Sero-epidemiological study on Maedi-Visna in selected areas of Ethiopia

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Abstract

The study was conducted to verify the existence of Maedi-Visna virus infection (Ovine Progressive Pneumonia- OPP) in exotic sheep breed in selected areas of Ethiopia. ELISA and Hematoxylin & Eosin (HE) staining techniques were employed to examine the serum and tissue samples respectively. ELISA test of serum samples showed 88%, 19%, 10%, 6%, and 0.8% infection rate for Debre Berhan sheep breeding center, and Arsi, Bale, North Omo and Gurage zones respectively. At necropsy 11% (4/36) of the sheep examined had characteristic gross pathological lung lesions of Maedi/OPP and 33% (12/36) indicated the presence of the disease through histopathological examinations. The major histopathological findings were peribronchial and interstitial lymphocytic infiltrations and/or follicle-like aggregations, interstitial connective tissue proliferation, hyperplasia of the smooth muscles in the ducts of terminal bronchioles and alveoli. The present findings provide a clear evidence for the dissemination of Maedi-Visna virus following the distribution of exotic rams from the breeding and multiplication center. Therefore, survey in other areas is also required to have broad picture of the disease in the country which will help to design a practical control strategy of the disease at the national level.

Keywords: Ethiopia, exotic sheep breeds, maedi-visna, sero-epidemiology

Introduction

Sheep population in Ethiopia is estimated at 25.02 million, roughly 75% of the population is found in the highlands and 25% in the lowlands (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 (Markos Tibbo, 2006). However, due to low productivities of the indigenous breeds, the Ethiopian government had been introducing sheep breeds of Hampshire, Corriedale

and Awassi from UK 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 Menz and Horro breed sheep in Debre Berhan 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 them were Agarfa (former farmers training center) and Arsi Rural Development Unit (ARDU), which owned the rams (Corriedale and Awassi) as of 1981.

Despite the genetic improvement, an incidence of a new case with undefined etiology characterized by a respiratory embarrassment appeared in Agarfa and ARDU sheep farms in 1990. The disease caused 10% mortality affecting mainly adults and had no response to antibiotic treatments. The chief veterinary office and laboratories in-charge tried to diagnose the disease; however, it was not easy to identify the etiology of the disease due to lack of facilities. 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 out of 43 tested samples (Ministry of Agriculture report, 1991, unpublished). Maedi/OPP is a slowly progressive disease of sheep and rarely goats reported first in the Iceland and distributed worldwide (Kahn *et al*., 2005; Radostits *et al*., 2000; Murphy *et al*., 1999; Vorster *et al*., 1996; Jones and Hunt, 1983). Since 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 (Moges Woldemeskel *et al*., 2002; Gelagay Ayelet *et al*., 2001; Markos Tibbo *et al*., 2001). Although farms and breeding centers have been reporting Maedi-Visna cases, the extent to which the disease disseminated in the country has not been established yet. This study was therefore designed to investigate the serological status of the disease in the areas where the exotic rams were distributed.

Materials and Methods

Study area

The study areas were selected based on retrospective data showing the history of introduction of cross breed sheep. The areas identified were selected peasant

associations (PAs) and farms in North Shewa, Arsi, Bale and North Omo zones (Table 1). All the areas were highlands known for sheep rearing practices during the study period (2005-2006).

Study Animals

The animals considered for the study were crosses reared in breeding centers and progenies originated from these centers. Exotic breeds introduced as of 1979 include Awassi, Corriedale and New Hampshire. The sheep sampled were all above 6 months of age and had exotic blood level of up to 87%. The numbers of sheep sampled were: in Debre Berhan breeding center 217 sheep; and in Ardaita, ARDU and in PAs around these farms were 180, 90 and 741 sheep respectively. In PAs around the former Agarfa farmers training center and in selected PAs of Omo and Gurage zones the numbers of sheep sampled were 258 and 302 respectively. In all the PAs samples were taken either from sheep originated from breeding centers or those that had contact with them. Sheep included in different breeding centers were owned by the government while those in PAs were by small scale farmers. In the breeding centers sheep were fed on pasture during the day time and they were fed on hay (made of natural pasture and oats) in properly sheltered pens during the night. Sheep owned by individual farmers were managed under traditional grazing system. They spent all the day on grazing pasture and sheltered during the night without any feed supplement.

Table 1. Serum sample collection area and sero-positivity for maedi-visna virus infection

Sample origin					No of samples	No of positive	% positive
Region	Zone	District	PA	Farms			
Amhara	North Shewa	-	-	Debre-Berhan*	217	192	88
Oromia	Arsi	Gedeb Asasa	Asasa	-	180	60	33
			Odasha	-	83	26	31
			Ela	-	45	22	49
			Bucha	-	45	3	7
		Shirka	-	Ardaita	180	33	18
			-	ARDU	90	2	2
			Gebre Kistos	-	135	43	32
	Bale	Degalo Ticho	Murkicha	-	97	7	7
		Bokoji	Limu Bilbilo	-	156	7	5
		Agarfa	Ambentu	-	103	15	14
S.N.N.P.R.	North Omo	Kokossa	Kokossa	-	155	11	7
		Chencha	Yirya	-	41	8	19
		Gofa	Bulqui	-	115	1	0.9
	Gurage	Chencha Gumer	Dokomesha	-	26	2	8
			Janboro	-	120	1	0.8
Total					1788	433	24.2

* Debre Berhan showed significant difference in infection rate compared to Ardaita and ARDU sheep farms ($P<0.01$)
PA – Peasant Association
S.N.N.P.R. – Southern Nation and Nationalities Peoples Region

Serology

Blood samples were taken from the jugular vein of 1788 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 sera samples were tested for the presence of specific antibody against maedi-visna virus using ELISA, Maedi-Visna/CAEV serum verification version P/00302/04 kit and instructions of the manufacturer were strictly followed.

Gross and microscopic examination

Tissue specimens were collected from Debre Berhan breeding center during a slaughter program of culled sheep. Lung and lymph node specimens were collected from carcass of 36 animals with mild to severe gross pathological lesions. Tissue specimens were preserved in 10% neutral buffered formalin until processed. Tissues were processed using paraffin wax method and sectioned at 3µm thickness. Hematoxylin & Eosin (HE) method was employed for staining of tissue sections.

Data analysis

Data were stored using Excel Microsoft Program and the infection rate of the disease was computed and compared in respect to sex and areas using Statistical Analysis System (Stat 8 statistics analysis, 1993). Proportion, one way analysis of variance (ANOVA) and chi-square test were employed for analysis. 5% and 1% confidence levels were taken for significance level determination.

Results

Serology

ELISA test showed high infection rate (88%, 192/217) in the Debre Berhan breeding center. Despite the variation in infection rates, the disease was insidiously disseminated to all the other study areas. As per zone, the infection rate was 20% in Arsi, 10% in Bale, 6% in North Omo and 0.8% in Gurage. The infection rate in Debre Berhan breeding center was significantly higher compared to other areas ($p<0.01$). Next to Debre Berhan sheep breeding center, Gedeb Asasa district of Arsi zone showed higher sero-positivity 31% (111/353) (Table 1; Figure 1). The difference in infection rates between ARDU (2%) and Ardaita (18%) farms were significant ($p<0.01$). Difference in sero-positivity due to sex was significant at 5% significant level (Table 2); however, it was difficult to see the difference in various age groups due to lack of data.

Figure1. Sero-prevalence rate of Maedi/OPP in different areas

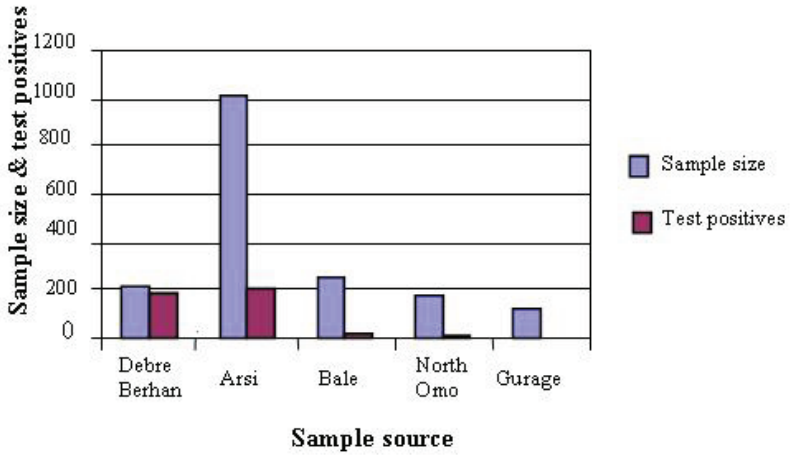

Table 2. Maedi-Visna virus infection rate on the basis of sex

Sex	Test positive	Test negative	Total
Male	116 (71.1)	34 (78.9)	150
Female	121 (165.9)	229 (184.1)	350
Total	237	263	500

Number in brackets are expected values, $\chi^2 = 77$, $p<0.05$

Gross and microscopic lesions

Among the sheep examined (n=36) at necropsy, four sheep (11%) showed grayish raised areas of consolidation mainly on the craneo-ventral and dorsal part of the lungs and the cut surface appears moist on incision. The pneumonic lesions observed were greatly enlarged weighing up to 4.5kg (two-three folds the normal weight of the species) and lymph nodes (bronchial and mediastinal) were enlarged and highly edematous. The histopathological results revealed about 44% (16/36) of the slaughtered sheep showed suggestive lesions of maedi-visna infection. The findings were lymphoid cell infiltration (in some follicular) in the peri-bronchial, peri-bronchiolar and peri-vascular sheaths; hyperplasia of the smooth muscles in the terminal bronchioles and alveolar ducts; and thickened

alveolar septa due to massive infiltration of lymphocytes and hyperplasia of smooth muscles (Table 3). All the sheep that showed pneumonic lesions were positive for the serological test; however, not all sero positive sheep showed pneumonic lesions.

Table 3. Descriptions of gross and microscopic lesions of maedi-visna in selected sheep cross (Awassi x Menz)

Tag number	Exotic blood level (%)	Age (year)	Gross lesions	Microscopic lesions
2351	75	4	Non-collapsed lungs, enlarged grayish brown in color, hepatization with irregular white or grayish raised areas of consolidation in the craneo-ventral & dorsal part of the lungs, on incision the cut surface appear moist with no fluid coming out. Enlarged edematous mediastinal and bronchial lymph nodes. Lung weighed 4.5 kg.	Peribronchial and interstitial lymphocytic infiltrations (severe interstitial pneumonia), aggregation (folloicle-like) in some areas, connective tissue proliferation in the interstitia, hyperplasia of the smooth muscles in the terminal bronchiolar and alveolar ducts.
0762	87	6	Grayish consolidation mainly on the area of craneo-ventral part of the lungs, and the other parts were soft (rubbery) in consistency. Lung weighed 4kg,	Proteinaceous exudates in some alveolar foci, peribronchiolar and interstitial infilitrations of lymphocyes(severe interstitial pneumonia),, connective tissue proliferation of the interstitia.
2669	75	4	Grayish consolidation in craneo-ventral lung and lesions were very similar to that observed sheep No 0762. Lung weighed 3.5kg,	Peribronchiolar and interstitial infilitrations of lymphocyes (severe interstitial pneumonia),, serious exudates in some alveolar spaces.
0617	87	6	Red hepatization, lesions similar to that of sheep No 2351. Lung weighed 4kg.	Follicular aggregates or diffuse infiltrations of lymphocytes in the peribronchial areas, peribronchiolar smooth muscle hyperplasia, interstitial connective tissue proliferation. .

Discussion

In the present study serological survey, gross and histopathological examinations revealed the presence of maedi-visna virus infection in sheep flocks. According to the OIE (2008), a definitive diagnosis of this disease is made based on the test results by AGID and ELISA, and characteristic gross and microscopic lesions in conjunction with a supportive clinical history of the disease. In this study ELISA and HE staining techniques were employed to examine the antibody and tissue samples respectively.

Based on the ELISA test results the infection rate of maedi-visna was 88% in Debre Berhan sheep breeding and multiplication center where the grand stock of sheep were reared. Previously Moges Woldemeskel *et al.* (2002) reported a mean infection rate of 76% in the center. Gelagay Ayelet *et al*. (2001) also reported infection rate of about 5.3% in small farms around this center showing the spread of the disease to the local farms as well. In the present study, the infection rate in Arsi, Bale, North Omo and Gurage zones respectively were 20%, 10%, 6% and 0.8%. The infection rate of the disease was significantly higher in Debre Berhan breeding and multiplication center than other areas in the current study ($p<0.01$). This could be associated to the higher susceptibility of sheep to the maedi-visna virus infection that had higher exotic blood level in this farm. Among the districts, Gedeb Asasa and Shirka showed higher sero-positivity 31% (111/353) and 32% (43/135) respectively. These areas are known for dense sheep population and retrospective data showed that numerous cross-rams were distributed to these localities compared to others (Figure1). This report showed the insidious spread of the disease in 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). The present study showed a significant difference in sero-positivity between males (71.1) and females (165.9) ($p<0.05$), which is in agreement with previous report by Legesse Garedew *et al.* (2010) and Gelagay Ayelet *et al*. (2001). Where as, the report by Moges Woldemeskel *et al.* (2002) showed no difference in infection rate of the disease. Female sheep usually are required to stay longer until they get older than males in the flock as they are required for rearing purpose and thus have greater chance in older sheep to detect the antibody for maedi-visna (Legesse Garedew *et al.,* 2010). The discrepancies of findings among different authors, however, may be due to differences in sample size, breed and management system of the respective study animals.

The gross pathological lesions found were enlarged non-collapsed lungs, retained rib impressions on the lungs, heavy meaty mottled gray-brown color with firm or rubbery texture of the lungs weighing up to two-three folds of the normal, enlarged and edematous mediastinal and bronchial lymph nodes. Among the sheep examined at necropsy, the characteristic gross pathological lung lesion of the maedi-visna was observed only in 11% (4/36); however, higher proportion of the sheep examined (44%, 16/36) showed suggestive lesions of the disease on histopathological examination. This indicates that histopathological examination is more sensitive and recommendable to observe lesions which one cannot appreciate macroscopically.

Major histopathological findings observed were severe interstitial pneumonia characterized by thickened alveolar septa due to massive infiltration with mononuclear cells mainly lymphocytes, hyperplasia of smooth muscle cells and connective tissues; obliterated alveoli in severe cases; peribronchial and perivascular lympho-follicular infiltrations. The development of lympho-proliferative changes in maedi-visna infected lungs is regarded to the replication of maedi-visna virus infection in the alveolar macrophage and subsequent presentations of viral antigens together with Class-II Major Histocompatibility Complex. The histopathological findings were in agreement with previous reports (Moges Woldemeskel *et al*., 2002; Juste *et al*., 2000; Hirsh and Zee, 1999; Vorster *et al*., 1996; Sharma and Adlakha, 1994; Gonzalez *et al*., 1993; Dawson *et al*., 1990).

In the present study all sheep that showed pneumonic lesions were tested positive for maedi-visna antibody by ELISA test, but the reverse was not true. Legesse Garedew *et al.* (2010) reported the possibility of encountering characteristic maedi-visna pneumonic lesions without sero positivity. This might be due to the fact that antibody against the maedi-visna virus accumulates over time and hence makes difficult to detect antibody at earlier stage of infection than at later stage of the disease (Radostits *et al.,* 2000).

Maedi-visna (Ovine Progressive Pneumonia) virus infection has neither appropriate treatment nor vaccine developed. Hence, control of the disease is very difficult and costly. De-stocking flocks or culling of sero-positive sheep and their progenies is a better choice for the control and prevention of the disease (Sihvonen *et al*., 2000; Williams-Fulton and Simard, 1989; Houwers *et al*., 1984).

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