Case Report

Clinical, Pathological and Molecular Investigations of Peste des Petits Ruminants Virus Infection in Goats from Turkana County in Kenya

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Abstract | Peste des petits ruminants (PPR), an economically important morbillivirus infection of sheep and goats, is widely distributed in Middle East, Central and South Asia, China and Africa. Whereas PPR antibodies were first reported in Kenyan small stock as early as 1995, the clinical disease was observed later in 2006 following dramatic and devastating PPR outbreaks in Turkana County. In this context, an outbreak of PPRV was observed in Tukana County in 2011 and the current study detail the clinical, pathological and laboratory findings of this outbreak. The disease was clinically manifested by the depression, diarrhea, difficult breathing, muco-purulent ocular-nasal discharges with matted eyelids and encrusted nostrils and finally death. Clinical outcome, gross lesions and histological observations were suggestive of PPRV infection, which were confirmed by the application of a reverse transcription–polymerase chain reaction (qRT-PCR). Collectively, results indicate the continuous persistence of PPRV in Kenya.

Editor | Muhammad Munir, The Pirbright Institute, Compton Laboratory, UK
Received | September 11, 2014; **Accepted** | September 25, 2014; **Published** | October 10, 2014
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Citation | Kihu, S.M., Gitao, G.C., Bebora, L.C., John, N.M., Wairire, G.G., Maingi, N., Wahome, R.G., Karanja, D.N., Oyugi, J.O., Lutomia, E., 2014. *British Journal of Virology,* 1(3): 98-102.

Peste des petit ruminants (PPR) is a highly infectious and often fatal disease of sheep, goats and wild small ruminants. The disease occurs in Middle East, Central and South Asia, China and Africa (Barnyard, et al., 2010; Munir, et al., 2013). Clinically PPR is characterized by the sudden onset of depression, fever, discharges from the eyes and nose, sores in the mouth, disturbed breathing and cough, foul-smelling diarrhea and death within 10 to 12 days post infection (Diallo, et al., 2007). Major pathological features are erosive, and ulcerative stomatitis; necrotic tonsillitis; fibrino-haemorrhagic enteritis; and broncho-interstitial pneumonia (Bundza, et al., 1988; Troung, et al., 2014). PPRV infection has characteristic histopathologic findings that are syncytial cells in affected oral mucosa and lungs, as well as eosinophilic nuclear and cytoplasmic inclusion bodies, especially in the respiratory and alimentary tract epithelia (Kul, et al., 2007).

PPR was first suspected in Kenya in 1992 (FAO, 2008) with further serological reports being made by Wamwayi, et al. (1995). Clinical confirmation of PPR was first reported in Turkana County of Kenya to Office International des Epizooties (OIE) in Jan-

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uary 2007 (OIE, 2014). Disease assessments carried out in mid-2007 have established that the PPR had managed to spread beyond Turkana County into the other arid and semi-arid pastoral counties in Northern and Southern parts of Kenya (FAO, 2009; Kihu, et al., 2012).

This study was carried out in Kakuma administrative division of Turkana County in a particular village herd named Lotakaa GPS position (N 03° 38 390; S 034° 50 987). The study respondents demonstrated having observed several suspect cases of PPR (Lomoo, local name of PPR) and narrated a history that showed 21 kids, 24 adult goats and one sheep had died from the suspect PPR infection two weeks prior to the study visit. Laboratory samples that included lung tissues, mesenteric and mediastinal lymph nodes; and colon were collected from carcasses of goats suspected to have died of PPR during this wave of outbreaks. Samples for histopathology analysis were preserved in 10% formalin while tissue samples for molecular analysis were transported under ice in cold boxes to University of Nairobi, Department of Veterinary Pathology, Microbiology and Parasitology Laboratory and later stored in refrigerated repository at -30°C.

Laboratory investigations involved preparation of histology slides for histological examination through the standard paraffin process and stained with Hematoxylin and Eosin stain (Luna, 1968). Tissue slides were then examined under light microscope. To perform molecular analysis on 18 frozen tissues samples, RNA extraction from each sample was performed as outlined by RNeasy® mini kit (QIAGEN® 2010). The extracted RNA samples were analyzed in a MicroAmp® Optical 96-well reaction plate by Abiprism® 7500 (Applied Biosystems) thermocycler using a specific qRT-PCR assay called TaqVet™ Peste des Petits Ruminants Virus kit which had a set of primers/probes designed for the N-gene of PPRV using a a protocol detailed in a report by Laboratoire Service International (LSI) (2011), supplier of the assay kit. The forward primer matched positions 483 > 508 in the (5-AGAGTTCAATATGTTRTTAGCCTCCAT-3); the TaqMan® probe positions 551 > 576 (FAM-5-CACCGGAYACKGCAGCTGACTCAGAA-3- MGB) and the reverse primer was located at positions 603<624 (5-TTCCCCARTCACTCTYCTTTGT-3) (Batten, et al. 2011).

To ensure quality and integrity of this research study consent was sought from Directorate of Veterinary Services who granted the permission for collection of field laboratory samples on PPR vide letter referenced "Ref.Meat/Vol.XIV/42" dated 1st July 2011. Consent was also sought from Turkana herders for voluntary presentation of their small stock for collection of samples which they granted and facilitated the exercise.

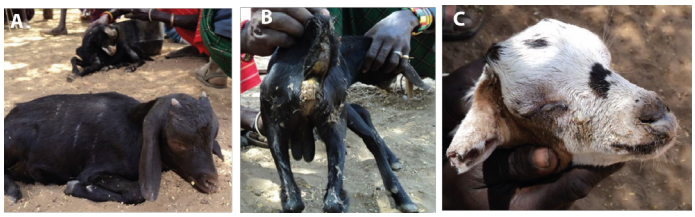

Figure 1: *A. Goat suspected to have PPR infection in a depressed state, B. Diarrhoea and soiled anal region and hind legs, C. Showing muco-purulent ocular nasal discharges, matting of eye lids and encrusted.*

Cases observed during this study were mainly from kids of four to six months old. The clinical signs presented included: depression (Figure 1A), diarrhea (Figure 1B), emaciation, difficult breathing, elevated body temperatures of 41 °C, serous and muco-purulent nasal discharges, and encrusted peri-nasal areas (Figure 1C). Serous and muco-purulent ocular discharges matting the eye lids at the inner eye canthus and the hair below the eye was also observed (Figure 1C).

Death due to this infection was observed in three kids and they were all necropsied for examination. All necropsied goats showed pneumonic lesions in the lungs. Inflammation of the apical lung lobe revealed red hepatized and congested area (Figure 2A). The large intestines showed moderate to severe hemorrhagic enteritis characterized by hemorrhagic intestinal mucosa (Figure 2B). Intestinal blood vessels were congested with blood and the mesenteric lymph nodes were enlarged and swollen (Figures 2C).

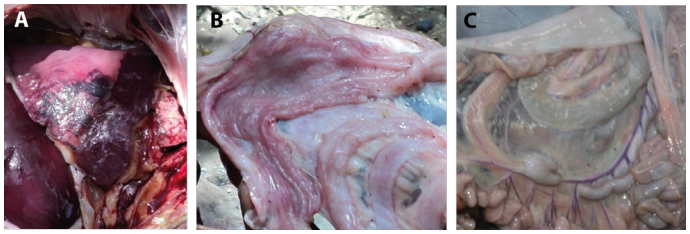

Figure 2: *A. Inflamed lung showing hepatized apical lobe in goat, B. Hyperaemic intestinal mucosa showing hemorrhagic points, C. Swollen mesenteric lymph node (arrow) and congested mesenteric veins.*

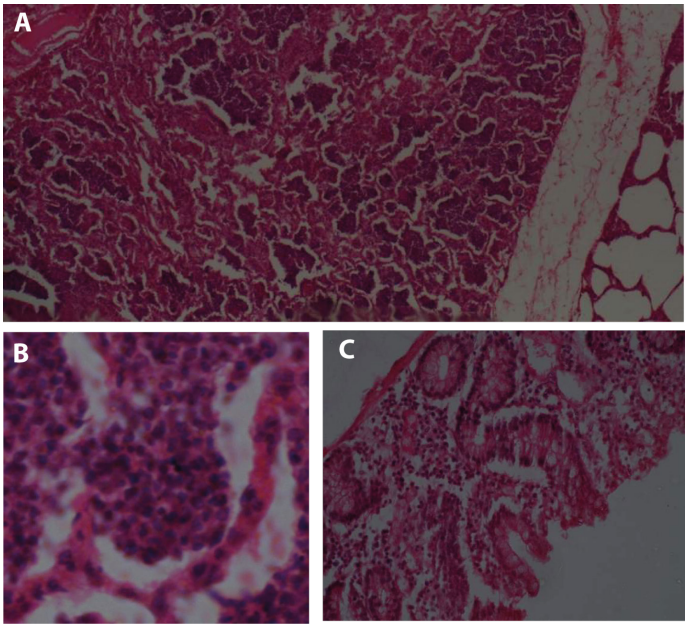

Figure 3: *A. Lung of goat showing collapsed alveoli, B. infiltration with mononuclear cell in the alveoli and multinucleated syncitia, C. Large intestine showing proliferation of goblet cells and infiltration of inflammatory cells.*

Table 1: *Results of qRT-PCR analysis for the frozen tissues.*

Goat Sampled	Sample Type	Frozen Tissues			
		Tissues Label	Inocula Label	Ct positive samples	Results
Goat 1	Mediastinal lymph node	1F		29.17	Positive
	Lung	2F		26.89	Positive
	Lung		I2	37.95	Positive
	Colon		I3	38.65	Positive
	Mesenteric lymph node	4F		27.04	Positive
	Mesenteric lymph node		I4		Negative
Goat 2	Mediastinal lymph node	5		21.99	Positive
	Mediastinal lymph node		I5	3.28	Positive
	Mesenteric lymph node	6		20.14	Positive
	Mesenteric lymph node		I6	29.29	Positive
	Lung	7F			Negative
	Lung		I7	28.37	Positive
	Colon	8F		20.46	Positive
	Colon		I8	31.28	Positive
Goat 3	Mediastinal lymph node		I9		Negative
	Lung	10F		22.60	Positive
	Lung		I10	40.56	Positive
	Mesenteric lymph node	11F		20.82	Positive

Histopathology slides of large intestines and lungs were prepared and examined. The lesions present in the lungs were characteristic of broncho-interstitial pneumonia (Figure 3A). Infiltration of the alveoli by mononuclear cells was evident in addition to multinucleated syncitia in the alveolar epithelium (Figure 3B). Lesions in the large intestines revealed proliferation of goblet cells, congestion of blood vessels, edema and infiltration of lamina propria by inflammatory cells (Figure 3C).

Molecular analyses for detection PPR virus RNA was carried out in samples listed in Table 1. The samples were in duplicates comprising inocula prepared from original frozen tissues samples and the original frozen samples. All original tissue samples tested, gave positive results for the presence of PPR virus RNA except sample labeled 7F. All the innocula samples

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tested returned positive results for presence of PPR virus RNA except inocula prepared from mediastinal lymph node from goat three (labeled I9) and mesenteric lymph node labeled I4.

In summary PPR clinical disease has been observed in Kenya since 2006 and its occurrence has been considered of major significance due to high morbidity and mortality that occasion serious socio-economic loses to the affected pastoral communities (FAO, 2012). However the clinical PPR disease outbreaks in Kenya have not been described except in unpublished administrative reports (Kihu, et al., 2012). This report presents a clinical picture of a confirmed PPR outbreak in a flock of goats in Turkana. The predominant age group affected with PPR was kids of four to six months. The description of the clinical presentation in the effected and observed kids as well as the post mortem finding and histopathological lesions were suggestive of PPR infection which was confirmed by detection of PPR virus genome in 17 tissues samples out of 18 samples presented for qRT-PCR assay.

Acknowledgements

This is to acknowledge "The Regional Universities Forum for Capacity Building in Agriculture" (RUFORUM) (RU/CFP/CGS/TADS/09/1) for financial support as well as Vetworks Eastern Africa for their logistical assistance towards this study. Acknowledgement also goes to University of Nairobi Institute of Tropical & Infectious Diseases (UNITID) for providing their molecular laboratories to carry out the molecular analysis of this study which was part of corresponding author's PhD research work.

Authors Contribution

Kihu S.M., Oyugi J.O., E. Lutomia and Gitao C.G. collected the data, created the electronic database and cleaned and processed and analyzed the molecular experiments. Kihu S.M., Karanja D.N. and Bebora L.C. conceived study analysis methods. Kihu S.M. drafted the manuscript assisted by Gitao C.G., Bebora L.C., Njenga M.J., and Wairire G.G. Gitao C.G., Bebora L.C., Njenga M. J., Wairire G.G., Maingi N. and Wahome R.G. raised funding for the study and assisted its coordination. All authors helped with the interpretation of the results and read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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