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RESEARCH ARTICLE OPEN ACCESS

A CROSS-SECTIONAL INVESTIGATION ON SERO-PREVALENCE OF PESTE DES PETITS RUMINANTS IN ADDIS ABABA MUNICIPAL ABATTOIR, ADDIS ABABA, ETHIOPIA

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I. ABSTRACT

The main objective of this study was to investigate the Sero-prevalence of PPR in small ruminants and the disease association with different risk factors at Addis Ababa municipal abattoir. A cross sectional survey was carried out to investigate the prevalence of antibodies and for this a total of 400 serum samples from sheep (191) and goat (209) were collected and tested from February to August 2013. These samples were subjected to monoclonal antibody based Competitive ELISA (C-ELISA) for specific measurement of antibodies to PPR virus in AU-PANVAC Laboratory. Out of these 400 samples, 82 sera were found to be positive for PPR antibodies with cELISA (Seroprevalence = 20.5%). Further findings suggested that the seropositive cases were higher in goat (21.1) than in sheep (19.9) but was not significant (p>0.05). It was concluded from this study that keeping in view the high risk of PPR, control strategy should be adopted and further study needs to be undertaken to ascertain the extent of PPR virus circulating in the population of goats and sheep. Under existing production and marketing system, sanitation and preventive vaccination should be the two practicable options for control of PPR.

Keywords: Peste des petits ruminants, Competitive-ELISA, Seroprevalence, Sheep, Goat, Addis Ababa municipality Abattoir.

II. INTRODUCTION

Livestock is a major part of African agricultural sector and plays an important role in food and economic security through provision of a variety of products and services including hides, skins, meat, manure, fiber, and energy and capital accumulation. Besides its significant contribution to agricultural gross domestic product (GDP) and to food security in many countries, livestock is an intrinsic part of people’s identity and way of life. Small ruminants are integral part of livestock keeping in Sub-Saharan Africa (SSA) that are mainly kept for immediate cash sources, milk, meat, wool, and manure. Small ruminants also have various social and cultural functions that vary among different cultures, socio-economies, agro-ecologies, and locations in tropical and sub-tropical Africa [36].

Ruminant livestock population of Ethiopia is the largest in Africa and 10th in the world [30]. They are important components of the livestock subsector and are sources of cash income and play a vital role as sources of meat, milk and wool for smallholder keepers in different farming systems and agro-ecological zones of the country [27]. They are also sources of foreign currency. However, the economic gains from these animals remain insignificant when it is compared to their huge number. There are various factors that contribute for low productivity: feed shortage both in quality and quantity, poor feeding, health constraints and management [59].

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The sheep and goat industry is threatened by several diseases, of which peste des petits ruminants (PPR) is one of the most important. Peste des Petits Ruminants(PPR) or goat plague is an acute and highly contagious viral disease of small ruminants such as sheep and goats [12]. This disease is characterized by high fever, ocular and nasal discharge, pneumonia, necrosis and ulceration of the mucous membrane and inflammation of gastro-intestinal tract leading to severe diarrhea [48].

The causative agent of this economically important disease of small ruminants is a *Morbillivirus*. These viruses are enveloped, non-segmented negative strand RNA viruses and constitute a genus within the family *Paramyxoviridae* and the order Mononegavirales [14].

The virus is closely related to Rinderpest virus (RPV), another member of *Morbillivirus* genus, which causes similar disease in large ruminants. The virus is also serologically related to Measles and Canine distemper virus. The virus may survive at $60^{o}C$ for 60 minutes, remain stable from pH 4.0 to 10.0 and is killed by most of the disinfectants but have long survival time in chilled and frozen tissues [5]. PPR virus is transmitted between animals such as sheep and goats through inhalation of aerosols and direct contact with ocular/nasal secretions, faeces, contaminated water and feed troughs [31]. Specific clinical signs of PPR include sudden pyrexia (40-$41^{o}C$), purulent ocular/nasal discharge with congested conjunctiva, erosions, respiratory distress, sneezing in an attempt to clear nose, ulceration of mucous membranes and gastroenteritis. Morbidity and mortality vary considerably, depending on the susceptibility of the small ruminants' population in an area, animal husbandry, breed and age [31]. Clinically, PPR is similar to Rinderpest (RPV). Initial diagnoses were made using Agar Gel Immunodiffusion (AGID), Counter-Immunoelectrophoresis (CIEP), Enzyme Linked Immunosorbent Assays (ELISA) and Virus Neutralization assays (VNT) [20].

PPR was clinically suspected for the first time in Ethiopia in 1977 in a goat herd in the Afar region, east of the country. Clinical and serological evidence of its presence has been reported in 1984 and later confirmed in 1991 with cDNA probe in lymph nodes and spleen specimens collected from an outbreak near Addis Ababa [51].

Generally PPR is OIE notifiable disease of small ruminants that seriously hinder sheep and goat production in Ethiopia and other part of the world. Knowledge of the disease in terms of the symptoms observed and prevention methods is important in combating the disease and consequently improve sheep and goat productivity. Therefore the objectives of this study were:- to investigate the Sero-prevalence of Peste des petits ruminants in Addis Ababa municipal abattoir and to determine the risk factors association and interaction with the sero-posetivity of animals tested.

III. MATERIAL AND METHODS

A. *Study area and Study population*

The study was conducted from February to August 2013 at Addis Ababa municipal Abattoir and it was purposively selected based on accessibility and capacity to slaughter large amount of small ruminants than other abattoirs to

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be included in active study. The Addis Ababa municipal abattoir is located roughly 3km to the NE part of Addis Ababa, the capital city of Ethiopian [42]. The study population comprised apparently healthy small ruminants, coming to the abattoir in the study period, irrespective to their age and sex.

B. Study Design and Sampling Methods

A Cross sectional study was carried out in Addis Ababa municipal abattoir. The abattoir was purposively selected based on its accessibility and capacity to slaughter large amount of small ruminants. Systematic random sampling was used to select the study animal which is conducted to determine the Sero prevalence of PPR in small ruminants coming to the abattoir using serological techniques: Competitive ELISA test. Epidemiological risk factors such as species, sex and age of animals were assessed for the presence of association with the prevalence of PPR.

C. Sample size and Collection

For the cross-sectional study to determine sero prevalence of PPR in sheep and goats using serological tests the formula for random sampling of [69] was used. For sample size calculation, 95% CI and 5% desired absolute precision, and expected prevalence of 50% herd prevalence was used.

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

Where: - n= required sample size

Pexp = expected prevalence of 50%

d^2 = desired absolute precision of 5%

1.96 = Z value of the 95% CI

Thus, the sample size of 384 for all species will be used to determine the sero prevalence of PPR; proportional sampling for age and sex was also considered. However for the sake of accuracy an ooverall of 400 animals (191 sheep and 209 goats) were sampled by selecting every five animals, from 955 sheep and 1045 goats using their Id nº, as representative from those that came to the abattoir during the study period. The animals were restrained by the attendants during every blood sample collections. Around 5-7ml blood samples were randomly collected by Jugular vein puncture using venoject needle and vacutainer tubes from each study animal at the larage.

The blood samples were transported to African Union- Pan African Vaccine Center (AU-PANVAC) virology laboratory and allowed to clot for 16-24 hrs at room temperature. The serum was centrifuged to remove the remaining red blood cells before being transferred to 2ml cryovials or serum tubes under laminar air flow hood in ordered to avoid contamination and the serum was kept (preserved) at -20°C temperature until processed. Each sample was labeled appropriately for identification. All the samples were subjected to Competitive Enzyme Linked Immuno Sorbent Assay (c-ELISA).

D. Methodology

The research approach during the study was abattoir investigation and laboratory examinations mainly serological test. Laboratory examination was conducted at African Union Pan African Vaccine Canter (AU-PANVAC) Bishoftu, Ethiopia.

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1) *Serological Testing*

Competitive Enzyme Linked Immuno Sorbent Assay (c-ELISA)

For detecting antibody seroprevalence, Competitive Enzyme Linked Immuno Sorbent Assay (c-ELISA) was employed which is supplied by PANVAC. The c-ELISA kit comprising, PPR antigen (75/I) strain, anti – PPRV monoclonal antibody, anti-mouse conjugate, control sera, substrate and chromogen [39].

Fifty µl of diluted PPR antigen was poured in all wells of the micro titration plates and incubated at 37°C for 1 hour in shaker. Then plates were washed with washing buffer and 45 µl blocking buffer was added to all wells of the micro titration plates. Five µl of test serum and 50 µl of anti – PPRV mono clononal antibody was added to all wells and incubated at 37°C for 1 hour in a shaker. Negative, weak and strong positive controls were also maintained. After washing, 50 µl of anti-mouse conjugate was dispensed to all the wells and incubated again for 1 hour at 37°C. After incubation, the plates were washed with washing buffer and 50 µl of substrate / chromogen was dispensed to all wells. Fifty µl of stopping solution was also added to all the wells to stop the reaction [39].

The ELISA micro-plate was read with an immuneskan reader with an inference filter of 492 nm. The reader was connected to computer loaded with ELISA Data Interchange (EDI) software that was used to automate reading and calculation of percentage Inhibition (PI) values. Test sera showing mean PI values of 55% or greater was considered as positive, while the test sera demonstrating mean PI values less than 55% was considered as negative [39].

E. *Data Management and Analysis*

Data from abattoir survey and serological test results were entered on Microsoft excel data sheet, coded and analyzed using statistical package: Epi Info and SPSS. Statistical testes such as descriptive statistics, chi-square test, and logistic regression, and other which were assumed important for the data were applied. For statistical significance, 95% CI and P value of 0.05 was considered.

IV. RESULTS

In the present study, c-ELISA was employed to investigate the antibody Seroprevalence of PPR. For this purpose, a total of 400 sera were collected from animals at Addis Ababa municipal abattoir and tested at AU- PANVAC laboratory. The variables included in the dataset and the number of observations for each variable is available in bar graph and the results are presented in the following sections.

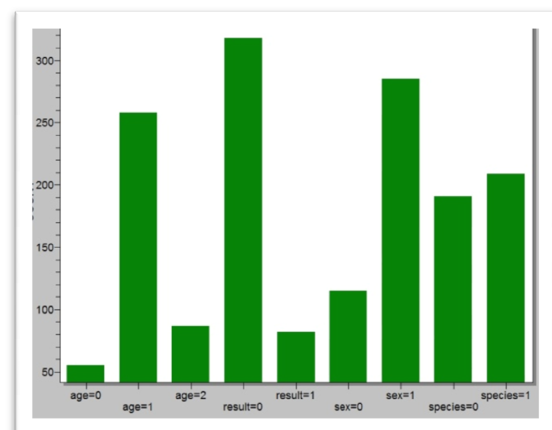

Figure 1: Frequency of the outcome and exposure variables

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A. Serologic Status of Sampled Animals

A total of 400 animals were sampled for antibodies to PPRV. The overall percentages of antibody response (Seroprevalence) to PPRV were 20.5% (Table 1). In general, the level of PPRV antibody response was different in goat and sheep; i.e., of 191 sheep examined, 38 (19.9%) and of 209 goats examined, 44 (21.1) were seropositive for PPRV.

TABLE 1. Antibody Response (Seroprevalence) of PPRV

	RESULT		
Species	**0**	**1**	**TOTAL**
0	153	38	191
Row %	80.1	19.9	100.0
Col %	48.1	46.3	47.8
1	165	44	209
Row %	78.9	21.1	100.0
Col %	51.9	53.7	52.3
TOTAL	318	82	400
Row %	79.5	20.5	100.0
Col %	100.0	100.0	100.0

Key:-Species: - 0 is sheep and 1 is goat, Result: - 0 is negative and 1 is positive

B. PPRV antibody status by age of animal

TABLE 2: PPRV Antibody Status by Age of Animals

Age	№ Sampled Sera	RESULT	
		Negative	Positive
< 1.9 years	55(13.8%)	48(87.3%)	7(12.7%)
2-3.9 years	258(64.5%)	202(78.3%)	56(21.7%)
>4 years	87(21.8%)	68(78.2%)	19(21.8%)
Total	400(100%)	318(79.5)	82(20.5)

The 400 sera of small ruminants that were sampled was within the ages; below 1.9 year (n = 55), 2-3.9 years (n = 258), and above 4 years (n = 87) respectively. The PPRV seroposetivity was; below 1.9 year 7 (8.5%), 2-3.9 years 56 (68.3%), and above 4 years 19 (23.2%). The above results show that seroposetivity rose with age up to 2 years and thereafter decline. (Table 2)

C. PPRV antibody status by sex of animal

Among 400 sera of small ruminants that were sampled from the abattoir; females were (n =115) and Male were (n = 285). Therefore PPRV seroposetivity was; 26(22.6) and 56(19.6).

TABLE 3. Analysis of Antibody Response Against Peste Des Petits Ruminants Virus(PPRV), by Sex.

Total No. Tested	Sex Distribution		% Positive Sera	Sex Distribution of positive sera	
	Female	Male		Female	Male
400	115	285	82(20.5)	26(22.6)	56(19.6)

D. Associations of Risk factors for positive serological status against PPR

In the univariate analysis, goats were 1.07 more likely to be seropositive for PPR than sheep (category combining both sheep and goats). Regarding the age of the animals, the highest prevalence was observed among animals between 2-4 years, 56(21.7%) of which were seropositive. Results of the logistic regression assessing the relationship between species, age and sex and serological status are presented in Table 4. There was no factor significantly associated with the logodds of positive serological status.

The logistic regression model used to assess the association between the potential risk factors sex, age and species and the outcome variable PPR serological status. There is no factor significantly associated with the odds of positive serological status (P > 0.05). Regarding the results of logistic

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regression the logodds of suffering by PPRV in goats is greater than by 1.07 in sheep, in which there is almost no association between the variable species and the disease PPR (p> 0.05).

TABLE 4.Results of a logistic regression of sex, age and species on serological status against PPR.

Variable	OR	95% CI	P-value
Sex			
Female	Ref		
Male	0.8371	0.4949 - 1.4159	0.5073
Age			
<1.9	Ref		
2-3.9	1.9010	0.8154 - 4.4317	0.1369
>4	1.9160	0.7469 – 4.9150	0.1761
Species			
Sheep	Ref		
Goat	1.0737	0.6600-1.7466	0.7746

After removing the effect of second variable sex the logodds of suffering by PPRV in species is 1.06, however, it is not significant (P>0.05).

TABLE 5: Logistic Regression of the Variable Species After Removing the Effect of Variable Sex.

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
Sex	0.8407	0.4964	1.4238	-0.1735	0.2688	-0.6454	0.5186
Species	1.0625	0.6523	1.7308	0.0607	0.2489	0.2437	0.8075
CONSTANT	*	*	*	-1.2656	0.2659	-4.7598	0.0000

Finally, the result of interaction between the study variables shows that there was significant interaction (P<0.05) only between sex and age (1/0). This shows that sex and age (1/0) together have significant effect on the seroposetivity of animals for PPRV. (Table 9)

V. DISCUSSION

In the present study, the collected serum samples were screened for the presence of antibodies against PPRV using C- ELISA. The overall Seroprevalence was 20.5% and specifically the level of PPRV antibody response was different in goat and sheep; i.e., of 191 sheep examined, 38 (19.9%) and of 209 goats examined, 44 (21.1) were seropositive for PPRV. This may indicate previous exposure to the virus either by natural infection or, most probably, by vaccination, since all animals in the study were >6 months of age. The seroprvalece in this study was higher than that reported in some other contries. In Saudi Arabia, using a microtiter neutralization assay (known for its low sensitivity[7]. In Yemen the seroprevalence of PPR was 15% in sheep and 18% in goats [67]. [57] reported a seroprevalence of 36.3% in sheep and 32.4% in goats which is higher than the current study and this higher seroprevalece might be due to the sample size difference and might be the study conducted during atime when the highest outbreak occurred.

The disease increase to reach high level in recovered and clinically healthy animals in which previous infection were more likely to be occurred specially in endemic area and this may explain the drastic melody recoded specially PPR is immunosuppressive virus in nature as revealed by [21] and can occasionally overcome the resistance of large ruminants and lead to the development of clinical signs [23].

In sheep, the antibody seroprevalence was 19.9% which is slightly lower than [8]. They recorded

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29% seroprevalence in sheep, while in another study [63 and 6] reported seroprevalence 45.78% and 57% respectively which might be due to the migration of a large number of clinically negative and seropositive sheep entering from neighboring areas.

TABLE 6. Interaction between the study variables age and sex.

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
age (1/0) * sex	6.7731	1.1230	40.8511	1.9130	0.9168	2.0865	0.0369
age (2/0) * sex	7.2381	0.9465	55.3508	1.9794	1.0379	1.9070	0.0565
CONSTANT	*	*	*	-0.6931	0.6124	-1.1319	0.2577

In present study, antibody sero-prevalence in goats was 21.1% which is not in line with the findings of [63, 50 and 8] who reported 0.93%, 9.2% and 49%, respectively. The high prevalence of PPR antibody could be attributed to the variation in sample size.

There was high sero-prevalence of PPR antibody in goats than that in sheep. These findings are similar to previously reported epidemiological studies [21 and 47], while in Saudi Arabia, the prevalence of PPR in sheep and goats were 3.1% and 0.6%, respectively by using a microtitre neutralization assay [7]. This may be due to low sensitivity of test or low prevalence of PPR disease in this area. While in other study [1] the antibodies in goats (9%) were slightly lower than in sheep (13%) which may have resulted from the lower number of samples investigated or from the fact that goats were more susceptible and may have died from the disease, whereas sheep may have survived.

The finding of this study suggests that animals between 2 and 4 year have a better chance of seroposetivity to PPR. This finding agrees with previous reports [47 and 57]. It is assessed that young animals are more susceptible than adults, which matches the serological profile reported by [72 and 52] and in accordance to [2] who reported that goats were more prone to PPR than sheep and young animals of both species were at high risk than adults. Similar finding were observed by [37] who reported significantly higher case fatality rate in kids.

Regarding the sex, in this study 26(22.6) females and 56(19.6) males were positive for PPRV out of 115 and 285 animals respectively. This result shows females have higher seroposetivity than males which might be due to the sample size difference taken from both sexes however, most of studies have shown that the prevalence of PPR antibodies in males is higher than females [49].

VI. CONCLUSION

The details of small ruminants' sera samples were collected from Addis Ababa municipal abattoir for the present study; a total 400 serum samples were screened using c-ELISA and per cent inhibition (PI) more than 55% were considered as positive reactions. Therefore, 82 sera were found to be seropositive and were obtained as serological evidence from the sampled sera which gives an overall prevalence of 20.5%. Specifically the seroposetivity for goat and sheep were 21.1 and 19.9 respectively.

From the obtained results of the present study, the following recommendations are forwarded:

- ✓ Proper Inspection before and during slaughtering of sheep and goat flocks should be undertaken thourouly.

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- ✓ Further Epidemiological surveillance and abattoir investigations should be undertaken in order to determine other risk factors that potentiate the occurrence and distribution of the disease.

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