Clinico-pathological findings of the 2011 outbreak of Peste des Petits Ruminants (PPR) in Tandahimba district, southern Tanzania

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Abstract

Although PPR outbreaks were reported in Northern Tanzania since 2008, there has been no description of the clinical or pathological manifestation of the disease, an important criterion in guiding veterinarians and farmers on proper recognition and diagnosis of the disease. A study was therefore conducted to investigate and describe clinical signs and pathological lesions associated with 2011 Peste des petits ruminants (PPR) outbreak in goats and sheep in Tandahimba district located in Southern Tanzania. The investigation involved taking history and conducting clinical examination of PPR suspected cases (25 goats and 3 sheep) in the study district which had neither a history of vaccination against PPR nor previous illness due to PPR. This work was complemented by collection of pathological samples and specimens for laboratory examination. A detailed post-mortem was performed on three sacrificed animals followed by collection of specimens including lungs, liver, spleen and lymph nodes for histopathological examination. Clinical samples from 30 animals which included swabs from ocular, nasal and mouth lesions were also collected for confirmation of PPR through detection of PPR ribonucleic acid using reverse transcription polymerase chain reaction (RT-PCR). Clinical examinations of the cases showed signs suggestive of PPR including severe depression, high fever (41°C), anorexia, muco-pulurent nasal discharge, erosive and necrotic stomatitis, mild diarrhoea and skin nodules. Post mortem examination showed evidence of pneumonia including lung congestion and consolidation, increased thickness of inter-alveolar walls, moderate infiltration of inflammatory cells in bronchiolar subepithelial and perivascular layers. Overall 56.7% of the samples (n=30) tested were positive for PPR by RT-PCR. This study has confirmed and described the presence of PPR in southern Tanzania. A more detailed study including other districts is recommended to provide more information regarding the magnitude and factors associated with PPR in Southern Tanzania.

Keywords: PPR, skin nodules; pneumonia; PPRV, morbillivirus


Introduction

Peste des Petits Ruminants (PPR) is an acute, highly contagious and infectious disease specific to small ruminants and small wild stocks (Nussieba et al., 2009a). The disease is caused by Peste des Petits Ruminants virus (PPRV). PPRV is a member of the order Mononegavirales, Family Paramyxoviridae, subfamily Paramyxovirinae, and genus morbillivirus. Paramyxoviridae are...
enveloped and contain single stranded non-segmented negative sense RNA (Enveloped (-) ssRNA) genomes of approximately 16 kb (Chauhan et al., 2009) with a single serotype.

Transmission of PPR is achieved by direct contact from infected to susceptible animals by close contact or through respiratory and oral routes. Healthy animals inhale fine infected droplets containing the virus that are released into air from secretions and excretions when infected animals cough or sneeze (Khan et al., 2008; Chauhan et al., 2009).

PPRV targets epithelial cells and pneumocytes leading to pathological findings of PPR are observed in the digestive and respiratory systems. The respiratory lesions include interstitial pneumonia and bacterial bronchopneumonia or fibrinoïd pneumonia (Aruni et al., 1998). Another study showed lung with subcapsular areas and medullary sinuses (Nussieba et al., 2009b). The lymph nodes are characterized by oedema in the cortical and medulla and infiltration of mononuclear cells as well as giant cells in subcapsular areas and medullary sinuses (Nussieba et al., 2009b).

The disease is endemic and common in goats (Capra hircus) and sheep (Ovis aries) in Asia, China, Middle East, Eastern parts of Europe, West, Central and East Africa (Banyard et al., 2010). The principle hosts for PPR are goats and sheep whereby goats are frequently more severely affected than sheep (Nussieba et al., 2009b). In naïve population PPR occurs in an epizootic form with a morbidity of 80-90% and mortality between 50 and 80% (Lefevre and Diallo, 1990; Chauhan et al., 2009).

In Tanzania PPR outbreak was first reported in 2008 in Northern parts of the country (Swai et al., 2009) and it was believed to have been introduced from Kenya. Recently, PPR broke out in the southern parts of Tanzania in early 2010, threatening a local population of over 13.5 million goats and over 3.5 million sheep (FAO, 2010).

Sheep and goats constitute more than 30% of the domestic meat utilization in Africa. Provided that small ruminants cover the family running income for most pastoral households; largely their present and future income generating ability is affected resulting in negative impact on the livelihoods and food security of pastoral community (Banyard et al., 2010). Women and children are even more affected since they are more involved in the sheep and goats production (Kumar et al., 2003). Besides, the disease impacts negatively the local and international livestock trade markets.

Although PPR disease outbreaks in Tanzania have been reported in Northern Tanzania since 2008, there has been no description of clinical or pathological manifestation of the disease which is important in guiding veterinarians and farmers to identify and diagnose PPR cases. When a new disease is introduced in an area, it is likely to be confused with other diseases and also lack of laboratory facilities necessitates use of clinical signs for field-based diagnosis. Clear picture on signs and lesions (standard case definition) that can be used to define a disease are important during disease investigation and control.

The overall objective of this study was to carry out a descriptive study on PPR based on disease outbreak in southern Tanzania in 2011 and describe clinical manifestations and pathological lesions associated with clinical cases as well as confirmation of PPR by detection of virus ribonucleic acid. Findings of this study would help veterinary practitioners, livestock keepers and other stakeholders at regional and national level to better understand, recognize and institute appropriate disease control measures to prevent spread of the disease and to reduce the impact of the disease on livelihoods and food security in the region.

Materials and Methods

Study Area

The PPR disease investigation was carried out in Mkulung’ulu and Bondeni villages, Mahuta ward in Tandahimba district of Mtwara region. The study area is located in southern Tanzania (Fig 1). Selection of villages was based on the presence of active suspected PPR cases as reported to the Tandahimba District Agriculture and Livestock Development Office (DALDO). The study area lies between Latitude 10°42’ and 10°52’ South of Equator, and Longitude 39°24’ and 39°47’ East of Greenwich at an elevation of 100-800 m above sea level. The rain is monomodal with rainy season starting in November to May and a total rainfall averaging 600–1000mm. The mean monthly temperature varies from 23°C to 27°C and relative humidity varies between 79% and 87%. Statistics based on figures available in the District Council Offices (DVO) show that Tandahimba district has population of 203,837 and livestock population of about 149,945 goats and 2,348 sheep. This district borders Mueda district of the Cabo Delgado province of Mozambique.

Animals and samples collection

Goats and sheep owners who participated in this study were purposively selected based on the presence of suspected PPR cases in their flocks. History taking involved among others questions on introduction of new animals and animal bought from live animal...
markets; morbidity and mortality; any treatment and its effects; previous similar incidences and clinical signs; grazing system; presence and frequency of veterinary services and any vaccination done. During clinical examination animals were well identified by owners and were given case numbers during bio-data taking. Animals were first visually inspected at a distance and environment was examined. Then sick animals were restrained by owners for detailed clinical examinations as well as recording rectal temperature using a clinical thermometer. The number of animals clinically examined for PPR signs were 75 goats and 11 sheep. Among the studied population 25 (33.3%) goats and 3 (27.3%) sheep showed clinical signs of which 7 (28.0%) goats died.

Detection of PPRV by Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Samples for virological examination from nasal and eye discharges as well as saliva from oral ulcers were collected from clinically sick animals (n=30) using sterile swabs (BD, Maryland, USA). The swabs were placed in a universal viral transport media (BD, Maryland, USA) for viruses, chlamydiae, mycoplasmas and ureaplasmas. The samples were transported chilled on ice for further analysis.

Pathological and clinical samples from 30 animals were collected including lungs, liver, spleen, lymph nodes and swabs from ocular, nasal and mouth. Clinical samples from each animal were pooled together for virological analysis using RT-PCR to confirm presence of PPRV in suspected cases.

The RNA extraction from samples was done using a commercial RNA extraction kit (Nucleospin RNA virus, Macherey-Nagel, GmbH, Duren, Germany). RNA was converted to DNA using a reverse transcriptase enzyme (Superscript III Platinum One-Step qRT-PCR System, Invitrogen, Carlsbad, CA) at 55°C for 30 minutes.

Afterwards, a 350 bp DNA fragment of the nucleoprotein (NP) gene was amplified by PCR with the set of specific primers NP3 (5′ – TCT CGG AAA TCG CCT CAC AGA CTG – 3’) and NP4 (5′ – CCT CCT CTT GGT CCT CCA GAA TCT – 3’) as described by Couacy-Hymann et al., (2002). The PCR amplification reaction was carried out in a DNA thermal cycler (Step One Real time PCR systems, Applied Biosystems, Singapore) with an initial denaturation at
95°C for 10 min followed by 40 cycles with denaturation at 94°C for 30s, annealing at 55°C for 30s and extension at 72°C for 30s. The amplification reaction was completed by a final extension at 72°C for 7 min.

The PCR products (amplicons) were analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide, visualized under a UV transilluminator and photographed.

**Histopathological study**

Detailed autopsy was conducted on three severely sick animals that were sacrificed. Owners of the sacrificed animals were compensated. Pathological samples were collected including lungs, liver, spleen, lymph nodes and intestines. Samples were preserved in 10% neutral buffered formalin and subsequently embedded in paraffin wax. Haematoxylin and Eosin staining was done on 5µm thick tissue sections. Stained sections were examined under light microscope attached to a digital camera and photographs were then taken.

**Results**

The outbreak suspected to be PPR in goats and sheep in the study villages was first reported in March 2011. History taken indicated that sporadic deaths of goats were observed prior to the outbreak. Later, an abrupt increase in mortality among goats of different age groups was recorded in the month of March at the onset of rains. Sheep were also affected but to a lesser extent compared to goats. All animals examined had no history of vaccination against PPR or history of previous illness due to PPR. History taking revealed that sick goats were treated with antibiotics as well as multivitamins but no significant response was observed. Sheep generally recovered with supportive treatment.

**Clinical signs**

Clinical signs observed in sick animals included high fever (41°C), depression, anorexia, purulent lacrimation, reddening of conjunctiva and matting of the eyelids. Other signs observed were purulent nasal discharges, respiratory distress and coughing, ulceration of oral mucous membrane with foul smelling. Some animals had severe nasal wounds. One goat was so severely affected that the nose sloughed off during handling (not shown). There were nodules all over the body observed in majority of the examined animals (Fig 2). Diarrhoea was noted only at the initial stage and two households reported abortions in pregnant goats. The owners of the flocks noted that kids had the highest rate of deaths compared to older animals.

**Gross lesions**

Purulent froth was present in the mouth, nostrils and trachea of dead animals with severely congested and consolidated, firm lung parenchyma (Fig 3). Lymph nodes particularly retropharyngeal lymph node and spleen were distended, congested and oedematous. Lung lesions were variable with other animals showing hyperaemic lungs with dark red and atelectasis. In all animals examined, lung lesions occurred in cranial ventral lobes. Ulcers were found on soft and hard palates, on nose, lips and face. In addition, nodules were found all over the body and those nodules were firm, freely movable with the skin, and were not painful on palpation (Fig 2). The animals had poor body condition. Orchitis and posthitis were noted in animals with ulcerated nodules on the scrotum and prepuce respectively. Gross lesions observed in goats were similar to that observed in sheep but were less severe compared to that of goats and were found mainly around the oral commissures.

**Histopathological findings**

Lungs showed increased thickness of inter-alveolar walls, infiltration of mononuclear cells and moderate number of neutrophils in the alveolar walls and bronchiolar subepithelial layer (Fig 4). Splenitis was evident with the capsule and trabeculae infiltrated with mononuclear cells. Congested splenic pulp hyperplastic reticulo-endothelial cells, macrophages, plasma cells and giant cells were noted. Sinusoids were dilated and lined by hypertrophied cells and necrosis of cells. Enlargement of lymphatic sinuses lined by hypertrophied endothelial cells were noted. The cortical nodules were replaced by broad sinusoids with a minimal lymphocyte cells. Focal necrosis of trabeculae, lymphatic nodules was replaced by thick sinusoids with a few lymphocytes. Histologically non ulcerated skin nodules showed lymphocytic inflammation accompanied with other mononuclear cells mainly found in the endodermis (Fig 5). Macrophages and few lymphocytes were also present in the dermis (Fig 5). Ulcerated nodules had thick crust covering ulcerated area which had a mixture of lymphocytes, macrophages and degenerated neutrophils. Luminal (arrow) and mural (arrow head) folliculitis dominated by lymphocytic infiltration; other mononuclear cells and degenerated neutrophils were also present (Fig 5).

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<th>Table 1: Distribution of RT-PCR results by animals sampled in Tandahimba</th>
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* All young animals examined were kids
Fig. 2: Severe ulcers on nostril, lips and face with eyelids matting and nodular skin lesions in goat

Fig. 3: Lung hyperaemia and consolidation during necropsy

Fig. 4: Acute Pneumonia - inter-alveolar walls thickened (arrow head), infiltration of inflammatory cells in bronchiolar subepithelial layer (arrow)

Fig. 5: H&E staining of skin lesion showing thick crust covering luminal (arrow) and mural (arrow head) mononuclear infiltration of inflammatory cells.

RT-PCR results
Out of 30 samples subjected to RT-PCR, 17 (56.7%) were successfully amplified confirming that the sick animals were PPR cases (Fig 6). Distribution of PCR results by age category, sex and animal species is summarized in Table 1.

Discussion
The clinical signs exhibited by the goats and sheep were similar to those reported in other studies (Ahmad et al., 2005; Das et al., 2007; Chauhan et al., 2011) except for pronounced nodular skin lesions. Data collected from the study area in Tandahimba district showed 73.1%, 37.4% and 51.2% disease morbidity, crude mortality and case fatality percentages respectively (Muse et al., 2012). Goats were more affected compared to sheep in the present outbreak a phenomenon noted elsewhere (Aruni et al., 1998; Kumar et al., 2004; Chauhan et al., 2011).

All age, sex and breed groups were affected. Pregnant animals were observed to abort during this outbreak as reported by Kulkarni et al. (1996) and Baron et al. (2011). High case fatality observed is attributed to bronchopneumonia.

In this study there was not much of enteric involvement and the symptoms mainly were focused on
respiratory system as also observed by Aruni et al. (1998). Clinical onset of the disease was seen to be sudden with pyrexia, intense discharge from the eyes and purulent exudates from nostrils. There were more deaths in young animals as noted in other studies (Abdollahpour et al., 2006; Nussieba et al., 2009b).

Although in this study, skin nodules were present all over the body, others have reported small skin nodules outside the lips around the muzzle (FAO, 1999; Baron et al., 2011). In this outbreak, the nodules covered the whole body but the udder had no lesions. In goat pox, nodules affect hairless areas including the mammary gland, mucous membrane and mild infection occur in adults (Rao and Bandyopadhyay, 2000). There were no nodular lesions in internal organs that are found in goat and sheep pox probably because the disease was noticed for the first time in naive population.

Post mortem showed consistent findings such as the frothy exudate in trachea, severe consolidation of lung parenchyma and atelectasis in the current outbreak that were suggestive of PPRV involvement as observed by others (Kumar et al., 2004; Toplu, 2004; Chauhan et al., 2009; Chauhan et al., 2011).

The epidemiological status of this disease in the study area showed no record of PPR in the past. Back tracing revealed that the source of the first outbreak was attributed to the purchase of diseased stock from livestock Market in Dar es Salaam (Muse et al., 2012). Movement of the animals predisposed them to outbreaks throughout the district especially from local market and mixing of animals during grazing.

Virus presence was confirmed in ocular, nasal and oral swabs and tissue samples. Overall 57.6% (n=30) of the samples were positive with RT-PCR analysis technique. Presence of virus and hence excreted through ocular discharge, saliva at the onset of clinical signs is the most important epidemiological aspect in spread of the disease.

Results of a monoclonal specific antibody (MAb) based competitive Enzyme Linked Immunosorbsent Assay (cELISA) revealed the presence of serum antibodies in recovered and in-contact clinically healthy animals. The data from serum samples collected from goats and sheep showed that the prevalence of PPRV antibodies was 55.5% in Tandahimba district (Muse et al., 2012).

PPR is an important disease which now threatens the million of small ruminant’s population in southern Tanzania and neighbouring countries. PPR is one of the priority animal diseases whose control is considered important for poverty alleviation (Chauhan et al., 2009). More research into understanding the disease dynamics is urgently required. PPR is given an even higher priority disease due to the point that PPRV can infect buffaloes, camels and small wildlife stocks.

**Conclusion and Recommendations**

The diagnosis of PPR was based on clinical examination, gross pathology, histological findings that were suggestive of PPRV and laboratory confirmation of the virus was done using RT-PCR for ribonucleic acid detection.

Control and prevention of PPR outbreaks depends on animal movement control combined with the use of vaccine. The main control method involves vaccination campaign that is specifically directed to young animals, 3-4 month of age (Aruni et al., 1998; Ahmad et al., 2005), proper disposal of carcass and contact fomites, decontamination and restriction on importation of sheep and goats from affected areas.

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References


Kumar, S., Vihan, V.S. and Deoghare, P.R. 2003. Economic implication of diseases in goats in India with references to implementation of a health plan calendar. Small Ruminant Research, 47: 159–164.


