

Research article

Assessing adoptability of diagnostic technique and modifiable risk factors for clinical peste des petits ruminants

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ABSTRACT

Application of hospital register data in veterinary medicine, in particular in Bangladesh, is very rare. In this case – control study, S. A. Quaderi Teaching Veterinary Hospital (SAQTVH) of Chattogram Veterinary and Animal Sciences University (CVASU) register data were used to describe the clinical features and epidemiology of peste des petits ruminant virus (PPRV) infection. Initially, the clinical diagnostic protocol was validated and followed for the diagnosis of the PPR patients at the SAQTVH, CVASU by antigen detection technique. The Cohen's kappa statistics was applied to check the agreement between two diagnostic protocols. A very good agreement was found between the techniques (Cohen's kappa=0.801, CI [0.635 – 0.966]). To identify the risk indicators, binomial probability test were used where a test proportion of 0.50 and a significant level of $p < 0.05$ was used. A good disparity was observed between sex (males are more susceptible than females), age groups (< 1 year or ≥ 2 years], $p = 0.005$), flock size ([Small or Large], $p < 0.05$), grazing pattern ([Free or Confined], $p = 0.006$), breed ([Black Bengal and its cross or Jamnapari], $p = 0.042$) and history of new introduction ([Yes or no], $p = 0.005$). The epidemic curve explored three epidemic peaks between August and September. One of the peaks corresponds to the 'Manasha Puja', goat sacrificing festival of the Hindu religion. Temporal autocorrelation confirmed the annual cyclic nature of the epidemic of PPRV in Chattogram region of Bangladesh. This present study is an example of the use of Veterinary Hospital register data for the descriptive clinical and epidemiological studies for the understanding the frequency and pattern of the infectious diseases.

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1. INTRODUCTION

Peste des petits ruminant (PPR) is a highly viral contagion of goat and sheep (Amjad et al., 1996; Roeder et al., 1994). The causal agent of the disease is peste des petits ruminant virus (PPRV) that is a morbillivirus of the paramyxoviridae family (Gibbs et al., 1979). In

non – protected animals the morbidity rate is 90-100% and in severe outbreaks mortality can reach up to 100% (Radostits *et al.*, 2000; Lefevre and Diallo, 1990). The disease was first described in early 1940s in West Africa (Gargadennec and Lalanne, 1942). The disease has recently spread over a very large geographic area. The rise in the number of endemic

countries throughout the period suggests that the disease is prevalent and expanding over equatorial Africa, the Arabian Peninsula, and a portion of the Indian subcontinent (Dhar et al., 2002).

PPRV infection almost always shows significant clinical signs and findings in clinical phase of the infection on which a proper diagnosis can be made. This febrile disease is clinically characterized by high fever, pneumonia, mucopurulent ocular and nasal discharge, necrosis and ulceration of the oral mucosa, profuse diarrhea and dehydration (Amjad et al., 1996; Roeder et al., 1994; Gibbs et al., 1979). Clinical findings in PPR is unequivocal and may be sufficient for the diagnosis PPR in endemic areas and virus isolation and histopathology is essentially suggested for previously unaffected areas and areas where the disease is sporadic (Scott, 1990).

Bangladesh faced the first outbreaks of peste des petits ruminants (PPR) in the year 1993, a serious outbreak had been occurred in the border belt of the south eastern districts of Bangladesh (Dhar et al., 2002; Debnath, 1995; Sil, 2000). The outbreak which was resembled to Rinderpest was confirmed later by the reference virology laboratory of Pirbright, UK as PPR (Debnath, 1995). Since 1993, following the first outbreaks recorded in Bangladesh PPR became endemic and remains as a major disease problem in the goat population in Bangladesh. Although PPR is prevalent in Bangladesh from about two decades very little information is known about the epidemiology of this disease in Bangladesh (Chowdhury et al., 2009). Moreover, the country is facing a significant economic loss every year due to the high morbidity and case fatality rate that is not estimated yet.

S. A. Quaderi Teaching Veterinary Hospital (SAQTVH) of Chattogram Veterinary and Animal Sciences University (CVASU) is a general veterinary hospital and referral center for the Chattogram Metropolitan City (CMC) and rest of the Chattogram district in Bangladesh. Significant number of outpatients of SAQTVH, CVASU is goat of different breeds with different diseases and disorders of which PPR is one of the common diseases

(Chowdhury et al., 2008). Diagnosis of the patients at SAQTVH, CVASU is mainly done by anamnesis, signalment, clinical findings and if required by laboratory findings. Individual patients in this hospital are registered with unique patient identification number. Each clinical case is recorded in the prescribed case register with animal history, population history, clinical sign, clinical findings, laboratory findings and presumptive or confirmatory diagnosis.

The objectives of the present study were to validate the clinical diagnostic protocol and epidemiology of PPR in CMA of Bangladesh, through the study of goat patients that were registered and clinically diagnosed as PPRV infection at the SAQTVH, CVASU in Bangladesh; and to consider the necessity and determine the existing gap of information relating to the risk factors and their association with PPRV infection.

2. MATERIALS AND METHODS

Sample

The retrospective study was conducted over 50 (Fifty) weeks in the year 2019 at S. A. Quaderi Teaching Veterinary Hospital (SAQTVH), CVASU, Bangladesh, on goat patients of any kind of various age and sex that were brought to the veterinary hospital over the study period. The animals were examined clinically and any animal with clinical signs and symptoms (Pawaiya et al., 2004; Roeder and Obi, 1999) resemble to peste des petits ruminants were sampled by swabbing from oral mucosa for antigen detection (Saliki et al., 1994). Therefore, tissue sample from oral mucosa was selected as for detection of PPRV infected goats.

Antigen detection

Sil et al. (2001) described an antigen detection technique where they directed monoclonal antibody against non-overlapping antigenic domain on the haemagglutinin (H) protein of PPR virus. The method described by Sil et al., (2001) was followed to detect the PPR specific H antigen in this study.

Enzyme immuno slide assay (EISA) by Sil et al. (2001) was conducted in samples coated glass

slides (Acetone – fixed) or in glass plates (12 – wells). Monoclonal antibody (Mab) against PPR and RP virus specific (Anderson et al., 1990, Libeau et al., 1994) were as reference antibodies (1:100 in blocking buffer, PBS, Tween – 20 and negative serum) and 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 both the PPR and RP antigens (Reference) were kept as positive control. For each sample, at least duplicate slides were prepared so that each slide contained one smear against PPR Mab, one against RP Mab and rest one against blocking buffer as plate control. Slides / plates were incubated either at humid chamber or at room temperature for an hour and washed with PBS (1:5) and finally air dried using table fan. Then 50 µl of anti – mouse IgG conjugate (1:100 in buffer solution) was added for an hour. The plates were then washed three times gently with washing buffer and dried in air. Fifty µl of ortho – phenyldiamine (Sigma, UK) mixed with hydrogen peroxide (1:200) and added to each well and incubated for 15 minutes at room temperature. The reaction was stopped by the addition of 50 µl of sulfuric acid (6.8%) and examined by naked eyes or optical densities of the samples were measure at 492 nm with a computerized ELISA Reader (Immuno – skan). EISA was also performed using supernatants and infected tissue. The positive/negative cut-off OD for the test was taken as three times of OD values of conjugate/plate control. Experimental infection with Bangladeshi strain of PPR virus showed the shedding of viral Ag at the one set of clinical disease (5 – 8 days after infection and samples like discharges) became positive in the EISA.

Validation of the clinical diagnosis

The clinical disease which is acute, and after an incubation period of 3 – 6 days, the clinical symptoms include high rise of body temperature, oral and ocular discharges, necrotic stomatitis, severe pneumonia, dyspnea, coughing, enteritis, severe diarrhea, and ultimate outcome is death (Pawaiya et al., 2004; Roeder and Obi, 1999). The clinical diagnostic protocol used for diagnosing the PPR at SAQTVH, CVASU was validated with the antigen detection technique described as EISA on 100

goats in a different setup. Agreement between the two methods was evaluated using Cohen's Kappa statistics (Cohen, 1960).

Population parameters and Clinical presentations of PPR cases

This study carefully reviewed the clinical case sheets and summarized possible risk indicators and their categories. Then this study retrieved the data from the patient register to describe the variations in the frequency of the PPR cases according to the categories. Application of binomial probability test saw the variations between the categories. A test proportion of 0.50 and a significant level of $p < 0.05$ for the binomial probability test were considered. Overall frequency of the presentation of clinical signs and symptoms was described as frequency distribution with the percentages.

Definition of case and control

The sequence of steps leading to detection of case and control are depicted in Figure 1. A total number of 100 patients were sampled during the study period suspected as PPR. Suspected samples tested positive for antigen detection of PPR virus were enrolled as cases ($n = 40$). The samples tested negative were considered as controls of this study ($n = 60$). 20 cases and 20 controls (1:1) were tested randomly for the current study.

Questionnaire and Risk factors with epidemic curve

A questionnaire was used to address the various demographic and management issues that might be potential risk factors for PPRV infection. The questionnaire included question about 7 potential risk factors. All the cases and controls were examined for any difference in demographic and management attributes that included age, sex, breed, grazing pattern, live animal market and introduction of new animals in the herd. Vaccination history was excluded because of the lack of proper vaccination record and chance of recall bias. Questionnaire was filled in for each suspected patient whilst samples were collected for antigen detection. A graphical presentation was done which was related to epidemic features of clinical PPR recorded in the SAQTVH, CVASU from

January to December, 2019. This was used to show in which month the clinical recorded PPR cases were peaking in the SAQTVH, CVASU. The epidemic curve and trend line were constructed by using the Excel software (Microsoft, Redmond, WA, USA), STATA 9.2 (Stata Corp., College Station, TX, USA) that were used for calculation of autocorrelation function and performed the binominal probability test.

Statistical analysis

Evaluation of differences in exposure to demographic and management factors in case versus control was analyzed using χ^2 test of homogeneity with a significant level $p < 0.05$. All the statistical analyses were performed in SPSS 13.0 for windows (SPSS Inc., USA).

3. RESULTS

Table 1. Agreement between two diagnostic protocols.

	EISA		Total	Cohen's Kappa (CI)
	+	-		
Clinical diagnosis	+	35	40	0.747 (0.513-0.980)
	-	3	60	
	Total	38	100	

Results of the diagnosis of same goats by both clinical protocol and EISA have been shown in Table 1.

A good agreement ($\kappa=0.801$, CI [0.635 – 0.966]) was observed between clinical diagnostic protocol and EISA. From January to December 2019, a total of 100 PPR cases were presented. Among them 38% were female and 62% were male. The age distribution of the cases was: 70 % goats of 1-12 months of age group, 21.0% goats of 12 -24 months of age group and 9% of them was of more than 24 month age group. The patient characteristics and the binomial test results are presented in Table 2.

The Hosmer and Lemeshow statistics for goodness of fit of the final model was 6.6 (df = 5, $p=0.252$) which is considered to be acceptable. No interaction and confounding were observed during model building procedure.

Table 2. Univariate association between PPRV infection and the selected factors.

Risk indicators		N= 100	Test Prop.	‡P
		Observed Proportion		
Sex	Male	38 (0.38)	0.50	<0.005
	Female	62 (0.62)		
Age	1 year ≤	30 (0.30)	0.50	<0.005
	1 year ≥	70 (0.70)		
Flock size	Small	58 (0.58)	0.50	0.006
	Large	42 (0.42)		
Grazing	Free grazing	85 (0.85)	0.50	<0.005
	Confined	15 (0.15)		
Breed	Black Bengal and Cross	56 (0.56)	0.50	0.042
	Jamnapari and sheep	44 (0.44)		
New introduction	Yes	79 (0.79)	0.50	<0.005
	No	21 (0.21)		

‡ Asymptomatic significant (two tailed) based on 'z' approximation

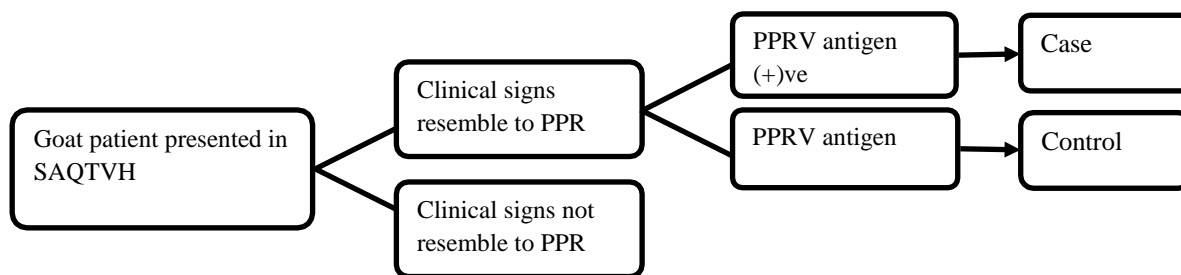


Figure 1. Scenario tree for selection of case – control in the current study.

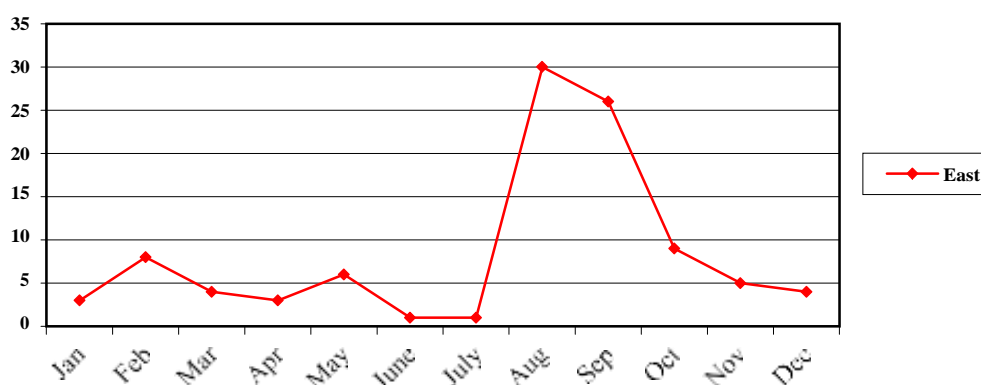


Figure 2. Temporality of the PPR cases: The epidemic curve and over all linear trend line of the recorded PPR cases at SAQTVH, CVASU

4. DISCUSSION

Diagnosis of PPR cases in most instances was based on clinical history and clinical examination at SAQTVH, CVASU. No prior validation study for the clinical diagnostic protocol was performed. Although the clinical signs sought sufficient in the diagnosis of PPR in the endemic areas (Scott, 1990) but still this study cannot ignore the chance of error due to the observer. Hence, we checked the agreement of the clinical diagnosis with EISA technique applying the kappa statistics. The kappa value obtained ($\kappa=0.747$ in this validation process shows a higher degree of agreement which supports the accuracy of the clinical diagnosis.

The risk indicator analysis using the binomial test highlights the significant differences in PPR patient frequency by sex, age group, flock size, grazing pattern, breed, and new introduction history. These elements are not always the cause of PPR infection, but they are unquestionably

candidates to be taken into account while analyzing the risk factors of PPR virus infection. The classical clinical features of the PPRV infection described earlier as high fever, pneumonia, mucopurulent ocular and nasal discharge, necrosis and ulceration of the oral mucosa, profuse diarrhea and dehydration (Gibbs et al., 1979; Radostits et al., 2000; Lefevre and Diallo, 1990). The research observation revealed the same clinical features among the PPR patients presented at SAQTVH, CVASU.

The observed temporal pattern consisting of the three distinct epidemic peaks. Epidemic peaks were observed between the month of August and September around the ‘Manasha Puja’, a festival of the Hindu Religion. This finding provides some interesting insights into the epidemic. The festival is known for sacrificing huge number of goats by the believers. To meet the demands of the increase number of the goats a huge supply comes from India by legal and

illegal trade. The goats imported from India are probably contributing to the epidemic peaks. It is reported that the PPRV is in epidemic state in India in the month of August and September (Taylor et al., 2002; Kumar et al., 2001). Besides, temporal autocorrelation function confirmed fixed annual cyclic pattern of the epidemic curve of PPRV in Chattogram region. This is another important finding from our study. Based on this finding it can formulate the mass vaccination schedule for PPRV in this region.

From the case-control study it was observed that the Black Bangle goat breed is more susceptible to PPR infection than the other exotic and cross bred goats. The higher prevalence (Jana and Ghosh, 2002) of PPR infection noticed in Black Bengal breed earlier. The susceptibility of the Black Bengal goat to PPRV infection needs to investigate in more depth.

Additionally, we found that animals raised in communal grazing were more susceptible to PPR infection than those raised in enclosed stalls or gidding. The transmission of the virus is well documented via direct contact. The direct contact between the susceptible and infected animals or by aerosol way is the common route of transmission (Jana and Ghosh, 2002; Scott, 2000). The communal grazing permits the susceptible animal to come in contact with the infected animal and increase the chance of transmissibility. Black Bengal goats are very important economic animal for Bangladesh. This research also suggests the vaccine efficacy trial for the selected areas.

5. CONCLUSION

This study is an example of the use of Veterinary Hospital register data for the descriptive clinical and epidemiological studies for the understanding the frequency and pattern of the infectious diseases in a particular region of a country. Though the Veterinarian can handle the PPR case by the clinical symptoms with proper treatment protocol, while a Veterinarian can use the results of this case – control study for further formulation of the future analytical study that can explore more details of the epidemiology of the PPRV in Bangladesh.

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