Seroprevalence and risk factors for peste des petits ruminants in goats and sheep in selected districts of Tanzania

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SUMMARY

Peste des petits ruminants (PPR) is a highly contagious and devastating viral disease of goats and sheep caused by Peste des petits ruminants virus (PPRV). Despite of its impact on the livelihood of rural African communities, insufficient epidemiological information hampers the implementation of effective PPR control strategies. This study was carried out to determine seroprevalence and risk factors for PPR infection in sheep and goats in Longido, Simanjiro, Ngorongoro, Monduli, Kongwa and Mlele districts using a competitive Enzyme–Linked Immunosorbent Assay and questionnaire to detect PPR antibodies, and to collect information related to the potential risk factors for PPR respectively. A total of 583 serum samples from sheep (n=248), and goats (n=335), and 40 households were involved in questionnare. This study confirmed presence of antibodies to PPRV in sheep and goats in all districts studied; and identified management systems related to movement control to be an important risk factor for the spread of PPR. The results suggest that controlling animal movement and limiting interaction with wildlife could PPR transmission.
Keywords: cELISA, PPR, Seroprevalence, Risk factors, Small ruminants

INTRODUCTION

Peste des petits ruminants (PPR) is a devastating disease of goats and sheep caused by Peste des petits ruminants virus (PPRV) under the family Paramyxoviridae (Kardjadj *et al.*, 2015). Peste des petits ruminants seropositivity has also been reported in cattle, camels, pigs, and wild ruminants with no clinical signs (Balamurugan *et al.*, 2014; Asil *et al.*, 2019; Berkowitz *et al.*, 2019). It is a transboundary animal disease with a significant socio-economic impact when it occurs in areas where small ruminants contribute significantly to community livelihoods (Diallo *et al.*, 2019; Idoga *et al.*, 2020). Peste des petits ruminants is placed as one of the most important diseases affecting goats and sheep (OIE and FAO, 2015). Peste des petits ruminants was first described in Ivory Coast in the 1940s (Munir, 2015), and since then, it has spread to more than 70 countries worldwide across Asia, Africa, and in the Middle East, having reached to Europe in 2016 (OIE and FAO, 2015; Diallo *et al*., 2019).

In Tanzania, PPR was reported in 2008 in Ngorongoro district, Arusha region (Swai *et al*., 2009). The disease was introduced from Kenya due to cross-border livestock movements (Karimuribo *et al.*, 2011; Muse *et al.*, 2012; Torsson *et al.*, 2016). Peste des petits ruminants was also introduced in Zambia and Comoro island from Tanzania causing massive deaths of goats and sheep (Kwiatek *et al.*, 2012; Chazya *et al.*, 2014; Ruget *et al.*, 2019). The transmission of PPRV is by direct contact between infected and healthy animals (Chauhan *et al.*, 2009). Introduction of new animals in the flock and presence of mixed animal species such as flocks of goats and sheep were reported as important risk factors for the spread of the disease in nomadic herding, pastoral farming and livestock trade (EMPRES, 2009).

Animal congregations in search for pasture or water or in livestock markets contribute to the spread of the disease to healthy farms (Chota, 2019). Infected animals can spread the viruses via ocular/nasal secretions and excretions in faeces (Ezeibe *et al.*, 2008;

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Diallo *et al.,* 2019). Affected animals are characterized by high fever (41 °C), and depression, accompanied by eye and nose discharges. Animals become anorexic, with painful erosive lesions in their mouth; and may suffer from severe pneumonia, and diarrhoea. Abortions and high mortality rates were also reported in some of the affected animals (OIE and FAO, 2015; Woma *et al*., 2016). Efforts were taken to contain the spread of disease following an incursion in Tanzania in 2008, like mass vaccination of goats and sheep in the Northern and Lake zones bordering Kenya (Karimuribo *et al*., 2011; Mdetele *et al*., 2015). Vaccination continued in small ruminants along livestock marketing routes in 2011, and in some herds closer to wildlife areas (Roos, 2016). However, the current status of the infection is not known. Given the transboundary nature and devastating effects, the disease is one of the greatest concerns in small ruminants rearing countries, and that requires a continuous monitoring (OIE and FAO, 2015; Jones *et al.,* 2020). However, controlling PPR requires a thorough understanding of its epidemiology, including predictors for its occurrence in different settings and farming systems. The knowledge of PPR epidemiology in Tanzania is currently not sufficiently available, and this study aim to address this knowledge gap by exploring the seroprevalence and associated risk factors of PPRV in goats and sheep in different districts spanning different regions of Tanzania.

MATERIALS AND METHODS

Study Area

The study was conducted in January 2020 in six districts namely; Longido, Simanjiro, Ngorongoro (Arusha region), Monduli (Manyara region), Kongwa (Dodoma region) and Mlele (Katavi region). The districts were purposively selected based on their popularity in goats and sheep production as well as areas with maximum livestock-wildlife interaction. The first three are occupied mostly by pastoral and the latter by agro-pastoral communities.

Study Design and Sampling Strategy

A cross-sectional study and multistage sampling procedure were employed where six districts were purposely selected. From each district, two to three villages were randomly selected for the study. In total 15 villages were selected. From each village, two to four households were randomly selected and recruited based on their willingness to participate in the study, and with flock size greater than 10. Fourty households with variable sheep and goat flock sizes participated in the study.

The sample size of animals to be included in the study was estimated according to Thrusfield (2007), where $n = Z^2 P (1-P)/ d^2$, and **n** is the required sample size, **z** = 1.96 (critical value for a 95% confidence level), p is the expected seroprevalence and 50% was assumed to get the maximum sample size, **d** = precision level or allowable error (estimated at 10%). A total of 10 to 16 goats and sheep were randomly selected from each flock and each household. A minimum of 96 goats and 96 sheep were included from each district studied. Total sample size from the six districts studied was 583 (335 goats and 248 sheep).

Data Collection

Prior to sample collection, all animals were examined for their health status including establishing the presence or absence of PPR related signs on the head, nostril, muzzle, eyelids, genital organs, skin; and other information including breed, age and sex, and body conditions. Animals with poor body conditions and those showing some clinical features such as diarrhoea, nasal and ocular discharges, coughing and oral lesions were considered as sick animals and those showing no clinical features were considered to be clinically healthy. Samples were collected from both healthy and sick animals.

A total of 583 whole blood samples (335 from goats and 248 from sheep) were collected using plain 10 ml vacutainer tubes and 19 gauge sterile needles from the jugular veins of non – vaccinated goats and sheep. Collected samples were kept overnight at room temperature to allow serum separation. Serum was decanted and transferred to labelled and chilled 1.5 ml cryovials (tubes) before being transported to Central Veterinary Laboratory (CVL) where they were stored at -20˚C waiting for diagnostic testing by a Competitive Enzyme-Linked

Immunosorbent Assay (cELISA). The c-ELISA is based on the use of monoclonal antibody (mAb) anti-nucleoprotein and a recombinant nucleoprotein produced in the baculovirus. The test depends on inhibition of the binding of the mouse monoclonal antibody (mAb) to a PPR-specific epitope in the presence of a positive serum. Inhibition is detected as a reduction in the optical density (OD) reading obtained with mAb alone thereafter followed by the addition of peroxidase labelled anti-mouse conjugate and substrate/chromogen mixture (Pathotrop *et al.*, 1995; TVLA-CIDB, 2020). Additional animal and household data were collected using a structured questionnaire administered to 40 household representatives (father, wife, son, mother, or livestock attendant). Information collected were: animal species (goats, sheep), breed of animal (indigenous, exotic, cross), flock size (11 – 50, 51 – 100, >100), sex (male and female), and age of animals (young, grower or adult).

Other data collected included the health status of animals (sick or healthy) based on clinical signs observed (ocular discharges, nasal discharges, salivation, oral discharges, respiratory signs), vaccination history, exposure status (if animals/farms were previously infected with PPR), use of animal (meat, milk, dual-purpose), source of animals (bought or raised), and type of flock (single species - sheep only or goats only; or mixed species - sheep and goats). Others were animal management systems (pastoral, agro-pastoral and ranching), contact point (grazing, watering points, marketing, wildlife proximity), name of the owner, date of visit, regions, districts and village name.

Data Analysis

Data were cleaned in Microsoft Excel and analysed using STATA version 14. During data cleaning 15 cELISA results were found to be doubtful and these were considered as negative as also suggested by Torsson *et al.* (2019). Frequencies and proportions were computed to determine seroprevalence of the disease, and comparisons were made using Chi-square test. Assessment of risk factors were performed using a univariate and multivariate logistic regression analyses. In the univariate analysis, a significant level was set at $p < 0.1$ based on the likelihood ratio, and variables that passed this cut-off point were utilized in the multivariate analysis to explore the effect of multiple factors.

Multivariate logistic modelling was performed employing a backward selection method which starts with a full (saturated) model. The criterion for staying in the model was set at $p < 0.2$. Odds ratios at p values < 0.05, with 95% confidence intervals (CI) were considered significant

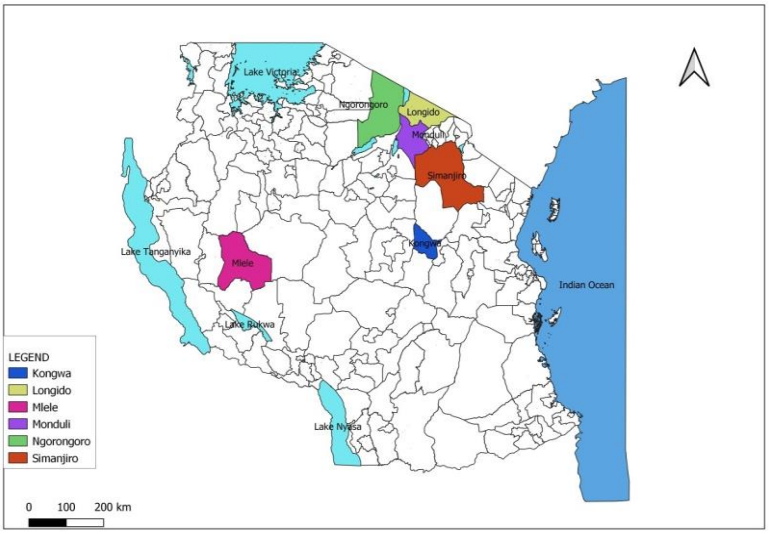

Figure 1. Map of Tanzania showing districts where research was conducted

RESULTS

Seroprevalence of PPR in Study Districts

Simanjiro district had the highest seroprevalence of 60.2% followed by Longido and Ngorongoro. Relatively lower seroprevalence was observed in Monduli, Kongwa and Mlele districts, with Mlele having the lowest seroprevalence, as shown in Figure 2. Antibodies to PPR were detected in all six districts. Out of 583 samples tested,

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103 (30.7%) from goats and 79 (31.9%) samples from sheep tested positive for PPR, yielding an overall seroprevalence of 31.2% [95% CI: 27.4% - 34.9%] as shown in Table 1).

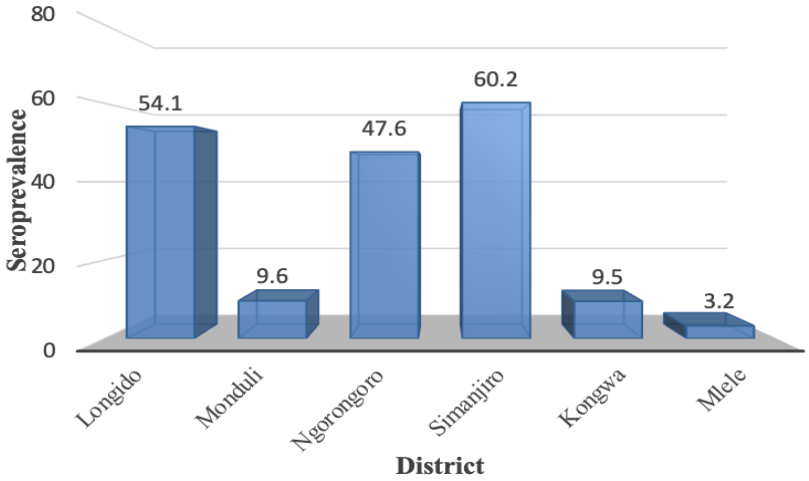

Figure 2. Seroprevalence of PPR in sheep and goats by district

Table 1. Seroprevalence of PPR for sheep and goats in the study districts, stratified in different categories

Variables	Level	Tested animals	Positive samples	Seroprevalence %
Species	Sheep	248	79	31.8
	Goat	335	103	30.7
Sex	Female	424	138	32.5
	Male	159	44	27.7
Breed	Crossbreed	122	54	44.3
	Indigenous	461	128	27.8
Age	Young	38	18	47.4
	Grower	63	7	11.1
	Adult	482	157	32.6
Use of animals	Dual	306	97	31.7
	Meat	277	85	30.7
Health status	Healthy	538	147	27.3
	Sick	45	35	77.8
Exposed	No	422	105	24.8
	Yes	161	77	47.8
Source of animal	Raised	569	181	31.8
	Bought	14	1	7.1
Flock size	11 – 50	113	21	18.6
	50 – 100	139	51	36.7
	>100	331	110	33.2
Management	Agro pastoral	358	169	47.2
	Pastoral	161	12	7.5
	Ranching	64	1	1.6
Type of flock	Mixed	488	179	36.7
	Single	95	3	3.2
Proximity to wildlife	No	95	9	9.5
	Yes	488	173	35.5
Watering points	No	252	61	24.0
	Yes	331	121	36.6
Marketing	No	8	2	25
	Yes	575	180	31.3

Seroprevalence: The percentage (%) of animals that developed antibodies against PPR virus suggesting that they have been exposed to PPR virus. Positive: Animals tested and discovered to have developed antibodies against PPR virus.

Table 2. Univariate analysis for potential risk factors associated with PPR seropositivity in goats and sheep

Variables	Level	χ^2	p value
Species	Sheep	0.08	0.775
	Goats		
Sex	Female	1.28	0.258
	Male		
Breed	Cross	12.2	0.000***
	Indigenous		
Age	Young	16.9	0.000**
	Grower		
	Adults		
Use of animals	Dual	0.08	0.792
	Meat		
Health status	Healthy	49.2	0.000***
	Sick		
Exposed	No		
	Yes	28.6	0.000***
Source of animal	Raised	3.87	0.049**
	Bought		
Flock size	11 – 50		
	51 - 100	11	0.004**
	>100		
Management system	Agro-pastoral		
	Pastoral	111	0.000***
	Ranching		
Type of flock	Mixed	41.6	0.000***
	Single		
Proximity to wildlife	No		
	Yes	24.9	0.000***
Watering points	No		
	Yes	0.15	0.702
Marketing	No		
	Yes	10.2	0.001**

*All statistically significant factors that are marked as ** and those marked as *** are highly significant based on p-value. P-value: Statistical measurement used to validate a hypothesis against observed data. Measures the probability of obtaining the observed results, assuming that the null hypothesis is true.* χ^2*: : Chi-square*

Risk Factor Analysis

An initial exploratory univariate analysis was conducted where 15 predictor variables were examined and the results are presented in Table 2. Ten potential risk factors qualified for inclusion in the multivariate analysis and these were breed, age, health status, history of exposure to PPR, animal source, flock size, management, type of flock and proximity to wildlife (Table 3). Multivariate analysis using logistic regression modelling identified six risk factors associated with the PPR seroprevalence. Distance from wildlife seemed to be a protective factor [p = 0.002, OR = 0.37 CI: 0.004 – 0.308] as animals in areas in contact with wildlife were 25 times more likely to get infected. Being a crossbred animal was a risk factor [p=0.000, OR = 3.04, CI = 1.79 – 5.1] as crossbred animals had 3 times higher odds of being ELISA positive as compared to indigenous animals. Growers were found to be in a lower risk of getting infected as compared to adults [p=0.011, OR=0.25; CI: 0.088 – 0.73]. No significant difference was observed between young and adult animals. Health status was found to be a good predictor of infection, as animals which were observed to be sick were 7 times more likely to test positive on ELISA as compared to animals which were apparently healthy [p=0.000, OR = 7.11, CI: 2.53 –

19.98]. Animals kept in a pastoral system had 68 times higher odds of infection as compared to animals kept in an agro-pastoral system [p=0.005, OR = 68.19, CI: 9.34 – 497.7], while animals in a ranch were about 97% less likely to test positive on ELISA as compared to those in the agro-pastoral system [p=0.005, OR = 68.19, CI: 0.003 – 0.4]. Flock size was a significant predictor factor. Animals in medium and small flock sizes were about 82% and 80% less likely to be infected as compared to those in large flock sizes [p=0.044, CI: 0.034– 0.951; p=0.05, CI: 0.039– 0.998) as shown in Table 3.

Table 3. Multivariate analysis for putative animal level and herd level risk factors associated with PPR seropositivity for sheep and goats.

Variable	Level	OR [95% CI]	p-value > |Z|
Breed	Indigenous	Ref.	
	Crossbreed	3.04 [1.79 – 5.16]	0.000***
	Adults	Ref.	
Age	Grower	0.25 [0.08 – 0.73]	0.011**
	Young	0.45 [0.16 –1.24]	0.121
Flock size	11 – 50	0.18 [0.03 – 0.95]	0.04**
	50 – 100	0.19 [0.03 – 0.85]	0.05**
	> 100	Ref.	
Health status	Healthy	Ref.	-
	Sick	7.11 [2.51– 19.98]	0.000**
Management system	Agro-pastoral	Ref.	-
	Pastoral	68.2 [9.34 – 497.75]	0.000***
	Ranching	0.033 [0.00 – 0.36]	0.005**
Proximity to wildlife	Yes	Ref.	-
	No	0.04 [0.004 – 0.31]	0.002**

Factors that are statistically significant were marked as **, and those marked as *** were highly significant.

DISCUSSION

The established an overall seroprevalence of PPR in the studied areas indicates the wide-spread of PPR infection. The observed PPR seroprevalence conforms to what was reported in previous studies in the country (Muse *et al.,* 2012; Kgotlele *et al.,* 2016). On the other hand, higher prevalence of about 45.8% was reported in the Northern zone (Swai *et al.,* 2009). The cause of this variation is unknown, we speculate that it could be related to differences in PPR control measures. Interestingly, both sheep and goats were found to be equally at risk as seroprevalence was not statistically different between the two species. This is contrary to the findings of other studies elsewhere where the prevalence in sheep was higher compared to goats (Özkul *et al.*, 2002; Abraham *et al.*, 2005 and Sow *et al.,* 2008). This differences could be partly explained by the management style of sheep and goats in Tanzania where you find that both sheep and goats are reared and grazed together, hence share a relatively equal chance of exposure. Furthermore, this study observed a slightly higher overall PPRV sero-positivity in female (32.5%) compared to males (27.7%), although, the difference was not statistically significant.

Similarly, Swai *et al.* (2009) reported lack of significant difference between sexes in reference to the infection rate. However, Acharya *et al.* (2018) reported a higher likelihood of infection in females (4.0 times) than in males [OR = 3.82; 95% CI 1.51 - 9.67]. This difference was attributed to the differences in livestock breeding patterns whereby most farmers keep females for longer periods for the reproduction purposes while most males are castrated and disposed in relatively younger ages. The longer animals are kept the more chances of

exposure to the infection (Acharya *et al.*, 2018). Other studies linked the differences between male and females to the existence of production and reproduction stress among females which makes them more prone to infection (Munir *et al*., 2008). Apart from sex, age is another important factor that can influence the spread of diseases. It was observed in this study that; growers were found to be 69% less likely to get infected as compared to adults. This result is in agreement with what was reported by Tonkara *et al.* (1996) who reported higher seroprevalence of PPR in older ruminants.

In addition to the factors described above, location and purpose for keeping animals can also play part in the spread of diseases. It was observed that districts differed significantly in PPR seroprevalence. An important finding in this study is the low seroprevalence in Mlele district, the area which had previously been reported to be PPRV-free, and was considered as a favorable district for zoning and exportation of animals (Mdetele *et al.*, 2020). Hence, more studies should be carried out to identify the risk factors for the introduction of PPR in Mlele district.

When the purpose of keeping sheep and goats was evaluated, the study showed that there was no statistically significant difference between dual-purpose animals (31.7%) and those used for meat only (30.7%). The cause of the difference is not known, mixed grazing can be a contributing factor as both animals have an equal chance of being infected.

Among the factors evaluated, management was found to have a relatively significant contribution on PPR seropositivity compared to other factors. For example, animals kept in a pastoral system had 68 times higher odds of being seropositive compared to animals kept in an agro-pastoral system where animal's movement is relatively restricted to protect crops.

Likewise, previous studies demonstrated higher PPR seroprevalence in pastoral than agro-pastoral management system (Swai *et al.*, 2009; Kivaria *et al.*, 2013; Herzog *et al.*, 2019). Pastoral system has been associated with an increased risk of PPR spread in Sudan and Ethiopia (Ahmed *et al.*, 2014) due to the mixing of flocks from different farms as reported by Kusiluka *et al.* (1994). Petits *et al.* (2013) and Mahapatra *et al*. (2015). Furthermore, consistent with other studies (Sacker *et al.*, 2011; Ahmed *et al.*, 2014; Acharya *et al.*, 2018), a higher odds of infection was observed in crossbreed animals than in indigenous breeds. It is not uncommon for genetic variations of animals to influence the health status. For example, Guinean breeds of goats (West African dwarf, Iogoon, Kindi and Djallonke) and the dwarf breeds were reported to be highly susceptible (Lefèvre and Diallo, 1990) to PPR, whereas the Sahelian breeds are considered to be more resistant to PPR (Couacy-Hymann *et al.*, 2007).

Lastly, the study confirms the already known existence of PPR in Tanzania, and reveals the spread of PPR to areas that were previously considered to be free of the disease. The risk factors for PPR spread identified in this study provides an invaluable contribution to the current body of knowledge and will inform the current and future PPR control and intervention strategies in the overall country goal of eradicating PPR by 2030

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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