SERO-PREVALENCE AND ASSOCIATED RISK FACTORS OF MAEDI-VISNA VIRUS IN SHEEP POPULATION OF SELECTED AREA OF EASTERN AMHARA, ETHIOPIA

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Abstract

Maedi-visna virus (MVV) causes a significant economic loss through morbidity, mortality and carcass weight loss in sheep worldwide. This cross-sectional study was conducted to determine the prevalence and identify associations with potential risk factors of MVV virus infection in the selected area of the eastern Amhara region, Ethiopia. A total of 494 sheep blood sera were collected during the period from November, 2017 to October, 2018 and examined using Indirect enzyme linked immuno-sorbent assay (I-ELISA) to screen specific antibodies against MVV. From the total tested sample 3.24% (16/494) were positive for the presence of antibodies against MVV in the area. The sero-prevalence of MVV was not significantly different between associated risk factors of breed, sex, age, production system, flock size and body condition score ($P > 0.05$). Awassi cross sheep distributor farm and ranch were incriminated as a source for Maedi-visna virus infection and effective control measures should be implemented through appropriate way of testing and culling mechanisms of all sero-reactor ewes and their progeny. High sensitive screening test should be practiced and implemented during introduction of new flocks from abroad and before distribution of Awassi cross breed rams from ranches and multiplication center to smallholder farms and individual farmers. In addition further epidemiological study (research) should be done in sheep producing areas of the country to know the level of infection at national and country level.

Key words: Eastern Amhara, Maedi-visna virus, Risk factors, Seroprevalence, Sheep

INTRODUCTION

Sheep production plays a great economic role in the small holder farmers of Ethiopian. Sheep population in Ethiopia is estimated about 31.30 million, out of which, 99.81% is indigenous breeds (CSA, 2017/18). More than 75% of the population is located in the highlands and less than 25% in the lowlands. The sheep population in the Amhara regional state is about 11, 086,083. This contributes 35.4% to the total population of the country (CSA, 2017/18).

Sheep are main sources of income and provide food proteins in most parts of Ethiopia of rural society. Their growing rate relatively fast and good proficiency rates and have a capacity to give mostly twins and triple births with short lambing period as compared to cattle. In Ethiopia they contribute a significant value of the national meat and skins production (Tibbo, 2006). However, relatively due to low productive potential of the local indigenous breeds, the

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Ethiopian government had been introducing high potential sheep breeds of Hampshire, Morrisdale, Awassi and Dorper from UK, SA and Israel since the early 1970's to upgrade the genetic makeup of the local sheep breeds (Gizaw *et al.*, 2013; Getachew *et al.*, 2016).

Imported sheep were stocked and crossed with the local Menz and Horro breed sheep in Debre Birhan and Amed Guya sheep breeding and multiplication centers and cross-rams were selected for distribution. The distribution was primarily for sheep farms established by peasant associations in different parts of the country with the intention that associations could distribute to other local breeders easily. Among the established association Agarfa which was used as the former farmers training center in the country and Arsi Rural Development Unit (ARDU), which owned the rams of Corriedale and Awassi in 1981 was used as the center for cross breed ram multiplication and distribution to other individual breeder farmer (Gizaw *et al.*, 2013; Getachew *et al.*, 2016).

Despite the genetic improvement, an occurrence of a new case with undefined etiology characterized by a respiratory embarrassment appeared in Agarfa and Arsi Rural Development Unit (ARDU) sheep farms in 1990 (BoA, 2000; Tibbo *et al.*, 2001). The disease caused 10% mortality affecting mainly adults and had no response to antibiotic treatments. Finally, sera samples were sent to Pirbright laboratory (UK) and specific antibody for the Maedi-visna virus (Ovine Progressive Pneumonia-OPP) was detected in 90.7% (39/43) tested samples. Therefore; the occurrence of MVV in Ethiopia was first detected in imported breed in 1986 at Agarfa sheep ranch Bale province, Ethiopia (Ayelet *et al.*, 2001).

MVV/OPP is a slowly progressive disease of sheep and rarely goats reported first in the Iceland in 1939 and subsequently eradicated,

has been reported in major sheep rearing countries throughout the world except Australia and New Zealand. (Jones and Hunt, 1983; Vorster *et al.*, 1996; Murphy *et al.*, 1999; Radostits *et al.*, 2000; Kahn *et al.*, 2005). MVV is a chronic disease of adult sheep characterized by progressive interstitial pneumonia and other syndromes such as meningo-encephalitis, indurative mastitis and arthritis (Lujan *et al.*, 1994). It is caused by a non-oncogenic retrovirus agent, which belongs to the subfamily *Lentivirinae*. Transmission occurs more readily between dams and lambs *via* colostrum and milk, and among confined individuals probably *via* respiratory secretions (Preziuso *et al.*, 2009).

In Ethiopia the detection of the virus, since 1986-1989, it has been assumed that MVV is an emerged disease introduced to the country through the imported sheep breeds. Previous study reports from the assessment of the disease in and around the stocking and rearing centers of North Shewa showed that the disease became one of the most important diseases of respiratory system of sheep in the central Ethiopia. The infection is persistent, so antibody detection is a valuable tool for identifying virus carriers (Ayelet *et al.*, 2001; Tibbo *et al.*, 2001; Woldemeskel *et al.*, 2002).

In the country, outbreaks of unidentified diseases often occur and a considerable number of sheep die with signs of respiratory embarrassment. When MVV is introduced into a new area, the mortality rate may reach 20-30%. The mortality rate is low in regions where MVV is endemic; annual losses rarely exceed 5% in a flock, even when nearly 100% of the flock is infected (Peterhans *et al.*, 2004). Still there is a paucity of information regarding the presence and significance of MVV in Ethiopia. Although few research work, particular farms and breeding centers have been reporting MVV cases in eastern Amhara, Ethiopia, the extent

to which the disease disseminated has not been established yet and there was no information regarding to this type of disease in farmer level as well.

Therefore this study was designed to estimate the sero-prevalence status of the MVV infection and to determine the potential associated risk factor in the study areas.

MATERIALS AND METHODS

Study area: Two administrative zones (South Wollo and North Wollo) were selected purposively based on the history of Awassi cross sheep population numbers. From each zone representative districts were again selected purposively based on their location, proximity and accessibility to road. Usually Awassi cross sheep were distributed around the highland areas of eastern Amhara and study districts were selected based on Awassi cross sheep distribution which practiced the crop – livestock mixed production system.

Legambo district is situated about 500 Km North of Addis Ababa at a Latitude, 11° 00' 0.00" N and Longitude, 39° 00' 0.00" E and an elevation of range from 1500 to 3700 meter above sea level. The sheep populations of the area were 120, 993. The annual rain fall of the area ranges from 950-1200 mm. The mean annual minimum and maximum temperatures are 1.5°C and 23.3°C, respectively and the area experiences a bimodal rain fall patterns with a short rainy season which occurs from January to March and long rainy season which starts at the end of June and ends at early November (Tefera and Mulate, 2016).

Gazobelay district (Wadila former) is found about 600 Km of Addis Ababa at a latitude, 11°50' N and longitude, 38°50' E and an elevation of ranges from 700 to 3200 meters above sea level. The sheep populations of the area were 143, 133. The rainfall pattern is

bimodal, with two-rainfall season, *belg* (February/March - April) and *meher* (July - October/November) and the mean annual rainfall amount is on average about 950 mm. This area has three seasons of sub-humid agro-climatic zone with mean daily temperature ranges from 16 - 21°C. Months of June to August are main rainy seasons; months of September to February are dry seasons, while months of March to May are short rainy season (Zegeye *et al.*, 2014).

Study animals and their management: The animals used for this study were local indigenous and Awassi cross bred sheep. Sheep above six months of age were sampled. In this study the production system was classified into two based on management systems of sheep owners in the study areas. In extensive system, sheep were spent all the day on grazing pasture on fallow lands and crop residues usually with no extra-supplement and sheltered during the night. This management system was basically practiced in small holder sheep producers. In semi-intensive production system owners were supplemented extra feed sources in addition to grazing. This production system was practiced by the sheep multiplication stations.

Study design and sampling strategy: Cross-sectional sero-epidemiological study was conducted from November, 2017- October, 2018. Multistage stratified cluster sampling methods were used in Legambo and Gazobelay districts and respective three peasant associations from each district were selected purposively based on accessibility and the history of Awassi cross ram distribution and high population of sheep. Finally, study units were sampled using simple random sampling method and a representative sampled animal were selected.

Sample size determination: Since this study was the first in these area, the total sample size

were determined using the formula for simple random sampling technique (Thrusfield, 2007) with 5% desired absolute precision level, 50% expected prevalence and a 95% confidence interval. Accordingly, 384 sheep samples were selected. But to increase the precision and accuracy, the sample size were maximized to 521. The number of animals sampled from each districts was proportionally distributed based on sheep population in the study districts. From a total sampled animal 27 serums were discarded due to different factors (hemolysis, less amount). Over all 494 serum samples were tested, analyzed and interpreted.

Blood sample collection: Blood samples were taken from the jugular vein of sampled sheep. Sterile vacutainer tubes and needles were used for each animal and about 5 mL blood sample was taken. Each sample from each animal was labeled by using codes describing the specific animal. The tubes were kept overnight at a room temperature to allow clotting. Next morning the clotted bloods in the tubes were centrifuged at 3000 rpm to obtain clear serum. Then serum were separated into 1.8 mL cryo-vial and were preserved at -20°C in Kombolcha Regional Animal Health Diagnostic Laboratory and Sirinka Agricultural Research Center until they were processed and analyzed in National Animal Health Diagnostic and Investigations Center, Sebeta, Ethiopia (Tefera and Mulate, 2016).

Serological examination and analysis: The sera samples were tested for the presence of specific antibody against Maedi-visna virus using Indirect enzyme-linked immune sorbent assay test (I-ELISA), MVV/CAEV serum verification version VISNAS ver 1217 EN (IDvet, 310, Rue Louis Pasteur – Grabels–France). The test was performed according to the manufacturer's manual. The results of the test were considered valid only if optical density of a positive control serum (OD_{PC}) was higher than 0.350 and OD_{PC} was more than three times

higher than optical density of a negative control serum (OD_{NC}). The optical density of a serum sample (OD_{sample}) was recalculated into percentage of OD_{PC} (S/P%) adjusted by OD_{NC} with the formula: $S/P\% = (OD_{sample} - OD_{NC}) / (OD_{PC} - OD_{NC}) \times 100\%$. The interpretations was samples presenting as S/P%, equal or below 50% are considered as negative, between 50% and 60% are considered as doubtful and equal or above 60% are considered as positive. The manufacturer cut-off of 50% sensitivity and specificity were 91.7% and 98.9% respectively (Nowicka *et al.*, 2014).

Data management and analysis: All data collected for this study were entered in Ms-Excel spread sheet, arranged and analyzed using STATA version 14.0 software. Descriptive statistics were used to estimate the sero-prevalence of Maedi-visna virus antibodies in the area. Risk factors such as breed, age, sex, body condition score, flock size and production system, were considered and their difference with sero-positivity was tested by chi square (χ^2). The relationship of associated risk factors with positive serological test result was analyzed by logistic regression. When the P value less than 0.05, it was considered as statistically significant.

RESULTS

In this study a total of 494 sheep serum samples, 149 from local and 345 from Awassi cross breed were collected from two district of North Wollo (Gazobelay) and South Wollo (Legambo) zones to identify specific antibodies of maedi visna using I-ELISA serological test. From a total sample tested 3.24% (16/494) were positive for the identification of antibodies against Maedi visna virus (MVV) in the area. There was no significance difference ($P > 0.05$) in sero-prevalence among both districts and all peasant associations; however there was slight numerical difference across each district and peasant associations in presence MVV

infection. The differences between MVV sero-prevalence in sheep per districts and peasant associations as well as their associations are summarized (Table 1).

The logistic regression odd ratio analysis of attribute risk factors indicated no significance

difference ($P > 0.05$) in sero-positivity between sheep of different breed, sex, age, body condition score, and production system and flock size. During the statistical analyses of all risk factors, the first level of each independent variable was used as a reference category (Table 2).

Table 1. Mean sero-prevalence of Maedi-visna virus in sheep and their associations with risk factors

Variables		No. of sample	No. of positive	% (Prevalence)	χ^2 (P- value)
Districts	S. Wollo (Legambo)	221	4	1.8	2.61 (0.14)
	N. Wollo (Gazobelay)	273	12	4.4	
Peasant association	Gimba (023)	116	3	2.6	4.33 (0.50)
	Chiro (025)	42	1	2.4	
	Dembesh (026)	63	-	-	
	Tachtalet (011)	101	4	4	
	Lay talet (012)	133	7	5.3	
	Shriya genet (07)	39	1	2.6	
Total		494	16	3.24	

Table 2. Logistic regression analyses (LR) of risk factors with dependent Maedi-visna virus sero-positivity in sheep of study area

Risk factors		No. of sample	No. of positive	Prevalence (%)	P-value	OR	CI 95%
Breed	Local(Indigenous)*	149	4	2.7	0.65	-	0.37 - 6.87
	Awassi cross	345	12	3.5		1.61	
Sex	Female*	306	12	4	0.27	-	0.17 - 2.41
	Male	188	4	2.12		0.64	
Age	< 2 years (young)*	238	7	3	0.91	-	0.26 - 2.95
	2-4 years (adult)	179	6	3.5		0.87	
	> 4 years (old)	77	3	3.9		0.82	
Body condition score	Poor*	104	6	5.8	0.25	-	0.15 - 1.55
	Moderate	290	7	2.4		0.48	
	Good	100	3	3		0.80	
Production system	Extensive	468	16	3.24	-		
	Semi- intensive	26					
Flock size	< 13 (small)*	140	5	3.6	0.14		0.06 - 1.08
	13-28 (medium)	229	4	1.7		0.26	
	> 28 (large)	125	7	5.6		0.93	
Total		494	16	3.24	0.79		

* Reference category

DISCUSSIONS

Maedi visna causes a significant economic loss through morbidity, mortality and carcass weight loss in sheep worldwide. Seroprevalence of Maedi visna virus (MVV) has been established at different times from various countries including Ethiopia. The result of the present study conducted in two districts and six peasant associations of eastern Amhara region, Ethiopia showed an overall sero-prevalence of 3.24% MVV infection in sheep. This sero-prevalence found in our study area indicating the occurrence and wide distribution of MVV infection in sheep production systems in selected area of eastern Amhara region, Ethiopia.

The 3.24% prevalence of MVV in this study was in line with the reports of Mahin *et al.* (1984) 2.7% in Morocco, 2.41% in Manitoba, Canada, Sihvonen *et al.* (2000) 1.6% in Finland, Aslantas *et al.* (2002) 1-5-2.6% in Hatay region, turkey, Getnet *et al.* (2010) 6% in north Omo, Ethiopia, Shuaib *et al.* (2010) and Tefera and Mulate (2016) 3.2% in Eastern Amhara, Ethiopia. However, the sero-prevalence result of the present study is much lower than many of the previous reports in Ethiopia, viz. 74% in central Ethiopia (Woldemeskel *et al.*, 2002), 62.5% in central cool highland (Garedew *et al.*, 2010), 88% and 20% in Debre-Brihan sheep breeding center and Arsi, respectively (Getnet *et al.*, 2010), 70.4% in Sheno agricultural research center (Seyoum *et al.*, 2011) and 15.6% in eastern Amhara region (Tsegaw and Ademe, 2012).

The findings in this study were also much lower than in other countries of the world. For instance, a prevalence of 19% in Canada (Simard and Morley, 1991), 15.6% in culled ewes in Alberta, Canada (Fournier *et al.*, 2006), 50% in Palestine (Hananeh and Barhoom, 2009), 28.8% in Germany (Hüttner *et al.*, 2010), 15.3% was reported in Turkish sheep (Preziuso

et al., 2010), 19.4% in Kirikkale district, Turkey (Azkur *et al.*, 2011), 18% in Wyoming sheep, USA (Gerstner *et al.*, 2015) and 29.6% in Khorasan-e Razawi province, Iran (Norouzi *et al.*, 2015). Such inconsistency (variation) in the prevalence rates of MVV might be the variation in the diagnostic tests, sampling method used, the prevalence variability within the population studied, the characteristics of the animals forming the population, susceptibility of different breeds to the disease, management practices and measures taken to control the disease.

This study showed a variation in sero-prevalence of MVV between different study districts (1.8% to 4.4%). Similar results were obtained in different parts of Quebec (14.5% to 69%) (Shuaib *et al.*, 2010), in turkey (3.8% to 41.2%) (Alkan and Tan, 1998), in Iran (6.7% to 72.2%) (Norouzi *et al.*, 2015) and indifferent parts of Ethiopia (0.6% to 88%) (Getnet *et al.*, 2010). The spatial difference in distribution of positive cases might be explained by the introduction of carrier animals from an infected area to disease free zones, the management practices and the bio-security implemented by farm owners. The sero-prevalence finding in Legambo (1.8%) and Gazobelay (4.4%) districts was quite interesting. These zones are geographically located far from severely affected sheep ranches and it is suggested that the disease might have been spread along with the distribution of Awassi crossbred rams.

There was no significant difference in sero-prevalence between male and female sheep ($P > 0.05$), which was in agreement with findings of Woldemeskel *et al.* (2002), Seyoum *et al.* (2011) and Tefera and Mulate (2016). The breed related sero-prevalence of MVV infection in present study showed no statistical significant difference between breeds ($P > 0.05$) which was in line with Tefera and Mulate (2016). The possible explanation for this similarity could be

the fact that animals rearing condition in the study areas was similar (extensive) and different breeds were herded together without separation and sheep of different breeds were in direct contact with each other. In contrast to our finding, susceptibility difference was reported between Menz and Awassi sheep breeds (Seyoum *et al.*, 2011; Tsegaw and Ademe, 2012). This breed susceptibility difference could be related to the influence of traits of particular family lines, the strain of the virus and the result of one or more recessive genes (Simard and Morley, 1991).

In this study result indicated that no significant variation in sero-prevalence among age groups ($P > 0.05$), among body condition score ($P > 0.05$), between production system ($P > 0.05$), among flock size ($P > 0.05$). However, there was a little numerical deviation. These findings were disagreed with the report of Ayelet *et al.* (2001), Getnet *et al.* (2010), Tsegaw and Ademe (2012) and Tefera and Mulate (2016) which were reported that the sero-prevalence had significantly differed in the mentioned risk factors. This was due to the management system, sample size and the proportion of sampled animal across each risk factor difference.

Even though, the finding of the present study prevalence was low it revealed that MVV is widely distributed in eastern Amhara region, Ethiopian high sheep population areas. However, there was no statistically significance difference across breed; the frequency was higher in Awassi cross sheep as compared to the indigenous local breeds and the economic

losses could be huge due to low production and productivity from MVV influence in cross breed. Hence, the finding of MVV infection does not only suggest the occurrence of the disease in sheep population of the study area, but also give attention for the presence of foci of infection that might serve as source of infection for the distribution of the disease into free animals around and elsewhere in the sheep producing areas for upgrading purpose of local sheep and also through marketing practice. Awassi cross sheep distributor farm and ranch were main challenged site as a source for maedi visna virus infection and effective control and prevention measures to be implemented through appropriate way of testing and culling mechanisms of all sero-reactor ewes and their progeny. High sensitive screening test should be practiced and implemented during introduction of new flocks from abroad and before distribution of Awassi cross breed rams from ranches and multiplication center to smallholder farms and individual farmers. In addition further epidemiological study should be done in sheep producing areas of the country to know the level of infection at national and country level.

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