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PATHOLOGICAL INVESTIGATION OF PESTE DES PETITS RUMINANTS (PPR) IN GOATS

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ABSTRACT

In pathological investigation of peste des petits ruminants (PPR), a total of 11 Black Bengal goats of both sexes about 6-12 months old were included. Six goats out of 11 were suspected to be of natural infection with PPR and 5 goats used for experimentation. Four experimental goats out of 5 were inoculated with PPR virus isolated from natural outbreak and one was used as uninoculated control. The diagnosis of PPR virus infection both in natural outbreak and experimental cases were based on clinical signs, gross pathology, histopathology and ELISA test for confirmation of the PPR virus. Clinical signs included anorexia, depression, fever (106 ± 1^{0}F), oculonasal discharges, diarrhoea with soiled hind quarter, sunken eyes, coughing, respiratory distress and prostration and/or death in natural cases. In experimental infection the goats showed the 5 phases of PPR virus infection cycle which included varying incubation period, prodromal phase, pneumonic phase, diarrhoeic phase and prostration and/or death. Average duration of incubation period was 4 days, prodromal phase 3 days, the pneumonic phase and diarrhoeic phase started at day 5 and 7 of inoculation, respectively and continued till death. Necropsy of both natural and experimental goats revealed stomatitis, congested and/or consolidated pneumonic lungs, generalized enlargement of lymphnodes accompanied with necrosis and congestion of some lymphnodes, atrophied congested spleen and haemorrhagic gastroenteritis. Congestion of the urinary bladder, uterus and vagina in experimental goats and intestinal intussusception in dead goats of natural infection were also found. Histopathological study of both natural and experimental cases revealed congestion and edema of lungs in some cases but in other cases there were network of fibrin infiltrated with neutrophils, formation of syncytia, gaint cell and presence of pink color bacterial colony. There was infiltration of neutrophils and mononuclear cells within the alveoli, bronchioles, alveolar wall and interstitium of lungs. Lymphoid organs showed necrosis and depletion of lymphoid cell; congestion, mononuclear and neutrophilic infiltration in the lamina propria and submucosa of the abomasum, intestine, uterus and urinary bladder; loss of intestinal villi; congestion of cortical blood vessels and glomeruli of kidneys were recorded. Samples of both natural and experimental cases were confirmed as PPR by ELISA test. In this investigation, it was observed that clinical signs, gross and microscopic findings were more severe in experimental PPR infected cases than that of natural cases.

Key words: Black Bengal goats, PPR, pathology, ELISA

INTRODUCTION

Peste des petits ruminants (PPR) is a highly contagious viral disease of small ruminants, particularly in goats in Bangladesh (Islam *et al.*, 2001). The disease is caused by PPR virus (PPRV) of the genus *Moribillivirus* of the family paramyxoviridae (Paul *et al.*, 1979). The virus is efficiently spread by aerosol and probably only small amount of virus is required to infect susceptible individuals (Radostits *et al.*, 2000). The virus replicates in the upper respiratory tract and then spreads to local lymphnodes (Radostits *et al.*, 2000). Amplification of virus in local lymph nodes produces primary viremia that result in the spread of virus to other lymphoid tissues and other organs including skin, kidney and gastro-intestinal tract (Radostits *et al.*, 2000; Taylor and Ali, 1984). In these various organs, the virus replicates in endothelial cells, epithelial cells and monocytes/macrophages (Taylor and Ali, 1984). The secondary viremia is associated with the onset of prodromal phase of infection. The incubation period is 4-5 days in a sero-negative herd but may range between 6-10 days. In Bangladesh, the presence of rinderpest like disease in goats was detected by FAO expert team in 1993. Disease investigation among organized goat farm in Bangladesh showed that outbreaks were always associated with introduction of new goats to the farm. Occurrence of PPR in an epidemic form has a drastic effect on the goat population in Bangladesh (Sil *et al.*, 1995). Because of high susceptibility of Black Bengal goats to PPR virus, the morbidity rate has been estimated to be 100% accompanied by mortality rate of 50-90%. Laboratory based works on some aspects of PPR, specially its isolation, identification and vaccine development were carried out in Bangladesh (Sil *et al.*, 2000-2001, 1995). To our knowledge, experimental pathogenesis and pathology of PPR in Black Bengal goats have yet not been performed in Bangladesh. Therefore, this investigation has been carried out, to determine the pathogenesis and pathology of PPR in Bangladesh.

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MATERIALS AND METHODS

Goat

A total of 11 Black Bengal goats of both sexes about 6-12 months old were used in the present investigation. Six goats out of 11 were suspected to be of natural infection with PPR. Attempts were taken to isolate PPR virus from natural suspected outbreak. Thereafter, 5 goats were used for experimentation with suspected PPR virus from natural cases.

History and clinical signs of suspected natural PPR infection in goats

An outbreak of a disease occurred in a goat farm of Bangladesh Agricultural University (BAU) campus. Six goats (3 dead and 3 moribund) were submitted to the Department of Pathology, BAU, Mymensingh for diagnosis of the disease in June 2004. After recording the history and clinical signs, the goats were necropsied and tentatively the disease was diagnosed as PPR.

Gross pathology and collection of samples

The lesions to different organs and tissues were recorded and then a representative tissue samples were collected from each goats.

Virological samples and processing for ELISA Test

For virological examination lymphnodes, a part of lungs and spleen were collected aseptically in separate sterile plastic tube and were preserved at -20^0C until processed. Each of the virological specimens was processed separately to make a 20% suspension with phosphate buffered saline (PBS) and supernatant was collected. A part of each supernatant was used for virus detection by ELISA test and other part was preserved at -80^0C.

Enzyme Linked Immunosorbent Assay (ELISA)

The ELISA test was performed using a PPR detection ELISA kit (BDSL, Flow lab., Led. & the Institute for Animal Health, Pirbright, UK) as per instruction of manufacturer in Virology Laboratory, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh. A total of 22 samples from 11 goats were tested. The results of OD (Optical Density) values were analyzed as per instruction of manufacturer.

Histopathology

For histopathology, a part of lungs, liver, spleen, lymphnodes, abomasum, intestine, kidneys, urinary bladder and uterus were collected in 10% neutral buffered formalin and allowed to be fixed. These formalin fixed tissues were processed, sectioned and stained with hematoxylin and eosin (H & E) for histopathological study according to Luna (1968).

Infection to experimental goats

The experimental goats were divided into two groups. Four goats were inoculated intramuscularly with lung tissue supernatant @ 1ml / goat after treating the supernatant with antibiotic and antimycotic (Ismail and House, 1991) and one goat kept as uninoculated control. After developed of clinical signs, all the goats were necropsied. The gross lesions of different organs and tissues were recorded and representative samples were collected for both virus re-confirmation by ELISA and histopathology. Samples were also collected from uninoculated control goat by sacrificing it. The ELISA test and histopathology of samples of experimental goats were performed as described above.

RESULTS AND DISCUSSION

History and clinical signs

History and clinical signs recorded in present investigation in goats of suspected natural intention were anorexia, depression, fever (106 ± 1^0F), oculonasal discharges, stomatitis, diarrhoea that soiled hind quarters, dehydration, emaciation, coughing, respiratory distress and prostration and/or death. These above clinical sings were also showed by experimentally infected goats along with straining and locked anal sphincter. These clinical sings recorded in natural and experimental PPR infection were similar to that described previously (Bundza *et al.*, 1988; Mondal *et al.*, 1995; Sergany *et al.*, 1992).

Experimental PPR infection cycle showed 5 phases which included incubation period, prodromal phase, pneumonic phase, diarrhoeic phase and prostration and/or death. Average duration of incubation period was 4 days and prodromal phase 3 days. On the other hand, pneumonic phase and diarrheic phase started at day 5 and 7, respectively and continued till death which correspond to the findings of Sil *et al.* (1995).

Necropsy findings

All the goats suspected to be infected naturally with PPR virus revealed emaciated and dehydrated carcass, congested and/or consolidated pneumonic lungs (Fig. 1), generalized enlargement of lymphnodes (Fig. 2) accompanied with necrosis and haemorrhage particularly of mesenteric lymphnodes, atrophied congested spleen and hemorrhagic gastroenteritis. Suspected materials from natural outbreak cases were inoculated to goats that also revealed similar lesions described in natural cases. In addition to these lesions, urinary bladder, uterus and vagina showed necrotic foci and congestion in experimental goats. Intussusception was recorded only in dead natural infected cases but not in moribund goats. Experimentally infected goats did not reveal intussusception like natural moribund stage goats. These necropsy findings both in natural and experimental cases with rare exception were almost similar to that of previously described by Sergany *et al.* (1992), Islam *et al.* (2001), Dhand *et al.* (2003) and Pawaiya *et al.* (2004). Though the necropsy findings in both natural and experimental cases were more or less similar but the lesions were more severe in experimental cases, might be due to young age of goats, massive load of infection, route variation or poor defense mechanism of experimental goats.

Fig. 1. Lungs of PPR virus infected goat showing congestion and consolidation.

Fig. 2. Intestinal tract showing congestion in intestinal tract, generalized enlargement of lymphnodes accompanied with necrosis and haemorrhage of some lymphnodes.

Histopathology

Both naturally and experimentally PPR virus infected goats' lungs revealed edema and congestion; some cases fibrin network infiltrated with mononuclear cells, congested alveolar wall and infiltration of reactive cells within the interstitium, lumen of alveoli, bronchioles and bronchi (Fig. 3); some cases along with preceding lesions formation of syncytia, giant cells and presence of pink color bacterial colony. Formation of bacterial colony in lungs indicated secondary infection following PPR virus infection. Lymphoid organs showed necrosis and depletion of lymphoid cells. Intestine showed loss of villi, congestion and mononuclear and neutrophil infiltration in the lamina propria and submucosa. Congestion, mononuclear cells and neutrophil infiltration in the mucosa and sumucosa of abomasum, urinary bladder and uterus. Kidneys in experimental cases showed congestion of cortical blood vessels and glomeruli (Fig. 4). Microscopic changes of one or more organs and tissues described in this investigation correspond with the findings by others (Bundza *et al.*, 1988; Brown *et al.*, 1991; Sergany *et al.*, 1992; Islam *et al.*, 2001; Kumar *et al.*, 2002; Dhand *et al.*, 2003; Pawaiya *et al.*, 2004). Microscopic lesions of kidneys in PPR infection recorded by the present study were not described previously. Grossly necrosis and hemorrhages in lymphnodes and microscopically necrosis and depletion of lymphoid cells in lymphoid organs could indicate immunosuppression in PPR virus infection. Congestion to almost all organs both in natural and experimental infection and especially in glomeruli and cortical blood vessels might be due to involvement and damage of endothelial cells in the pathogenesis of PPR virus infection (Taylor and Ali, 1984).

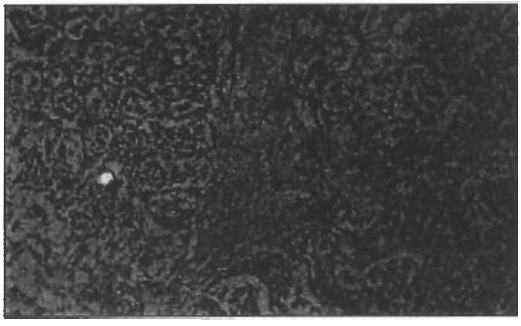

Fig. 3. Section of experimentally PPR virus infected lungs showing fibrin network associated with mononuclear cells within the interstitium, lumen of alveoli, bronchioles and bronchi (H & E, × 82.5).

Fig. 4. Section of experimentally PPR virus infected kidney showing congestion of glomeruli and cortical blood vessels (H & E, × 82.5).

Table 1. Results of ELISA in samples of natural and experimental cases of PPR virus infection

Natural cases			Experimental cases		
Name of the sample	Optical density (OD)	Remarks	Name of the sample	Optical density (OD)	Remarks
Positive (+ ve) Control	1.183	+ ve (Control)	Positive (+ ve) Control	1.183	+ve(Control)
Negative (- ve) Control	0.097	-ve (Control)	Negative (- ve) Control	0.097	-ve (Control)
Test samples			**Test samples**		
Goat-1 (lymphnode)	0.403	+ ve	Goat-1 (lungs)	0.586	+ ve
Goat-1 (lungs)	0.769	+ ve	Goat-1 (spleen)	0.752	+ ve
Goat-2 (spleen)	0.890	+ ve	Goat-2 (lungs)	0.536	+ ve
Goat-2 (lungs)	0.752	+ ve	Goat-2 (spleen)	0.616	+ ve
Goat-2 (lymphnode)	0.453	+ ve	Goat-3 (lungs)	0.953	+ ve
Goat-3 (lungs)	0.732	+ ve	Goat-3 (Spleen)	0.702	+ ve
Goat-3 (Spleen)	1.093	+ ve	Goat-3 (Spleen)	1.093	+ ve
Goat-4 (lungs)	0.973	+ ve	Goat-4 (lungs)	0.574	+ ve
Goat-5 (lungs)	0.503	+ ve	Goat-4 (lungs)	0.878	+ ve
Goat-6 (lungs)	0.694	+ ve	Goat-c (lungs)	0.087	- ve

OD value ≤0.097 is negative and >0.097 is positive, Goat-c = Control.

ELISA test findings

The OD values of samples of natural infected cases and that of experimental cases were shown in Table 1. The ELISA was used for both confirmation and re-confirmation of PPR virus infection as it was found most sensitive, reliable and appropriate method in routine diagnosis of field samples reported by Sil *et al.* (2000-2001) and Libaeu *et al.* (1994).

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