Serological surveys of *maedi visna virus* in sheep population of selected areas of eastern amhara, ethiopia

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Abstracts: Maedi-Visna (MV) causes a significant economical loss through morbidity, mortality and carcass weight loss in sheep worldwide. A cross-sectional study was conducted to determine the prevalence and associations with potential risk factors of Maedi-visna virus infection in the selected areas of the Eastern Amhara. A total of 323 Awsi cross sheep blood sera were collected in the period from November, 2017 to October, 2018 and examined using indirect enzyme linked immune-sorbet assay (i-ELISA) to screen antibodies against *Maedi-Visna virus*. From a total sample tested 4.0% (13/323) were positive for the presence of antibodies against *Maedi visna virus (MVV)* in the area. The seroprevalence of *Maedi visna* was statistically no significantly different between associated risk factors of age ($\chi 2$=2.193, p=0.139), sex ($\chi 2$=0.288, p=0.591), body condition score ($\chi 2$=1.378, p=0.502). This study showed relatively low seroprevalence against *Meadi Visna* in sheep population in these study areas of the country. Due to difficulty in clinical diagnosis, chronic course of the disease, the absence of effective vaccine and treatment and huge economic loss, a comprehensive epidemiological study all over the country in high sheep population areas should be taken without delay to depict the real picture of the disease in the country.

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1. Introduction

Sheep production plays a great economic role in the small holder farmers of Ethiopian. Sheep populations in Ethiopia are estimated about 27.3 million, out of which, 99.9% are indigenous breeds (CSA, 2014). Roughly 75% of the populations are found in the highlands and 25% in the lowlands. The sheep populations in the Amhara regional state are about 8,987,694. This contributes 23% to the total population of the country (CSA, 2009). Sheep are good sources of income and food proteins for rural farmers in most parts of Ethiopia. They have fast growing and return rates and are able to give twins and triple births with short lambing intervals. In Ethiopia they provide a significant value of the national meat and skins production (Tibbo, 2006).

Despite their importance as a component of the Ethiopian farming system, their contribution to food production, rural income and export revenue are far below than their expected potential. This is because small ruminant production is constrained by the compound effects of disease, poor feeding and poor management (Getachew, 1995). Among the different disease problems of sheep, Meadi Visna is an important disease, which causes a significant economical loss through morbidity, mortality and carcass weight loss in sheep worldwide.

Maedi visna/ ovine progressive pneumonia (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 (Kahn *et al*., 2005; Radostits *et al*., 2000; Murphy *et al*., 1999; Vorster *et al*., 1996; Jones and Hunt, 1983). Maedi-visna (MV) is a chronic disease of adult sheep characterized by progressive interstitial pneumonia and other syndromes such as meningo encephalitis, indurative mastitis and arthritis. It is caused by a non-oncogenic retrovirus, which belongs to the subfamily lentivirinae.

The incubation period of this subclinical infection is usually more than two years and its clinical signs appear when the animal is 3 to 4 years old (OIE, 2012). Consuming contaminated colostrum and milk and inhalation of infectious respiratory secretions in close contact are the main routes of transmission (Brodie SJ et al., 1998). Generally, both horizontal and vertical transmission has been proposed for MV virus (Blacklaws *et al.,* 2004). After entry of the virus into the body, the host is infected lifelong (OIE, 2012).

Since 1988-1989 the detection of the virus in Ethiopia, it has been assumed that Maedi-Visna is an emerged disease introduced to the country through the imported sheep breeds. Previous 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 (Woldemeskel *et al*., 2002; Ayelet *et al*., 2001; Tibbo *et al*., 2001; *Getnet et al.2011*).

The economic losses of the disease are due to mortality associated with clinical disease, poor value of the removed animals and reduction of economic life.6 Effects of subclinical infection on the reproductive potential should also be added to the economic losses (Cutlip RC *et al.,* 1982). There is no treatment and effective vaccine against the disease but by increasing the quality and efficiency of diagnostic tests, there is the possibility of eradicating the disease (Shuaib M *et al.,* 2010).

So far in Etghiopia, outbreaks of unidentified diseases often occur and a considerable number of sheep die with signs of respiratory embarrassment. Although farms and breeding centers have been reporting Maedi-visna cases, Eastern Amhara, Ethuopia, the extent to which the disease disseminated has been established, but There was lack of information on the status and losses associated with Maeddi visna and very little attention has been given to the role of maedi visna virus as the cause of disease and production losses in sheep in Ethiopia. Therefore, taking into account the significance of the disease as one of the most important causes of economic losses and the scarcity of information in the country, the present study was designed:

- To investigate the serological status of the disease in the selected areas of North Shoa.
- To design a practical control strategy of the disease at the regional and national level.

2. Materials And Methods

2.1. Study areas

The study areas were selected based on retrospective data showing the history of introduction of Awassi cross rams. In previous certain years period (2008-2019) about more than 5000 cross rams originated from sheep multiplication and research centers were distributed in different districts of South Wollo and North Shoa zones of Amhara region to upgrade the genetic potential of the local indigenous sheep breeds (BoARD, 2011). Of these districts, faji from North Shoa zone and Legambo from South wollo zone were selected purposively for this study. Faji is found in the Basona werana werda of North Shoa zone of the Amhara region. It is located at a distance of 110- 130 Kms North of Addis Ababa at a latitude between 90 30'' 26'' to 90 64''92''N and 39014'' 32'' to 390.27'' 37''E longitude. Legambo the other study sites are situated about 500 Km North of Addis Ababa at a latitude between 100 32'86" to 100 57'81"N and 39014'' 32'' to 39026'' 13'' E longitude. The study district is found in central highland of the country at an altitude of above 2770 m. The annual rain fall of the study area ranges from 950-1200mm. The mean annual minimum and maximum temperatures are 1.5 and 23.30C, 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.

2.2. Study Animals and their management

The animals used for this study were Awassi cross breed sheep. The sheep sampled were all above 6 months of age. Sheep owned by individual farmers were managed under traditional grazing (extensive) system 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 while in semi intensive they were extra supplemented in addition to grazing.

2.3 Study Design, Sampling Strategy and Sample Size Determination

Cross-sectional sero- epidemiological study was conducted from November, 2017- October, 2018 to detect and estimate the prevalence of Maedi-visna infection in the area. The sampling method employed in this study was multistage cluster methods. Two districts; Faji and Legambo from north shoa and South Wollo zone respectively were selected purposively based on accessibility and the history of Awassi and their cross ram distribution and had high population of sheep. From each districts a representative sampled animal were selected. All animals above six months of age were kept for breeding purpose was sampled in simple random sampling from the purposive selected area for blood collection.

Since there was a previous study conducted in the study areas with a prevalence rate of 4% by Nigussie and Belay (2016). So the desire sample size for this study was determined based on the previous prevalence 4%, the 5% desired absolute procession and 95% confidence interval (CI)) according to Thrusfield (2005).

$$n=\frac{(1.96)^2 p_{exp}(1-p_{exp})}{d^2} =$$

Where

n = required sample size

P_{exp} = Expected prevalence

d = Desired absolute precision

1.962 = the value of z at 95% Confidence level

Accordingly, 59 sheep sample was required. But to increase the precision and accuracy, the sample size were maximized to more than five folds, 323.

2.4. Data Collection and Serological Examination

Samples were taken from the jugular vein of sampled sheep aged over 6 months. Sterile vacutainer tubes and needles were used for each animal. While collecting blood samples, data related to age, body condition score, origin, and sex, of each sampled animal were recorded properly. 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 to obtain clear serum. Then serums were separated into 2ml crayo-vial and were preserved at -20ºC in Debre Birhan Agricultural Research Center Animal Health Laboratory until it were processed and analyzed. The test was performed at National Animal Health Disease Investigation Center (NAHDIC), Sebeta, Ethiopia.

To determine the presence of antibodies against Maedi-visna virus, the instructions of the manufacturers' manual were strictly followed. The sera samples were tested for the presence of antibody against Maedi-visna virus using Indirect Enzyme-linked immune sorbent assay test (I-ELISA), *Maedi-Visna/CAEV* serum verification version *VISNAS ver 1217 EN* (IDvet, 310, Rue Louis Pasteur – Grabels – France) according to the protocols recommended by OIE (2008), 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}) × 100%. The interpretations was samples presenting an 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.**

3. Data Management And Analysis

Data collected during sampling and laboratory results were entered in Ms-Excel spread sheet and analyzed by using SPSS-20 software version. Descriptive statistics were used to approximate the seroprevalence for MVV antibodies in the area. Risk factors such as breed, age, sex, body condition score, and origin were considered and their difference with seropositivity was tested by chi square (X^2). The relationship of associated risk factors with positive serological test result was analyzed by logistic regression. A test value at $P < 0.05$ was taken as statistical significant.

4. Result

In the present study a total of 323 sheep serum samples were collected from two districts of North Shoa and South Wollo zones respectively to screen antibodies for *Meadi Visna Virus* using i-ELISA serological test. Of total samples tested, 13(4.0%) were positive for the presence of antibodies against *Meadi Visna Virus*. The highest and the lowest sero-positivity rate were 6.1% and 0.8% in Legambo and Faji districts respectively (Table 1).

Table 1: Sero-positivity to MVV antibodies in sheep detected by i-ELISA from study districts

Study District	No. Sampled	No. Positive (%)
faji	125	1 (0.8)
legambo	198	12 (6.1)
Total	323	13 (4.0)

Number and proportion of seropositive animals with respect to different levels of independent variables and the results of analysis showing the association of each independent variable with seroprevalence of *Meadi Visna* virus. The analysis of associated risk factors indicated no significance difference in sero-positivity between sheep of different age groups ($\chi2=2.193$, $p=0.139$), sex ($\chi2=0.288$, $p=0.591$), body condition score ($\chi2=1.378$, $p=0.502$) (Table 2).

Table 2: Sero-positivity to MV infection in sheep based on various risk factors

Variables		**No. sampled**	**Positive (%)**	**χ2**	**p-value**
Sex	Female	254	11 (4.3)	0.288	0.591
	Male	69	2 (2.9)		
Age	Adult	278	13 (4.7)	2.193	0.139
	Young	45	0(0.0)		
Bcs	Good	23	0 (0.0)	1.378	0.502
	Medium	235	11 (4.7)		
	Poor	65	2(3.1)		
Over all		323	13 (4.0)		

5. Discussion

Maedi visna causes a significant economical loss through morbidity, mortality and carcass weight loss in sheep worldwide. The result of the present study conducted in two districts faji and legambo of north shoa and south wollo, Ethiopia respectively. The present findings disclosed an overall sero-prevalence of 13(4.0%) Maedi-visna infection in sheep population.

The sero-prevalence result of the present study is in line with the reports of Nigussie and Belay (2016) 4% in four districts of eastern Amhara region, Ethiopia. The findings in this study were also is in line with other countries of the world. For instance, Shuaib *et al.,* (2010) 2.41% in Manitoba, Canada, Aslantas *et al., (*2002) 1-5-2.6% in Hatay region, turkey, 2.7% in Moroco, and Sihvonen *et al.,* (1999) 1.6% in Finland. However, the sero-prevalence result of the present study much lower than of the previous reports in Ethiopia, viz., Tsegaw and Ademe (2012) 15.6% in eastern Amhara region, Ethiopia, 70.4% in Sheno agricultural research center (Seyoum *et al.,*2011), 62.5% in central cool highland (Garedew et. al., 2010), 20% in Arsi, Ethiopia (Getnet, et. al., 2010), and 88% in Debre-Brhan sheep breeding center (Getnet, et. al., 2010), and 74% in central Ethiopia (Woldemeskel et. al., 2002). The findings in this study were also much lower than in other countries of the world. For instance, Gerstner *et al., (*2015) 18% in Wyoning sheep, USA, Norouzi *et al., (*2015) 29.6% in Khorasan-e- Razawi province, Iran, Azkur *et al., (*2011) 19.4% in Kirikkale district, Turkey, Hüttner et al., (2010), 28.8% in Germany, Preziuso *et al., (*2010) 15.3% in Turkish sheep, Hananeh, (2009) 50% in Palestinem, Fournier *et al., (*2006), 15.6% in culled ewes in Alberta, Canada, Simard and Morley, (1991) 19% in Canada.

Such inconsistency in the prevalence rates of MV may be due to 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 survey showed a variation in sero-prevalence of *Meadi visna* between different study districts (5.2% to 63.5%). Similar results were obtained indifferent parts of Ethiopia (0.6% to 88%) (Getnet et. al., 2010) and 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% to72. 2%) (Norouzi *et al.,* 2015). This geographic difference in distribution of positive cases could be explained by the introduction of carrier animals from an infected area to disease free zones, the management practices and the bio-security followed by farm owners.

The seroprevalence of the present finding in the study districts were quiet interesting. These districts 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 cross breed rams as other study investigated seroreactor rams in the villages obtained from ranches a year ago.

In the present study, an attempt was carried out to know whether the associated potential risk factors influence or not on prevalence of *Meadi Visna virus* infection in sheep. In this finding, there was no statistically significant difference in seroprevalence of *Meadi* Visna between sex ($\chi 2=0.288$, $p=0.591$), which is in agreement with findings of Woldemeskel *et al.,* (2002), Seyoum *et al., (*2011) and Tefera and Mulate (2016), Nigussie and Belay (2016). There was no significant difference in seroprevalence among age ($\chi 2=2.193$, $p=0.139$. This finding was disagreed with the reports in Canada (Arsenault et. al., 2003; Simard and Morley, 1991), in Ethiopia (Nigussie and Belay, 2016, Ayelet et al., 2001), in Turkey (Preziuso *et al.,* 2010) and in Iran (Norouzi *et al.,* 2015). The body condition score related seroprevalence of MV infection in present study showed no statistical significant difference among body condition score ($\chi 2 =1.378$, $P=0.502$). This finding was disagreed with the report of Tefera and Mulate (2016), Tsegaw and Ademe, (2012), Getnet *et al.,* (2010), Ayelet *et al.*, (2001), and Pritchard and Dawson (2000). This variation was due to the management system, sample size and the proportion of sampled animal across each categories of body condition factor.

6. Conclusion And Recommendation

In conclusion, our serological findings suggested that MVV is relatively less prevalent in North Shoa and South Wollo of Amhara Regional State; however the economic losses could be enormous. Hence, the finding of positive serological reactors does not only suggest the occurrence of the disease in sheep population of the study area, but also indicates the presence of foci of infection that could serve as source of infection for the spread of the disease into unaffected animals around and elsewhere in the sheep producing areas through marketing. This study also gives a clue what has to be done in the future to control the disease spreading from different breeding and multiplication centers to the farmers and then the centers distribute genetically improved and infection free cross-breed rams to the farmers. In light of this the following recommendations are forwarded.

- Detail nationwide epidemiological investigations in sheep producing areas should be conducted.
- Screening test should be carried out during introduction of new flocks and before distribution cross breed rams from different ranches and multiplication center to smallholder farms
- Unless and otherwise a sheep is a valuable progeny, all sero-positive animals should be culled and annual or semi-annual testing testing of the animals should be practiced until a flock is free from infection.

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