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Original Article

Sero-Epidemiological Investigation on Peste Des Petits Ruminants in Black Bengal Goats

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A natural outbreak suspecting PPR (peste des petits ruminants) was thoroughly investigated and confirmed by monoclonal antibody (mAb)-based enzyme immuno slide assay (EISA). Nasal discharge in early stage of disease course, diarrhoeic faeces and lung as a post-mortem sample was a source of sufficient virus to be detected by this technique. Convalescent polyclonal sera from the recovered animal diagnosed as PPR by EISA revealed high antibody titre by competitive-ELISA. It was found that EISA is suitable, sensitive and specific to confirm PPR infection in both field and laboratory conditions especially in developing country. In the affected houses morbidity and mortality rate was 74.13% and 54.83% respectively and observed high in the age group of 5-8 weeks, but sex difference was not significant. Early rainy season (July 2006) was the period of the present outbreak. Sero-positive animal closer to the outbreak area concluded that virus was circulating in the experimental area of Mymensingh district. Vaccinated sero-nagative animal could withstand the natural disease onset. Purchase of new animal from market and grazing in the same field with infected goats was the source of present outbreak.

Introduction

Clinically, peste des petits ruminants (PPR) is characterized by erosive stomatitis, enteritis with diarrohea and pneumonia. The outbreaks of a rinderpest like disease later confirmed by World Reference Laboratory to be PPR have been occurring in goats since 1993 in Bangladesh and found that the virus has a close relation with the Indian isolates (West Bengal) of PPRV at a cluster with Asian group[1]. The overall sero-prevalence was 36.00, 49.17 and 19.05% in sheep, goat and cattle respectively in Bangladesh[2]. Prevalence is higher in indigenous Black Bengal goats than exotic breed like Jamunapari[3]. Immunosuppression is commonly found in morbillivirus infection and this is thought to contribute susceptibility to secondary infections that accounts for most of the mortality due to PPR infection[4]. A monoclonal antibody based enzyme immuno slide assay (EISA) has been developed on glass slide for the rapid and accurate detection of PPR virus from excretion and pathological homogenized tissue sample of infected animals[5]. In this paper a suspected PPR outbreak was studied using EISA technique to further check the suitability of the method using field samples and later confirmed by Competitive ELISA**.** The epidemiological factors related to disease outbreak were thoroughly investigated to set up a possible control strategy.

Materials and Methods

Study population

The study was carried out in six houses in a village (Napterali) at Mymensingh district with a natural field outbreak suspected to be peste des petits ruminants (PPR) in goats. Apparently healthy goats (n = 38) without vaccination history were also investigated in nearby houses with a distance of 0.5 to 1 kilometres.

Clinical study

The disease courses of the infected animal were observed and recorded. Investigation of clinical signs and symptoms was concluded primarily as PPR. Among the studied population, 23 goats were found to be infected and later 17 goats died.

Test sample

Nasal and oral swabs were taken on cotton swabs at 4-6 days of the disease onset. Faecal swabs were collected after onset of diarrhoea. Necropsy examination of dead goats was performed and lung, spleen, lymph nodes and intestines were collected aseptically and kept at -80ºC. Polyclonal test sera of six convalescent goats and healthy goats were collected and stored at -20ºC until further use.

EISA technique

Reference PPR virus as antigen and monoclonal antibodies used for the study were obtained from C-ELISA kit (IAEA and BDSL,

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UK). EISA test was conducted in samples coated glass slides or in 12 wells glass plates after acetone fixation[5]. Smears were prepared using nasal, oral discharges and diarrhoeic faeces. Cotton swabbing of the infected tissue samples (lung, spleen, lymph nodes and intestines) were performed aseptically before smearing on slide or plate. The smear was air dried and fixed in ice cold acetone for 15 min, used immediately or kept at 4ºC for further use. In brief, monoclonal antibodies against PPR virus (1:100 in PBS) was added at an amount of 50 µl/smear/well, while plate or negative control was kept using 50 µl/well blocking buffer solution. For every assay the PPR antigens (reference) were kept as positive control. Slide/plates were incubated at 37ºC for an hour and wash with PBS (1:5) and air dried. After drying, 50 µl of anti-mouse IgG conjugate (1:1000 in buffer solution) was added to all wells. The slide was then incubated at 37°C for 1 h. Fifty microlitre of ortho-phenylendiamine (Sigma, UK) mixed with hydrogen peroxide (1:200) was added to each well and incubated for 15 min at room temperature. The reaction was stopped by the addition of 50 µl of sulphuric acid (6.8%) and examined by naked eyes (golden yellow colour change in positive cases) or optical densities of the samples were measured at 492 nm with a computerized ELISA reader.

Competitive-ELISA

Sera collected from the convalescent animals after field outbreaks from PPR infection (antigen detected by Mab-based EISA) were tested for the presence of PPR specific antibody to confirm results obtained by EISA using Mab-based competitive ELISA as per technique described elsewhere[6]. Serum antibody titres of apparently healthy goats before and after vaccination were also measured.

Vaccination

Vaccination of the sero-negative goats from the nearby houses was performed. Live attenuated PPR vaccine produced in Livestock Research Institute (LRI) Mohakhali, Dhaka was used for this study. The vaccine was given subcutaneously in the cervical region at the dose rate of 1 ml per animal. Vaccinated animals were observed for any natural disease outbreak.

Results

Clinical and post mortem findings of PPR suspected goats

Fever, dry muzzle, anorexia followed by serous to mucopurulent nasal discharge, severe stomatitis with necrotic lesions in the buccal cavity, diarrhoea and respiratory distress were recorded as the main clinical signs. Dyspnoea with cough, frothy salivation, conjunctivitis, depression etc. were also manifested. Abrupt rise of body temperature (103-107ºF), which is the most striking feature of PPR infection and the temperature rising was occurred from first day of infection.

Four dead goats revealed severely dehydrated and emaciated carcasses following post-mortem examinations. Haemorrhages on the mucosa of the jejunum, ileum and colon and the pathognomonic lesion 'Zebra stripe' were observed in caeco-colic junction in all goats. Spleen of goats was atrophied and lymph nodes were found enlarged. There were eroded and ulcerated mouths and the tracheas were congested along with frothy mass in the lumen of tracheas. Lungs showed congestion and consolidation. The morbidity and mortality was recorded 74% and 55% respectively in the present study.

PPRV detection from discharges and post-mortem sample

High level of PPR antigen was detected from nasal discharges ranging from 0.449 to 0.945. The average OD values of nasal, oral and faecal discharge were 0.77 ± 0.13, 0.27 ± 0.04 and 0.62 ± 0.11 respectively. Optical density values varied from 0.307 to 0.892 for different postmortem tissue sample. There was found that PPRV concentration was higher in lung (0.85 ± 0.06) compared to intestine (0.75 ± 0.04), lymph nodes (0.62 ± 0.08) and spleen (0.46 ± 0.15). The optical density (OD) values less than or equal to 0.097 were considered as negative.

Serological status of PPR antibodies in recovered goats, apparently healthy goats and determination of antibody responses to PPR vaccine

Convalescent sera of the recovered animal after a field outbreak were tested by C-ELISA against PPR antigen and found high level of PPR specific antibody (average percent of inhibition [PI] was 81.99 ± 3.82). During outbreak of PPR the sera were collected from 38 goats from the houses with a distance of 0.5 to 1.0 kilometre of the affected houses. The mean antibody titres of sera were 29.95 ± 12.14 and 56.45 ± 5.02 in case of sero-negative (PI value <50%) and sero-positive (PI value ≥50%) goats respectively. Percent of sero-positive goats was 60.53 and in case of sero-negative 39.47. After vaccination of sero-negative goats, average antibody titres were 62.6 ± 5.61 at 14th day and 73.25 ± 3.23 at 28th day post vaccination.

Discussion

A naturally occurring peste des petits ruminants (PPR) of goats was investigated. Clinical signs and postmortem features were considered for primary clinical diagnosis of PPR. In this study smear was prepared by discharges, faeces and swabbing of crude tissue sample from suspected field outbreak showed that EISA was able to detect PPR antigen from nasal and oral discharge, faecal sample and postmortem tissues. This test is rapid, economic and can be performed at field condition, keeping sample at ambient temperature for one week (data not shown). These results have later been confirmed by C-ELISA using convalescent sera against PPR antigen. The high level of PPR specific antibody of recovered animal supports the specificity of EISA as one of the major diagnostic tools for epidemiological investigation of PPR infection. Thus after the disease course the animal posses a durable immunity and remain resistant to subsequent infection[7]. The clinical signs and symptoms observed in the present outbreak were similar to those reports in case of PPR of goats from different geographical areas[3,8-11].

In the early stage of disease course, nasal discharge showed higher level of viral antigen than oral discharge and faecal sample considered as a suitable virus antigen source for diagnosis after onset of diarrhoea. Similar results were obtained using Immuno Capture ELISA (ICE)[12] and EISA[5]. Nasal discharge sampling seemed to be less traumatic and remain as a good source of antigen until mucopurulent. Smear prepared from lung samples revealed more deep golden brown colour change in EISA test. Less quantities of virus antigen was detected in spleen than intestine and lymphnodes confirming result previously obtained elsewhere[13].

The morbidity and mortality rate was 74 and 55% respectively. Age group 5-8 weeks was found highly susceptible and mortality also observed more. The results were in agreement with the results obtained by others researcher[7,14-16]. The Morbidity and mortality of male goats were more but this sex difference was not significant due to less population studied[11]. The present outbreak of PPR in Napterali village was occurred in July, 2006 (rainy season)[7]. At the time of disease outbreak, the mean of PPR sero-positive antibody titre of apparently healthy goats from nearby house was 56.45 ± 5.02. It could be concluded that virus was circulating in the experimental area of Mymensingh district[5,17]. Vaccination of the sero-negative goats from the neighbour's houses was performed with live attenuated PPR vaccine. No disease of vaccinated animals was observed subsequently. Thus vaccination could protect the susceptible animal nearer to an outbreak.

There was a history of PPR outbreak in the neighbouring village Batipara and Khasiarchar before and after the studied area respectively. It was also found from the interview of the farmers that the goats of a certain area generally grazed in one or two common grass field. Farmers are not well concerned about the pitfall of mixing sick and healthy animal for grazing. In case of village practice it is found that diseased goats and in contact goats usually sold to the market if any outbreak occurred. Before this outbreak a no of goats were also procured from local market and mixed with home stock. The purchase of new stock from market and the return of unsold animals often precipitated severe outbreaks[18,19]. So awareness program should be taken targeting smallholder goat and sheep farmers regarding large scale vaccination against PPR in a certain area, proper quarantine after purchasing new animals from village market, separation of sick animals and proper hygienic measure in the house of affected flock along with full course treatment to combat the secondary infection. In the livestock market of urban and semi-urban area saleable animal also should be checked with trained personnel before mixing with healthy stocks.

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