Equine herpesvirus 1 and/or 4 in working equids: seroprevalence and risk factors in North Shewa Zone, Ethiopia

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Abstracts

A cross-sectional study was conducted in selected districts of North Shewa Zone of Amhara Region with the objectives of estimating the seroprevalence of equine herpesvirus 1 and/or 4 and to identify possible risk factors for the occurrence of the disease. A total 397 working equids sera were examined for the presence of antibodies against equine herpesvirus 1 and/or 4 using indirect enzyme-linked immunosorbent assays. Risk factors associated include geographic location, species, age and sex of equines. The overall sero-prevalence of equine herpesvirus 1 and 4 was 71.9% (95% CI: 67-76%). The sero-prevalence of equine herpesvirus 1 and 4 was 50% (95% CI: 22%-78%) in mules, 66.7% (95%CI: 57%-76%) in horses and 74.7% (95%CI: 70%-80%) in donkeys. Variation in prevalence was observed among age groups. The sero-prevalence in older donkeys (more than eight years) was 94.7 % (95%CI: 75%-98%) while in donkeys aged less than three the prevalence was 63.7 % (54%-73%) (p=0.002). In conclusion, this study revealed that the seroprevalence of Equine herpesvirus 1 and/or 4 in North Shewa Zone was high and prevalent. Thus, further epidemiological studies in Ethiopia on equine herpesvirus and implementation of prevention measures are recommended.

Keywords: Ethiopia; Equine; Equine herpesvirus; Indirect ELISA; Seroprevalence

Introduction

Herpes virus infections caused by equine herpes virus type 1 (EHV-1) and equine herpes virus type 4 (EHV-4), members of Alpha herpes virinae (Patel and Heldens, 2004) are endemic in domestic equine populations worldwide (Ataseven *et al*., 2009) and are considered to have wide host range such as horse, donkey and mules (Roizman, 1996). EHV-1 or EHV-4 causes upper respiratory tract disease; also, EHV-1 is associated with abortion, perinatal death, or paralytic neurological disease in equines. EHV-4 has also been sporadically associated with abortion and neonatal infection in horse (OIE, 2015; Tekelioglu *et al*., 2005). Furthermore, EHV-1 and EHV-4 infections affect welfare in the affected equines, leading to death of working equids in some cases (Lopez, 2014).

Ethiopia has the largest equine population in the world, including 7.43 million donkeys, 2.03 million horses and 0.4 million mules (CSA, 2015). These equines play a significant role in the livelihood of small scale farmers, mainly used for transportation, draught power and other purposes. In northern Ethiopia, especially in Amhara Region respiratory problems and abortion cases of unknown aetiology in working equids are frequently reported. In Amhara Region, during 2011-2014 outbreaks of working equids neurological problems were reported and EHV-1 has been isolated and genetically characterized from these outbreaks in working equids of Ethiopia (Haileleul Negussie *et al*., 2015).

EHV-1 and EHV-4 were found to be prevalent in working equids in central Ethiopia (Getachew Mulugeta *et al*., 2014) but, no previous investigation has been carried out to determine the prevalence of EHV-1/-4 infection in North Showa Zone, Northern Ethiopia. On the other hand, prevention and control strategies for EHV-1 and EHV-4 infections should be in place due to lack of a specific treatment and the speed at which EHV-1 and EHV-4 spread within an equine population (Lopez, 2014). Therefore, understanding the existing situation about the occurrence of EHV-1/-4 infections and the associated risk factors of exposure to EHV-1 and EHV-4 in working equids is essential. This study was conducted to estimate the seroprevalence and assess risk factors for the occurrence of EHV-1/-4 infections in working equids of the study areas.

Materials and Methods

Study area

The study was conducted in Menz Gera Midr and Basona Werana districts of the North Shewa Zone of Amhara Regional State. North Showa Zone of Amhara Regional State is located between 8°44' and 10°44' North latitude and 38° 40' and 40° 6' East longitude. Menz Gera Midr district is located 282 km northeast of Addis Ababa, Ethiopia. The district has an altitude ranging from 1680 to 3600 meters above sea level (m.a.s.l.) and minimum and maximum annual rainfalls of 800mm and 1600mm, respectively. Its annual average temperature ranges from 8 °C to 18 °C. The equine population of the Menz Gera Midr district includes 13,949 donkeys, 3,630 horses and 1,479 mules Menz Gera midr Wereda Agricultural and Rural Development Office (MGWARDO, 2009).

Basona Werana district is located 130 km northeast of Addis Ababa. The district has an altitude ranging from 1500 to 3400 meter above sea level and its average annual minimum and maximum rainfall are 800mm and 1000mm respectively. The mean annual maximum and minimum temperatures are 24 °C and 18 °C respectively. The equine population in the district includes 29,857 donkeys, 9,184 horses and 103 mules Basona Werana Woreda Agricultural and Rural Development Office (BWWARDO, 2009).

Study population and study design

A cross-sectional study was carried out in the North Shewa Zone of Amhara Regional State. The study population comprised horse, donkeys and mules that are dwelling in Menz Gera Midr and Basona Werana both districts of North Shewa Zone. The Menz Gera Midr and Basona Werana districts were purposively selected based on the reports of the respiratory problems and abortion cases in working equids in these two districts (Kombolcha regional laboratory outbreak reports).

Sampling and sample size determination

Four villages namely: Tsahayi Sina, Sina Amba, Amedwasha and Alpha Midr and three villages comprising Birbirsa, Washwash and Asofi were purposely selected from Menz Gera Midr and Basona Werana districts respectively based on the equine population and accessibility. Because the previous prevalence of the disease in the zone was not known, 50% expected prevalence and a 5%

absolute level of precision was considered to calculate the number of animals to be sampled according to formula given by Thrusfield (1995) as follows:

$$n = \frac{1.96^2 \times P_{exp}\left(1 - P_{exp}\right) \times 100}{d^2}$$

Where n is required sample size; is expected prevalence; d is desired absolute precision, for n = 384. A total of randomly selected 397 equids of which, 281 donkeys, 103 horse and 13 mules of different sex and age categories were included in this study. The age of study animals were categorized into three groups as less than 3 years old, between 3 to 8 years old and more than 8 years of age based on dentition (Crane, 1997; Martin *et al.*, 1999). All the equines population of the study areas were not vaccinated against EHV-1 and EHV-4 (Ministry of Agriculture, Ethiopia, 2016).

Sera sample collection and serological testing

Following proper restraining of the animals, about seven milliliters (7 ml) whole blood was collected from jugular vein of non-vaccinated working equids into plain vacutainer tubes and the tubes were coded. During the blood sample collection data on district, villages, equines species, sex and age of the animals were recorded. After clotting overnight at room temperature sera samples were carefully collected into serum tubes and transported in cold chain condition to Nation Animal Health Diagnostic and Investigation center (NAHDIC). Samples were then frozen at -20 ^{0}C until testing. The serological technique used for the analysis of antibodies against EHV-1/- 4 in this study was an enzyme-linked immunosorbent assay (ELISA) commercial kit (Ingezim Rinoneumonitis 14.HVE.K1®, Ingenasa,Spain) and it is based on an indirect ELISA technique which uses a monoclonal antibody (MAb) specific for equine IgG. The test was performed in accordance with protocol recommended by manufacturer. The results were evaluated by reading of plates in ELISA reader at 450 nm.

Statistical analysis

Collected data were entered into Microsoft Excel 2003 spreadsheets (Microsoft Corp., Redmond, WA, USA) and analyzed using SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). The animals were divided into different groups according to species namely: horse, donkey and mules; based on sex as female and male; age groups that is; less than 3 years old, between 3-8

years old and more than 8 years of age. Twenty sera samples which presented equivocal or inconclusive results were omitted from data analysis since it was not possible to repeat the test because of the shortage of the ELISA kit. Chi-square test analysis and logistic regression model were used to examine associations between sero-prevalence and location (districts and villages), species, sex, age of the working equids. Differences were considered significant at values of $p<0.05$.

Results

Out of 397 sera samples of 271 tested were positive for antibodies against EHV-1/-4, whereas 20 samples presented equivocal results. The overall sero-prevalence of equine herpes type 1 and 4 (EHV-1/-4) in the study area was 71.9% (95%CI: 67%-76%). Table 1 shows sero-prevalence of EHV-1/-4 in working equids base on different variable categories.

Table 1. Overall seroprevalence of EHV-1/4 in working equids based on different variables

Variable	Category	No. equines tested	EHV-1/-4 + (%)	x 2 (p-value)	OR	95% CI OR	P value
District	Menz Gera	252	183(72.6)	0.2(0.72)	0.86	0.37-2.02	0.72
	Basona Warana*	125	88(70.4)		1	-	-
Species	Mules*	12	6(50)	5.18(0.08)	1		0
	Horse	96	64(66.7)		2.64	0.71-9.78	0.15
	Donkeys	269	201(74.7)		3.89	1.13-13.34	0.03
Sex	Female	253	182(71.9)	0.001(1)	0.94	0.56-1.57	0.82
	Male*	124	89(71.8)		1		
Age	<3 years*	137	86(62.8)	8.85(0.01)	1		
	3-8years	202	156(77.2)		1.99	1.2-3.3	0.01
	≥8 years	38	29(76.3)		1.98	0.82-4.8	0.13

OR: Odds ratio; *: Reference category

The sero-prevalence of EHV-1/-4 in mules, horses and donkeys were 50% (95% CI: 22-78%), 66.7% (95%CI: 57-76%) and 74.7% (95%CI: 70-80%), respectively. It was observed that the sero-prevalence of EHV-1/-4 did not significantly varied among mules, horses and donkeys ($p>0.05$). Age of the working equines

was found to be significantly ($p<0.05$) associated with overall sero-prevalence of EHV-1/-4, where increase in sero-prevalence was associated with increase in age of the working equids.

The distribution of the EHV-1/-4 antibodies positive samples in the sampled villages is summarized in Table 2.

Table 2. Overall seroprevalence of EHV-1/4 in equines in the sampled villages

Villages	No. equines Tested	EHV-1/-4 + (%)	χ 2 (p-value)	OR	95% CI OR	P value
Tsahayi Sina	60	43(71.7)	7.29(0.29)	1.67	0.8-3.56	0.20
Sina Amba*	58	35(60.3)		1	-	-
Amedwasha	94	74(78.7)		2.43	1.18-5.00	0.02
Alpha Midr	40	31(77.5)		2.26	0.91-5.62	0.08
Birbirsa	33	25(75.8)		2.05	0.79-5.33	0.14
Washwash	33	23(69.7)		1.51	0.61-3.76	0.75
Asofi	59	40(67.8)		1.38	0.65-2.96	0.40

Results of Chi square analysis and binary logistic regression to evaluate the odds of sero-positivity to EHV-1/-4 in donkeys based on location (districts and villages) and host related factors (age and sex) are summarized in Table 3. Age of the donkeys had a significant ($p<0.01$) effect on the seroprevalence of EHV-1/-4 where it was highest 94.7% (95%CI: 75%-98%) in donkeys aged more than eight years and the lowest 63.7% (95%CI: 54%-72%) sero-prevalence of EHV-1/-4 was recorded in donkeys less than three years old. In line with this, binary Logistic regression pointed out that donkeys of age more than eight years were 8.56 times more likely to be EHV-1/-4 seropositive compared to those less than three years of age category.

Table 3. Distribution of antibody positive samples in donkeys as tested by indirect ELISA based on different variables

Variable	Category	No. equines	EHV-1/-4 + (%)	X 2 (p-value)	OR	95% CI OR	P-value
District	Menz Gera*	201	150(74.6)	0.004(0.95)	1	-	-
	Basona Warana	68	51(75)		1.52	0.44-5.30	0.5
Sex	Female*	173	127(73.4)	0.44(0.56)	1	-	-
	Male	96	74(77.1)		1.23	0.65-3.54	0.53
Villages (location)	Tsahayi Sina	41	29(70.7)	8.79(0.19)	1.44	0.59-3.46	0.42
	Sina Amba*	51	32(62.7)		1	-	-
	Amedwasha	83	67(80.7)		2.49	1.13-5.46	0.23
	Alpha Midr	26	22(84.6)		3.27	0.98-10.92	0.05
	Birbirsa	16	12(75)		1.78	0.50-6.32	0.37
	Washwash	24	16(66.7)		1.19	0.43-3.30	0.74
	Asofi	28	23(82.1)		2.73	0.89-8.38	0.08
Age	<3 years*	102	65(63.7)	12.5(0.002)	1	-	-
	3-8years	148	118(79.7)		2.13	1.2-3.9	0.02
	≥8 years	19	18(94.7)		8.56	1.06-68.81	0.04

OR: odds ratio; *: Reference category

Of the sampled horses 66.7% (95% CI: 56.7%-75.3% were EHV-1/-4 seropositive. None of the considered risk factors had caused a significant variation in seroprevalence of EHV-1/-4 in horses (p>0.05) Table 4.

Table 4. Distribution of antibody positive samples in horses as tested by indirect ELISA based on different variables

Variable	Category	No. equines	EHV-1/-4 positives (%)	$\chi 2$ (p-value)	OR	95% CI OR	P value
District	Menz Gera*	39	27(69.2)	0.19(0.66)	1	-	-
	Basona Warana	57	37(64.9)		1.45	0.25-8.28	0.68
Sex	Female	72	51(70.8)	2.25(0.13)	1.73	0.64-4.71	0.25
	Male*	24	13(54.2)		1	-	-
Villages (location)	Tsahayi Sina	17	13(76.5)	5.88(0.4)	4.33	0.67-28.12	0.12
	Sina Amba*	7	3(42.9)		1	-	-
	Amedwasha	6	4(66.7)		2.67	2.77-25.64	0.39
	Alpha Midr	9	7(77.8)		4.67	0.53-40.89	0.16
	Birbirsa	17	13(76.5)		4.33	0.67-28.12	0.12
	Washwash	9	7(77.8)		4.67	0.53-40.89	0.16
	Asofi	31	17(54.8)		1.62	0.31-8.48	0.57
Age	<3 years	29	18(62.1)	1.34(0.57)	1.24	0.31-4.9	0.76
	3-8years	50	36(72)		1.89	0.56-6.42	0.31
	≥8years*	17	10(58.8)		1	-	-

OR: odds ratio; *: Reference category

Discussion

In this study, a total of 397 serum samples were tested and the overall seroprevalence of EHV-1/-4 infection was 71.9% (95%CI: 67%-76%). The sero-prevalence of EHV-1/-4 was 72.6% (95%CI: 67%-78%) and 70.4% (95% CI: 61%-78% in Menz Gera Midr and Basona Warana districts respectively; there was no statistically significant difference in sero-positivity based on sampled districts (P>0.05). In Ethiopian, EHV-1 has been isolated and characterized from clinically equine herpes virus myeloencephalopathy affected horses, donkeys and mules (Haileleul Negussie *et al*., 2015). High sero-prevalence of EHV-1 and EHV-4 in equines was also reported in central Ethiopia using type specific indirect ELISA method (Getachew Mulugeta *et al*., 2014).

The equines involved in this study were not vaccinated against equine herpes viruses because there is no vaccine against any of the herpes viruses in Ethiopia (Haileleul Negussie *et al*., 2015) and EHV-1 and 4 establishes long-lasting latent infections which can reactivate following stress or pregnancy in equines

(OIE, 2015). The seropositivity identified in sampled equines was considered to have arisen from infection. The results of this study indicate the natural occurring prevalent EHV-1/-4 infection in equine population of the study areas and this might be one of explanations among several reasons, for respiratory problems and abortion cases in working equines which were reported from the study areas.

The overall sero-prevalence of infection was 71.9% (95%CI: 64%-80% in male and 71.8% (95%CI: 66%-77%) in female animals and there was no statistically significant difference ($p > 0.05$) in the sero-positivity based on sex. In this study the overall sero-prevalence of EHV-1/-4 among the age categories of the working equids significantly varied ($p<0.05$). Significantly higher 77.2% (95%CI: 71%-83%) seroprevalence was recorded in working equids of age group between 3 to 8 years followed by 76.3% (95%CI: 63%-90%) in animals older than 8 years. Whereas, significantly lower 62.8% (95%CI: 56%-71%) seroprevalence of EHV-1/-4 was recorded in equines less than 3 years of age. This is in agreement with studies by Haileleul Negussie *et al*. (2015) in northern part of Ethiopia, who reported that the highest proportion of equines were affected at the age ranging from 7 to 10 years, because as animals stays longer in the environment chance of exposure to get the infection increases. The sero-prevalence of EHV-1/-4 did not significantly varied among the sampled villages ($P>0.05$). This study revealed an overall sero-prevalence rate of 50% (95%CI: 22%-78%) of EHV-1/-4 for mules, 66.7% (95%CI: 57%-76%) for horses and 74.7% (95%CI: 70%-80%) for donkeys and the difference was no significant ($p>0.05$). However, logistic regression analysis revealed that donkeys were 3.84 times more likely to be infected by EHV-1/-4 than mules and horses were 2.64 times more likely to be infected by EHV-1/-4 than mules (95%CI: 71% – 97.8%). These results are in agreement with pervious report by Haileleul Negussie *et al*. (2015) who reported that EHV-1 affected large proportion of donkeys than horse and mules. These could be due to the effects of breed (Barbic *et al*., 2012)

The EHV-1/-4 seroprevalence of 74.7% (95%CI: 70%-80%) in donkey in this study is slightly higher than 63% reported using the same method in Bulgaria (Chenchev *et al*., 2011). In Ethiopian, Haileleul Negussie *et al*. (2015) reported that large proportions of donkeys were affected by EHV-1 infection than horses and mules where of the total of 91 clinically EHV-1 infected equines, 82 were donkeys. An outbreak of neurological syndrome in donkeys in Amhara regional state of Ethiopia was reported and confirmed to be caused by EHV-1 (Getachew Mulugeta *et al*., 2014). The overall sero-prevalence of 66.7%

(95%CI: 0.57-0.76%) in horses in this study is lower than 76% reported in the Netherlands (De Boer *et al*., 1979) but, is higher than 53.6% reported in central Spain (Lopez, 2014), all that reported using the same methods. The variations in prevalence in this study and those reported from other countries could be due to differences in agro-ecological systems, seasons of the sampling, equine production and management systems.

In this study EHV-1/-4 seroprevalence in donkeys significantly varied among the age categories of the sampled donkeys. Considerably, higher prevalence of 94.7% (95%CI: 75%-98%) was recorded in donkeys more than eight years of age compared to those less than three years old. In horses, there was no statistically significant variation ($p>0.05$) in seroprevalence between the male and female or horses of different age categories. These findings do not accord with that of Lopez (2014), who reported a significantly higher seroprevalence in stallion and significantly varied seroprevalence among age categories of horses in central Spain. These differences might be due to breed difference, variations in animal production and management systems and climatic conditions.

In conclusion in sampled working equids unvaccinated against EHV-1/-4, the overall sero-prevalence of EHV-1/-4 was 71.9%, indicating widely distributed EHV-1/-4 infections throughout the equine population including in mules, horses and donkeys in study areas. Thus, considering the effect EHV-1 and EHV-4 infections on health and welfare of working equids and significant cause of economic loss to the equine industry due to EHV-1 and EHV-4 infections further researches are recommended to elucidate epidemiology of equine herpes virus type 1 and equine herpes viruses type 4 in Ethiopia.

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