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SHORT COMMUNICATION

Seroprevalence of Bluetongue Virus in Small Ruminants of Krishna District, Andhra Pradesh, India by Competitive ELISA

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ABSTRACT

Bluetongue is an infectious, noncontagious, vector borne viral disease causing heavy morbidity and mortality. Disease is prominent in sheep with apparent clinical signs while goats and bovines may serve as reservoir hosts. Most of the times field veterinarians diagnose bluetongue based on clinical signs only. The serological tests like competitive ELISA (c- ELISA) are helpful in diagnosis and prevalence studies of bluetongue. Sero-surveillance of bluetongue virus in sheep (n=350) and goat (n=100) of Krishna district (AP) was conducted using commercially available c-ELISA kit. The results revealed that among 450 serum samples, 62.66% (63.71% in sheep and 59% in goat) were detected positive for bluetongue virus antibodies.

Keywords: Blue tongue, c- ELISA, prevalence, sheep, goat

Blue tongue (BT) is an infectious, noncontagious, rapidly spreading, economically important, hemorrhagic, *Culicoides* borne viral disease affecting domestic and wild ruminants like sheep, goat, cattle, buffaloes *etc.* (Joardar *et al.* 2013). Disease is mostly prominent in sheep with distinct clinical signs accompanied by heavy losses in the form of morbidity and mortality. Clinical form of the disease has not been reported till now in cattle and buffaloes, whereas in goats sporadic occurrence was reported (Maheshwari, 2012). Bovines were reported to serve as reservoir hosts for BTV (Krishnamohanreddy *et al*. 2008). Fever, depression, nasal discharge, drooling of saliva, oral lesions, facial edema, hyperemia of coronary bands and muscle weakness are characteristic clinical signs noticed in BT affected sheep (Afshar, 1994). In 1964, first report on the prevalence of BT was reported causing heavy losses in sheep (Sapre, 1964). Since then there are numerous reports of BT occurrence in southern and western parts of India (Ilango, 2006). There is no known zoonotic potential even though some species of *Culicoides* feed on human blood (Maheshwari, 2012; Baten *et al*. 2013).

Blue tongue virus (BTV) is an arbovirus (arthropod borne), belongs to genus *Orbovirus*, family *Reoviridae* and transmitted by hematophagous midges of the genus *Culicoides* and family *Ceratopogonidae* (Mertens and Diprose, 2004). Sometimes it can be transmitted by oral route or vertically in sheep and cattle (Wilson *et al*. 2009; Machlachlan and Guthrie, 2010). BT serogroup contains 26 serotypes as of now (BTV1- BTV26) with recent addition of 25th serotype (Toggenburg orbovirus) from Switzerland in goat and 26th serotype from Kuwait in sheep and goat (Hoffmann *et al*. 2008; Mann *et al*. 2011, 2012; Batten *et al*. 2013; Bitew *et al*. 2013) among which 23 were reported in India (Sairaju *et al*. 2013). BTV is a small icosahedral virus with double layered protein coat with a 10 segmented, double stranded RNA genome that encode 4 nonstructural (NS1- NS4) and seven structural (VP1-VP7) proteins (Schwartz-carnil *et al*. 2008). Very low level of cross protection among BTV serotypes is making vaccination strategies and control programmes a difficult task (Hoffmann *et al*. 2008; Eschbaumer *et al*. 2009). As per the 19th livestock census of India, Krishna district (AP) is having 5

lakh sheep population and 1.5 lakh goat population. There is dearth of literature regarding prevalence of bluetongue antibody in this area. In this regard, a study was undertaken to assess the prevalence of BT among small ruminants in Krishna district of Andhra Pradesh.

MATERIALS AND METHODS

Samples

A total of 450 blood samples (350 from sheep and 100 from goat) were collected among various flocks in Krishna district, Andhra Pradesh, India during August to December, 2014. All samples were collected randomly from clinically healthy animals (Table 1). 10 ml of blood sample was collected aseptically from each animal, from jugular vein using BD® vaccutainers, allowed to clot at room temperature, transferred to laboratory. The serum were separated and stored at -20°C until further use.

Competitive ELISA (c-ELISA)

IDEXX® blue tongue competition, an enzyme immunoassay for the detection of antibodies against VP7 protein from blue tongue virus in individual serum from ovine and caprine origin was used for diagnosing BTV as per manufacturer's instruction. Briefly, Serum samples were diluted 1:4 times with sample dilution buffer, dispensed into each well of pre-coated ELISA plate and incubated for 45 min at 37°C. Later diluted conjugate was added to each well and incubated again for 45 minutes at 37°C. The plates were washed thrice with wash solution and then the reaction was developed with TMB substrate for 10 minutes at 37°C. The reaction was stopped with the designated stopping solution and the ODs were read at 450nm with BioTek® microplate reader. The results were analysed with xChekPlus® soft ware.

RESULTS AND DISCUSSION

Results revealed that 63.71% (223/350) of sheep and 59% (59/100) goat were found positive for BTV with an overall prevalence of 62.66% (282/450) among small ruminants (Table 1).

Table 1: Details of samples screened and prevalence of BTV antibodies in small ruminants

S. No.	Species	No. of samples collected	No. positive		Percent Positive
			Female	Male	
1	Adult Sheep	300	179 (250) 71.60%	21(50) 42%	66.66%
2	Young Sheep	50	17 (30) 56.67%	6 (20) 30%	46%
	Total	350	196 (280) 65.33%	27(70) 38.57%	63.71%
3	Adult Goat	70	39 (60) 65%	4 (10) 40%	61.43%
4	Young Goat	30	11 (20) 55%	5 (10) 50%	53.33%
	Total	100	50 (80) 62.50%	9(20) 45%	59%

Blue tongue is an important disease causing huge economic losses in the small ruminant sector. Blue tongue is recognized as a multiple species disease by OIE, World Organization for Animal Health. Most of the times field veterinarians diagnose BT based on clinical signs only, which emphasize the need for rapid, reliable, sensitive and specific diagnostic method like c- ELISA. Overall prevalence of 62.66% was reported in this study, on contrary lower prevalence of 16.4% was reported by Reddy and Sushmita (2012) in Andhra Pradesh. Among other states prevalence of 28.6% in Ut ar Pradesh (Bitew *et al.* 2013), 30.3% in Rajasthan (Shringi and Shringi, 2005), 30.8% in Maharashtra (Deshmukh, 2009) and 33.16% in Madhya Pradesh (Sikrodia *et al.* 2011) were also reported by various authors. High prevalence of BT was observed in sheep (63.71%) compared to goat (59%), which was in agreement with the findings of various workers (Sreenivasulu *et al*. 2003; Chakrabarti *et al*. 2007; Shlash *et al*. 2012; Arun *et al*. 2014; Tigga *et al*. 2015).

Among 350 samples of sheep, 223 (63.71%) were found positive. Results observed in this study are proximate to the findings of various authors (45.71% in Andhra Pradesh by Sreenivasulu *et al*. 2003; 57.66% in West Bengal by Panda *et al*. 2011; 58.82% in Assam by Joardar *et al.* 2013). In contrast lower prevalence of 13.8% in Ut ar Pradesh (Bitew *et al.* 2013), 16% in Kerala (Arun *et al.* 2014), 23.5% in Haryana, Himachal Pradesh and Punjab (Naresh and Prasad, 1995), 25.66% in Tamilnadu (Selvaraju and Balasubramaniam, 2013), 33.75% (Mandal *et al.* 2011) and 34.47% (Chakrabarti *et al.* 2007) in West Bengal, 36.11% in Gujarat (Chauhan *et al.* 2004) and 43.68% in Jharkhand (Tigga *et al.* 2015) were reported. There is a huge variation between the findings of various authors regarding seroprevalence of BTV among small ruminants in various states of India. The difference in disease prevalence in various parts of the country may be due to varied climatic conditions, sheep population density and susceptibility of sheep breeds to BT (Rao *et al.* 2014).

Blue tongue outbreaks follow monsoons. In Southern parts of Andhra Pradesh, Karnataka and most of Tamilnadu, BT is observed in between October to December (Sreenivasulu *et al*. 2003). Cooler temperatures, humid climate and water logging due to heavy rainfall provides congenial breeding conditions for *Culicoides* and may be correlated with high prevalence observed in this study (Rao *et al*. 2014). 59% of goats were found to be positive for BTV. Infection rate of 54.5% in Ut ar Pradesh (Bitew *et al*. 2013) and 57.25% in Gujarat (Bhagat *et al*. 2014) were reported, proximate to the findings of this study. On contrary lower prevalence of 2.63% in Kerala (Ravishankar *et al.* 2014), 5.3% in Karnataka (Doddamani and Haribabu, 2006), 7.5% in Kerala (Arun *et al.* 2014), 24.03% in West Bengal (Chakrabarti *et al.* 2007), 31.79% in Assam (Joardar *et. al.*, 2013), 31.72% in Madhya Pradesh (Sikrodia *et al.* 2011), 39.61% in Gujarat (Bhagat *et al.* 2014), 43.33% in Jharkhand (Tigga *et al.* 2015), 43.56% in Andhra Pradesh (Sreenivasulu *et al.* 2003), 45% in Jammu (Singh *et al.* 2009), 47.25% in West Bengal (De *et al.* 2009) and higher prevalence of 66.95% in West Bengal (Panda *et al.* 2011) than the present study were also reported by various authors.

Competitive enzyme linked immunosorbent assay (c-ELISA), Compliment Fixation test (CFT) and Agarose gel immunodiffusion (AGID) have been recommended by OIE for screening of BT in international trade (OIE, 2008). Many authors declared the effectiveness of c-ELISA that detects antibodies directed against VP7 core protein, which is present in all 26 serotypes (Shlash *et al*. 2012). VP7 is found to be highly conservative and group specific antigen (Manjunatha *et al*. 2010). Vandenbussche *et al*. (2008) considered it as the first choice for serosurveillance of BT in susceptible animals.

High prevalence of BT among sheep observed in this study might be due to continuous exposure from goats as rearing sheep and goat together is a common practice. Bovines also might have served as reservoirs, leading to high prevalence in small ruminants. Even though high prevalence of BTV is observed in this study among healthy sheep and goat, no prominent clinical signs are noticed. There is a need to identify the circulating and pathogenic serotypes and evaluate herd immunity against those serotypes in this area by vector trap and sentinel systems in order to formulate an effective vaccine.

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