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Comparative Clinical, Histopathological and Molecular Approaches for PPR Diagnosis in Naturally Infected West African Dwarf (WAD) Goats

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Abstract

Objective: PPR is a highly contagious trans boundary viral disease of small ruminants, with high morbidity and mortality especially in young animals. Infections are easily spread by close contact in natural settings, and clinically characterized by erosive stomatitis, enteritis and pneumonia. It is endemic in many countries/regions, and poses a major limitation to small ruminant production as both morbidity and mortality are usually very quick, hence the need for accurate rapid diagnosis. Clinical diagnosis of PPR in WAD goats is, in practice, often limited to the observation of the associated clinical signs and post mortem lesions due to laboratory diagnostic constraints. This study investigated the comparative efficacy of routine clinical diagnostic methods and contemporary molecular diagnosis by PCR.

Methods: 15 WAD goats adjudged to be naturally infected with PPR were assessed for clinical signs and post-mortem lesions of the disease. Tissue samples were subjected to molecular detection of viral genome via RT_PCR.

Results: The results show that the observed clinical signs and post mortem lesions in all 15 animals were consistent with the established disease profile. However, only 66.7% of the animals had detectable viral genome in tissues.

Conclusion: Although molecular/PCR diagnosis is often considered to be highly sensitive and specific, several factors undermine its diagnostic efficiency and applicability for PPR diagnosis in WAD goats. Proper modifications and adequate attention to the limiting factors are recommended to improve the diagnostic efficiency of RT-PCR for PPR diagnosis in WAD goats to enhance prompt disease diagnosis, monitoring and control.

Keywords: PPR; West African dwarf goats; Natural infection; Molecular diagnosis; RT-PCR

Introduction

Peste Des Petits Ruminants (PPR) is a highly contagious transboundary viral disease of small ruminants caused by PPR virus, a morbilivirus of the family paramyxoviridae, with high morbidity and mortality [1–3]. The disease is clinically characterized by severe pyrexia, catarrhal ocular and nasal discharge and erosive stomatitis in early stages, which later develops into severe viral enteritis and pneumonia [4–6]. It is of high economic importance because of high mortality rates especially among young animals and restrictions on livestock trading [7,8]. It constitutes one of the major hurdles to the improvement of small-ruminant production in Nigeria and other countries where it is endemic [9–12].

PPR virus is a pleomorphic, single stranded negative sense RNA virus which is antigenically related to Bovine rinderpest, canine distemper, and human measles virus [13,14].The infection spreads readily from sick to healthy animals in close contact via aerosol and oral routes [15,16].Clinical signs of this disease are mainly pyrexia, mucopurulent nasal and ocular discharges, cough, dyspnoea, gastroenteritis, which then leads to diarrhea. At necropsy, gross lesions include congested lungs, congestion of gastrointestinal tract, especially the discontinuous streaks of congestion which is referred to as Zebra stripes or Zebra markings, edematous congested retropharyngeal and mesenteric lymph nodes and splenomegaly [17–19]. Histologically, there is an initial pulmonary congestion and edema with mild lymphocytic infiltration, hyperplasia of the bronchial and associated lymphoid tissue and goblet cell hyperplasia of the trachea. In the intestine, there is villous atrophy and matting, lymphocytic infiltration into the lamina propria and necrosis of the submucosal glands [20]. However, the disease is often complicated by secondary bacterial infections [21,22].

The disease can be diagnosed from its clinical signs, pathological lesions, and specific detection of virus antigen/antibodies/genome in the clinical samples by various serological tests and molecular assays [4]. Diagnosis of PPR remains a problem due to its rapid morbidity and mortality, bacterial co-infections, and cross reacting anti-bodies with other members of the genus Morbillivirus [10,23,24]. In Nigeria (and many endemic countries), majority of the reported diagnosis of PPR are based on clinical signs and pathological lesions [11,25–27]. Serological tests are often not used because in addition to its huge cost and inaccessibility of the requisite facilities, PPR is endemic in this area and most small ruminants would have been exposed and produced antibodies, leading to false positive results [28–34]. Since diagnosis by clinical signs and post mortem lesions is quite subjective, there is still need for a very specific and sensitive diagnostic tool for accurate diagnosis, prompt management and control of this disease. Molecular diagnosis by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) presents a contemporary possible solution. In this study, we assessed the diagnostic efficiency of the routine (clinical and postmortem) diagnosis of PPR and detection of viral genome using RT-PCR in naturally infected West African Dwarf (WAD) goats.

Materials and Methods

Experimental animals

Fifteen (15) male WAD goats less than 1-year-old were purchased from Orba market, Udenu Local Government Area, Enugu state,

Nigeria. Twelve (12) of them were apparently healthy and three (3) were showing clinical signs of PPR [Table 1]. The goats were kept together in a well aerated pen in the experimental animal house unit and acclimatized for 7 days prior to the commencement of the study. During the period of acclimatization, the animals were placed on Gentamicin sulphate (Inj 5 mg/kg IM 4/7), Ivermectin (Inj 0.04 mg/kg SC STAT), vitamin B complex (Inj 20 mg/kg IM 4/7). The animals were fed on cut fresh grasses consisting of Elephant grass (Pennisetum purpureum), Guinea grass (Panicum maximum), as is the usual practice of this eco-zone. Water and concentrates were also provided ad libitum.

Day	GT1	GT2	GT3	GT4	GT5	GT6	GT7	GT8	GT9	GT10	GT11	GT12	GT13	GT14	GT15
8							In				Oc			Di, de	
9	Fe				Fe		Fe	St, Fe	Fe	Fe, st			St, fe	D	Fe
10	St		Na, Sn	Oc, na Di			Na, Sn				Na, Oc, Fe	Na	Oc, co, na		Na, Sn
11	Na, Sn					na, Sn co, di	De, re, D	In, Di		Co	P(Oc, Na)				St, di
12		Fe	In, fe		In	In		Oc, na		sn					Oc, De
13	In, Oc Di	Oc, na	Oc	Oc, na	Oc, na				Oc, na, co, di	Na, mo		Oc, na, or, Sn			
14	We, De	Hy	P(oc	Mo, fe											
15		D	Sl	Hy									Co	P(oc, na)	Hy
16				Re, D		Mo							Di	Cor		
17	Bl		Mo, re D		Bl	Re, D			Re, Hy D				Hy	Re, di, Hy, D	D	P(oc, na)
18													We	D		We
19	Mo				Co, D			Hy					D			Co
20	P(oc, na)							D								Re
21																
22	D															D

Table 1: Sequential development of clinical signs in WAD naturally infected with PPR. Key: Fever (Fe), Starry hair coat (St), Coughing (Co), Sneezing (Sn), Nasal discharge (Na), Ocular discharge (Oc), Moist respiratory rales (Mo), Inappetence(In), Bloaty appearance(Bl), Weakness(We), Corneal opacity(Cor), Diarrhea (Di), Dehydration (De), Recumbency (Re), Hypothermia (Hy), Sleepy appearance (Sl), Oral lesion (Or), Profuse ocular and nasal discharge (P(0c,na), Death (D).

Morbidity, Clinical signs and Mortality

Physiological parameters such as pulse rates, respiratory rates and heart rates were monitored daily. Rectal temperature was also monitored during the morning hours between 6-8am daily. The animals were observed twice daily throughout the experimental period for clinical signs of PPR such as ocular discharge, nasal discharge, diarrhea, dehydration, appetite, color of mucus membranes, oral

lesions, palpable lymph nodes, body condition/weight changes, etc. Cases of mortality were also recorded.

Tissue sample collection

Duplicate samples of the lungs and a section of the intestines were collected at postmortem. One group of the samples were fixed in 10% buffered formaldehyde for histopathology, and processed following standard H&E staining procedures. The other set of postmortem samples were stored at -20ºC until processed for RT-PCR.

Sample processing for Reverse Transcriptase Polymerase Chain Reaction

1g of tissue samples collected were ground and 4ml of phosphate buffered saline added to them, to make a 20% homogenate, then centrifuged at 2000rpm for 5 minutes. RNA was then extracted from the supernatant using QIAamp Viral RNA Mini Kit following the manufacturer's protocol. RNA amplification was done as described by Couacy-Hymann et al and Elsheikh et al. [14,35]; by adding PPR RT-PCR master mix (containing RNAse-free water, DNTP, NP3 and NP4 specific primers, RNAse inhibitor, RT enzyme) to the extracted RNA in microcentrifuge tube. The mixture was centrifuged for 15 seconds at 1000rpm and 20µl of the mixture was collected into another microcentrifuge tube and set up in a thermocycler using one step protocol (Reverse transcription at 42ºC, 30 minutes; Initial denaturing at 94ºC, 2minutes; denaturing at 94ºC, 30 seconds; annealing at 55ºC, 30 seconds; extension at 72ºC, 30seconds and final extension at 72ºC for 7 minutes). Electrophoresis was done using 1.5% agarose gel and gel images taken using the gel documentation machine. Samples positive for PPR were observed to have bands matching that of the positive control sample (vaccine strain Nig 75/1).

Results

Clinical signs and morbidity

Clinical signs that are suggestive of PPR were observed from day 7 post exposure. There was varying rise in temperature at early phase of the disease, but hypothermia was observed at the terminal phase when the animals were diarrheic. There was also an initial rise in respiratory rate, which later dropped to near normal (20-30cpm) and subsequently rose again towards the end of the disease. A similar pattern was also observed for the heart rate, whereas the pulse rate showed an initial rise and subsequently decreased below normal towards the end of the disease. Other clinical signs observed include catarrhal nasal discharge, severe lachrymal and ocular discharges with conjunctival encrustation in the medial canthus and matting of the fur, corneal opacity (observed only in one goat), fever, anorexia, coughing, moist rales, ulcerative stomatitis and profuse diarrhea. Diarrhea was observed usually towards the terminal phase of the infection, and was observed to be fetid, watery, profuse, and sometimes projectile (Figure 1). Table 1 shows the sequential development of clinical signs of PPR in the experimental animals. Morbidity of the infection was 100%, and all the animals showed more than four clinical signs.

Figure 1: Some of the clinical signs of PPR observed in the experimental animals. A: bilateral nasal discharge, B: ocular discharge, C: corneal opacity with matting of the fur and D: profuse diarrhea.

Mortality of wad goats naturally infected with PPR

The mortality recorded from the time the animals were exposed to natural infection of PPR until the end of the study (day 0-23) is shown in Figure 2. No mortality was recorded in week 1, but 20% mortality was recorded in week 2. The highest mortality (66.67%) was recorded in week 3 (days 15-21), giving a total mortality of 86.7%. 100% total mortality was recorded within 22 days of exposure to the infection.

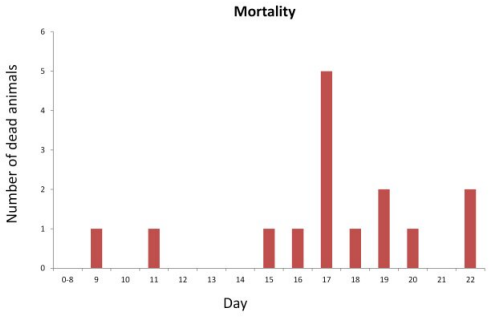

Figure 2: Mortality of West African dwarf goats naturally infected with PPR.

Gross and histopathological lesions

At necropsy, some of the gross lesions observed are shown in Figure 3. The respiratory airways of the animals contained moderate to profuse frothy exudates extending from the trachea to the bronchioles, with pulmonary congestion of the apical and cardiac lobe or entire lungs in some goats and focal consolidation of some portions of the lungs. The enlargement of the lungs was so severe in some animals that the impression of the ribs was clearly marked on the lungs (Figure 3B).

In the gastro-intestinal system, ulcerative and erosive lesions were predominantly found in the oral cavity, especially the soft palate. The rumen, reticulum, omasum and abomasum were filled with fetid watery fluid. The large intestines were severely affected with congestions, especially around the ileo-cecal valves and ceco-colic junction. On the mucosal surface, discontinuous streaks of congestion, often referred to as "Zebra stripes", were observed (Figure 3D). The mesenteric lymph nodes were enlarged, and the spleen was enlarged with congestion on its surface. There were hemorrhages on the ventral aspect of the liver.

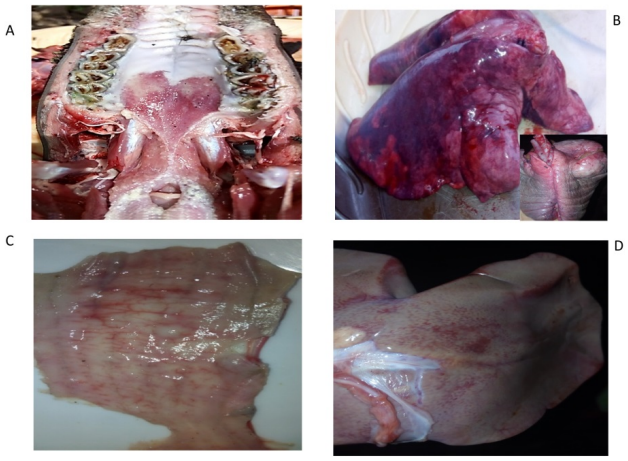

Figure 3: Gross post mortem lesions of PPR observed in the experimental animals. A: soft palate-showing ulceration of the epithelium. B: severe enlargement and generalized congestion of the lungs (inset shows rib impression due to severe enlargement). C: Colon showing linear discontinuous hemorrhages (zebra stripes). D: Liver showing hemorrhage on the ventral aspect.

Histopathological lesions observed include general infiltration of mononuclear cell (especially lymphocytes) into tissues. In the lungs, there was pulmonary congestion in interlobular spaces, infiltration of mononuclear cells into inter-alveolar and interlobular spaces and thickened interlobular septa (Figure 4), with the alveolar spaces, trachea and bronchi filled with exudates. The intestines showed ulceration of mucosal surface, focal areas of ulceration on the intestinal villi, hemorrhage on the lamina propria and infiltration of lamina propria by mononuclear cells. In the liver, there was sinusoidal hemorrhage and hemorrhage into portal tract. There was also mild thickening of the splenic capsule.

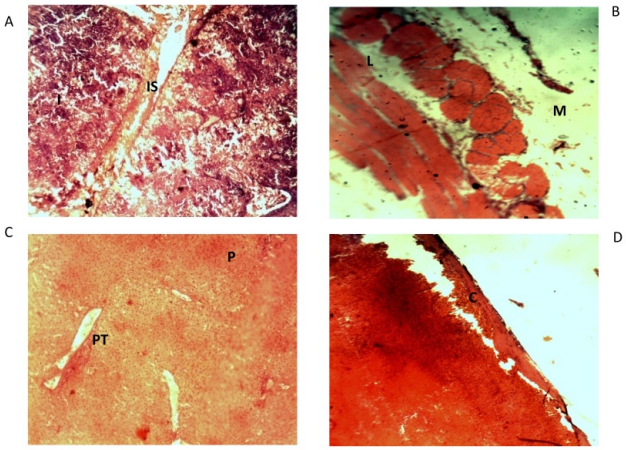

Figure 4: Histopathologic lesions of PPR observed in the experimental animals. A: lung tissue showing thickening of interlobular spaces (IS), pulmonary congestion in interlobular spaces, infiltration of mononuclear cells into inter-alveolar and interlobular spaces (I) B: Intestinal tissue showing ulceration of mucosal surface (M), focal areas of ulceration on the intestinal villi, hemorrhage on the lamina propria (L) C: Liver tissue showing hemorrhage into the portal tract (PT), mononuclear cell infiltration into the liver parenchyma (P) and sinusoidal hemorrhage D: Spleen showing thickening of the capsule(C) (x40)

Virus detection using reverse transcriptase-polymerase chain reaction

With RT-PCR, PPR virus-specific genes were detected in tissue samples of 10 out of the 15 animals used in this study (66.67%). A comparison of the results of routine clinical and postmortem diagnosis of PPR with molecular diagnosis using RT-PCR for each animal is presented in Table 2.

Animal Id	Clinical signs	Gross & Histopathologic lesions	RT-PCR
GT 1	+	+	+
GT 2	+	+	+
GT 3	+	+	+
GT 4	+	+	-
GT 5	+	+	+
GT 6	+	+	+
GT 7	+	+	+
GT 8	+	+	-
GT 9	+	+	-
GT 10	+	+	-
GT 11	+	+	-
GT 12	+	+	+
GT 13	+	+	+

GT 14	+	+	+
GT 15	+	+	+

Table 2: Comparison of the routine clinical and post mortem diagnosis of PPR with molecular diagnosis.

Discussions

Peste Des Petits Ruminants (PPR) is a highly infectious disease of small ruminants, and natural infection could be easily achieved by close contact [10], as was also observed in this study. Morbidity and mortality recorded in West African Dwarf (WAD) goats in this study was 100% within a period of 23 days (without supportive treatment). Similarly high mortality rates have been reported in other goat breeds [17,36]. PPR mortality has also been reported to be higher in goats than other small ruminants such as sheep, and especially in young animals [1,6,27,37]. The clinical signs observed in this study are consistent with literature records for different breeds of goats [5,6,20]. Each animal showed more than four clinical signs. Corneal opacity is a rare occurrence in Peste des Petit Ruminants, and was observed only in one goat with very profuse ocular discharge (GT 12). Also, both gross and histopathological lesions observed in this study agree with literature records in other breeds of goats [6,17,20,38].

Due to the subjective nature of clinical and postmortem diagnoses, there is a need for a highly sensitive and specific diagnostic technique for confirmation, especially for disease monitoring. False positive results abound with ELISA [28–30], making its use unsatisfactory. The use of molecular diagnostic tools (PCR) is increasing, hence the need to assess its diagnostic efficiency for PPR in this indigenous breed of goats and natural settings. The results of this study showed that PPR virus genes were detected (by RT-PCR) in only 10 out of 15 animals. Although all the animals showed the classical clinical signs and post mortem/histopathologic lesions often associated with PPR, PCR diagnostic sensitivity was 66.7% in this study conditions. This is an improvement from the 42% recorded by Woma et al. and 51.5% by Luka et al. [39,40]. Lower diagnostic sensitivity of PCR has also been recorded for PPR in other goat breeds [5,26,41].

The lower sensitivity of RT-PCR diagnosis in these studies could be attributed to several factors. Generally speaking, facilities for molecular diagnosis by RT-PCR are not readily available/accessible in many endemic areas like Nigeria [10], leading to challenges with sample storage and processing. Samples have to be stored for some time and transported to one of the few labs that have molecular diagnostic capacity. This could lead to sample and viral RNA degeneration during storage and transportation. An improvement in molecular diagnostic capacity of labs and sample storage facilities/conditions would certainly improve the molecular diagnostic efficiency. Similarly, storage facilities for reagents are often not reliable (especially with regards to power supply for cold storage). This may also contribute to the reduced sensitivity of the molecular diagnostic procedure. Again, it has been reported that PPR is often associated with secondary bacterial infections/complications [19,21]. In West African Dwarf goats, incriminated bacteria include Staphylococcus sp, Streptococcus sp, Neisseria sp, Pasteurella sp, Pseudomonas sp, Proteus sp and Corynebacterium sp. [22]. Therefore, it is also possible that some of the clinical presentations may arise from secondary bacterial infection that could also be spread in natural settings, giving rise to a PPR-like disease. In addition, it is also possible that animals originally infected with PPRV would mount an immune response capable of eliminating the virus from the tissues, with morbidity and mortality consequent upon secondary bacterial infection. These factors should be considered in the interpretation of the results of PPR molecular diagnosis in WAD goats, as well as in formulating strategies to achieve higher molecular diagnostic efficiency. Improved molecular diagnosis of PPR in WAD goats that would be promptly accessible at low cost is necessary for the successful implementation of PPR control measures in these endemic areas.

Conclusion

The clinical diagnosis of PPR in many endemic and technologically less advanced countries is mostly dependent on the observation of the associated clinical signs and lesions. As good and efficient as this may be, there is need for confirmatory laboratory diagnosis for prompt disease monitoring and control. The endemic nature of the disease and cross-reacting antibodies makes immunodiagnostic/serologic procedures unreliable, as most animals have circulating antibodies. Although molecular diagnosis by RT-PCR is presumed highly specific and sensitive, this study shows that conditions for molecular diagnosis by RT-PCR need to be optimized for PPR virus detection in WAD goats to achieve optimal sensitivity. This is important for proper interpretation of prevalence, surveillance and disease monitoring studies in such PPR endemic areas, in order to institute appropriate control measures where and when necessary.

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Conflict of Interest

None.

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