

On-station Seroprevalence And Associated Factors Of Maedi Visna Virus In Sheep Population Of North Shoa, Ethiopia

Enyiew Alemnew¹; Mesfin Zewdu², Wondoson Kinfe³; Tadiwos Asfew¹; Chekol Demis¹; Yeshtila Wondifra¹; H Abtamu Addis¹

1 Debre Birhan Agricultural Research Centre, PO. Box 112, Debre Birhan Ethiopia

2 Debre Birhan Sheep Breed Improvement And Multiplacation Center, Debre Birhan Ethiopia

3 Amed Guya Sheep Breed Improvement And Multiplacation Center, Debre Birhan Ethiopia

yohansaddis68@gmail.com

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 north shoa. A total of 1851 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 10.3 % (191/1851) were positive for the presence of antibodies against *maedi visna virus (mvv)* in the area. The seroprevalence of *maedi visna virus* was statistically significantly different between associated risk factors of age ($\chi^2=26.678$, $p=0.000$), sex ($\chi^2=14.202$, $p=0.000$), body condition score ($\chi^2=346.757$, $p=0.000$), study areas ($\chi^2=226.636$, $p=0.000$), and breed ($\chi^2=11.230$, $p=0.011$). Awassi cross and dorper cross sheep distributor farm and ranch were incriminated as a source for *maedi visna virus* infection and effective control measures should be implemented; through annual or semi-annual testing and culling of all seroreactor ewes and their progeny. Removal of lambs at birth before taking colostrum and raising them artificially in isolation either on pasteurized milk or milk substitutes. Simultaneously with regional government, screening test should be carried out during introduction of new flocks and before distribution of awassi cross breed and dorper cross breed rams particularly from ranches and multiplication center to smallholder farms. In addition; further epidemiological study all over the country in high sheep population areas should be implemented.

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Key words: Maedi-visna virus; risk factors, seroprevalence and sheep

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. 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 intervals. In Ethiopia they provide a significant value of the national meat and skins production (Tibbo, 2006).

However, due to low productivities of the indigenous breeds, the Ethiopian government had been introducing sheep breeds of Hampshire, Corriedale, Awassi and Dorper from UK, SA and Israel since the early 1970's to upgrade the genetic makeup of the local sheep breeds. Imported sheep were stocked and crossed with the local sheep in Debre Berhan agricultural research center, Debre Berhan and Amed Guya sheep breed improvement 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 them were Agarfa (former farmers training center) and Arsi Rural Development Unit (ARDU), which owned the rams (Corriedale and Awassi) (Ministry of Agriculture report, 1991, unpublished).

Despite the genetic improvement, an incidence 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. 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 (Ovine Progressive Pneumonia-OPP) virus was detected in 39 (90%) out of 43 tested samples (Ministry of Agriculture report, 1991, unpublished). Therefore; the occurrence of MVV in Ethiopia was

first detected in imported breed in 1986 at Agarfa sheep ranch (Bale province).

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).

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. Transmission occurs more readily between dams and lambs *via* colostrum and milk, and among confined individuals probably *via* respiratory secretions (Dawson, 1985).

So far in the country 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, North Shoa, Ethiopia, 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 determine the associated risk factors of *maedi visna virus* disease
- 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 purposely based on retrospective data showing the history of meadi visna disease in sheep of ranches and research center. Of these districts, Debre Birhan Agricultural Research Center On-Station, Debre Birhan Sheep Multiplication and Breed Improvement Center, and Amed Guya Sheep Multiplication and Breed Improvement Center in North Shoa zone were selected purposely for this study.

Debre Birhan Agricultural Research Center On-Station and Debre Birhan Sheep Multiplication and Breed Improvement Center are located around Debre Birhan town at a distance of 110- 130 Kms North of Addis Ababa at a latitude between 9° 30' 26" to 9° 64' 92" N and 39° 14' 32" to 39° 27' 37" E longitude. The study districts are found in central highland of the country at an altitude of above 2770 m. The annual rain fall of the study areas ranges from 950-1200mm. The mean annual minimum and maximum temperatures are 1.5 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. Amed Guya sheep multiplication and breed improvement center is also other stud area, which is situated in the Mehal Meda town of North Shoa zone of the Amhara region. It has a geographical coordinate of 10° 18' 0" north, 39° 40' 0" east with an altitude of 3132 above sea level. It is located 180 Km north of Debre Birhan town and 361Km north east of Addis Ababa. The average temperature and the average annual rain fall of the study area is 12.2°C and 1149mm respectively and the area experiences a bimodal rain fall patterns with a short rainy season which occurs in winter and long rainy season in summer.

2.2. Study Animals and their management

The study was carried out on 1851 (pure awasi, cross awasi, cross dorper, and local monz sheep above six months of age and kept under on-station. Of these sheep, 501 from Amed Guya Sheep Multiplication And Breed Improvement Center, 52 from Debre Birhan Agricultural Research Center, and 1298 from Debre Birhan Sheep Multiplication and Breed Improvement Center, North Shoa zone. Sheep were managed under semi-extensive system.

2.3. Study design and sample size determination

A cross-sectional serological study was carried out on three purposely selected districts of North Shoa to determine the prevalence of *Maedi-visna* infection in the areas. Census samplings were applied. All animals, above six months of age were kept for breeding purpose was sampled from Debre Birhan Sheep Multiplication and Breed Improvement Center, Amed Guya Sheep Multiplication and Breed Improvement Center. And Also Awasi crosses sheep

from Debre Birhan Agricultural Research Center on-station.

Since there was a previous study conducted in the study areas with a prevalence rate of 70.4% by (Seyoum *et al.*, 2011). So the desired sample size for this study was determined based on the previous prevalence 4%, the 5% desired absolute precision 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

Thus, the desired sample size for $P_{70.4\%} = 0.704$ is $n = 320$. However, to increase accuracy and precision, the sample size was increased near to six folds, 1851 sheep included in this study.

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, breed, 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 cryo-vial and were preserved at -20°C in Debre Birhan Agricultural Research Center Animal Health Laboratory until it was processed and analyzed. The test was performed at Kembolcha regional animal health disease investigation center and 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 ($\text{OD}_{\text{sample}}$) was recalculated into percentage of OD_{PC} (S/P %) adjusted by OD_{NC} with the formula: $\text{S/P\%} = (\text{OD}_{\text{sample}} - \text{OD}_{\text{NC}}) / (\text{OD}_{\text{PC}} - \text{OD}_{\text{NC}}) \times 100\%$. 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 *Meadi visna virus* 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 (χ^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 1851 sheep serum samples were collected from three districts of North Shoa zones to screen antibodies for *Meadi visna virus* using i-ELISA serological test. Of total samples tested, 191(10.3%) were positive for the presence of antibodies against MVV. The highest and the lowest sero-positivity rate were 63.5% and 5.2% in Debre Birhan Agricultural Research Center and Debre Birhan Sheep Multiplication and Breed Improvement Center 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 (%)
AGSMBIC	501	90 (18.0)
DBARC on-station	52	33 (63.5)
DBSMBIC	1298	68 (5.2)
Total	1851	191 (10.3)

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 significance difference in sero-positivity between sheep of different age groups ($\chi^2=26.678$, $p=0.000$), sex ($\chi^2=14.202$,

$p=0.000$), body condition score ($\chi^2=346.757$, $p=0.000$), study areas ($\chi^2=226.636$, $p=0.000$), and breed ($\chi^2=11.230$, $p=0.011$). (Table 2).

Table 2: Proportion of positive cases for different levels of independent variables and the association of each of the independent variables with disease caused by Meadi Visna virus in sheep.

Variables	No. sampled	Positive (%)	χ^2	p-value	
Sex	Female	1754	170 (9.7)	14.202	0.000
	Male	97	21 (21.6)		
Age	Adult	1581	187 (11.8)	26.678	0.000
	Young	270	4(1.5)		
	Good	223	10 (4.5) ^b		
Bcs	Medium	1447	90 (6.2) ^b	346.757	0.000
	Poor	181	91(50.3) ^a		
	Awasi Cross	1498	162 (10.8) ^a		
Breed	Dorper Cross	131	7 (5.3) ^b	11.230	0.011
	Monz	131	19 (14.5) ^a		
	Pure Awasi	91	3 (3.3) ^b		
	AGSMBIC	501	90 (18.0) ^b		
Study area	DBARC	52	33 (63.5) ^a	226.536	0.000
	DBSMBIC	1298	68 (5.2) ^c		
	Over all	1851	191 (10.3)		

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 three districts of North Shoa, Ethiopia. The present findings disclosed an overall seroprevalence of 191(10.3%) *Maedi-visna virus* infection in sheep population. The sero-prevalence result of the present study is in line with the reports of Tsegaw and Ademe (2012)15.6% in eastern Amhara region, Ethiopia, Prezioso, *et al.*, (2010) 15.3% in Turkish sheep, and Fournier *et al.*, (2006), 15.6% in culled ewes in Alberta, Canada.

However, the sero-prevalence result of the present study higher than of the previous reports of, Nigussie and Belay (2016) 4% in four districts of eastern Amhara region, Ethiopia, Shuaib *et al.*, (2010) 2.41% in Manitoba, Canada, Aslantas *et al.*, (2002) 1-5-2.6% in Hatay region, turkey, 2.7% in Morocco, Sihvonen *et al.*, (1999) 1.6% in Finland, and much lower than many of the previous reports in Ethiopia, viz. 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, 19% in Canada (Simard and Morley, 1991), Azkur *et al.*, (2011) 19.4% in Kirikkale district, Turkey and

Gerstner *et al.*, (2015) 18% in Wyoming sheep, USA, Norouzi *et al.*, (2015), 29.6% in Khorasan-e- Razawi province, Iran, Hüttner *et al.*, (2010), 28.8% in Germany, and Hananeh and Barhoom, (2009) 50% in Palestine. Such inconsistency in the prevalence rates of *Meadi visna* 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 seroprevalence 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% to 72.2%) (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. Even though; these districts are geographically located nearest each other, they had quite different disease distribution. it is suggested that the different might be due to those sheep multiplication and breed improvement centers were applied a continuous annual test and culling

system for the past five years as a result those disease prevalence were reduced from 88% (Getnet *et al.*,2010) to 5.2% in Debre Birhan Sheep Multiplication and Breed Improvement Center and 66.67% (Wondoson, 2014 unpublished) to 18.0% in Amed Guya Sheep Multiplication and Breed Improvement Center, but Debre Birhan Agricultural Research Center was not applied any measurement to control or prevent disease distribution, as a result there was no different between the previous (70.4% Seyoum *et al.*,2011) and present (63.5%) findings.

In the present study, an attempt was carried out to know whether body condition influence or not on prevalence of MV infection in sheep; and it was found that poor body condition animals 91(50.3%) were more likely to be infected as compared to good body condition animals 10 (4.5%). Our finding is in accordance with the finding of Nigussie and Belay, (2016) and Pritchard and Dawson (2000) they reported sever emaciation in sheep infected with *Meadi Visna*. This is supported by the fact that MVV targets the cells of the immune system leading to concomitant infectious diseases and ultimately weight losses.

There was statistically significant difference in sero-prevalence of *Meadi visna virus* infection between sex ($\chi^2=14.202$, $p=0.000$), (Table 2), which is in agreement with findings of Tsegaw (2012) and Simard and Morley (1991) reported a highly significant difference in seroprevalence between sexes, higher in male (24.1%) than female sheep (14.4%). This sex sero-prevalence difference can probably be rams are longer repeat exposure to different female flocks during mating, which may exposes for more horizontal transmission. In contrast to the above findings, Nigussie and Belay (2016), Woldemeskel *et al.*, (2002) and Seyoum *et al.*, (2011) reported there was no statistically significant difference in sero-prevalence between sexes.

The age related seroprevalence of *Meadi visna virus* infection in present study showed statistical significant difference between age groups ($\chi^2=26.678$, $p=0.000$) (Table 2), which disclosed that adult sheep were more likely to be infected as compared to younger sheep. In this regard, the finding of this study is consistent with the results reported elsewhere. viz, in Canada (Arsenault *et al.*, 2003; Simard and Morley, 1991), in Ethiopia (Ayelet *et al.*,2001, Nigussie and Belay, 2016), in Turkey (Preziuso *et al.*,2010) and in Iran (Norouzi *et al.*,2015). This age sero-prevalence discrepancy can probably be explained by the longer exposure to horizontal transmission and development of detectable levels of MV antibodies can vary from months to years (Radostits *et al.*, 2000). Thus, the older the animals, the greater the potential for a greater proportion of sheep to be become infected with MV.

The breed related seroprevalence of *Meadi visna virus* infection in present study showed statistical significant difference between breeds ($\chi^2=11.230$, $p=0.011$) (Table 2). In this regard, the finding of this study is in line with Seyoum *et al.*, (2011); Tsegaw and Ademe (2012), susceptibility difference were reported between different sheep breeds. 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 regard, the finding of this study is in contrast with the results reported by Nigussie and Belay (2016). The possible explanation for this similarity could be due to the fact that different breeds were herded together without separation and sheep of different breeds were in direct contact with each other.

6. Conclusion and recommendation

In conclusion, our serological findings suggested that MVV is relatively high prevalent in Debre Birhan Agricultural Research Centers and low in Debre Birhan Sheep Breed Improvement and Multiplication Center as a result 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 for upgrading purpose of local sheep and also through marketing. Sheep breeding ranches and centers were incriminated as a home-base for *Maedi visna virus* infection and effective control measures should be implemented. This study also gives a clue what has to be done in the future to control the disease spreading from different breeding and multiplication centres to the farmers and then the centres 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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