



Epidemiological investigation into the introduction and factors for spread of Peste des Petits Ruminants, southern Tanzania

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A study was carried out to confirm and identify sources and elucidate factors associated with the introduction of Peste des Petits Ruminants (PPR) in southern Tanzania. This study was conducted in Tandahimba and Newala districts of Mtwara region following suspected outbreak of PPR in the area. Qualitative data were collected using semi-structured questionnaires and in-depth interviews of key informants who included goat and sheep owners with suspected cases of PPR and animal health service providers as well as local administrative authority. Additionally, 216 serum samples and 28 swabs were collected for serological and virological laboratory disease confirmation. The results show that PPR was first introduced in Likuna village of Newala district in February 2009 through newly purchased goats from the Pugu livestock market located about 700 km in the outskirts of Dar es Salaam city. Factors which contributed to spread of PPR included communal grazing and the cheap prices of sick animals bought by livestock keepers for slaughtering in other villages. Laboratory findings confirmed presence of PPR in the area by RT-PCR and serological analysis revealed that seroprevalence was 31%. These findings have confirmed, for the first time, introduction of PPR in southern Tanzania. The presence of PPR poses high risk of southward spread of the disease to other southern African countries in the SADC region thus calling for concerted and collaborative efforts in prevention and control of the disease to avoid losses. Further elaborate studies on the spread, prevalence and risk factors associated with the disease should urgently be investigated.

Introduction

Peste des Petits ruminants is an acute, highly contagious infectious disease of small domestic ruminants and small wild ruminants, such as antelopes, impala and gazelles (Abu Elzein *et al.* 2004; Nussieba *et al.* 2009). The disease is caused by Peste des Petits Ruminants (PPR) virus (PPRv). PPRv belongs to a Morbillivirus genus of Paramyxoviridae family. PPRv is a single serotype that is differentiated into four (I-IV) lineages (Forsyth & Barrett 1995; Couacy-Hymann *et al.* 2002). The geographical distribution of PPR lineages varies as lineage I and II have been commonly reported in West Africa (Ashley *et al.* 2010), lineage III has been reported eastern Africa except for Sudan which has been found to harbour lineage IV in addition to lineage III (Khalafalla *et al.* 2010). On the other hand, lineage IV has been reported in Central and North Africa, Asia and China (Ozkul *et al.* 2002; Wang *et al.* 2009; Awa *et al.* 2000; Ayari-Fakhfakh *et al.* 2010; Balamurugan *et al.* 2010; Khalafalla *et al.* 2010).

The disease is transmitted by direct contact involving secretions or excretions from infected animals to healthy animals in close proximity. Clinically, PPR is characterised by sudden onset of depression, fever, lacrimation, sores in the mouth, dyspnoea and coughing, foul smelling diarrhoea and death. Post-mortem findings, normally restricted to the alimentary tract, consist of extensive erosive stomatitis and haemorrhagic gastro-enteritis, and often include streaks of congestion along the folds of the mucosa resulting in the characteristic 'zebra-striped' appearance (Chauhan *et al.* 2009). Secondary bronchopneumonia is common.

The disease is characterised by high morbidity and mortality (50% – 80%) in naive sheep and goats populations, impacting negatively on the livelihoods, food security and socio-economic activities of livestock keepers in affected areas. The disease also impacts negatively on the local and international livestock trade markets. In Tanzania the disease limits the efforts of farmers and government in attaining the millennium development goal for eradicating extreme poverty and hunger.

In Tanzania, PPR was first confirmed in 2008 in the northern areas (Kivaria *et al.* 2009; Swai *et al.* 2009), where it was confined until recently when it was suspected to have been introduced

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in southern areas in 2010 (FAO 2010). The presence of PPR in southern Tanzania would pose a high risk for spread to the whole of the Southern African Development Community (SADC) threatening to devastate the livelihoods and food security of millions of small herders and agro-pastoralists (FAO 2010). Until the current study was designed, no official confirmation of PPR in southern parts of the country had been carried out. The objectives of this study were:

- to confirm the presence of PPR in goats and sheep in Newala and Tandahimba districts, Mtwara region
- to identify sources and factors that contributed to the introduction and spread of the PPR in southern Tanzania
- to describe the epidemiological factors and losses in the affected villages.

The hypotheses were to confirm if suspected cases seen in goats and sheep in southern Tanzania were caused by PPRv and to find out which factors facilitate the spread of the disease in this zone.

Material and methods

Study area

The study was carried out in eight selected villages in two districts of Mtwara region, Newala and Tandahimba districts, in southern Tanzania between March and May 2011 (Figure 1). The estimated census for humans, goats and sheep in these districts are shown in Table 1. The study area was purposively targeted following reports of suspected PPR outbreak that decimated small ruminants in the districts. This area borders Mueda district of the Cabo Delgado province of Mozambique with a small ruminant population that is naive to PPR. The study area lies between 2° 11' and 6° 14' S, and 35° 11' and 38° 26' E at an elevation of 100 m – 800 m above sea level. The rainy season starts in November and/or December to April and/or May with an average annual precipitation of 893 mm – 1001 mm. The mean monthly temperature varies from 23 °C to 27 °C and relative humidity varies from 79% to 87%.

Study design and data collection

The current study employed a cross-sectional study design where selected villages were visited once between March 2011 and May 2011. Purposive sampling of animals was carried out in villages with suspected PPR cases based on opinion from local veterinary officials and leaders. Upon identification of household with suspected cases, the researcher (EAM) conducted detailed interviews with owners of goats and sheep. This was complemented with random selection of five animals, either goats or sheep, which were subjected to detailed clinical examination as well as sample collection.

Questionnaire survey

A semi-structured questionnaire was developed and field tested on a few households keeping goats and sheep in the two districts. The interviews focused on collection of information on flock size, species, age and sex, health and

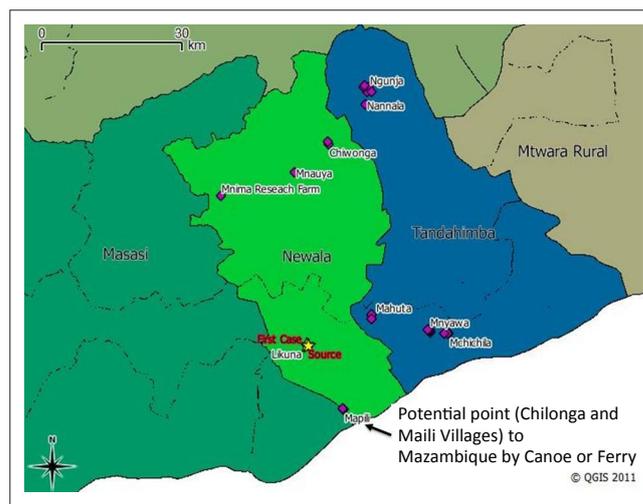


FIGURE 1: Showing sampling points and introduction of Peste des Petits Ruminants in Newala (star).

TABLE 1: The proportions of households, morbidity, mortality, chemotherapy, vaccination and statistics for goats, sheep and people.

Parameter†	Newala (n = 31)	Tandahimba (n = 48)	Overall (n = 79)
Households affected	19.4	81.3	57.0
Morbidity	4.8	73.1	48.9
Mortality (Crude)	4.4	37.4	25.7
Case fatality	92.9	51.2	52.6
Households treated (Chemotherapy)	22.6	43.8	35.4
Households vaccinated	0.0	0.0	0.0
Population statistics‡			
Goat	105 174	149 945	255119
Sheep	2085	2348	4433
Human	183 344	203 837	387181

†, Parameters are measured in percentage.

‡, Statistics are based on figures available in respective District Council Offices in Newala and Tandahimba districts during the study.

vaccination status, and management. Age was approximated and classified as kids or lambs (≤ 3 months), weaners (> 3 to ≤ 9 months) and adults (> 9 months). Health status data was collected by recording history of disease outbreak or occurrence, its clinical signs, overall number of sick animals (used to compute morbidity) as well as overall and specific deaths associated with observed clinical cases (used to compute crude and case fatality of PPR). Flock management data included access to animal health and extension services (presence, type and frequency of services), action taken after the outbreak, live animal market visiting frequency and addition of new animals, and suspected source of the infection.

In-depth interviews of key informants were conducted to obtain opinion from local livestock field officers (LFOs) as well as from local government officials in affected villages and wards. The district veterinary officer (DVO) of Newala District and veterinary officers at Mtwara Veterinary Investigation Centre (VIC) were also interviewed to ascertain where and when PPR was first introduced into southern Tanzania and which disease control measures were instituted before the current study.



Samples collection for Competitive Enzyme Linked Immunosorbent Assay Analysis

A total of 216 serum samples were collected from goats and sheep in Newala and Tandahimba districts. Initially, blood samples were collected from the jugular vein of each animal using plain Vacutainer tubes. The samples were labelled accordingly to allow identification of each animal and flock sampled and kept in a slanted position overnight to allow serum separation from clotted blood samples. Serum was decanted and aliquoted into 1.5 mL cryovials before being transported and stored temporarily at the VIC, Mtwara. Finally the serum samples were shipped in a cool box chilled on ice packs to the Laboratory at the VIC, Arusha where serological analysis was carried out.

A monoclonal antibody (MAb) based competitive Enzyme Linked Immunosorbent Assay (cELISA) (Diallo *et al.* 1995) was used for the detection of antibodies in sera to PPRv using approved competitive ELISA kit as described by Singh *et al.* (2004a) and Swai *et al.* (2009). According to the manufacturer, the sensitivity and specificity (for both animal and flock levels) of this cELISA are 99.4% and 94.5%, respectively. Briefly, the ELISA plates were coated with PPR antigen; the unbound antigen was washed away using buffer then samples were added; Rabbit antimouse-horseradish peroxidase (HRPO) conjugate was added and incubated with constant agitation in each stage. Substrate solution (O-phenylenediamine dihydrochloride containing H₂O₂) was added allowing for a colour reaction to develop which was halted with the addition of an equal volume of 1 M H₂SO₄. The ELISA micro plates were read with an immunoskan reader (Flow laboratories, UK) with an inference filter of 492 nm and connected to a computer loaded with ELISA Data Information (EDI) software for automated reading and calculation of the percentage inhibition (PI) values.

Samples collection for Reverse Transcriptase Polymerase Chain Reaction

Samples ($n = 28$) from nasal and eye discharges as well as saliva from oral ulcers were collected from clinically sick sheep and/or goats using sterile swabs which were placed in a viral transport media containing antibiotics and antifungals. The samples were transported on ice to Sokoine University microbiology laboratory for further analysis.

Virological analysis employed Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) to confirm involvement of PPR in suspected cases. Briefly, the test was carried out as follows: The RNA extraction from samples was done using commercial RNA extraction kit (Qiagen®); the RNA was converted to cDNA using a reverse transcriptase enzyme (Superscript III Platinum One-Step Quantitative, Invitrogen®). The cDNA was amplified using PPRv specific NP3 and NP4 primers as previously described by Couacy-Hymann *et al.* (2002). PCR products were analysed by electrophoresis and visualised in a UV transilluminator.

Statistical data analysis

Villages, farms and individual animal data were stored in Microsoft Excel 2007 (version 12). Descriptive statistics for the animal and flock level explanatory variables examined in the study were computed using Microsoft Excel. Proportions were calculated for seroprevalence and factors that included animal species, sex and age, location of the flock, health status, management and veterinary services provision. The statistical significance for the proportions was compared using Chi-square test in Epi Info software version 5 (Centre for Disease Control and Prevention). A confidence limit of less than 5% was used to indicate a significant level. Separate statistical analyses were performed for the data from the two species because previous studies have indicated that virus infection rate and epidemiology may be very different in the two species (Waret-Szkuta *et al.* 2008). Study village maps were created using Quantum GIS (QGIS 1.4.0 version) Enceladus (Open Source Geospatial Foundation).

Results

Disease prevalence

The presence of the PPR infection in southern Tanzania was confirmed by the RT-PCR test whereby 53.6% of the samples were positive from both sheep and goats.

The overall PPR seroprevalence was 31% (95% CI = 24.9% – 37.6%) in the two districts (Figure 2). Tandahimba district recorded higher seroprevalence (55.5%) compared to the seroprevalence in 5.7% in Newala district, the difference which was also statistically significant ($p < 0.001$). Mnyawa village recorded highest seroprevalence followed by Nannala, Ngunja, and Mchichira villages of Tandahimba district (Figure 2). Mnima Research Farm had high seroprevalence followed by Chiwonga, Mapili and Mnauya village in that order in Newala district. There was no statistical difference in the seroprevalence recorded in goats (35.3%) compared to that in sheep (30.7%). Similarly, there was no statistical difference in the PPR seroprevalence in female (36.8%) compared to that recorded in male (29.8%) animals sampled in this study. Age-wise, 32.1% of the adults and 22.7% of the kids or lambs were seropositive, however, there was no statistical difference.

The PPR prevalence based on reported cases observed by interviewees is shown in Table 1. Overall, 57% of 79 households visited had experienced suspected PPR cases in their flocks. Again, a significantly higher proportion of households in Tandahimba (81.3%) had PPR cases than those in Newala district (19.4%; $p < 0.001$). A similar trend was also observed with respect to morbidity of PPR when Tandahimba recorded higher morbidity (73.1%) compared to that in Newala district (4.8%; $p < 0.001$). Clinically, a significantly higher proportion of goats (90.9%) were reported to be sick compared to only 9.1% sheep which were sick ($P < 0.001$). A similar trend in mortality was recorded when the proportion of goats and sheep which died was 93.4% and 6.6%, respectively ($P = 0.059$). A good number of

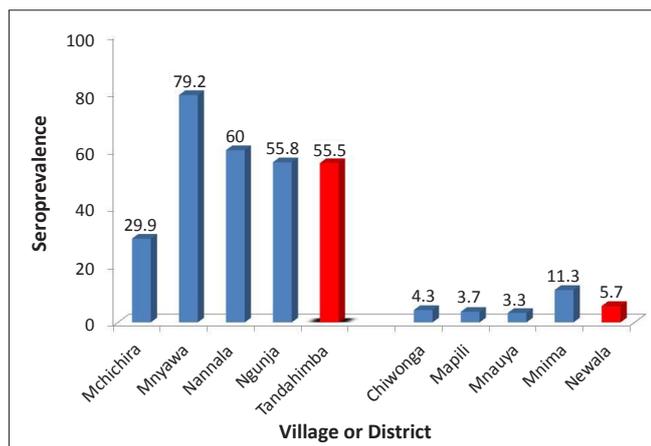


FIGURE 2: Seroprevalence by villages or district.

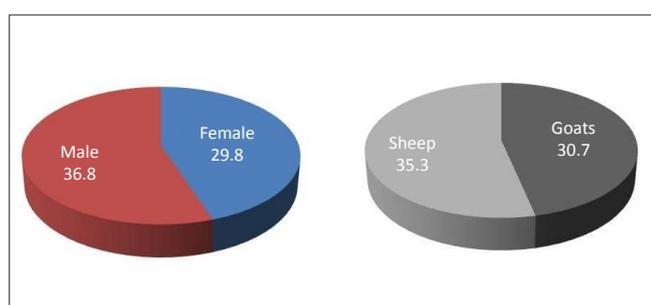


FIGURE 3: Proportions (%) for seroprevalence by sex (left) and species (right).

small ruminant owners also reported to treat their animals using antibiotics as shown in Table 1.

Management practices

It was observed that goats and sheep in the two districts are managed in two different ways. The first group keeps animals in small group in small houses on a raised floor at night and graze in a community land during day time. The second group manage animals collectively by keeping them together in groups in relatively large animal houses on ground floor and utilise communal grazing. Goats and sheep are kept for meat and for sale in order to generate income for the household.

There were more male respondents interviewed (90.0%) in comparison to female respondents (10.0%; Table 2). Only a few respondents in Newala district tethered their animals at night (10.1%) and most (94.9%) farmers kept their animals in a small raised house at night. All (100%) of the respondents utilised communal grazing areas (Table 2).

Introduction of Peste des Petits Ruminants in southern Tanzania

The in-depth interview revealed that PPR was introduced in the southern Tanzania for the first time in goats and sheep in February 2009. The current study managed to trace a village where sick animals started which was Likuna village of Newala district. It was observed that PPR introduction was through newly purchased goats from the Pugu livestock market located about 679 km in the outskirts of Dar es Salaam city. These animals were brought in about one week prior to the disease outbreak. Other villages in close proximity

of this village that reported outbreak of PPR in the same month were Kikuyu, Makote, Namiyonga, Lidumbe and Mkunya. It was confirmed that these five villages had also received some of these goats from Pugu Livestock Market. The disease spread to neighbouring Tandahimba District through buying sick and cheap animals from Newala district with the intention of selling them at Tandahimba live animal markets and butcheries.

Observed clinical signs

Different clinical signs were reported by livestock keepers in suspected PPR cases. The signs included diarrhoea, lacrimation, nasal discharges, respiratory distress, oral ulcers and skin nodules (Figure 4).

Economic losses

The economic loss due to mortality in two districts was 25.7% and case fatality rate was 52.6% (Table 1). Case fatality rate was higher in Newala district (92.9%) compared to (51.2%) in Tandahimba district ($P < 0.000$).

TABLE 2: Proportions of respondents' sex, animal management, awareness of the disease, source and spreading of the infection and veterinary extension services.

Parameter†	Newala (n = 31)	Tandahimba (n = 48)	Overall (n = 79)
Household head sex			
Male	32.9	57.0	89.9
Female	5.1	5.1	10.1
Animal management			
Animal tethering	10.1	0.0	10.1
Boma	36.7	58.2	94.9
Communal grazing	39.2	60.8	100
Livestock keepers' awareness			
Outbreak awareness	3.8	60.8	64.6
Seen affected animals	11.4	57.0	68.4
Own animals affected	7.6	49.4	57.0
Knowledge of what disease it was	1.4	1.4	2.9
Seen similar disease in past	1.3	5.1	6.5
Extension worker present	17.8	41.1	58.9
Regular animal inspection	17.6	35.3	52.9
Frequency of inspection			
At least once a month	15.4	24.6	40.0
Once in several months	3.1	10.8	13.8
No visit at all	24.6	9.2	33.8
Visit when called	0.0	12.3	12.3
Source of infection and spread			
Unknown cause	0.0	36.7	36.7
Change of weather	0.0	20.0	20.0
Communal grazing area	10.0	33.3	43.3

†. Parameters are measured in percentage.

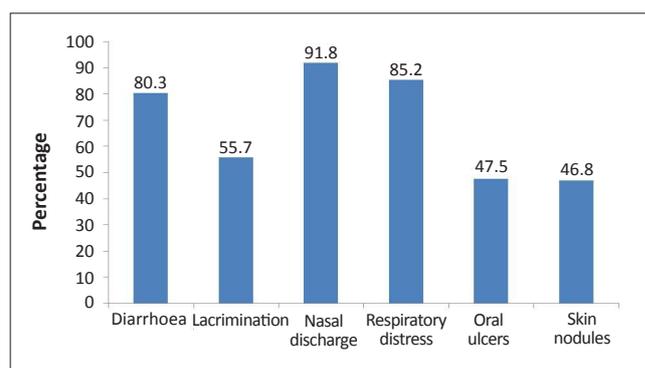


FIGURE 4: Clinical signs presented by Peste des Petits Ruminants disease.



Disease awareness and vaccination

Over 60.8% of respondents in Tandahimba were aware of the disease outbreak and 49.4% had their own animals affected (Table 2). Very few livestock keepers (2.9%) knew what disease it was and only (6.5%) thought that they had seen it in the past. During the outbreak, animal owners (35.4%) from the two districts treated their animals using chemotherapy whereas 22.6% were from Newala and 43.8% were from Tandahimba (Table 1). More than half (58.9%) had veterinary extension workers in their villages but half (52.9%) of the farmers reported regular health inspection for their animals, although the frequency of inspection varied (Table 2). All (100%) respondents had not vaccinated their animals (Table 1). The source of the infection and spread was suspected by farmers to be communal grazing and change of weather conditions, with more animals being affected during the rainy season.

Discussion

This study has confirmed the aetiology of the disease outbreak that occurred in the districts of Tandahimba and Newala in southern Tanzania to be PPR in March 2011. It has also identified the village where the first introduction and consequently, the source of PPR spread in southern Tanzania happened. Although it took only one year from first official confirmation of the disease in northern Tanzania to introduction in southern Tanzania, it has taken about two years to confirm this disease through efforts of the current study. This is consistent with the findings by Karimuribo *et al.* (2011) of delayed confirmation following introduction of PPR in the country. These findings point to the importance of having efficient disease surveillance and diagnostic capacity especially for emerging and re-emerging fatal diseases affecting animal populations.

In this study it was found out that the source of the disease was through the introduction of new animals purchased from live animal market. Similar sources of the disease have been implicated before (Singh *et al.* 2004a; Muhammad *et al.* 2009).

The proportions of sero-converted animals examined in the area under study was low (31.0%) compared to the reports from northern Tanzania (45.5%) (Swai *et al.* 2009) but at the flock level the prevalence was high (48.9%). Ozkul *et al.* (2002) in Turkey found comparable findings whereby the overall prevalence was low based on antibody test and higher based on clinical signs. Low PPR seroprevalence have also been reported in Tunisia (Ayari-Fakhfakh *et al.* 2010).

Although the seroprevalence was low in this study it has been shown elsewhere that seroprevalence can be as high as 45.5%, 78% and 92.5%, as reported in Cameroon, Nigeria and Sudan respectively (Ekue *et al.* 1992; Obidike *et al.* 2006; Osman *et al.* 2008) using neutralisation and haemagglutination tests. When the competitive ELISA method was used the seroprevalence was reported to be 51% and 50% (Khan *et al.* 2008; Misbah *et al.* 2009) in Pakistan. The inconsistency

in the seroprevalence of antibodies to PPRv in different areas is attributed to variations in a number of factors including the management system, levels of immunity, diagnostic test, sampling procedures used and technical know-how of the researchers (Singh *et al.* 2004b; Waret-Szkuta *et al.* 2008). In this study, goats were reported to show an acute form of the disease whilst sheep showed a sub-acute or chronic form; this finding is supported by other studies (Obi, Rowe & Taylor 1984; Swai *et al.* 2009).

Although, the differences observed between males and females was not significant Swai *et al.* (2009) observed sex differences in sheep whereby males were more affected compared to females. With respect to age category, the highest prevalence of PPR was observed in adults compared to other age category. This result is in agreement with other finds observed in Ethiopia and India (Singh *et al.* 2004a; Waret-Szkuta *et al.* 2008) where they reported high prevalence in adults. Association with seasonal (weather) changes observed by farmers has been reported elsewhere (Singh *et al.* 2004a; Muhammad *et al.* 2009).

Even though the PPR was introduced in Newala, it was more prevalent in Tandahimba district. The reasons are thought to be first selling of sick animals from Newala to Tandahimba animal markets and butcheries and secondly the livestock keepers in Tandahimba district managed their animals by utilising more (60.8%) communal grazing compared to Newala district (39.2%) (Table 2).

This study revealed that more than half of the farmers have little or no access to veterinary services. Lack of appropriate veterinary services and inadequate infrastructure especially in the local live animal markets in the country, may facilitate disease transmission. Extra attention from the government to upgrade the handling and penning facilities of these market places could result in reduced level of disease transmission. Efforts should be made to increase livestock and public awareness with respect to this new disease in the area. The current ongoing government efforts to perform PPR vaccination of goats and sheep in unaffected villages should be encouraged.

Conclusion

This study has confirmed, for the first time, the presence of the PPR in southern Tanzania. In addition, the study has identified the source of introduction of PPR to be newly purchased animals from Pugu Livestock Market. Given that no vaccinations had been carried out against PPR, our result confirms natural transmission of PPR virus under field conditions in the southern Tanzania. The spread of this disease to southern Tanzania poses a high risk of the disease spread to southern countries (SADC countries including Mozambique, Zambia and Malawi) with naïve goat and sheep populations. Further studies on virus isolation, disease status in wildlife and temporal trends events are required to define the epidemiology of PPR in large area of the southern Tanzania. National, regional and international collaborative efforts are required to contain and control the disease.



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