glanders was significantly higher in horses than in donkeys and mules ($p = 0.001$). Similar sero-prevalence of EHV-4 was observed among the different species of equids, while that of EHV-1 showed a significantly higher sero-prevalence in donkeys ($p = 0.001$). Compared to other diseases tested, EHV-4 showed a significantly higher sero-prevalence in all species of equids ($p = 0.001$). There was no significant variation in sero-prevalences of the tested diseases among the different age groups ($p=0.580$) and sexes ($p=0.320$) of equids. Infection of equids with more than one infectious disease was diagnosed. Out of the 208 equids tested for herpes 11.1% were infected with both EHV-1 and EHV-4. Co-infection of AHS and EHV1 (1%), AHS and EHV-4 (4%), Glanders and EHV-1 (2%) and Glander and EHV-4 (7.7%) were also observed. Although low numbers of equids were examined for Piroplasmosis, the overall sero-prevalence of *T. equi* (50%) was higher than that of *B. caballi* (15%). The number of equids examined and sero-prevalence of the tested diseases in the different agro-ecological zones are shown in Table 4. Significant variation of sero-prevalence of AHS, Glanders and Dourine was observed in the different agro-ecological zones. The sero-prevalence of dourine was significantly higher ($p = 0.001$) in the mid-lowland, while that of AHS and Glanders was higher in the highland regions ($p = 0.01$). Although, the numbers of equids examined for EHV-1 and piroplasmosis were not sufficient to statistically compare among the different regions, higher sero-prevalences of EHV-1, *T. equi* and *B. caballi* were found in the mid-lowland, lowland and highland regions, respectively. Similar sero-prevalence of EHV-4 was found across the different agro-ecological zones.

DISCUSSION

The present study has shown not only the prevalence of the different infectious diseases in the equine population but also their distribution across the different agro-ecological zones of the central regions of Ethiopia.

African horse sickness (AHS)

The present finding of AHS in all species is similar with the finding by Kassa (2006). However, a higher sero-prevalence of AHS was reported by Bitew, et al. (2011) in equids and by Teshome, et al. (2012) in donkeys and mules. These studies were conducted in regions where AHS is endemic and frequent outbreaks are reported compared to our study areas. This could be the reason for the observed differences in sero-prevalence. Although, the sensitivity and specificity of the tests they used was not specified, the methods of diagnosis could also be a factor. Although, the present study and studies by Bitew, et al. (2011) and Teshome, et al. (2012) have shown a high sero-prevalence of AHS in donkeys in Ethiopia, no clinical cases were reported in these equine species, unlike in horses. However, frequent outbreaks of suspected cases of AHS with typical clinical signs of the mixed form were observed in donkeys in Kenya; eighteen sera samples from some of these cases were diagnosed at CVRL in Dubai and revealed 10 (55.6%) sero-positives for AHS (M Getachew, unpublished data). Although, it is generally accepted that donkeys, particularly African donkeys, are resistant and most infections are sub-clinical

Table 3. Number of each equine species examined and % serologically positive for the different diseases tested in the central regions of Ethiopia.

Disease	Equids	% sero-positive	95% CI*
AHS	Donkeys (n =165)	8.5	4.24-12.76
	Mules (n =18)	33.3	26.42-40.18
	Horses (n = 45)	8.9	0.58-17.22
	Total (n = 288)	10.5	6.52-14.48
Dourine	Donkeys (n = 662)	1.1	0.31-1.89
	Mules (n = 51)	0	-
	Horses (n = 284)	0	-
	Total (n = 997)	0.7	0.18-1.22
Glanders	Donkeys (n = 657)	0.2	0.14-0.54
	Mules (n = 48)	0	-
	Horses (n = 277)	12.3	8.4-16.17
	Total (n = 982)	3.8	2.6-5.0
EHV-1	Donkeys (n = 104)	20.2	12.48-27.92
	Mules (n = 4)	0	-
	Horses (n = 100)	7	2.0-12.0
	Total (n= 208)	13.5	8.86-18.14
EHV-4	Donkeys (n = 104)	84.6	82.37-86.83
	Mules (n = 4)	100	100-100
	Horses (n = 100)	91	85.39-96.61
	Total (n = 208)	88	83.58-92.42
EIA	Donkeys (n = 662)	0.2	-0.68
	Mules (n = 51)	0	-
	Horses (n = 289)	0	-
	Total (n= 1002)	0.1	-0.4

*95% confidence interval.

clinical (Mellor and Hamblin, 2004; Guthrie, 2007; OIE, 2013a), the epidemiological role they may play is not well known. Moreover, their susceptibility to the 9 different strain of AHSV has not been investigated. Given the current high sero-prevalence, the large donkey population and absence of vaccination against AHS in donkeys in Ethiopia, and the fact that they could act as silent reservoirs like zebras do, and may play a significant role for the virus transmission to other equids (Hamblin et al., 1998; Guthrie, 2007) consideration should be given to the inclusion of donkeys in the vaccination programme. The practice of vaccinating in horses, irregular vaccination of mules and no vaccination in donkeys (Aklilu et al., 2014; Ayelet et al., 2013) could be one of the major risk factors of having frequent outbreaks of AHS in horses in Ethiopia.

Dourine

Dourine, caused by *Trypanosoma (T) equiperdum*, is among the Non-Tsetse Transmitted Animal Trypanosomosis (NTTAT) (OIE, 2013a). Although, low in sero-prevalence, the finding of dourine only in donkeys in the present study is quite interesting. Dourine was reported only in horses from the highland regions of Ethiopia (Gari et al., 2010; Hagos et al., 2010). However, these studies did not include donkeys and other studies by Mekuria et al. (2010) and Eyob et al. (2011) were focused on detecting tsetse transmitted trypanosomosis. The present finding from the mid-lowland region is the first report, showing the spread of the disease from the highland to the mid-lowland regions. This could be associated with the movement of animals through trade routes of equids from the highland to mid-lowland regions. As *T. equiperdum* is a sexually transmitted disease, and donkeys are more resistant than horses and may remain as apparent carriers, it can spread not only among the donkey population but also to horses in areas where mule breeding is practiced. Although, the area of study was not inhabited by camels, and Surra, caused by *T. evansi*, seem to be restricted to arid and semi-arid areas of Ethiopia where it is endemic in camel population (Tefera and Gebreab, 2004; Kassa et

Table 4. Number of equids examined from the different agro-ecological zones and % serologically positives for the different diseases tested in central regions of Ethiopia.

Disease	Agro-ecological zone	% sero-positive	95% CI*
AHS	Highland (n =188)	9.6	5.39-13.81
	Lowland (n = 40)	15	3.93-26.07
Dourine	Highland (n = 636)	0.2	-0.7
	Midland (n = 210)	2.9	0.63-5.17
	Lowland (n = 151)	0	-
Glanders	Highland (n = 621)	4.5	2.87-6.13
	Midland (n = 210)	1.4	-3.18
	Lowland (n = 151)	2.4	-4.88
EHV-1	Highland (n = 159)	12	6.69-17.05
	Midland (n = 19)	31.6	10.7-52.5
	Lowland (n = 30)	10	-21.48
EHV-4	Highland (n = 159)	88.1	83.07-93.13
	Midland (n = 19)	84.2	67.8-100.0
	Lowland (n = 30)	90	9.26-100.0
EIA	Highland (n = 641)	0	-
	Midland (n = 210)	0.5	-1.9
	Lowland (n = 151)	0	-

*95% confidence Interval

al., 2011), now-a-days dromedaries are forced to move to the mid-lowland areas in search for feed during the dry season. These might predispose equids in the region to Surra complicating the diagnosis. Because *T. equiperdum* is genetically and antigenically similar to *T. evansi*, and CFT cannot differentiate between the two infections (Claes et al., 2003). The current finding therefore, should be cautiously interpreted. This indicates the need of further epidemiological studies to establish the extent of the distribution of dourine and surra in the different agro-ecological zones and their prevalence in the equine population in general and donkeys in particular using highly sensitive and specific diagnostic methods that can differentiate between the two infections (Sumbria et al., 2014).

Glanders

Apart from fragmented reports (OIE, 2013a), and anecdotal information based on clinical signs, the epidemiology of Glanders has not been previously investigated in Ethiopia. This is the first report of serological evidence of the disease in Ethiopia. The serological finding of this disease in all agro-ecological zones shows its wide distribution among the equine population in the central regions of the country. Donkeys are reported to be the most susceptible equids often developing the acute form of the disease, which is often fatal within days (Wernery, 2009; Khan et al., 2012). Survival bias could be the reason for the low sero-prevalence in donkeys as compared to in horses in the present study. The crowding condition of equids in public places, such as market, flour mills, traditional ceremonies and the trade routes across the country with poor hygienic and stressful conditions could be the major factors for its spread. Moreover, the highly prevalent harness inflicted wounds and fly population may play a significant role in the transmission of the disease among equids. As CFT can give some false-positive or false-negative results (OIE, 2013b), the present serological finding of Glanders should be interpreted carefully. Study made by Turnbull et al. (2002) showed that donkeys which were positive with CFT were later confirmed to be negative with the Mallein test and at necropsy, indicating a cross-reaction to other related microorganisms. *Burkholderia mallei* and *Burkholderia pseudomallei* are antigenically closely related and cross-reactivity was noted (Anuntagool and Sirisinha, 2002; Wernery, 2009). Studies have shown that combined use of CFT and Mallein test, or CFT with a more specific and complement independent tests increases the detection rate of Glanders (Neubauer et al., 2005; Khan et al., 2012). Therefore, further epidemiological study using a more sensitive and specific tests are recommended to

establish the true prevalence of Glanders among working equids in Ethiopia to help develop strategic control programme.

Equine infectious anemia (EIA)

Although EIA has a worldwide distribution (OIE, 2013a; van Maanen, 2013), there is no previous report as to its presence in Ethiopia. Despite the conducive environment for the insect vectors in the low lying and swampy areas in the mid-lowland regions, from where some of the samples were collected, the finding of only one sero-positive donkey (0.2%) out of the 1002 sera-samples tested may show that this disease is rare in the study areas. Moreover, we could have seen more infection prevalence given the possibility that once equids are infected they become carriers and remain infectious for life and with rare exception yield a positive serological test result (OIE, 2013b; Issel et al., 2013). Similar studies conducted in UAE (Turnbull et al., 2002), in Bulgaria (Chenchev et al., 2011) and Turkey (Ataseven and Arslan, 2005) showed the absence of this disease in the donkey population.

Equine herpes viruses

The finding of high sero-prevalence of both EHV-1 and EHV-4 in the different agro-ecological zones shows the wider distribution of these viruses among working equids in the study areas. Similar high sero-prevalence of both herpes infections was reported in Ethiopia (B. Endebu, unpublished data). As EHV4 and EHV1 establish persistent lifelong latent infections and ELISA is type specific (Crabb and Studdert, 1995; Patel and Heldens, 2005), and vaccination against any of the herpes virus is unknown in Ethiopia, the present finding indicates the natural occurrence of these viral infections in the equine population of the study areas. Working equids are often exposed to stressful and harsh conditions from overworking, malnutrition, parasitic infections and to other management factors. This may predispose them to frequent relapse and diseases as herpes viruses establish lifelong infection. It is known that EHV-4 and EHV-1 cause acute respiratory disease, viral-induced abortion and myeloencephalitis, respectively, in equids throughout the world (Patel and Heldens, 2005; Pronost et al., 2012). The high sero-prevalence of EHV- 4 and EHV-1 in the present study areas might be one of the major reasons for the high respiratory problems and abortion rates seen in working equids, particularly in donkeys (Donkey health and welfare project, Ethiopia, personal communication). An outbreak of a neurological syndrome in donkeys in the Amhara region of Northern Ethiopia was recently reported, which was later diagnosed to be due to EHV-1 (T. Worku, personal communication). The present high sero-prevalence of both EHV-1 and EHV-4 shows the need to conduct further epidemiological researches to determine the extent of its distribution and associated problems in different parts of the country to help develop sound control and prevention strategies.

Equine piroplasmosis

The present study showed high sero-prevalence of both *T. equi* and *B. caballi* although the numbers of samples examined were small. Studies made by Gizachew et al. (2012), Mekibib et al. (2010) and Tefera et al. (2011) showed a much lower prevalence of these parasites. This variation could be attributed to differences in the geographical location and methods of diagnosis employed. Apart from study by Gizachew et al. (2012), the others studies were based on parasitological methods. Given the difficulty in demonstrating the parasites in blood, especially when the parasitaemia is low in carrier animals, false negatives are common using parasitological methods. As *T.equi* can be transmitted transplacentally from carrier mares to offspring (Chhabra et al., 2012), a thorough investigation using serological and molecular methods is required in combination with parasitological diagnosis (George et al., 2011). The high sero-prevalence in the present study indicates the presence of suitable tick vectors capable of transmitting the parasites (Pegram et al., 1981; Gizachew et al., 2012) and high degree of exposure of equids to the infection. Therefore, further epidemiological studies are required to more precisely determine the extent of the distribution of this pathogen within the equine population in different agro-ecological zones.

Conclusions

The present study has shown from high sero-prevalence to rare presence of the investigated infectious diseases in the equine population with a wide spatial distribution. Although, not investigated here, the high presence of multiple infectious diseases could contribute to the low productivity, low work performance and early demise observed in working equids in these areas. This further indicates the potential of these infectious diseases in causing welfare problems and economic losses. The wide distribution of most infectious diseases in the donkey population needs further investigation. Donkeys are particularly neglected when it comes to disease investigation, control and prevention. Although reported to be resilient to the effect of many infectious pathogens, they can be carriers and serve as source of infections for other equids. The high population density of equids, particularly donkeys, in public places, such as markets, local flour mills, watering points and ceremonial events,

and increased movement of animals between different regions due to trade could contribute to transmission and hence the high sero-prevalence, multiple infections and wide spread of most infectious diseases in the different agro-ecological zones. These pose special threat and demonstrate some important considerations in the practice of disease control.

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Conflict of Interest

The author(s) have not declared any conflict of interests

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