



SHORT COMMUNICATION

Bluetongue in Bovines: A serological Survey in Punjab, India

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ABSTRACT

The present study was conducted to ascertain the seroprevalence of bluetongue in cattle and buffalo population in Punjab, India. Total 92 serum samples were collected from female bovines belonging to 3 different agroclimatic zones of Punjab and subjected to cELISA test for the detection of anti-bluetongue antibodies. The overall apparent seroprevalence of bluetongue was 8.7%, while true serological prevalence was calculated 7.8%. There is no clinical report on bluetongue in small and large ruminants in Punjab. Though, a very high seroprevalence (53.0%) was reported in sheep (58.6%) and goat (50.6%) population of the state in a previous study. The prevalent serotypes of BTV circulating in this region are needed to identify in further study.

Keywords: Bluetongue, bovines, ELISA, prevalence, Punjab

Bluetongue is an infectious, non-contagious, arthropod borne viral disease of domesticated and wild ruminants caused by bluetongue virus (BTV) of genus *Orbivirus* in the family *Reoviridae*. At present, 26 serotypes have been reported throughout the world (Maan *et al.* 2011) of which 21 serotypes (except 19, 22, 24, 25 and 26) have been reported from different states of India (Rajkhowa *et al.* 2008, Joardar *et al.* 2009, Joardar *et al.* 2012).

Bluetongue is clinically characterized by fever, depression, nasal discharge, drooling of saliva, oral lesions, facial edema and hyperemia of coronary bands, and is transmitted by *Culicoides spp.* The worldwide economic losses is about \$3 billion annually in the form of death, abortion, weight loss, reduced milk yield, meat efficiency and export restrictions for live animals, their semen and products such as fetal bovine serum. There is no literature available regarding the prevalence of bovine bluetongue in Punjab. Therefore, it was decided to employ competitive enzyme linked immunosorbent assay (cELISA) to establish seroprevalence of bluetongue in the bovine population of Punjab, India.

Total 92 serum samples were collected from adult female bovine population (cattle-80, buffalo-12) during the year

2015. All the sera samples were screened for antibodies against bluetongue virus by using commercially available cELISA kit (Bluetongue virus antibody test kit, VMRD) to establish the seroprevalence of bluetongue. Briefly, 25µl of samples including positive controls, negative controls and test serum samples were loaded in each well of antigen coated microplate and incubated for 15 minutes at room temperature (25°C). This was followed by addition of 25µl of antibody- peroxidase conjugate to each well. After incubation for 15 minutes, the plate was washed thrice with 100µl of wash solution. Further, 50µl of substrate solution was added and the plate was again incubated for 10 minutes at room temperature and 50µl of stop solution was added to all the wells and mixed well by tapping. Finally, optical density was measured at 630nm in iMark Microplate Reader (BIORAD) and test samples were considered positive or negative if optical density was less or more than 50% of the mean of the negative controls, respectively.

By analyzing the results, overall apparent seroprevalence of bluetongue in bovine population was found 8.7%. With sensitivity and specificity of bluetongue virus antibody test, cELISA reported as 100% and 99% and true prevalence



was calculated 7.8% (Cameron, 1999). Both cattle and buffalo population were found positive for antibodies against bluetongue virus, though overall serological prevalence was reported to quite low in comparison to sheep (58.6%) and goat (50.6%) population in Punjab (Singh, 2015). Similar findings were also reported by Arun *et al.* (2014).

In a previous study, the seroprevalence of bluetongue virus was 19.4% (Adam *et al.*, 2014). Older cattle (>2 years age) were four times more susceptible to BTV than young cattle (OR=4.31, CI=1.94-9.57, p-value=0.01). There is no clinical case report of BT in bovines in Madhya Pradesh (India), but, serosurveillance revealed a very high prevalence (73.9%) in the bovine population (Kumari *et al.* 2010). Similarly, Joardar *et al.* (2013) also reported a very high seroprevalence of bluetongue in sheep (58.8%), goat (31.8%) and cattle (70.0%) in Assam.

The distribution and intensity of infection in different regions is determined by the climate, geography and altitude that might affect the occurrence and activity of the *Culicoides spp.* vectors (Erasmus and Potgieter, 2009). The climate being the major risk factor as *Culicoides*, require warmth and moisture for breeding and calm, warm and humid weather for feeding. Bluetongue is endemic in many parts of India. In Tamil Nadu, a total of 258 outbreaks were reported between 1986 and 1995 (Sreenivasulu *et al.*, 2004). An outbreak in Tamil Nadu (1997-98) caused the death of 300,000 sheep and goats (Ilango, 2006). Some sporadic cases were reported in northern states of India including Rajasthan and Uttarakhand (Mehrotra *et al.* 1995).

Economic significance of the disease was reviewed by many workers (Prasad *et al.* 1992, Maheshwari, 2012, Chand *et al.* 2015) and complete genome of bluetongue virus serotype 16 of goat origin from India was sequenced by Prasad *et al.* (2012).

Despite high seroprevalence of bluetongue in Punjab, there are no clinical reports on bluetongue in small and large ruminants. This might be due to presence of less virulent strains, different *Culicoides spp.* or lower population of sheep. Although, BTV has been isolated from *Culicoides midges*, but the particular species responsible for transmission has not yet been identified (Prasad *et al.* 1992). The studies on BT in India are in preliminary stages, which may open fields for future investigation.

Unlike sheep, bluetongue virus causes inapparent clinical signs in cattle and goats, so the disease is rarely observed. Goats and cattle are considered as reservoir hosts, and thus play an important role in the epidemiology of BTV. Detection of virus specific antibody in animals indicates an indirect evidence of virus in Punjab. This implies that the cattle population may act as carrier of bluetongue virus and thus plays an important role in its dissemination.

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