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Short Communication

MAIDEN REPORT ON SEROPREVALENCE OF BLUETONGUE IN RUMINANTS OF SIKKIM

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The study was undertaken to assess seroprevalence of bluetongue (BT) infection, if any, in the state of Sikkim, a north-eastern state of India. BT has not been reported in ruminants of Sikkim. Serum samples were collected from apparently healthy goats and cattle from different places of Sikkim. Anti-BT antibodies were assessed in serum samples using indirect enzyme linked immunosorbent assay (iELISA). Out of total 151 samples (cattle-134 and goat-17) tested, 8 cattle (5.97%) and 5 goats (29.41%) were found to possess anti-BT antibodies. This study revealed seroprevalence of bluetongue in goats and cattle of Sikkim for the first time, indicating presence of potent vector midges and circulating bluetongue virus (BTV) in the state.

Key words: Antibodies, Bluetongue, iELISA, Seroprevalence, Sikkim, Virus

Bluetongue (BT) is an arthropod-transmitted non-contagious viral disease of domestic and wild ruminants, caused by Bluetongue virus (BTV) of the genus *Orbivirus*, and family *Reoviridae* (Attoui *et al*., 2009). Although the principal hosts of BT are sheep and some wild ruminants, goats, cattle and some other ruminants may also carry the pathogen without clinical manifestation (MacLachlan and Dubovi, 2011). In India, BT was first reported from Maharashtra with considerable mortality (Sapre, 1964). Since then southern and western states of India experienced several incidences and/ or outbreaks of BT and the three southern states, especially Andhra Pradesh, Tamil Nadu and Karnataka are considered endemic for BT incidences (Joardar *et al*., 2009). Out of 27 serotypes distributed globally, 22 have been reported

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from various parts of the country and 13 different serotypes (BTV1-4, 6, 9, 10, 12, 16-18, 21, 23) have been isolated so far (Chand *et al*., 2014). India being tropical with high to moderate rainfall, ruminants of major parts of India is susceptible to BT infection (Prasad and Srivastava, 1995). However, eastern and north-eastern parts of India are considered to be an un-affected region in terms of active disease prevalence (Rajkhowa *et al*., 2008 and Joardar *et al*., 2009). Among different serological tests performed for detection of anti-BT antibodies, indirect enzyme linked immunosorbent assay (iELISA) is one of the specific and sensitive tests (Hubschle *et al*., 1981). Almost from all parts of Indian subcontinent seroprevalence of BT was reported except some of the north-eastern states including Sikkim (Joardar *et al*., 2012 and Halder *et al*., 2016). In this context, it is expedient to undergo an investigation on prevalence of bluetongue disease in ruminants of Sikkim.

Prevalence of anti-BT antibodies in cattle and goats of Sikkim was assessed in the present study using the serum samples that were collected randomly from apparently healthy cattle (n=134) and goats (n= 17) from different districts of Sikkim (Table 1). Samples were collected from adult animals (more than one year in case of cattle and more than 3 months in case of goat) of both sexes. Collected sera were stored at -20ºC till further use.

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

After screening the samples by iELISA, 5 samples (29.41%) were found positive for goats and 8 (5.97%) were positive for cattle (Table 1). In the present study, overall

Table 1. Assessment of anti-bluetongue antibodies in serum samples of Sikkim by iELISA

Species	Number of samples tested	Name of district	Number of positive samples
Goat	**17**	Eastern Sikkim	**5 (29.41%)**
Cattle	54	Southern Sikkim	5 (9.26%)
	40	Northern Sikkim	2 (5.00%)
	40	Eastern Sikkim	1 (2.50%)
Total (Cattle)	**134**		**8 (5.97%)**
Grand total (Goat + Cattle)	**151**		**13 (8.60%)**

8.60% seroprevalence of BTV group specific antibodies were detected in goats and cattle. Such low seroprevalence might be due to several factors, viz. low population of competent vector, non-conducive environment for vector propagation, low susceptible animal population etc. These probable factors are to be examined in Sikkim in due course of time. The present overall seroprevalence value corroborates with the results of Arun *et al*. (2014) who reported seroprevalence (9.3%) in ruminants of northern Kerala, although they used competitive ELISA to assess anti-BT antibodies in ruminants. Earlier, several workers reported high seroprevalence in ruminants of different states of India. They used either cELISA or iELISA while screening their samples. Both forms of ELISA are suitable for serrmonitoring of BT, however, cELISA possesses more potential that use monoclonal antibody (Afsher *et al*., 1987). Sreenivasulu and Subba Rao (1999); De *et al*. (2008); Tigga *et al*. (2015) and Joardar *et al*. (2016) reported 42.31% seroprevalence in Andhra Pradesh, 47% in sunderban area of West Bengal and 45.83% in Jharkhand and 43.07% in Tripura, respectively. Chauhan *et al*. (2004) and Panda *et al*. (2011) reported still high i.e. 50.85% and 60.26% seroprevalence of anti-bluetongue antibody among ruminants using cELISA and iELISA in Gujarat and West Bengal, respectively. The higher seroprevalence indicates presence of potent vectors and circulating virus in the ruminant population of the region (Tigga *et al*., 2015).

In case of goat, the seroprevalence observed was 29.41% in the present study. This value is considered as moderate in case of BT infection. This finding is corroborated with the findings of Joardar *et al*. (2013 and 2014) where seropositivity of goat was found to be 31.25% and 31.79% in two eastern and north-eastern states viz. Orissa and Assam, respectively. However, Tigga *et al*. (2015) recorded higher sero-positivity (43.33%) in Jharkhand. Variation in seroprevalence in goat might be due chance of exposure of BTV through competent vector and other factors like season of sample collection, suitable agro-climatic environment etc. However, low sample size being weakness of the present study warrants its further validation with large goat serum samples in future occasion. In case of cattle, the percent seropositivity detected in the present study is low (5.97%) that is corroborated with the findings of Arun *et al*. (2014) where they reported 6.9% sero-positivity in cattle. Earlier, low sero-positivity (16.21%) was recorded in West Bengal by Chakrabarti *et al*. (2007). However, Panda *et al*. (2011) and Joardar *et al*. (2016) reported that cattle were 52.00% seropositive in West Bengal and 42.37% in Tripura, respectively. Dayakar *et al*. (2001) reported much higher seropositivity viz. 65.91% in Andhra Pradesh, 79.15% in Karnataka and 80.95% in Tamil Nadu. Joardar *et al*. (2013) also reported a high prevalence of anti-

bluetongue antibodies (70.00%) in cattle serum samples of Assam while performing i-ELISA. It is interesting to note that quite low seroprevalence in cattle is observed in Kerala and Sikkim, although they are positioned in completely two different agro-climatic zones. This might be due to non-availability of competent vector population in the regions. However, it needs further investigation before coming to a conclusion.

The seroprevalence of BT in goats and cattle 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 northwest, humid and sub-humid east and sub-temperate Himalayan region (Dubey *et al.*, 1987; Govindarajan *et al.*, 2002 and Singh *et al.*, 2009) in addition to different methods of investigation (Shringi and Shiringi, 2005 and Prasad *et al.*, 1992).

Proper seromonitoring can forecast the possible future outbreaks. The seroprevalence observed in the present study may be considered as an indication of possible outbreaks in future. It may be mentioned here that the virus has evolved globally with the emergence of BTV serotype-25 which is more pathogenic to goats (Hofmann *et al.*, 2008). There are reports of clinical disease in experimentally infected goats (Backx *et al.*, 2007). Hence, it cannot be ruled out that the virus in circulation for quite some time in goat and cattle in Sikkim may cause disease in these species.

In conclusion, this study shows seroprevalence in bluetongue infection in goats and cattle in Sikkim, although active disease has not been reported in this state so far. The findings indicated that further studies are needed to identify *Culicoides midges* (vector of BT) form various districts of Sikkim and to identify different BTV serotypes that are circulating in the state.

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