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ORIGINAL RESEARCH

Seroprevalence of bluetongue in north eastern Indian state- Assam

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Abstract

Aim: The study was undertaken to assess presence of sub-clinical bluetongue in the state of Assam, one of the un-affected north-eastern state of India.

Materials and Methods: Serum samples were collected from apparently healthy as well as suspected sheep, goat and cattle from different districts of Assam encompassing various agro-climatic zones. Anti-BT antibodies were screened in sera using indirect enzyme linked immunosorbent assay (iELISA).

Results: Out of total 313 animal serum samples screened (sheep-68, goat-195, cattle-50), 58.82% of sheep, 31.79% of goat and 70.00% of cattle serum samples were found positive. The prevalence of anti-BT antibodies in different agro climatic zones ranged between 31-50%.

Conclusion: This study revealed high seroprevalence of bluetongue in cattle, sheep and goats in Assam. Incidence of bluetongue in animals of Assam being not reported so far; the present seroprevalence status of bluetongue in Assam indicates presence of sub-clinical infection in the state for the first time.

Keywords: Antibodies, Assam, bluetongue, iELISA, seroprevalence, virus

Introduction

Bluetongue is an economically important disease affecting susceptible ruminants in the semi-tropical and temperate regions of the world. Bluetongue virus (BTV), the type species of the *Orbivirus* genus and *Reoviridae* family, is the causative agent of bluetongue [1]. BTV can infect ruminants, camelids, and occasionally large carnivores [2]. The virus is transmitted by biting midges (*Culicoides* spp.) in which it also replicates. It can sometimes also be transmitted either via an oral route, or vertically in sheep and cattle [3,4]. BTV is only enzootic in areas where continuous series of virus infection cycles in vector and vertebrate host are maintained. In ruminants, it may cause severe systemic disorders with moderate to high mortality. The infected bovines exhibit prolonged viraemia compared to sheep and may act as reservoir host for BT virus [5]. BTV infection of ruminants is often sub-clinical, but outbreaks of severe disease occur with regular frequency especially at the upper and lower limits of the virus' global range where infection is highly seasonal–occurring in the late summer and autumn [6].

Twenty four distinct BTV serotypes have been recognized for decades, any of which is thought to be capable of causing BT in ruminants. However, two further BTV serotypes, BTV-25 (Toggenburg orbivirus, from Switzerland) and BTV-26 (from Kuwait) have recently been identified in goats and sheep, respectively [7]. Since 1998, multiple BTV types have emerged within Europe, events that have been linked to international trade and climate change in the region, raising concerns about possible future threats posed by bluetongue and other related orbiviral diseases [8]. BTV has now been identified on all continents except Antarctica [9]. The first confirmed outbreak was reported in island of Cyprus [10]. In India, the first report of the disease was from Maharashtra in 1963 that cause a heavy loss in sheep [11]. Since then Southern and Western states of India experienced several incidences and/ or outbreaks of BT from time to time. Out of 26 serotypes distributed globally, 21 serotypes have been reported from various parts of the country [12]. Major part of India being tropical with high to moderate rainfall, the huge population of ruminants is susceptible to BT infection.

However, eastern and north-eastern part of India are considered un-affected region in terms of active disease prevalence as no such disease incidence/ outbreaks are reported so far in any Government documents or literature [13,14]. Absence of any bluetongue disease incidence/outbreak in entire eastern and north-eastern India was documented in the Progress Report (2002-2004) of All India Network Programme on Bluetongue Disease [15].

Della-Porta and co-workers [16] reported BT infection in sheep, goat and cattle by serological methods. With the rapid development in serological techniques like AGPT, CIE, SNT and more recently

Table-1. Seroprevalence of bluetongue in different animal species in Assam

Species	Male	Female	Total number of samples assessed	No. positive	Positivity (%)
Sheep	5	63	68	40	58.82
Goat	47	148	195	62	31.79
Cattle	5	45	50	35	70.00
Total	57	256	313	137	43.77

Table-2. Seroprevalence of bluetongue in animals of different Agro-climatic zones of Assam

Agro-climatic zones	Districts	Total number of samples assessed	No. positive	Positivity (%)
Upper Brahmaputra Valley	Golaghat, parts of Karbi Anglong	29	14	48.24
Central Brahmaputra Valley	Morigaon	62	27	43.55
Lower Brahmaputra Valley	Nalbari, Bongaigaon, Goalpara, Kamrup Metro, Kamrup Rural	142	71	50.00
Hills	Karbi Anglong, parts of Kamrup Metro	80	25	31.25
Total		313	137	43.77

various forms of ELISA, serological diagnosis of BTV infection in both small and large ruminants has become easy [17]. Among different serological tests performed for detection of anti-BT antibodies, iELISA is one of the specific and sensitive tests [18]. Almost from all parts of Indian subcontinent seroprevalance of BT was reported except from the North-Eastern states including Assam [19]. To know the presence of sub-clinical infection, if any, prevalence of anti-BT antibodies in sheep, goat and cattle of different districts of Assam was ascertained in the present study using serum samples of randomly collected suspected as well as apparently healthy animals.

Materials and Methods

Sera: Total 313 numbers of serum samples were collected randomly from apparently healthy as well as suspected sheep (68), goat (195) and cattle (50) having high-rise of temperature (above 105ºF), inflammation of buccal mucous membrane, gums, lips and tongue etc. from different districts of Assam encompassing various agro-climatic zones. Samples were collected from adult animals of both sexes, viz. more than one year in case of cattle and more than 4 months in case of sheep and goat. Collected sera were stored at -20ºC till further used.

Indirect enzyme linked immunosorbent assay (iELISA): For detection of anti-bluetongue antibodies in test serum samples, iELISA was performed using the test protocol as per De and coworkers [20] with slight modification in blocking time i.e. for overnight at 4^0C. The BTV antigen was supplied from the collaborating centre of All India Network Programme on Bluetongue (AINP-BT) at Mukteswar. Briefly, 50µl of diluted BT viral antigen (rVP7) was added to coat each well of the plate. After blocking the uncoated portions of the wells with blocking buffer, 50µl of diluted known positive, known negative and test serum were put into the wells and kept in room temperature for 1 hour. After washing the plate three times, 50µl of diluted conjugate was added to all wells except the conjugate control and kept for 1 hour at room temperature. Then, 50µl of chromogen-substrate (ortho-phenylene diamine, OPD) solution was added to all wells. It was kept for 10min in dark till the colour develops and then 50µl of stop reagent (1M H_2SO_4) was given to all wells. Finally, reading was taken in an ELISA plate reader (ECIL) at 492 nm. The average optical density (O.D.) values of negative control is calculated and compared with the test O.D. values. The O.D. values of tests that were higher than the average O.D. values of the negative control were considered as positive for anti-BT antibodies.

Meteorological information: The overall climate of the study area is temperate. The average temperature of the area remains between 35-38^{0}C in summer and 5-10^{0}C in winter. Average rainfall is 600-1700 mm per anum and relative humidity lies between 65-85%. [21].

Results and Discussion

In this study, out of total 313 serum samples (sheep-68, goat-195, cattle-50) screened, 40(58.82%) of sheep, 62 (31.79%) of goat and 35 (70.00%) of cattle serum samples were found positive. The results are presented in the Table-1 and Table-2. Presence of anti-bluetongue antibody was found highest in cattle population followed by sheep and goat. The prevalence in different agro-climatic zones ranged between 31-50% (Table-2). It was highest (50%) in Lower Brahmaputra Valley and the lowest (31.25%) in Hills.

The seroprevalence of BT in sheep, goat and cattle in different states of India showed wide variation, as they represented different agro-climatic zones, covering the sub-temperate south, semi-arid north and north-west, humid and sub-humid east and sub-temperate Himalayan region [22-27] and different methods of investigation [17,28]. However, in our study we found a significant difference between the species. Cattle population has high seropositivity followed by sheep and goat. This data indicates that the cattle and sheep population are highly vulnerable in comparison to goats. This also possibly implies that the cattle population acts as major carrier of virus and thus plays an important role in its dissemination. Cattle are considered to be the reservoir hosts of BTV because the viraemia is prolonged and the majority of infections are subclinical [29]. Oberoi and others [30] demonstrated

the BTV antibodies in 70% of cattle sera in Punjab state which were having a resemblance with our findings from cattle samples. In case of sheep, Panda and other workers [31] found 79 (57.66%) samples positive out of 137 sheep serum while performing iELISA in West Bengal. In our study, the seropositivity of goats was low than that of sheep and cattle. Such low seropositivity of goat (31.79%) than sheep (58.82%) was in accordance with the findings of Chakrabarti and coworkers [32] where they detected 25 positive goat serum samples out of 104 (24.03%) and 111 positive sheep serum samples out of 322 (34.47%) from West Bengal.

Conclusion

Incidence of bluetongue in sheep, goat and cattle of Assam being not reported so far; the present seroprevalence status of bluetongue in Assam is the first record of its kind. This study reflected high seroprevalence of bluetongue infection in cattle, sheep and goats in Assam. The results indicated that further studies are needed to identify the vector from different agro-climatic zones of Assam and to determine the BTV serotypes that are and have been circulating in Assam.

Author's contribution

SNJ, BB, AH and DS implemented the study design and carried out the experiment. SNJ and BB analysed the data. SNJ, BB and CL drafted and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

Authors declare that they have no competing interests.

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