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Seroprevalence of Goatpox in Assam

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A B S T R A C T

Goatpox and Sheeppox are highly contagious, trans-boundary viral diseases of sheep and goats and are economically important causing high morbidity and mortality along with huge production loses. The disease was recorded for the first time from Assam in 2016 with high morbidity and mortality. No systematic vaccination policies are being followed so far against this disease. Therefore, the present study was undertaken to study the sero-prevalence of goatpox in goat population of Assam. Out of 220 serum samples collected from different parts of Assam, 157 (71.36%) were found positive for goatpox antibody by Indirect ELISA. The difference in prevalence rates among the various districts was statistically significant ($p<0.05$). However, the difference in prevalence rates between young and adult animals and male and female animals were statistically not significant ($p>0.05$). The present study concluded that goatpox infection is endemic in Assam indicated by high sero-positivity (71.36%).

Introduction

Goatpox and Sheeppox are highly contagious, trans-boundary viral diseases of sheep and goats, respectively, caused by goatpox virus (GTPV) and sheeppox virus (SPPV) of the genus *Capripoxvirus*, sub-family *Chordopoxvirinae* of family *Poxviridae* (Van Regenmortel *et al.,* 2000). GTPV is closely related to other members of the genus such as SPPV and lumpy skin disease virus (LSDV). Diseases caused by members of the genus *Capripoxvirus* (*Poxviridae*) are Office Internationale des Epizooties (OIE) notifiable diseases (Bhanuprakash *et al.,* 2011). Goatpox is often a great threat to goats and sheep and characterized by pyrexia, lacrymation, secondary bronchopneumonia with nasal discharges and generalised pock lesions with lymphadenopathy causing high

mortality (50-100%) and morbidity upto 100% (Bhanuprakash *et al.,* 2006; Babiuk *et al.,* 2008). The disease is not distinguishable from sheeppox serologically but possible only by molecular technique (Hosamani *et al.,* 2004). Indigenous sheep and goats exhibit some natural immunity, while the European breeds of sheep and goats are more susceptible to infection with these viruses (Heine *et al.,* 1999). Goatpox and sheeppox infections are endemic in India and regular reports of outbreak episodes are available (Bhanuprakash *et al.,* 2006, Venkatesan *et al.,* 2010, Bhanuprakash *et al.,* 2010, Verma *et al.,* 2011, Bora *et al.,* 2018).

In Assam, goat population showing pox like disease have been reported (Hopker *et al.,* 2019) and tested positive for goatpox. The mortality and morbidity rate recorded was very high, up to 60-70% and 100% respectively (Unpublished data).

The disease has been reported for the first time in Assam and as such, no systematic vaccination policies are being followed so far against this disease. In such situations, a sero-prevalence study on goatpox among the goat population of Assam may give an indication about the status of the disease and will help in formulating the control strategy to be applied against this disease.

Materials and Methods

Serum sample

Blood samples (n=220) were collected from naturally infected and in contact apparently healthy goats of different parts of Assam and serum was separated and transferred immediately to -20°C freezer for further investigation. Samples were collected throughout the year 2016-2018 to study the prevalence of the disease.

Reference virus

Goatpox virus (GTPV/Uttarkashi/P60) working seed strain in freeze dried form obtained from Pox Virus Laboratory, Indian Veterinary Research Institute, Mukteshwar Campus, Nainital, Uttarakhand was used in the present study.

Revival and bulk production of goatpox reference virus

Goatpox reference virus (GTPV/Uttarkashi/P-60) received in lyophilized form was revived in Vero cell line following the guidelines of OIE (OIE, 2010). Identity of the reference virus was checked based on characteristic CPE in Vero cells, amplification of *Capripoxvirus* specific full length *P32* gene and PCR-RFLP based on full length *P32* gene (Hosamani *et al.,* 2004). Bulk production of the reference virus was carried out in confluent vero cell culture flasks (300 cc).

Purification of goatpox virus

Goatpox virus was concentrated and purified by sucrose gradient centrifugation following standard method (Burleson *et al.,* 1992). Briefly, the harvested cell culture fluid was subjected to centrifugation at 6000 rpm for 10 minutes and supernatant was collected into a fresh container. PEG 6000 (Polyethylene glycol) was added to the collected supernatant at the rate of 8.0% (w/v) and subjected to constant mixing under magnetic stirrer at 4°C overnight. The sample was subjected to centrifugation at 6000 rpm for 30 minutes and the resulting pellet was collected. The pellet was reconstituted in 8ml of 1X TE buffer (10mM) for homogenization and again centrifuged @ 7000 rpm for 4 minutes. The sample was overlaid onto 36% sucrose followed by ultracentrifugation at 85,000 x g for 1 hour. The resultant pellet was collected and again overlaid onto 60% and 36% sucrose

gradient and subjected to ultracentrifugation @ 80,000 x g for 1 hour. The translucent layer interfacing the 60% and 36% layers containing the desired virus was collected and pelleted after diluting in 1X TAE buffer and stored at -80°C till further use.

Hyperimmune serum

Anti Goatpox hyperimmune serum, raised in rabbit obtained from Pox Virus Laboratory, Indian Veterinary Research Institute, Mukteshwar Campus, Nainital, Uttarakhand was used in the present study.

Indirect ELISA for detection of antibodies

The prevalence of GTPV specific antibody in serum samples were tested by Indirect ELISA as per the method of Bhanuprakash *et al.*, (2006) with some modifications. Briefly, the 96 wells microtitre ELISA plates (M/s Nunc, Polysorp) were coated with purified Goatpox virus with 1:1000 dilution (approx 1µg/well) in Carbonate-bicarbonate buffer (pH 9.6). The diluted antigen (50µl) was added to all the wells except antigen negative (Ag-ve) control wells, where 50µl of PBS was added.

The plates were incubated for 1 hour at 37°C and kept overnight at 4°C. After incubation, the plates were washed thrice with washing buffer, PBS-T containing 0.05% Tween-20. Test samples at 1:50 dilution, diluted in blocking buffer (PBS-T with 3% LAH and 2% Skimmed Milk Powder) was used. 50µl of diluted serum samples were added in duplicates into the sample wells and incubated at 37°C for 1 hour. (Wells A12 and B12 were kept as positive controls, C12 and D12 were kept as negative serum controls, E12 and F12 were kept as negative conjugate controls and G12 and H12 were kept as negative antigen controls). After incubation, the plates were washed thrice with washing buffer.

50µl of diluted anti-goat HRPO conjugate (1:5000 dilution in blocking buffer) was added to each well except the negative conjugate control wells (E12 and F12) where 50µl of blocking buffer was added. The plates were again incubated at 37°C for 1 hour and washed thrice. 50µl of freshly constituted substrate solution was added to each well and incubate at 37°C for 15 minutes.

After 15 minutes, colour reaction was stopped by adding 50µl of 1M H_2SO_4. Optical density (O.D) of the wells was measured at 492nm. Cut off value was based on negative serum reactivity as follows: (Mean O.D. value of test sample – Mean O.D. of negative sample) more than equal to 0.1 ($\geq$ 0.1) was considered as positive.

Results and Discussion

Goatpox is economically important disease in endemic regions like India. In Assam, we have recorded outbreaks of goatpox among the goat population of some districts (unpublished data). So, a seroprevalence study was designed to find out the overall prevalence of the disease. Clinically the disease is characterized by high fever, conjunctivitis and generalized pock lesions as well as associated with high morbidity and mortality (Bhanuprakash *et al.*, 2010; Bora *et al.*, 2018). In the present study, the reference goatpox virus (GTPV/Uttarkashi/P60) virus was revived and purified for use as a coating antigen. The twenty-four hours confluent vero cell monolayer infected with Goatpox reference virus (GTPV/Uttarkashi/P60) in 300cc flasks showed cytopathic changes. Initiation of CPE was observed on day 2 post infection (2dpi) and kept under incubation until cell degeneration was observed.

The CPE was characterized by ballooning, increased refractility, formation of syncytia and detachment of the cells from the surface

as reported earlier by many workers (Rao *et al.*, 2000; Dutta *et al.*, 2019; Bora *et al.*, 2018; Madhavan *et al.*, 2016). The propagation of the virus in the cells was further confirmed by amplification of partial P32 gene in the cell culture hervest which resulted in an expected product size of 390 bp (data not shown). Infected vero cells were harvested by three cycles of freezing and thawing and the aliquots were processed for purification of the virus. The cell culture harvest was subjected to centrifugation for clarification and final purification of virus was carried out by sucrose discontinuous gradient ultracentrifugation. A major purified virus band as a well-defined opalescent zone was seen between 60% and 36% sucrose layer. The virus pellet obtained was finally suspended in required volume of 1X TAE buffer, collected and stored at -20°C till further use for use as coating antigen. In the present study, blood samples were collected from naturally infected and in contact apparently healthy goats of Assam.

Table.1 Detection of Goatpox Viral Antibody in Serum Samples by Indirect Elisa

District	Total no. of samples	Total positive	% positivity
Kamrup	76	51	67.11
Karbi Anglong	66	50	75.75
Nagaon	17	11	64.71
Sonitpur	8	6	75
Dhubri	6	5	83.33
Morigaon	14	9	64.29
Darang	23	17	73.91
Nalbari	10	8	80
TOTAL	**220**	**157**	**71.36**

Table.2 Sex wise prevalence of goatpox viral antibodies

Sex	Number of samples Collected	Number of Positive Samples	Prevalence (%)
Male	86	58	67.44
Female	134	99	73.88

Table.3 Age wise prevalence of goatpox viral antibodies

Age	Number of samples Collected	Number of Positive Samples	Prevalence (%)
Adult	145	93	64.13
Young	75	64	85.33

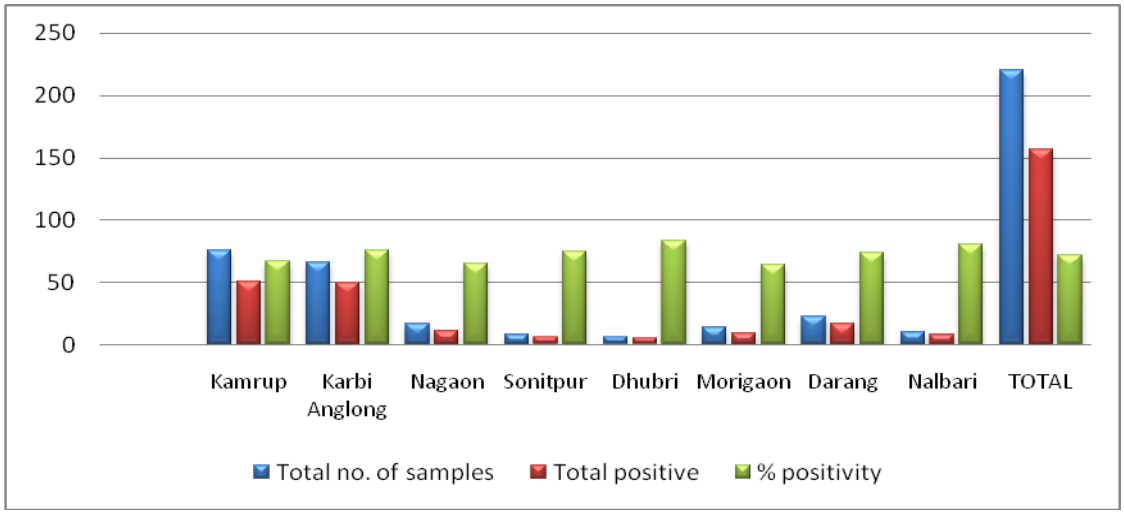

Fig.1 Graph representing place of collection, total number of positive serum samples and percent positivity of prevalence of goatpox viral antibody from different districts of Assam

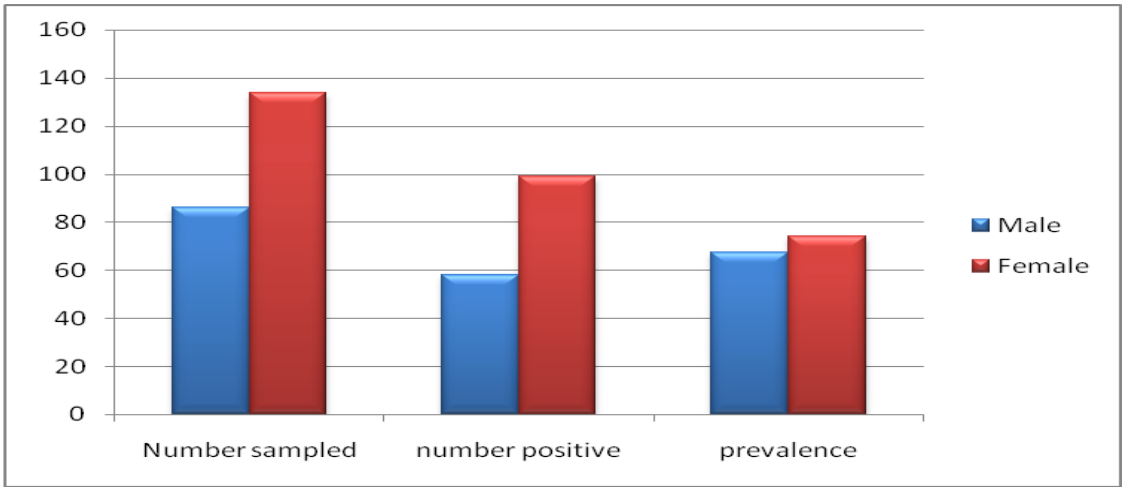

Fig.2 Graph representing sex-wise prevalence of goatpox viral antibody

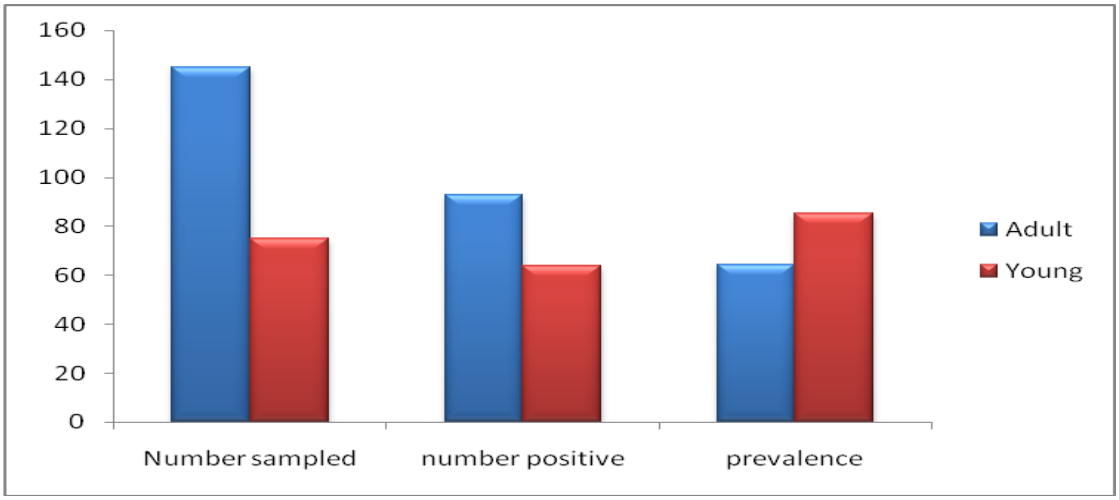

Fig.3 Graph representing age-wise prevalence of goatpox viral antibody

A total of 220 serum samples (Table 1) were collected from 8 districts of Assam, out of which 157 were found positive for Goatpox viral antibody by Indirect ELISA with a percent positivity of 71.36 % (Table 1, Fig. 1). Highest percentage of samples having positive Goatpox viral antibody was recorded from Dhubri district of Assam (5/6) with a percent prevalence of 83.33% and lowest was recorded from Morigaon district of Assam (9/14) with a percent positivity of 64.29%.

The difference in prevalence rates among the various districts was statistically significant at 5% level of significance ($p<0.05$). Age and sex wise study revealed that, goatpox was more prevalent in young animals (85.33%) as compared to adults (64.13%) (Table 2, Fig. 2) while prevalence was higher in females (73.88%) as compared to males (67.44%) (Table 3, Fig. 3). However, the difference in prevalence rates between young and adult animals and male and female animals were statistically not significant ($p>0.05$).

Indirect ELISA and immune precipitation tests were optimized to detect goatpox virus specific antibody as well as virus antigen (Sharma *et al.,* 1988, Bhanuprakash *et al.,* 2006b). In our study, indirect ELISA was applied to screen the prevalence of goatpox in the goat population of Assam. However, the study was confined only to those districts where clinical cases of goatpox were recorded, depicting a clear picture of seropositivity of goatpox antibodies among the goat population.

Goatpox was recorded in Assam for the first time in 2016 (unpublished data), and as such no systematic vaccination is followed by the farmers. The presence of GTPV specific antibody in sera indicates the occurrence of goatpox in these animals. In seroprevalance studies, applications of indirect ELISA have been well documented (Bora *et al.,* 2016, Garam *et al.,* 2016). In the present study, the overall seroprevalence of the goats against GTPV was considerably higher (71.36%), which may be due to the exposure of these animals to the virus, recovered or becoming symptomless carrier. Similar observations were also recorded by previous workers (Bora *et al.,* 2016, Gokce *et al.,* 2005). Considering the age group, the highest prevalence of GTPV-specific antibodies was found in the young age group (85.33%) in comparison to adults.

Similaly, sex wise, GTPV specific antibodies were more prevalent in females (73.88%) than males. However, the difference in prevalence rates between young and adult animals and male and female animals were statistically not significant at 5% level of significance ($p>0.05$). The present study is a preliminary one involving few districts of Assam. Collection of more number of samples from goat covering all the districts will be required to elucidate the exact epidemiological picture of goatpox in Assam.

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