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Article

Management of an outbreak of peste des petits ruminants with antibiotic combined hyperimmune serum therapy

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Abstract: Peste des petits ruminants (PPR) is a highly contagious viral disease associated with high morbidity and mortality in goats of Bangladesh. Though there is no known effective drug against PPR virus, Animal Health Research Division (AHRD), Bangladesh Livestock Research Institute (BLRI) has developed a treatment technology "antibiotic combined hyperimmune serum therapy (ACHST)" against the deadly PPR virus. This article described the management of a huge outbreak of PPR by ACHST at goat farm of BLRI regional station, Naikhongchari, Bandarban, a hilly area of Bangladesh. Seventy two goats out of 159 goats were infected with PPR virus naturally. The clinical history and necropsy findings were recorded and nasal swabs were collected for diagnosis of PPR virus by RT-PCR. Thirty goats among 72 goats were died before providing treatment and the rest alive 42 goats were received ACHST. Each of the goats was treated with PPR specific hyperimmune serum @ 4-5ml/goat slow IV for once, Amcicoli-D (Ampicillin trihydrate, Colistin sulfate and Dexamethasone acetate) @ 1ml/10kg body weight daily IM once for 3 days and metronidazole @ 500mg/35kg body weight orally thrice daily for 3 days. After treatment, 38 goats (93.23%) were recovered out of 42 goats. The comparative evaluation of ACHST at different stages of naturally PPR infected goat's shown highest recovery rate in incubation (100%) and prodromal (100%) phases followed by pneumonic (91.67%) and diarrhoeal (81.25%) phases. It may be concluded that the ACHST could be used as therapeutic interventions for PPR infected goats.

Keywords: goat; PPR virus; antibiotic; hyperimmune serum

1. Introduction

Peste des petits ruminants (PPR) is a highly contagious viral disease of goats. The causal viral agent is a member of the genus Morbillivirus under the family of Paramyxoviridae (Gibbs *et al.*, 1979). The disease is now widespread in tropical and sub-tropical countries, particularly in sub-Saharan Africa, Middle East and western and southern Asia (Dhar *et al*., 2002) .The PPR virus was indentified in Bangladesh after a severe outbreak in 1993 from the border belt areas of southwestern districts and the disease is now endemic in this country. Outbreaks of PPR in a organized goat farm is generally associated with introduction of new goats from outside. The disease is characterized by mainly four symptoms like discharge (nasal, ocular, and oral), diarrhea and death, hence it is called 3D disease. The other symptoms are high fever (104^0 to 107^0 F), eroded stomatitis, pneumonia and gastrititis (Radostits *et al.*, 2000; Islam *et al.*, 2001; Kul *et al*., 2007; Rahman *et al.*, 2011). PPR

occurs mainly in three forms, peracute, acute and subclinical. Peracute and acute form of the disease are seen in 4 phases include incubation, prodromal, pneumonic and diarrhea/ death (Islam *et al.,* 2001). The affected animal standing apart with impaired appetite accompanied with poor rumination and constipation. 2-5 days after onset of fever, mucosal erosion as pin heads of necrotic epithelium on the mucous membrane lining of mouth, nasal passages and urogenital tracts. After two three days of mucosal erosion the fever regress and pneumonia accompanied with diarrhea. The peri-oral and peri-nasal areas are encrusted with mucopurlent discharges (Radostits *et al.,* 2000; Singh *et al.,* 2000). The high morbidity (100%) and mortality (50 to 90%) rates in goats caused by PPR have been described in Bangladesh (Rahman *et al.,* 2011; Islam *et al.,* 2003; Sil *et al.,* 2000, 2001). It is known that there is no specific treatment for PPR disease. Islam *et al.,* (2003) described a treatment technology called "antibiotic combined hyperimmune serum therapy (ACHST)" for the treatment of PPR disease; they got 90.63% success in incubation phase. Hyperimmune serum and supportive treatment with fluid therapy for dehydration and antibiotics to prevent secondary bacterial infection could be used to save the life of the infected goats. This paper describes the therapeutic use of antibiotic combined hyperimmune serum against clinical PPR disease in goats and also reports an investigation of a PPR outbreak including pathological observations and molecular detection of the virus (PPR).

2. Materials and Methods

The study was conducted on a natural outbreak of PPR in July 2015 at the goat farm of BLRI regional station, Naikhongchari, Bandarban, Bangladesh. Detailed history and clinical features of the outbreak were recorded daily.

2.1. Clinical sings and pathological investigation

In this study, the clinical sings and management practice (feeding, vaccination status, biosecurity pattern etc.) were recorded. Routine necropsy was performed on dead goats and gross pathological changes were recorded.

2.2. Sample collection and molecular diagnosis

Four nasal swabs were collected from infected goats with viral transport media (VTM) for molecular detection using RT-PCR technique. Collected samples were transported to the SAARC Regional Leading Diagnostic Laboratory for PPR, Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka-1341, Bangladesh maintaining cool chain.

A reverse transcription polymerase chain reaction (RT-PCR) was adopted for the detection of PPR virus. Total RNA was extracted from the nasal swabs of clinically affected goat using RNeasy mini kit (Qiagen, Germany) as per the manufacturer's instruction. The extracted RNA was evaluated both quantitatively and qualitatively using Nanodrop machine. One-step RT-PCR (Qiagen, Germany) kit was used for preparing master mix and 20µl was dispensed to each PCR tube. Then 5µl extracted RNA template was added to the respective tube and the PCR tubes were placed in the thermocycler. The thermal cycler was 35 cycles programmed. As briefly, Reverse transcription at 50ºC for 30 min, initial denaturation at 95ºC for 30 min, denaturation at 94º for 30sec, annealing at 55ºC for 30 sec, elongation at 72ºC for 30sec, final elongation at 72ºC for 10 min, held at 4^{o}C. PCR products were analyzed by electrophoresis on 1.5% agarose gel, stained with ethidium bromide and examined against UV light using an image documentation system and the images were captured. The oligonucleotide primers were selected from published literature to detect of PPR virus (Table 1).

2.3. Therapeutic intervention to affected goats

The antibiotic combined hyper immune serum therapy (ACHST) was applied on 42 clinically infected goats aged between 2 to 10 months. These selected 42 goats were divided into four groups viz. A, B, C, and D on the basis of infection phases. Group-A (Incubation phase) consisting of 6 goats, Group-B (Prodromal phase) consisting of 8 goats showed high fever, ocular and nasal discharges, Group-C (Pneumonic Phase) consisting of 12 goats showed only signs of pneumonia and group- D (Diarrhoeal phase) consisting of 16 goats with diarrhea and pneumonia. The serum against PPRV was collected from the PPR vaccinated goats of AHRD and high level of antibody was detected in serum by cELISA (ID screening® PPR competition kit, ID.vet, France). Each goat of all the four groups was treated with antibiotic combined hyperimmun PPR serum with or without addition of metronidazole and the treatment schedule is presenting in Table 2.

3. Results and Discussion

The infected goats shown the clinical signs included fever (up to 107ºF), depression, and anorexia, serous to mucopurulent nasal discharge, severe dehydration (Figure 1), erosions and ulcerations in the buccal cavity, diarrhoea (Figure 1) and respiratory distress. Frothy salivation and conjunctivitis were also found.

Table 1. Specific primers, their sequences and size of RT- PCR amplicons for the detection of PPR viruses in goat.

Primer	Target gene	Sequence (5´-3´)	Position	Product Size	Reference
NP3	N	-TCTCGGAAATCGCCTCACAGACTG-	1232-1255	**351bp**	Couacy-Hyman
NP4	gene	-CCTCCTCCTGGTCCTCCAGAATCT-	1583-1560		*et al.*, 1995

Table 2. The therapeutic prescription for PPR infected goats.

Therapy/ status	Dose and route	Group-A (n=16) Incubation	Group-B (n=12) Prodromal	Group-C (n=8) Pneumonic	Group-D (n=6) Diarrhoeal
Hyperimmune serum[1]	4-5 ml/goat slow IV at a time	+	+	+	+
Antibiotic[2]	1 ml/10 kg bwt IM 2nd dose after 24 hours	+	+	+	+
Metronidazole[3]	500mg/35 Kg bwt orally for 3 days.	-	-	-	+
Oral saline[4]	Quantity sufficient	-	-	-	+

n= No. of goat treated, [1]obtained from PPR vaccinated goats, [2]Amcicoli-D (Komi Pharm International Ltd), [3]Dirovet (The ACME Laboratories Ltd, Dhaka), [4]ORS (SMC, Dhaka).

Table 3. Efficacy of hyperimmune serum combined with antibiotics at different age group of PPR infection in goats.

Age groups (months)	Incubation		Prodomal		Pneumonic		Diarrhoeal	
	No. treated	No. survived	No. treated	No. survived	No. treated	No. survived	No. treated	No. survived
2-4	2	2	3	3	4	4	5	4
5-7	2	2	3	3	5	4	7	6
8-10	2	2	2	2	3	3	4	3
Total	6	6(100%)	8	8(100%)	12	11(91.67)	16	13(81.25%)

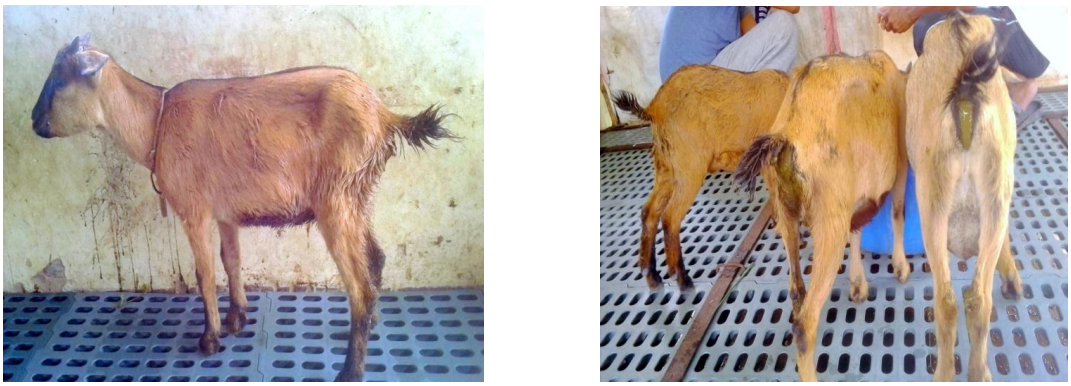
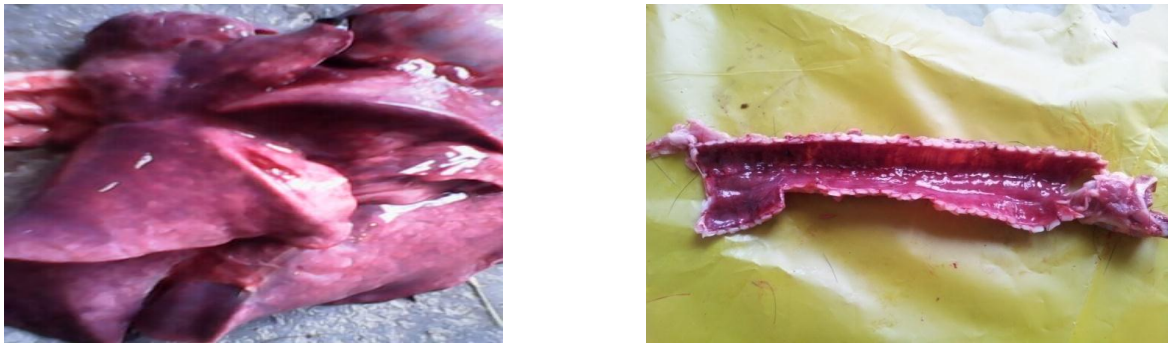


Figure 1. The PPR infected goat at Naikhongchari goat farm with severe dehydration and diarrhea.

At necropsy, stomatitis with erosions, ulcerations and necrotic lesions in the buccal cavity, erosions on the pillars of rumen, severe congestion throughout the intestinal tract, zebra stripes in caeco-colic junction, and pale and moderately enlarged liver with distended gall bladder were the main gross lesions in the digestive system.

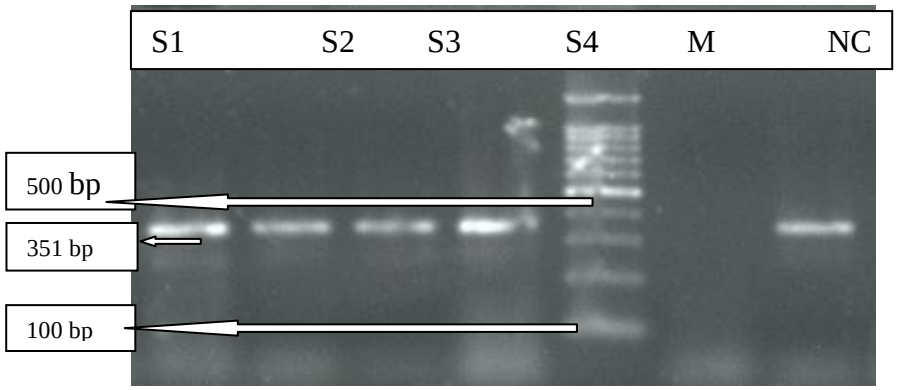
Congested and consolidated lungs and hemorrhagictreachea (Figure 2), enlarged and oedematous lymph nodes, paintbrush haemorrhages in the heart and atrophied spleen were found.



A **B**

Figure 2. Congested and consolidated lungs (A) and hemorrhagic trachea (B) of PPR infected goats.

An RT-PCR method was successfully adopted to amplify a 351 bp fragment of N gene of PPR virus. The test shown all clinical samples were positive (Figure 3) where as other authors also found the expected band from the clinical tissue samples (Rahman *et al.*, 2011).



M=Marker, PC= positive control, NC= Negative control, S1-S4=Samples.

Figure 3. Amplification of the fragment of N gene of PPRV by RT-PCR and stained with ethidium bromide.

Outbreaks of the PPR have been reported to be associated with morbidity rate was 45.28%. Before treatment the case fatality rate was 47.22% and after treatment the case fatality rate was 9.52% among affected goats. After applied ACHST (PPRV specific hyperimmune serum combined with antibiotic) in 42 PPR infected goats of BLRI Regional Station at Naikhanchari of Bangladesh. Thirty eight goats were recovered among the 42 affected goats. The comparatively higher recovery rate (93.23%) (Table 3) of PPR infected goats due to their better hygienic care and management along with the therapy, where as the previous authors found the recovery rate was 68.75% (Islam *et al.*, 2003). The PPRV specific hyperimmune serum and supportive treatment with fluid therapy for dehydration and antibiotics to prevent secondary bacterial infection could be used to save the life of the infected goats.

The recovery rate at different clinical phases of PPR infected goats like incubation, prodromal, pneumonic and diarrhoeal phase were 100%, 100%, 91.67% and 81.25%, respectively (Table 3). The present study revealed that ACHST at incubation and prodomal phases of PPR infection provides better recovery rate than other phases of infection. The findings of this study is better than the findings of (Anene *et al.*, 1987 and Islam *et al.*, 2003) who studied the appraisement of the treatment of naturally occurring PPR in goats with hyperimmune serum, oxytetracycline, chloramphenicol 25% aqueos solution and metronidazole in different groups at the recommended dose rates and found recovery rate of 14.29% and 68.75%, respectively.

4. Conclusions

It may be concluded from this study that ACHST can be used successfully to limit the spread of virus and to recover the PPR infected goats that are under incubation and in early stage of infection. Hygienic and good management practice can improve the recovery rate.

Conflict of interest

None to declare.

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