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SHORT COMMUNICATIONS

Seroprevalence of bluetongue and presence of viral antigen and type-specific neutralizing antibodies in goats in Tripura, a state at Indo-Bangladesh border of northeastern India

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Abstract
Bluetongue (BT) is a notifiable multiple species transboundary viral disease of domestic and wild ruminants. Though the disease is enzootic in India, little is known of the disease burden and prevalent serotypes in Tripura, a hilly state of northeastern India sharing a vast porous border with Bangladesh. A surveillance study was conducted to understand the disease burden in goats in Tripura. Serum ($n = 1240$) and blood ($n = 194$) samples were collected during the year 2014 to 2017 from all the eight districts of Tripura. The overall prevalence of BT seroconversion was 47.58% whereas the presence of viral antigen was 20.61% at the individual level. Percent seroconversion was found more (50.47 ± 4.00, CI 41.31 to 49.47) in adult goats in comparison to the younger animals where it was 45.39 ± 2.08, CI 42.63 to 58.31. Presence of neutralizing antibodies in selected serum samples ($n = 72$) was investigated by serum neutralization test (SNT) against six bluetongue virus (BTV) serotypes and BTV-1 was found as most predominant (65.27%) followed by BTV-16 (26.38%), BTV-10 (20.83%), BTV-9 and 23 (13.88%), and BTV-2 (6.94%). To the best of our knowledge, this is the first study conducted in Tripura to investigate the presence of BTV antigen and type-specific neutralizing antibodies in apparently healthy goats.

Keywords Bluetongue · Seroprevalence · Neutralizing antibodies · Tripura · Northeast India

Introduction

Bluetongue (BT) is an infectious, non-contiguous, arthropod-borne viral disease caused by bluetongue virus (BTV) which is the prototype species of the genus *Orbivirus*, family Reoviridae. BTV affects both wild and domestic ruminants such as sheep, goats, cattle, buffaloes, deer, and various other artiodactyla species (Darpel et al. 2007; Mauroy et al. 2008). BTV occurs in multiple serotypes (27) with scarce serological cross-reactivity amongst them (Zientara et al. 2014). BT is enzootic in India and several efforts have been made to understand the prevalence of the disease in various states of India (Joardar et al. 2013; Sairaju et al. 2013; Rao et al. 2016). However, comprehensive studies on the disease epidemiology are scanty, especially for the states of northeastern Indian. Tripura (23.9408° N, 91.9882° E) shares 856 km of porous border with Bangladesh covering the western, northern, and southern parts (Fig. 1), which makes this state vulnerable to an incursion of many transboundary diseases. Keeping the above circumstances in mind, the present study was conducted to generate data on seroepidemiology and antigenic evidence and also to investigate the presence of type-specific neutralizing antibodies in goats in the state of Tripura. The outcomes of the study provide new insight into the disease status, prevalent virus serotypes, and

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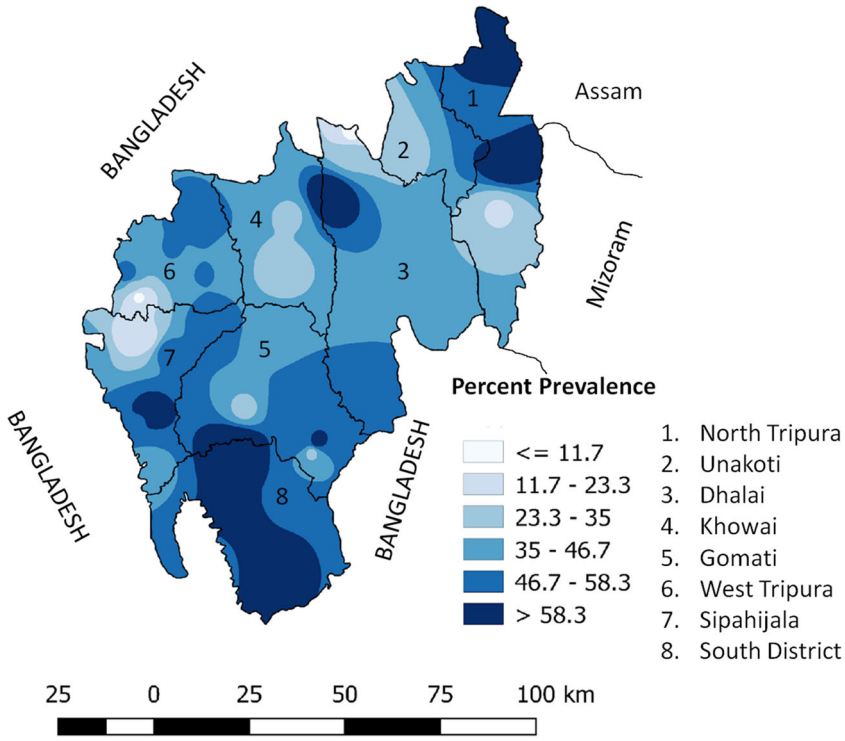

Fig. 1 Geographical distribution patterns of seroprevalence to BTV across the different districts of Tripura, India. Numbers denoting different districts are mentioned in the parenthesis. Differences in the intensity of the darkness on the map represent different percentages of seroprevalence

disease epidemiology which will help to develop appropriate intervention strategies to control BT in the northeastern states of India and in the neighboring country.

Materials and methods

Blood and serum samples were collected during the period of 3 years from 2014 to 2017. Sampling plan for sero-surveillance of BT antibodies in goats was prepared based on village wise small ruminant population of all 1038 villages of Tripura. A random sampling technique with probability proportional to size (PPS) without replacement was followed. The serum samples ($n = 1240$) were collected from organized as well as unorganized farms, located at different parts of all eight districts of Tripura. Random blood samples ($n = 194$) from all the eight districts of the state were also processed separately for detection of BTV antigen.

The serum samples were screened for group-specific antibodies to BTV by an indirect ELISA (iELISA) described earlier (De et al. 2009). Results obtained by the iELISA were statistically analyzed for determination of the disease prevalence. Testing of the significance by analysis of variance and odds ratio of age, district, and year of the collection at 95% confidence interval was carried out using binary logistic regression model (Software SPSS, Version 17.0.1). The presence of group-specific BTV antigen in whole blood samples was determined by a sandwich ELISA (sELISA) described earlier by Chand et al. (2009).

Serum samples ($n = 72$) showing strong positive reactivity in iELISA were selected randomly to investigate the presence of neutralizing antibodies against BTV serotypes 1, 2, 9, 10, 16, and 23. The beta SNT was performed according to the method described earlier (Oura et al. 2009; Batten et al. 2012) with minor modifications. Briefly, 100 $TCID_{50}$/50 μl/well of the virus was incubated with 50 μl of diluted serum at 37 °C for 1 h. Upon incubation, 100 μl of freshly cultured BHK-21 cell (approximately 10^6 cells/ml) suspension was added to each well of the 96-well culture plates. The cells were observed for 3 days for BTV-specific cytopathic effect (CPE). Presence of type-specific antibodies in serum was determined based on ability of a serum to neutralize the virus that reflected as the absence of virus-specific CPE at serum dilution 1:16 or above.

Results

The serum samples ($n = 1240$) tested for group-specific antibodies to BTV revealed 47.58% overall seroprevalence in goats in Tripura. The highest seroprevalence was observed in South Tripura district (59.5%), whereas it was the lowest (40%) in West Tripura district (Fig. 1). The seroprevalence in younger animals (below 6 months) was found to be lower (45.39 ± 2.08, CI 42.63 to 58.31) than that in the animals of above 6 months of age where it was 50.47 ± 4.00, CI 41.31 to 49.47 (Table 1). The overall prevalence during 2014–2015, 2015–2016, and 2016–2017 was found to be 48.3%, 52.0%,

Table 1 Seroprevalence of BTV in goats in different districts of Tripura during 2014 to 2017

District	No. of samples tested	No. of positive samples	Apparent prevalence (%)	True prevalence (%)	Age-wise prevalence	
					< 6 months	> 6 months
West Tripura	220	88	40.0	38.79	58/151 (38.41%)	30/69 (43.48%)
North Tripura	120	67	55.8	55.76	41/80 (51.25%)	26/40 (65%)
South Tripura	220	131	59.5	59.73	73/143 (51.05%)	54/78 (69.23%)
Sepahijala	180	80	44.4	43.51	44/95 (46.32%)	37/85 (43.53%)
Khowai	180	80	44.4	43.51	30/82 (36.59%)	50/98 (51.02%)
Unakoti	80	36	45.0	44.16	17/35 (48.57%)	19/45 (42.22%)
Gomati	100	48	48.0	47.38	23/53 (43.40%)	26/47 (55.32%)
Dhalai	140	60	42.9	41.90	34/66 (51.52%)	28/73 (38.36%)
Total	1240	590	47.58	46.93	320/705	270/535
Mean ± SE			47.58 ± 2.38	46.85 ± 2.25	45.39 ± 2.08	50.47 ± 4.00
			CI 42.92 to 52.24	CI 42.44 to 51.26	CI 41.31 to 49.47	CI 42.63 to 58.31

Confidence interval (CI) at 95% level

and 40.2%, respectively. The results of the binary logistic regression of BT with different variables studied are depicted in Table 2.

Amongst the blood samples ($N = 194$) tested, 20.61% of the samples were found positive for the BTV antigen. The percentage of viraemic samples was found to be higher in the samples collected from Khowai (40%) followed by West Tripura (35.2%), Gomati and Dhalai (25.0%), Unakoti (20.0%), North Tripura (15.0%), South Tripura (12.5%), and Sepahijala district (9.0%).

Presence of neutralizing antibodies in serum against BTV-1 was found as most predominant (65.27%) followed by BTV-16 (26.38%), BTV-10 (20.83%), BTV-9 and 23 (13.88%), and BTV-2 (6.94%). Many of the serum samples tested neutralized more than one BTV serotype at a dilution of 1:16 or higher. Out of 72 samples, positive for group-specific antibodies to BTV, 16 samples (22.22%) could not neutralize any of the six BTV serotypes used in the SNT (Fig. 2).

Discussion

Tripura is a hilly state of northeastern India having goat population of about 6.2 lakh (19th Livestock Census), mostly held as backyard farming. Here, we first report serologic and antigenic evidence of BTV in goats across the districts and

Table 2 Binary logistic regression model determining the relationship between categorical predictors and seropositivity in goats

	B	S.E.	Wald	df	Sig.	Exp(*B*)	95% CI for Exp(*B*)	
							Lower	Upper
Dhalai	Reference							
West Tripura	0.094	0.233	0.162	1	0.687	1.098	0.696	1.732
North Tripura	0.670	0.264	6.450	1	0.011	1.954	1.165	3.276
South Tripura	0.780	0.224	12.121	1	0.000	2.182	1.406	3.384
Sepahijala	0.186	0.235	0.625	1	0.429	1.204	0.760	1.908
Khowai	0.151	0.237	0.404	1	0.525	1.163	0.731	1.850
Unakoti	0.192	0.286	0.452	1	0.501	1.212	0.692	2.124
Gomati	0.191	0.268	0.507	1	0.476	1.210	0.716	2.044
Age (> 6 months)	Reference							
Age (< 6 months)	− 0.272	0.119	5.211	1	0.022	0.762	0.603	0.962
2016–2017	Reference							
2014–2015	0.345	0.160	4.662	1	0.031	1.412	1.032	1.931
2015–2016	0.462	0.157	8.638	1	0.003	1.588	1.166	2.161
Constant	− 0.540	0.230	5.511	1	0.019	0.583		

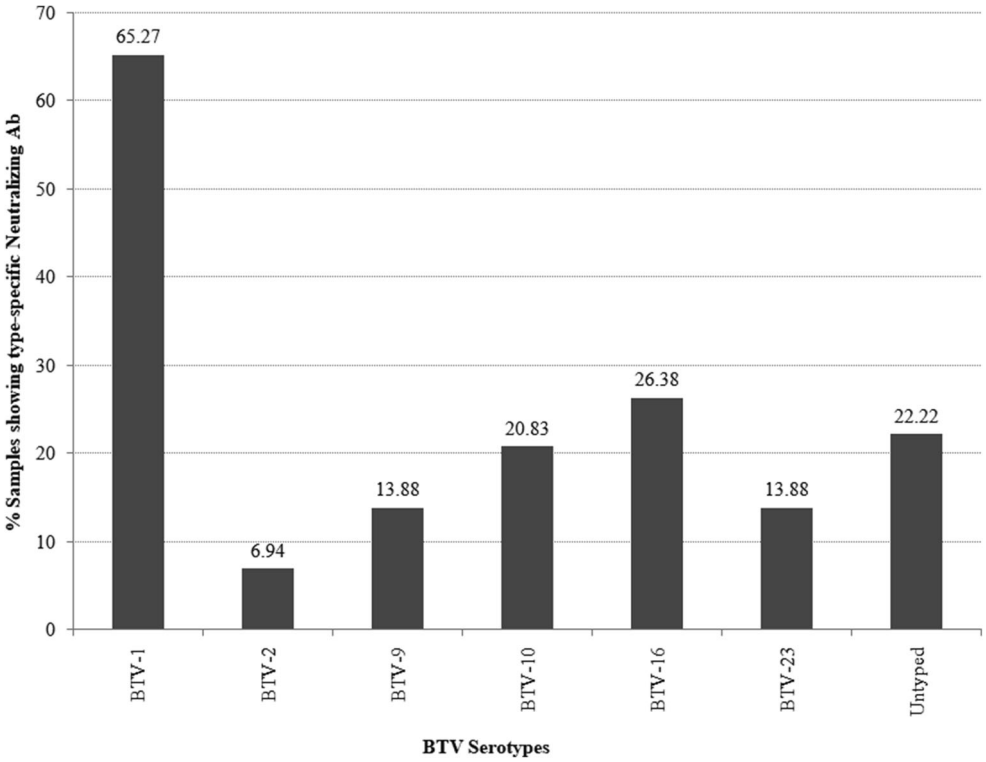

Fig. 2 Presence of neutralizing antibodies against different BTV serotypes in the seroconverted animals. The graph shows percentage of samples positive for neutralizing antibodies to different BTV serotypes tested

presence of type-specific neutralizing antibodies in the seroconverted animals to represent the disease burden in Tripura. In the present report, 47.58% seroconversion to BTV and presence of viral antigen in 20.61% of blood samples at the individual level is an important evidence for circulation of BTV in Tripura where no vaccination against the disease is practiced. A higher percentage of seroconversion was observed amongst the adult animals (more than 6 months) in comparison to the younger animals which may be due to continuous presence of the virus or because of reinfection (Ma et al. 2017). Out of 194 blood samples tested from eight districts of the state, 40 (20.61%) were found positive for BTV group-specific antigen, indicating active circulation of the virus.

Presence of neutralizing antibodies against BTV-1 was predominant (65.27%) followed by BTV-16 (26.38%), BTV-10 (20.83%), BTV-9 and 23 (13.88%), and BTV-2 (6.94%). The findings support the notion that BTV-1 is the most prevalent serotype in India whereas BTV-2 is mostly reported from the southern and western part of the country (Sairaju et al. 2013; Rao et al. 2016).

The contemporary circulation of multiple BTV serotypes within the same territory can imply the co-infection of the ruminant and/or the vector populations (Martinelle et al. 2016; Guimaraes et al. 2017). It is, therefore, possible that animals in such a complex epidemiological niche may harbor type-specific antibodies against multiple serotypes as a response to the superinfections as found in the present study. Despite this high prevalence of BTV antibody in goats in Tripura, there is no clinical report of bluetongue. The possible reasons may be but not only limited to the probable circulation of less virulent virus strains, prevalence of different vector species, and host immune status (Sharma et al. 2016). Factors related to lack of routine monitoring system, difficulties in clinical diagnosis, and misdiagnosis with similar viral diseases are also not to be ignored. However, a higher percentage of seroconversion and presence of the viral antigen in the animals implies that the goat population may act as a potential carrier of BTV and thus plays an important role in its dissemination in the susceptible animals in this region and in the neighboring country. The present study will help to understand the BT burden and to devise appropriate intervention strategies to control the disease. Further studies are needed for a comprehensive understanding of the molecular epidemiology of BT in this region.

Conclusion

The present study concludes a wide prevalence of bluetongue in goats across Tripura. The overall prevalence of BTV seroconversion in 47.58% and presence of viral antigen in 20.61% of the animals at individual level indicate the disease burden in this region and neighboring country. BTV-1 may be the predominant serotype followed by 10, 23, 9/16, and 2 as predicted by the presence of neutralizing antibodies. Further investigations are necessary to investigate and characterize potential serotypes and vector species involved for implementing appropriate control strategies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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