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Maedi-Visna: Sero-prevalence and risk factors in sheep population of South Wollo and North Shewa Zones, Ethiopia

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Abstract

Maedi-Visna (MV) is a chronic disease of adult sheep characterised by progressive interstitial pneumonia and other syndromes such as meningo-encephalitis, indurative mastitis, and arthritis. The study was conducted to determine the serological epidemiology and associated risk factors of Maedi-Visna in the highland of the North Shewa and South Wollo zones of the Amhara region, Ethiopia. A total of 807 serum samples were collected randomly from purposely selected areas and examined using an indirect enzyme-linked immunosorbent assay to screen specific antibodies against Maedi-Visna. The data were analysed using logistic regression. The overall seroprevalence of Maedi-Visna was 11.7%. The highest and lowest seroprevalence were in Basona-Werena (19.2) and Legambo (6.1%) districts, respectively. As per peasant associations, the highest and lowest seroprevalence were in the Agricultural Research Centre (63.5%) and Kormargefya (0.8 %), respectively. Despite the variation in prevalence level, the disease was insidiously disseminated to all the other study districts and peasant associations. The final multivariable logistic model identified age OR = 5.04 (95% CI: 1.19–21.43), production system OR = 6.96 (95% CI: 3.79–12.78) and body condition score OR = 10.12 (95% CI: 3.63–28.19) as the most important risk factors in relation to MV seroprevalence. This finding showed a higher prevalence of Maedi-Visna in research centre and ranches, which can be a source of Maedi-Visna virus infection in other parts of sheep producing areas. Therefore, strict and regular screening tests should be carried out during the introduction of new flocks and before the distribution of crossbreed rams, particularly from ranches and research centres to smallholder farmers.

Keywords: Antibody, Maedi-Visna, Prevalence, Risk factor, Serology, Sheep

Introduction

Maedi-Visna (MV) is an insidious, lifelong, and eventually fatal disease syndrome in sheep that occurs in most sheep-keeping areas worldwide. The most common symptoms, chronic

respiratory disease and indurative mastitis, only become evident some years after infection and cause lost production and excess resource use (Blacklaws, 2012). MV is caused by the small ruminant lentiviruses (SRLV), the family retroviridae, recognised as a heterogeneous group of viruses that infect sheep, goats, and wild ruminants with evidence of cross-species infection (Leroux et al., 2010; Leroux et al., 1997).

Transmission of SRLV is now recognised as being primarily through inhalation of respiratory secretions (McNeilly et al., 2008; Broughton-Neiswanger et al., 2007) and readily between dams and lambs *via* colostrum and milk (Preziuso et al., 2009). There is no treatment or commercial vaccine for MV. Thus, an accurate diagnosis is the cornerstone for setting up an optimal control program for the infection and reducing its prevalence. Multiple diagnostic techniques can be used to detect SRLV infection. Direct methods to detect SRLV (PCR, indirect immunofluorescence, and in situ hybridization) are efficient diagnostic techniques (De Andrés et al., 2005). Indirect methods (AGID and ELISA) have been proposed as the most appropriate to detect infected animals, with ELISA having higher sensitivity and lower specificity than AGID (Larruskain and Jugo, 2013).

Maedi-Visna virus infection causes economic losses due to impairing the production and productivity of infected sheep. However, the infection has neither an appropriate treatment nor a vaccine (Sihvonen et al., 2000). Hence, control of the disease is difficult and costly. Destocking flocks or culling of sero-positive sheep and their progeny is a better choice for the control and prevention of the disease (Williams-Fulton and Simard, 1989; Houwers et al., 1984).

Maedi-Visna is of international significance, being present in most of the sheep-producing nations of the world, including Ethiopia, with the exceptions of Australia and New Zealand (Straub, 2004). In Ethiopia, MV is the most economically important disease of sheep (Getnet et al., 2010) and the cause of several sheep deaths with signs of respiratory embarrassment (Ayelet et al., 2001). The prevalence of MV has been reported in different localities in Ethiopia. Accordingly, a prevalence of 74% in central Ethiopia (Woldemeskel et al., 2002), 62.5% in central cool highland Ethiopia (Gardew et al., 2010), 6% in north Omo (Getnet et al., 2010), 70.4% in the Sheno-agricultural research center (Seyoum et al., 2011), 15.6% in highland sheep at ranches and smallholder farms in eastern Amhara Region (Tsegaw and Adem, 2012), and 4% in selected districts of the Amhara region (Tefera and Mulat, 2016).

Although previous works showed the existence of MV in different localities of Ethiopia (Tefera and Mulat, 2016; Tsegaw and Adem, 2012; Getnet et al., 2010), very little attention

Journal of Science and Inclusive Development Vol. 5, No. 1, DOI: 10.20372/jsid/2023-234

has been given to the role of MV as the cause of production losses in sheep industries in Ethiopia. To alarm the government of Ethiopia, full emphasis on the prevention and control of the MV disease epidemiological studies is necessary. Therefore, taking into account the significance of the disease as one of the most important causes of economic losses in the study areas, the present study was designed to estimate the sero-epidemiology of MV in sheep and its associated risk factors in the South Wollo and North Shewa Zones, Ethiopia.

Materials and methods

The study was conducted in four districts of the North Shewa Zone, namely Basona-Werena, Debre-Birhan town, Menz Gera, Menz Mama and Legambo districts of the South Wollo Zone of Amhara Region, Northeast Ethiopia (Figure 1). The study districts were purposefully selected based on the history of exotic sheep population in ranches and research centres, their distribution to small-holder farmers, and their proximity and accessibility to roads. Awassi and Dorper crossbreed sheep were distributed around the highland areas of Eastern Amhara which practiced the crop-livestock mixed production system. All the areas were highlands known for sheep-rearing practices (Getachew et al., 2016).

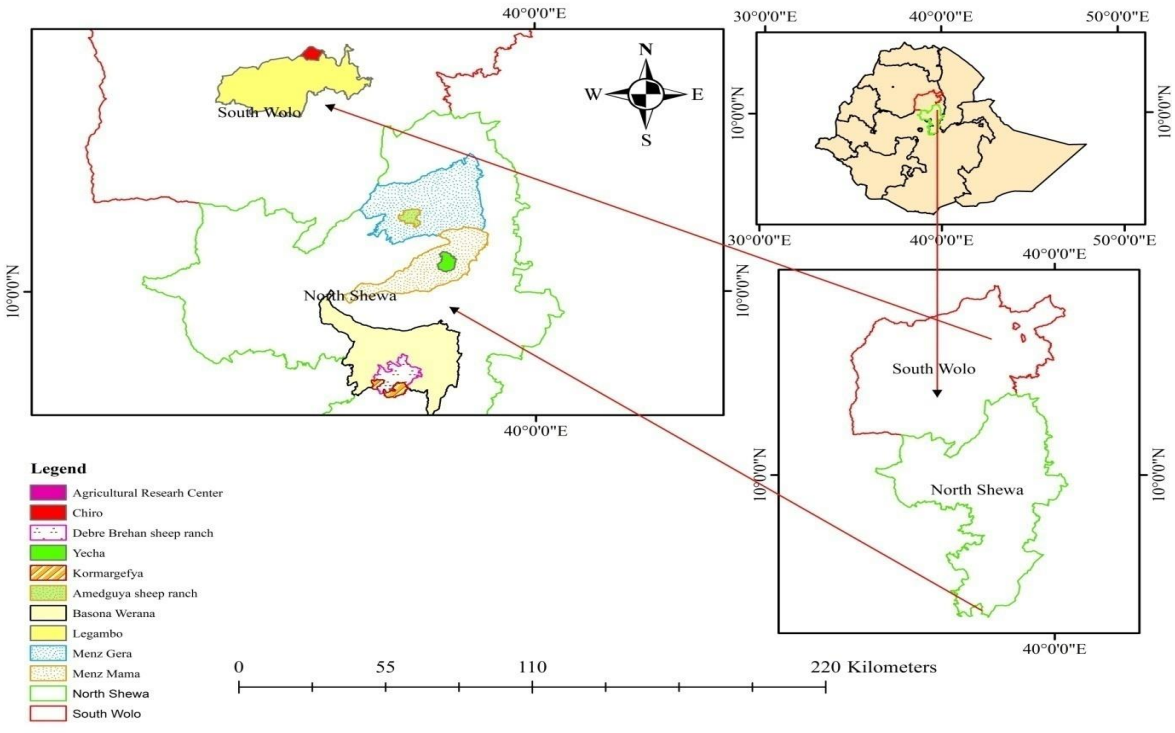

Figure 1. Maps of the study areas

Agricultural Research Centre and Kormargefya Peasant Association from Basona-Werana District, and Debre-Birhan Sheep Ranch from Debre-Birhan town are located at a distance of

110- 130 km north of Addis Ababa at a latitude between 90 30’ 26’’ to 90 64’92’’N and 90 3’ 26’’ to 390 27’ 37’’E longitude. These study areas are located in the central highland of the country at an altitude of above 2770m. The annual rainfall of the study areas ranges from 950-1200mm. The mean annual minimum and maximum temperatures are 1.5 and 23.30C, respectively. The area experiences a bimodal rainfall pattern with a short rainy season from January to March and a long rainy season from the end of June to early November. Yecha Peasant Association and Amed-Guya Sheep Ranch are located in the Menz-Mama and Menz-Gera Districts of the North Shewa zone, respectively. It has a geographical coordinate of 100 18’0" N and 390 40’ 0’’E with an altitude of 3132 above sea level and is located at a distance of 270 - 301 km north of Addis Ababa. The average temperature and the average annual rainfall of the area are 12.20C and 1149 mm, respectively. The area experiences bimodal rainfall patterns with a short rainy season (December to February) and a long rainy season (June - August) (DBARC, 2014; Menz Gera and Menz Mama Livestock Offices, 2014; Ethiopian Treasures, 2002).

Tuluawlya Peasant Association is another site around the Legambo district of the South Wollo Zone. It has a geographical coordinate of 100 32’86" to 100 57’81" N and 390 14’’ 32’’ to 390 26’’ 13’’E with an altitude of 2770 above sea level at a distance of 500 km north of Addis Ababa. The annual rainfall 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 rainfall pattern with a short rainy season (January to March) and a long rainy season (end of June to early November) (Tefera and Mulate, 2016).

Study populations

Animals used for this study were short fat-tailed indigenous breeds (Solomon et al., 2011), Awassi cross, Dorper cross, and pure Awassi sheep, and all were above six months of age. The age of each sheep was classified based on the dentition formula given by Geoff (2016) into young (≤ 1 year) and adult (> 1 year). They were grouped into good, moderate, and poor body condition scores (Russel, 1991.) Animals owned by smallholder farmers were managed under an extensive system; they spent all day on grazing pasture on fallow lands and crop residues, usually with no extra-supplement and were sheltered during the night. Whereas, in a semi-intensive production system (owned by ranches and research centre), owners supplemented extra feed sources in addition to grazing.

Journal of Science and Inclusive Development Vol. 5, No. 1, DOI: 10.20372/jsid/2023-234

Study design and sample size determination

A cross-sectional sero-epidemiological study was conducted on sheep kept in different management systems in the South Wollo and North Shoa Zones from March to December 2019 to determine the prevalence of Maedi-Visna and its associated risk factors. A simple random sampling method was used to select the study animals from the population. The sample size for this study was determined based on the expected prevalence of 4% (Tefera and Mulate, 2016) and the 5% desired absolute precision and 95% confidence interval (CI) according to Thrusfield (2005).

$$n = \frac{1.96^2 * Pex(1 - Pex)}{d^2}$$

Where, n - required sample size, Pex - expected prevalence (4%), d - desired absolute precision, and 1.96^2 - the value of z at 95% Confidence level.

Accordingly, the required sample size was 59 sheep, but to increase precision and accuracy, the sample size was maximized to 807, or about 14 folds.

Data collection and serological examination

Blood samples were taken from the jugular vein of 807 sheep aged over 6 months. Sterile vacutainer tubes and needles were used for each animal. The serum was separated into 2ml crayo-vial and preserved at -20^{o}C until analysis. The test was carried out at Kombolcha Animal Health Diagnostic and Investigations Laboratory, Amhara Region, and at the National Animal Health Diagnostic and Investigations Centre, Sebeta, Ethiopia.

The serum samples were tested for the presence of specific antibodies against Maedi-visna virus using I-ELISA, Maedi-Visna Caprine Arthritis-Encephalitis virus serum verification version VISNAS ver 1217 EN (IDvet, 310, Rue Louis Pasteur Grabels France) and the instructions of the manufacturer were strictly followed. This kit allows the joint detection of antibodies directed against Maedi-Visna virus and Caprine Arthritis-Encephalitis Virus (Nowicka et al., 2014). At the manufacturer cut-off of 50% sensitivity and specificity were 91.7% and 98.9% respectively (Nowicka et al., 2014). The test was performed according to the manufacturer`s instruction manual (OIE, 2008).

Data management and statistical analysis

All data collected for this study were entered into a MS-Excel spread sheet, arranged, and analysed using Stata 14 software. Descriptive statistics were used to estimate the seroprevalence of MV virus antibodies in the study areas. Univariable logistic regression was

used to identify significantly important risk factors such as age, breed, body condition score, sex, production system, and peasant association for the occurrence of MV virus antibody.

Age, body condition score, sex, production system, and peasant association from MV virus were selected for the multivariable model ($p < 0.25$). However, peasant association and production systems were highly correlated (correlation coefficient of ≥ 0.7), and based on their biological importance, production systems were selected and included in a multivariable logistic model. All two–way interactions between variables in the final multivariable models were tested, but no significant interactions were found. Confounding was checked during the model-building process by evaluating the change in the beta estimate of other variables when a variable was added to the models. If this change in beta estimate was >30%, the variable was considered a confounder, but no confounding variables were found.

Potential risk factors that were statistically significant at $p < 0.25$ in the univariable analysis were tested starting from the most significant variable by adding one variable at a time in the same multivariable logistic regression models using forward stepwise selection. The backward stepwise procedure was used for further selection of the variables. Correlations between pairs of independent variables were evaluated using Spearman rank correlations. If two variables had a correlation coefficient of ≥ 0.7, only one of the variables was included in the further multivariable analysis. All two-way interactions between variables in the final multivariable models were tested.

Confounding was checked during the model-building process by evaluating the change in the beta estimate of other variables when a variable was added to the models. If this change in beta estimate was >30%, the variable was considered a confounder (Dohoo et al., 2009). In all cases, differences between parameters were tested for significance at probability levels of 0.05 and a 95% confidence interval (CL).

Results

Of the total samples tested, 94(11.7%) (CI=11.6-11.8) were sero-positive for the presence of antibodies against MVv in the study areas. As per zone, the prevalence rate was 13.8% in North Shewa and 6.1% in South Wollo. In the present study, the highest and lowest seroprevalence were in Basona-Werena (19.2) and Legambo (6.1%) districts, respectively. Likewise, as per peasant association, the highest and the lowest seroprevalence were in Agricultural Research Centre (63.5%) (CI=1.11-64.348) and Kormargefya (0.8 %)

Journal of Science and Inclusive Development Vol. 5, No. 1, DOI: 10.20372/jsid/2023-234

(CI=0.003-0.168), respectively. Despite the variation in prevalence level, the disease was insidiously disseminated to all the other study districts and peasant associations, as shown in Table 1.

Table 1. Serum sample collection areas and sero-positivity for *Maedi-Visna virus* antibody

Zone	Districts	Peasant Association	No. Sampled	No. of positives	Prevalence (%)	CI (95%)	
						lower	upper
North Shewa	Basona-Worana	Kormargefya	125	1	0.8	0.003	0.168
		Agricultural Research Centre	52	33	63.5	27.803	1668.283
	Debre-birhan Town	Debre-Birhan Sheep Ranch	266	17	6.4	1.114	64.348
	Menz-Gera	Amedguya Sheep Ranch	102	27	26.5	5.943	335.307
	Menz- Mema	Yecha	64	4	6.3	0.904	75.533
South Wollo	Legambo	Tuluawlya	198	12	6.1	1.027	62.306
Total			807	94	11.7	11.6	11.801

CI: Confidence interval.

The univariable logistic regression odd ratio model analysis of attribute risk factors indicated a significant difference ($p<0.05$) in sero-positivity between age groups (OR=8.12, CI = 1.97–33.46); production systems (OR = 4.89, CI = 2.83–8.43) and among body condition scores (OR= 8.87, CI = 3.38–23.30); poorly conditioned animals (36.6%) were more likely to be seropositive as compared to well-conditioned animals (6.1%). Also, there was a significant difference among peasant associations. However, there was no significant variation in sero-positivity among different breeds ($p>0.05$) of sheep, as stated in Table 2.

Table 2. Univariable logistic regression model for potential risk factors of MV sero-positivity in sheep

Risk factors Category	Risk factors Category	No. Sampled	No. Positive	Prevalence (%)	*P* - Value	OR	CI (95%)	
							lower	upper
Age	Young*	109	2	1.8	-	-	-	-
	Adult	698	292	13.2	0.004	8.122	1.971	33.464
Breed	Pure Awassi*	20	1	5.0	-	-	-	-
	Dorper Cross	27	3	11.1	0.469	2.375	0.228	24.701
	Awassi Cross	675	181	12.0	0.357	2.591	0.342	19.614
	Local	85	9	10.6	0.455	2.250	0.268	18.863
Body Condition Score	Good*	82	5	6.1	-	-	-	-
	Moderate	580	36	6.2	0.969	1.019	0.388	2.676
	Poor	145	53	36.6	0.000	8.872	3.378	23.301
Sex	Female*	678	72	10.6	-	-	-	-
	Male	129	22	17.1	0.039	2.910	1.029	2.910
Production System	Extensive*	387	17	4.4	-	-	-	-
	Semi-Intensive	420	77	18.3	0.000	4.886	2.832	8.430
Peasant Association	Kormargefya*	125	1	0.8	-	-	-	-
	Tuluawlya	198	12	6.1	0.047	8.000	1.272	62..306
	Yecha	64	4	6.3	0.061	8.267	0.904	75.574
	Debre-birhan Sheep Ranch	266	17	6.4	0.039	8.466	1.113	64.348
	Amed-guya Sheep Ranch	102	27	26.5	0.000	44.640	5.043	335.307
	Agricultural Research Center	52	33	63.5	0.000	215.368	27.803	1668.283
Total		807	94	11.7				

*Reference, OR: Odds ratio; CI: Confidence interval.

Journal of Science and Inclusive Development Vol. 5, No. 1, DOI: 10.20372/jsid/2023-234

The final multivariable logistic model identified that age (OR = 5.04; 95% CI: 1.19–21.43), production system (OR = 6.96; 95% CI: 3.79–12.78) and body condition score (OR = 10.12; 95% CI: 3.63–28.19) are the most important risk factors for MV virus seroprevalence as shown in Table 3.

Table 3. Multivariable logistic regression model for potential risk factors of MV sero-positivity in sheep

Risk Factors		No. Sampled	No. Positive	Prevalence (%)	*P* - Value	OR	CI (95%)	
							lower	upper
Age	Young*	109	2	1.8	-	-	-	-
	Adult	698	292	13.2	0.029	5.04	1.185	21.426
Body Condition Score	Good*	82	5	6.1	-	-	-	-
	Moderate	580	36	6.2	0.746	0.85	0.313	2.300
	Poor	145	53	36.6	0.000	10.12	3.632	28.185
Production System	Extensive*	387	17	4.4	-	-	-	-
	Semi-Intensive	420	77	18.3	0.000	6.96	3.785	12.783
Total		807	94	11.7				

*Reference, OR: Odds ratio; CI: Confidence interval.

Discussion

Maedi-Visna (MV) is an insidious, lifelong, and eventually fatal disease syndrome in sheep that occurs in most sheep-keeping areas worldwide. A serological study on MV has been conducted at different times in various countries, including Ethiopia. The present study is conducted in purposefully selected sheep-producing highland areas of the South Wollo and North Shewa zones of the north-eastern parts of the Amhara region, Ethiopia. The present serological survey revealed the presence of Maedi-Visna virus antibody in sheep flocks with an overall sero-prevalence of 11.7%.

Based on the serological results, the prevalence level of MV was 63.5, 6.4, and 26.5% in the Agricultural Research Centre, DebreBirhan Sheep Ranch, and Amedguya Sheep Ranch, respectively, where the nucleus stocks of exotic sheep were reared. Also, the prevalence level of MV was 6.3, 6.1 and 0.8% in Yecha, Chiro and Kormargefya, respectively, in smallholder farms where exotic blood-level rams were distributed. Previously, Woldemeskel et al. (2002) and Getnet et al. (2010 reported an average prevalence rate of 76% and 88% in the ranch, respectively. Also, Ayelet et al. (2001) and Tefera and Mulat (2016) reported prevalence rates of about 5.3% and 4% in smallholder farms, respectively, around this ranch and research centre, showing the spread of the disease to the local farms as well. These reports showed the insidious spread of the disease at an alarming rate among the sheep population through carrier cross-rams in wider areas of the country. This is in agreement with the case of Finland as reported by Sihvonen et al. (2000). Also, this report is aligned with the finding of Yizengaw et al. (2020) in Eastern Amhara, Ethiopia.

This serological survey showed a variation in the sero-prevalence of MV between different study areas (0.8% to 63.5%). Similar results were obtained in different parts of Ethiopia (0.6% to 88%) (Getnet et al., 2010), in Quebec (14.5% to 69 %) (Shuaib et al., 2010), in Iran (6.7% to 72. 2%) (Norouzi et al., 2015), and in Eastern Amhara, Ethiopia (1.8-4.4%) (Yizengaw et al., 2020). This geographic difference in the distribution of seropositivity could be explained by the introduction of carrier animals from an infected area to disease-free sites, the difference in management practices, and the biosecurity followed by farm owners. The seroprevalence findings in small-holder farms in Kormargefya (0.8%), Yecha (6.3%), and Tuluawlya (6.1%) were interesting because these study areas are geographically located far from severely affected research center and sheep ranches, and it indicated that the disease might have been spread along with crossbred rams that were distributed for breeding purpose in the areas. This hypothesis is supported by the reports of Garedew et al. (2010) and Seyoum et al. (2011), who investigated sero-reactor rams in the villages obtained from sheep ranches a year ago.

The overall 11.7% prevalence of MV in this study is comparable with the reports of Tsegaw and Ademe (2012) (15.6%) in the eastern Amhara Region, Ethiopia; Preziuso et al. (2010) (15.3%) in Turkish sheep; and Fournier et al. (2006) (15.6%) in culled ewes in Alberta, Canada. But it is higher than the previous reports of

Journal of Science and Inclusive Development Vol. 5, No. 1, DOI: 10.20372/jsid/2023-234

Yizengaw et al. (2020) (3.24%) and Tefera and Mulate (2016) (4%) in the eastern Amhara Region, Ethiopia; Shuaib et al. (2010) (2.41%) in Manitoba; 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); 88% in Debre-Birhan sheep breeding center (Getnet et al., 2010), and 74% in central Ethiopia (Woldemeskel et al., 2002). The finding in this study is also relatively lower than those reports from other countries around the world. For instance, 19.4% in Kirikkale district, Turkey (Gerstner et al., 2015); 28.8% in Germany (Azkur et al., 2011); 19% in Canada (Hüttner et al., 2010); and 29.6% in Khorasan-e- Razawi province, Iran (Norouzi et al., 2015). Such inconsistency in the prevalence rates of MV may be due to the variation in the study populations, production system, diagnostic tests, and sampling method used.

The final multivariable logistic model identified age (OR = 5.04; 95% CI: 1.19–21.43), production system (OR = 6.96; 95% CI: 3.79–12.78) and body condition score (OR = 10.12; 95% CI: 3.63–28.19) as the most important risk factors for MV virus seroprevalence as shown in Table 3. The age-related seroprevalence of MV in the present study showed that adult sheep were about five times more likely to be seropositive compared to younger sheep. In this regard, the finding of this study is consistent with the results reported elsewhere. viz, in Ethiopia (Alemnew et al., 2021; Tefera and Mulate, 2016; Ayelet et al., 2001); in Turkey (Preziuso et al., 2010), in Iran (Norouzi et al., 2015). The higher seroprevalence in old age compared to young age might be explained by the fact that with the increase in age, small ruminants are repeatedly exposed to various stress conditions that can predispose them to disease. Further, with increasing age, the probability of encountering infectious agents also increases. Also, this age seroprevalence discrepancy can probably be explained by the longer exposure to horizontal transmission, and the development of detectable levels of MV antibodies can vary from months to years (Radostits et al., 2007).

This study also revealed that the production system was significantly associated with the occurrence of MV virus antibodies. Sheep kept under a semi-intensive management system were about seven times more likely to be infected than animals kept under an extensive management system. The present result is in agreement with previous findings by Ayelet et al. (2001), who reported a lower prevalence (3.7%) of MV in village flocks and relatively higher (7%) in on-station. Also, Woldemeskel et al. (2002) and Tsegaw and Ademe (2012) reported higher seroprevalence 74% and 30% of MV in clinically moribund sheep at ranches than in smallholder farmers, respectively. This seroprevalence difference between the two management systems might be associated with the flock size of the farms, the housing of the animals for longer hours during cold seasons, age groups, and keeping a high proportion of older animals in ranches. Baumgartner et al. (1990) have reported that unfavourable housing conditions such as insufficient room, bad climatic conditions, and

crowding behaviour in sheep promote a high incidence of the disease. But this finding disagrees with the report of Yizengaw et al. (2020), who reported no seropositivity difference between production systems.

In the present study, an attempt was made to know whether body condition influences or not the prevalence of MV virus antibody in sheep, and it was found that poor-condition animals were ten times more likely to be seropositive as compared to good-condition animals. Our finding is in accordance with the findings of Alemnew et al. (2021) and Tefera and Mulate (2016) since they reported severe emaciation in sheep infected with MV. This is also supported by the fact that the MV virus targets the cells of the immune system, leading to concomitant infectious diseases and ultimately weight loss. But the present finding disagrees with the report of Yizengaw et al. (2020).

A slightly higher prevalence of MV was observed in male (17.1%) than female (10.6%) sheep. However, multivariable logistic regression model was not indicated as potential risk factors for MV virus seroprevalence. This finding is in agreement with the findings of Yizengaw et al. (2020); Tefera and Mulate (2016); Seyoum et al.(2011); Woldemeskel et al. (2002), who reported that both sexes have an equal chance of having the infection. In contrast to our finding, Alemnew et al. (2021), Tsegaw and Ademe (2012), and Simard and Morley (1991) reported that there is significant difference between that sexes of sheep in the sero-positivity of MV. The variation between different reports with regard to sex as a risk factor of MV virus seropositivity might be due to variation in the study population/ flock composition of females and males, sampling type, and management system, among many other types of studies.

There was no significant difference in seroprevalence of MV among different breeds of sheep. This result is in line with the reports of Yizengaw et al. (2020) and Tefera and Mulate (2016), who reported no susceptibility differences among different breeds of sheep. In this regard, the finding of this study was in contrast with the results reported by Alemnew et al. (2021), Tsegaw and Ademe (2012), and Seyoum et al. (2011).

Conclusions

The current sero-epidemiological study of MV showed that the infection is distributed all over the study areas, with higher sero-positivity in sheep ranches and research centre. The finding of positive serological reactors does not only suggest the occurrence of the disease in the sheep population of the study areas but also indicates the presence of foci of infection that could serve as a source of infection for the spread of the disease into free animals around and elsewhere in the sheep producing areas for the upgrading purpose of local sheep and also through marketing. Therefore, effective control measures should be implemented through screening test before distribution of exotic breed rams from different ranches and research centre to smallholder farms, as well as annual testing and culling of all sero-reactor ewes and their progeny.

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Conflict of interest

The authors have not declared any conflict of interest.

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