# Detection of Bluetongue Virus Antibodies in Small Ruminants of Coastal Odisha

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#### ABSTRACT

This study aimed to detect the prevalence of bluetongue virus (BTV) antibodies in goats and sheep of coastal Odisha using indirect enzyme-linked immunosorbent assay (iELISA). Serum samples (n=504) were collected randomly from apparently healthy goats (n=382) and sheep (n=122) of seven districts of coastal Odisha during October 2011 and March 2012. iELISA was conducted for detection of antibodies to recombinant group specific VP7 antigen of BTV in the Tissue Culture Laboratory, Division of Virology, IVRI, Mukteswar. The apparent seroprevalence of 85.51% (431/504) was detected overall, with the prevalence ranging from 68.85% in sheep to 90.84% in goats. Ganjam district recorded the maximum number of infected animals (95.01%) and the least affected district was Jagatsinghpur (42.31%). Among goats, the most affected district was Ganjam (99.60%) and among sheep, Cuttack registered the maximum seroprevalence (88.46%). The seroprevalence differed significantly statistically with respect to the species (Chi Square Test, p<0.05) while the difference in prevalence was not statistically significant with respect to the districts (Chi Square Test, p>0.05). This study detects active subclinical BT infection in the small ruminants of coastal Odisha which if left uncontrolled might lead to widespread economic losses in the form of mortality, morbidity and production losses.

Keywords: Bluetongue, goats, Odisha, seroprevalence, sheep

Bluetongue (BT) is a disease of ruminants caused by Bluetongue virus (BTV) of Genus Orbivirus of Family Reoviridae (Chand et al., 2015). It is mainly a disease of improved breeds of sheep but it also infects cattle and goats which remain as asymptomatic natural reservoirs. The economic losses caused by BT is around 3 billion US\$ per year in the world which encompass direct losses due to death, abortions, weight loss and reduced milk and meat productions and indirect losses due to export restrictions of live animals, semen and foetal calf serum (Amin and Kilo, 2016). BT is classified as a notifiable disease to the World Organization of Animal Health (OIE), and BTV is transmitted among ruminants by biting midges of the Genus Culicoides (Samy and Petersen, 2016). This disease is endemic in the tropical, subtropical and temperate regions of the world between the latitudes of approximately 40° N and 35° N covering America, Africa, Australia and Asia where the vectors are present (Amin and Kilo, 2016). Worldwide, 28 different serotypes of the virus have been discovered till date (Savini *et al.*, 2017) with 22 serotypes circulating in India (Prasad *et al.*, 2016).

The emergence of BT occurs when susceptible animal species are introduced into areas with circulating virulent BTV strains, or when virulent BTV strains extend their range to previously unexposed populations of ruminants (Zientara *et al.*, 2010). Thorough clinical, serological and entomological investigations are imperative to ascertain the presence and extent of BT infection in an area (Amin and Kilo, 2016) so that effective control measures can be implemented. The serotypes responsible for causing outbreaks vary temporally and spatially so it is crucial



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to identify the circulating serotypes and include them in vaccine formulations (Rao *et al.*, 2016).

Odisha is a state located in the eastern coastal region of India between 17° 48' to 22° 34'N latitude and 81° 24' to 87° 24'E longitudes. About 72% of the population of Odisha lives in rural areas and 40% of this rural population is dependent on sheep and goat farming for their source of livelihood (Sheep and Goat Genetic Resources of Orissa). Diseases like BT causes widespread losses including productive and reproductive losses due to mortality, abortions, drop in body weight and milk yield. Although no clinical cases of the disease have been reported so far in the state of Odisha, the presence of BT antigen was demonstrated in the sheep and goats of Odisha by Pany et al. (2016) who conducted sandwich ELISA to detect the group specific VP7 antigen of BT. The status of BT infection in the state is ambiguous due to the lack of elaborate research work which urged us to undertake this study that investigates the extent of sheep and goats in the coastal regions of Odisha infected with BT.

### MATERIALS AND METHODS

#### Serum samples

A total of 504 serum samples were collected randomly from the sheep and goats of coastal districts of Odisha viz Ganjam, Dhenkanal, Cuttack, Khurda, Puri, Jagatsinghpur, Balasore, during October 2011 to March 2012, out of which 382 were of goat and 122 were of sheep. The serum samples were stored at -20°C in the Department of Veterinary Microbiology, C.V.Sc & A.H, Bhubaneswar till further use.

### **Indirect ELISA (iELISA)**

The serum samples were tested for the presence of BTV group specific antibody by iELISA as described by Biswas *et al.* (2005). Briefly, 50 ml of BTV VP7 recombinant antigen in PBS was coated on all except four (antigen control) wells of a flat bottomed microtitre plate (Maxi Sorp, Nunc, UK) and incubated at  $37^{\circ}$  C for 1hr in shaker incubator. After washing the plates thrice with washing buffer (PBS-T), 100 ml of blocking buffer was added to each well and incubated at  $37^{\circ}$  C for 1h. The plates were then washed and 50 ml known positive serum (1:10

dilution), known negative serum (1:10 dilution) and test serum (1:60 dilution) diluted in blocking buffer were added in the respective wells in duplicates and incubated at 37°C for 1h with continuous shaking. The pooled goat and sheep serum positive for anti-BT antibodies were used as positive control while healthy goat and sheep serum were used as the negative control. After washing, 50 ml of secondary antibody conjugate (Donkey Anti goat Horse Radish Peroxidase, Sigma) diluted 1:6000 in blocking buffer was added and incubated at 37° C for 1h and then washed thrice in PBS-T. Four wells were kept as conjugate control in which conjugate was not added. Thereafter, 50ml ortho-phenylenediamine dihydrochloride (OPD, Sigma) substrate solution was added and incubated at 37<sup>0</sup> C for 10 to 15 minutes till the colour developed. Absorbance in the form of optical density (OD) was measured at a wavelength of 492nm after stopping the reaction with 50 ml of stopping solution ( $1M H_2SO_4$ ).

The samples were classified as positive when the sample OD was greater than or equal to twice the OD of the negative control.

### Statistical analysis

The statistical significance of difference of the prevalence of BT in the small ruminants of Odisha with respect to the species and districts was tested at 5% level of significance employing the Chi Square test using online Social Science Statistics calculator (Accessed from http://www. socscistatistics.com/tests/chisquare2/Default2.aspx). A probability value (p-value) of less than or equal to the level of significance (0.05) indicated the statistical significance of the results.

#### **RESULTS AND DISCUSSION**

Bluetongue is an insect-borne disease, which is endemic and notifiable in India (Rao *et al.*, 2016). BTV infected sheep often show severe clinical signs, while cattle and goats are usually asymptomatic (Schulz *et al.*, 2016). BT is characterised by fever, nasal discharge, drooling of saliva, oral lesion, facial oedema, depression, anorexia, and muscle weakness in sheep and wild ruminants while goats and cattle are asymptomatic (Ma *et al.*, 2017). The definitive diagnosis of BTV is done mainly by serological tests, virus isolation either in chicken embryo or cell culture, and detection of viral nucleic acid. Although, competitive ELISA (c-ELISA) has been listed as the prescribed test for the detection of BT antibody by OIE, indirect ELISA (iELISA) is a comparatively cost effective test (De *et al.*, 2009) when large number of samples has to be screened, hence this study employed iELISA for detection of antibodies to BT in 504 serum samples (goats=382, sheep=122) of the sheep and goats of coastal districts of Odisha.

Table 1: Indirect ELISA results of goat and sheep samples

Sl. No.	Sample	Total	Positive (%)	Strong positive (%)
1	Total	504	431(85.51)	347(68.85)
2	Goat samples	382	347(90.84)	301(78.80)
3	Sheep samples	122	84(68.85)	34(27.87)

The overall apparent prevalence was found to be 85.51% (431/504) with the seroprevalence ranging from 68.85% (84/122) in sheep to 90.84% (347/382) in goats by indirect ELISA (Table 1, 2). Among the districts Ganjam (95.01%) recorded the maximum number of animals with antibodies against BT followed by Cuttack (92.65%), Khurda (72.41%), Dhenkanal (70.59%), Puri (68%), Balasore (66.67%) and Jagatsinghpur (42.31%, Table 2). In case of goats the prevalence ranged from 18.75% in Jagatsingpur to 99.60% in Ganjam district (Table 2). The prevalence registered in sheep ranged from a minimum of 53.85% in Dhenkanal district to a maximum of 88.46% in Cuttack district (Table 2). The prevalence of BTD antibodies was found to be statistically significant with respect to species

of animals (Chi Square Test, p < 0.05) and statistically nonsignificant with respect to the districts (Chi Square Test, p > 0.05).

The apparent prevalence of BT in small ruminants of coastal Odisha witnessed in the current study is 85.51% (goats = 90.84%, sheep = 68.85%). Previously, Behera et al. (1997) registered a lower prevalence of BT in Odisha by AGPT (sheep = 39.19%, goats = 10.6%) and CIE (sheep = 44.42%, goats = 24.02%). The present prevalence is higher than the percent prevalence of 20.3% (443/2187) in Tibetan sheep (Ma et al., 2017) and 69.01% and 60.53% prevalence in sheep and goats, respectively among the small ruminants in breed improvement centres in different parts of Ethiopia (Gizaw et al., 2016). As compared to this study a higher percentage of 96.7% of buffaloes and cattle in selected provinces in Lao People's Democratic Republic (Douangngeun et al., 2016) were found positive for antibodies to BT. So, it may be inferred that the differences in the diagnostic methods, climatic conditions, geographical conditions, species/breeds, sample sizes, and sanitation, contributed to such differences (Ma et al., 2017). Although no clinical report of BT has been ever made from Odisha, the detection of antibodies in the small ruminants confirms the exposure of the sheep and goats to the virus which might have been introduced due to transmigration of infected animals from the bordering states with reports of the disease (Rao et al., 2016).

The percentage of goats (90.84%) harbouring antibodies against BT is higher as compared to sheep (68.85%) in this study. The Chi Square test found that there was significant statistical variation in seroprevalence of

Table 2: District wise prevalence of Bluetongue virus antibodies in sheep and goats by indirect-ELISA

Sl. No.	District	Goats		Sheep		Total	
		Т	P (%)	Т	P (%)	Т	P (%)
1	Ganjam	249	248(99.60)	32	19(59.38)	281	267(95.01)
2	Cuttack	42	40(95.23)	26	23(88.46)	68	63(92.65)
3	Khurda	15	11(73.33)	14	10(71.42)	29	21(72.41)
4	Dhenkanal	38	29(76.32)	13	7(53.85)	51	36(70.59)
5	Puri	10	8(80)	15	9(60)	25	17(68)
6	Balasore	12	8(66.67)	12	8(66.67)	24	16(66.67)
7	Jagatsinghpur	16	3(18.75)	10	8(80)	26	11(42.31)
	Total	382	347(90.84)	122	84(68.85)	504	431(85.51)

T = Total no. of samples, P = No. of samples positive, %-Percentage

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bluetongue in goats and sheep due to their difference in species (p<0.05). So species of the animal is one of the risk factor concerning BTV infection which has also been reasoned by Ma *et al.* (2017). Similarly, Rao *et al.* (2016) also found that the prevalence of BT was higher in goats than sheep in Andhra Pradesh. On the contrary, the findings of Gizaw *et al.* (2016) show that sheep were more affected than goats. It may be assumed that the wool of sheep provided protection against the bite of the Culicoides vector thus decreasing the risk of transmission of the infection to the sheep. However it is also possible that the affected sheep which suffer from severe visible clinical signs are marked for slaughter and hence there are lesser chances of detection of the affected sheep (Pany *et al.*, 2016; Bhanuprakash *et al.*, 2008).

About 86.99% of goats and 40.48% sheep out of the total seropositive goats and sheep serum samples in this study were found to be strongly positive by iELISA. The high titre of antibodies may be ascribable to peaking of the antibody response to the initial infection usually 7-14 days post infection (OIE, 2014; Arun et al., 2014) or alternatively, to the flaring up of the already present BT infection in animals due to immune-suppression (Mondal et al., 2009) as a result of interplay of several other viral infections such as Foot and Mouth Disease (FMD) Virus (FMDV) and PPRV, Poxvirus which have been previously reported from this region although concurrent infection of viruses has not been reported. The higher antibody level in animals of this study indicates the presence of active infection in the small ruminants of Odisha. The higher titre of the infection so mounted also increases the risk of transmission of the disease by the vector as a small blood meal will be potent enough to positively infect the vector and initiate an infection (Koumbatia et al., 1999).

The samples in the present study were collected from apparently healthy sheep and goats indicative of subclinical infection of BT in small ruminants of Odisha. Rao *et al.* (2016), Najarnezhad and Rajae (2013) and Gizaw *et al.* (2016) have also uncovered in-apparent BT infections in domestic ruminants in Andhra Pradesh, North Iran and Ethiopia respectively. In support of the current study, Pany *et al.* (2016) reported that 52.43% goats and 44.94% sheep of central and coastal Odisha were positive for BTV antigen by sandwich ELISA in absence of any clinical signs. The carrier state of sheep and goats as detected in this study is crucial to BT epidemiology as in some circumstances local breeds of small ruminants may be important in maintaining the virus (Koumbatia *et al.*, 1999), thus acting as a reservoir of infection. On a different note, the absence of clinical disease suggests that indigenous local breed of sheep and goats have a high degree of innate immunity and are resistant to clinical disease of bluetongue infection or there may be circulation of mild virus strain in the population (Gizaw *et al.*, 2016).

The present study detected in-apparent BT infection in the sheep and goats of coastal Odisha. The presence of high titre of antibodies indicated a possible active infection of BT or eruption of infection due to immunesuppression. The silent infection of animals may further facilitate the spread of disease to healthy animals. So it is essential to ascertain the strain of the virus infecting the small ruminants of the state so that effective treatment and preventive measures are brought into action to check the disease.

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