

Short Communication



Seroprevalence of Bluetongue in Free-range Mithuns (*Bos frontalis*)

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Abstract | The present study was conducted to assess the seroprevalence of bluetongue (BT) in mithun (*Bos frontalis*). Serum samples were collected from apparently healthy mithuns from their original breeding tract of Nagaland with free range management. Anti-BT antibodies were detected in sera using indirect enzyme linked immunosorbent assay (i-ELISA). Out of total 50 serum samples screened, 19 serum samples (38%) were found positive for the presence of anti-BT antibodies. Incidence of bluetongue in animals of Nagaland being not reported so far, the present paper reports seroprevalence of bluetongue in this bovine species indicating bluetongue virus (BTV) infection in those animals. The study indicates presence of bluetongue virus in Nagaland that warrants further studies on potent vectors and BTV serotypes circulating in the state.

Keywords | Antibodies, Bluetongue, i-ELISA, Mithun, Nagaland, Seroprevalence

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Bluetongue (BT) is an infectious, non-contagious, insect-borne viral disease of ruminants and has been reported from most of the tropical and subtropical regions of the world. Bluetongue virus (BTV), the type species of the *Orbivirus* genus and *Reoviridae* family, is the causative agent of bluetongue (Attoui et al., 2009). 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 (Maan et al., 2011).

BTV has now been identified on all continents except Antarctica (Maclachlan, 2010). In India, the first report of the disease was from Maharashtra in 1963 that cause a heavy loss in sheep (Sapre, 1964). Since then Southern and Western states of India experienced several incidences and/ or outbreaks of BT from time to time. Major part of India being tropical with high to moderate rainfall, the huge population of ruminants is susceptible to BT infec-

tion. However, eastern and north-eastern part of India are considered un-affected region in terms of active disease prevalence (Prasad and Srivastava, 1995; Joardar et al., 2009; Tigga et al., 2015). The mithun (*Bos frontalis*) is a semi-wild bovine species found in the north eastern hilly region of India including Nagaland (latitude 25°10'N to 27°4'N and longitude 93°15'E to 95°15'E), Myanmar, Bhutan, China, Malaysia, Bangladesh. The mithun prefers a moderate climate, dense forest and steep slopes which are the general geographical features of north-eastern region of India.

In the present study, prevalence of anti-BT antibodies was assessed in one of the susceptible hosts of BT, mithuns of Nagaland, to know the presence of sub-clinical infection in this bovine species, if any.

In the present study, a total of 50 serum samples were collected randomly from apparently healthy mithuns from their native tract in Nagaland that maintains free range system of management. Samples were collected from var-

ious villages falling under three districts – Kohima, Phek and Tuensung having abundant tropical rain forests. Samples were collected from both calf (< 2yr old) and adult (> 3yr old) mithuns of both sexes separately. Collected sera were stored at -20°C till further used.

For detection of anti-BT antibodies in serum samples, i-ELISA was performed using BTV antigen supplied from the collaborating centre of All India Network Programme on Bluetongue (AINP-BT) at IVRI, Mukteswar following the standard protocol (De et al., 2008). The reading was taken in an ELISA plate reader (ECIL, India) at 492 nm and average optical density (O.D.) values of negative control was calculated and compared with that of test samples.

Chi-square test at two degree of freedom (5%) for detection of significant difference between positive serum samples of male and female animals and that of between age group > 3yr with <2yr old was performed in SPSS version 21 (SPSS Inc., Chicago, USA).

In this study, out of total 50 animal serum samples screened, 19 serum samples (38.00%) of mithun were found positive for anti-BT antibodies (Table 1).

Table 1: Assessment of anti-bluetongue antibodies in serum samples of mithun by i-ELISA

Type of animal	Number of samples tested	Positive samples	Positivity (%)
Male	22	8	36.36*
Female	28	11	39.28*
< 2yr old	24	9	37.50*
> 3yr old	26	10	38.46*
Total	50	19	38.00

*No significant difference at two degree of freedom (5%)

The seroprevalence of BT in ruminants in different states of India has shown 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 (Dubey et al., 1987; Chandel, 1996; Sreenivasulu and Subba Rao, 1999; Hinsu et al., 2000; Govindarajan et al., 2002; Singh et al., 2009; Joardar et al., 2012) and different methods of investigation (Shringi and Shringi, 2005; Prasad et al., 1992). In our study, we found an overall 38% seropositivity in this domesticated yet maintained in the wild bovine species (mithun). However, earlier report showed an overall much higher seropositivity (86%) in mithun (Rajkhowa et al., 2008). Moreover in the present study, % positivity found in male and female animals were 36.36% and 39.26%, respectively. Again younger animals of age <2-3yr had percent positivity of 37.50, but adult animals of age > 3yr had 38.46% seropositivity. The difference in % positivity

between males and females or due to age variation was not significant at two degree of freedom (5%). Similar higher % positivity was observed in male (89%) and female (85%) animals as also younger (<2-3yr old) (58%) and adult (> 3yr old) mithuns (86%). This might be due to difference in tests (i-ELISA vs. cELISA) employed in these studies and areas covered for sample collection. Sex of the animals was considered in the study with the idea that virus has potential to cause abortion and BTV exposed female animals might have more seropositivity. However, as the animals were from free ranged management, authors failed to get any data on history of abortion. There was no significant difference in seropositivity in younger and adult animals. Seroconversion in form of considerable high seropositivity in younger animals might be due to vertical transmission that are apparent in bluetongue. However, horizontal transmission also cannot be ruled out. Again, as the animals were in free range system, monitoring of the animals were not possible that hindered of getting clear picture of the BT infection in those animals. High and considerable low anti-BT antibodies were found in bovines of different regions. Oberoi and co-workers (1988) demonstrated anti-BTV antibodies in 70% cattle sera in Punjab and Joardar et al. (2013) in Assam state. However, considerable low seropositivity (2.69%) was reported in dairy Holstein cattle of central Iran by Noaman et al. (2013). Bovines are considered to be the reservoir hosts of BTV because the viraemia is prolonged and the majority of infections are sub-clinical (Maclachlan et al., 1992). These animals play an important role for dissemination of BT through potent vector-*Culicoides* spp. (Halder et al., 2013). The present study confirms the need of studying the vector biology of the area for better understanding the BT epidemiology of the state. It may be mentioned here that, mithun being wild and ferocious in free range form; practically it is quite difficult to get blood samples in large quantity. As such a small sample size may be considered useful for getting an indication of the alarming situation of BTV exposure in this bovine species that warrants future study encompassing the whole agro-climatic zone.

In conclusion, this study reflected the current status of seroprevalence of bluetongue infection in mithun of Nagaland although active disease outbreak/incidence has not been reported so far in ruminants of the state. The results indicated that further studies are needed to identify the competent vector(s) from Nagaland and to determine the BTV serotypes that are circulating in the state.

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CONFLICT OF INTERESTS

Authors declare that they do not have any conflict of interest.

AUTHORS CONTRIBUTION

Siddhartha Narayan Joardar and Sabyasachi Mukherjee designed the work. Arkendu Halder, Sabyasachi Mukherjee and Aupama Mukherjee performed the work. Siddhartha Narayan Joardar and Chandan Lodh analysed the data. Siddhartha Narayan Joardar, Arkendu Halder and Sabyasachi Mukherjee prepared the manuscript. All the authors approved the manuscript.

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