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Seroprevalence of Bluetongue among domestic ruminants in Northern Kerala, India

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Abstract. The objective of the present study is to assess the seroprevalence of bluetongue (BT) among domestic ruminants of Northern Kerala. Sera samples from cattle (82), goat (40) and sheep (50) collected from districts of Wayanad, Kozhikode and Palakkad respectively were tested using competitive enzyme linked immune-sorbent assay (cELISA). Out of the 172 samples tested, the overall BT seroprevalence was 9.3%. There is an increase in prevalence from previous reports which may indicate possible outbreaks in future.

INTRODUCTION

Bluetongue is caused by Bluetongue Virus (BTV) of the genus *Orbivirus*, placed under the subfamily *Sedoreovirinae* and family *Reoviridae*. The virus is transmitted between the vertebrate hosts by *Culicoides* spp. (Maclachlan & Dubovi, 2011). Bluetongue is listed as a multispecies disease by Office International des Epizootics (OIE). BT most commonly affects sheep and wild ruminants. Although goat and cattle are susceptible, the disease is usually subclinical or inapparent in these species. Cattle serve as the most important reservoir host of BT (Reddington *et al.*, 1991; Reddy *et al.*, 2008). The disease affects domestic and wild ruminants reared in regions between latitudes 40-50° North and 35° South.

The disease was first reported in India in 1964 (Sapre, 1964). There are reports of 26 serotypes of BTV worldwide, of which 21 serotypes have been reported in India either on the basis of virus isolation or serology (Prasad *et al.*, 1992; Sreenivasulu *et al.*, 2004; Hofmann *et al.*, 2008; Maan *et al.*, 2011).

Prevalence of antibodies against BT among sheep, goat and cattle were reported from many states of India (Prasad *et al.*, 1992; Bhanuprakash *et al.* 2008; Sikrodia *et al.*, 2012). Seroprevalence of BT in ruminants has been reported in Kerala (Ravishankar *et al.*, 2005; Sunilkumar *et al.*, 2005). However these two studies were carried out with the aim of establishing the prevalence of the disease in ruminants in Kerala for the first time. Thereafter no studies have been carried out in this direction. Hence this study was undertaken to assess the seroprevalence of the disease in these species after a five year period.

MATERIALS AND METHODS

The area of study encompassed three districts of Northern Kerala namely, Wayanad, Kozhikode and Palakkad. The state

of Kerala is geographically placed between the Western Ghats in the east and the Arabian Sea in the west. Wayanad district forms the southern tip of Deccan plateau with rugged terrain of Western Ghats with a major portion of forest cover, and borders the two neighbouring states of Tamil Nadu and Karnataka. Palakkad district has Coimbatore district of Tamil Nadu to its east and forms a gateway to Kerala through the Palakkad pass between the Western Ghats. The Kozhikode district is geographically located amidst the districts of Kannur, Wayanad and Malappuram, which have borders to the neighbouring states.

Sera: A total of 172 sera samples were assessed for the presence of antibodies against BTV. Eighty two were from cattle of Wayanad district, 40 from goats of Kozhikode and 50 from sheep of Palakkad district.

ELISA: An Ingezim BTV Compac ELISA kit (Ingenasa, Spain) which works on the principle of direct competitive ELISA was employed for the detection of BTV antibodies (Abs). The kit contained ELISA plates pre-coated with VP7 protein of BTV. After addition of the test serum, if it contained specific Ab, it will bind to the antigen. A specific monoclonal antibody (MAb) was then added and if the serum Abs were absent then, MAbs would bind to the antigen. If serum Abs were present, they would block the binding of MAb. After washing to remove the unbounded material, presence or absence of the labeled MAb was detected by the substrate in the presence of peroxidase.

Briefly, 50 µl of sample diluent was added in each well of the ELISA plate, followed by 50 µl of each test sera sample, positive and negative controls in duplicates in the designated wells and the plate was incubated at RT for 18 hours. After the overnight incubation, the plate was subjected to a washing process using 300 µl of washing solution in each well and the washing is carried out five times. Peroxidase labeled MAb, amounting to 100 µl was added in each of the wells and was incubated at 37°C for 30 minutes. Then, the plate was washed for six times. After washing, 100 µl of tetramethylbenzidine (TMB) substrate was added to each well and the plate was incubated at RT for 10 minutes. Finally a volume of 100 µl of stopping solution (sulfuric acid) was added and the plate was read in an ELISA reader at 450 nm.

- Cut off (negative) = 0.65 × negative control.
- Cut off (positive) = 0.6 × negative control.

Samples were considered positive when the optical density (OD) was equal or less than positive cut off value (60 per cent of negative control) and the samples were considered negative when OD was equal or greater than positive cut off value.

RESULTS

The ELISA test could detect positive samples in all the three species. The species-wise positivity was 6.9% (five out of 82), 16% (eight out of 50) and 7.5% (three out of 40) in cattle, sheep and goat respectively. The overall BT prevalence was 9.3%.

DISCUSSION

Bluetongue is an economically important arthropod borne viral disease mainly affecting sheep and wild ruminants. Bluetongue infection in cattle and goats is subclinical or asymptomatic. The global distribution of BTV infection has taken a drastic turn due to global climatic change, presumably affecting the distribution of the vector transmitting the virus. The virus has been isolated from all continents except Antarctica (Maclachlan *et al*., 2009).

Among the ruminants sheep has been observed to be the predominant host susceptible to BT (Shringi & Shringi, 2005). The clinical signs in sheep usually include fever, nasal discharge, frothy salivation, excoriation of oral mucosa and laminitis and some of these clinical signs overlap with that of foot and mouth disease (FMD) (Radostits *et al*., 2007). Serological response appears usually 7-14 days post infection and is generally long-lasting (OIE, 2013).

The bluetongue virus is a non-enveloped virus containing double stranded RNA. The viral genome is surrounded by two major structural proteins VP3 and VP7 and three minor structural proteins VP1, VP4 and VP6. This in turn is surrounded by outer capsid containing two structural proteins VP2 and VP5. The VP7 and VP3 polypeptides are predominant and constitute more than 50% of the total BTV protein structure. The VP7 antigen has been found to be a highly conservative group-specific antigen (Afshar *et al*., 1992; Mecham & Wilson, 2004; Manjunatha *et al*., 2010).

In India the occurrence of BT between different parts are dependent on the rainfall, with maximum number of outbreaks occurring during north-east monsoon, followed by south-west monsoon (Sreenivasulu *et al*., 2004). Seroprevalence of BT has been observed in many states of India including Kerala.

In the present study the percentage positivity in cattle was only 6.9% which is much below the overall prevalence of the disease previously reported in Kerala (Sunilkumar *et al*., 2005). However they had not surveyed cattle from Wayanad district. Though sheep and goat sera from Wayanad district were tested for BTV Ab, they were negative (Ravishankar *et al*., 2005). Hence this study has succeeded in establishing prevalence of the disease in cattle in this district. In India higher prevalence rate of the disease have been reported for cattle. Desai (2004) reported a high BT seroprevalence of 80% among cattle in two south Gujarat districts. A BT seroprevalence of 58.33% was observed in buffaloes in Gujarat (Chauhan *et al*., 2005).

In goats the BT seroprevalence was found to be 7.5%. A seroprevalence of 47% was observed among goats in the coastal saline areas of West Bengal (De *et al*., 2009). A BT seroprevalence of 16% among sheep was observed in the study. Bhanuprakash *et al*. (2008) reported the BT seroprevalence among sheep in five northern states of India. They were 59.5%, 72.6%, 21.4%, 35.4% and 21.4% in Maharashtra, Jammu and Kashmir, Gujarat, Rajasthan and Uttar Pradesh respectively. Among the three species tested in this study, the highest prevalence was seen in sheep. This may be due to the proximity of Palakkad district to Tamil Nadu, where a number of BT outbreaks are reported and also due to unrestricted entry of livestock from that state (Aruni *et al*., 1997; Reddy *et al*., 2008 2010; Venkataramanan *et al*., 2010).

An overall BT seroprevalence of 9.3% was observed in the present study. Ravishankar *et al*. (2005) conducted a similar study encompassing all the 14 districts of Kerala, where an overall BT seroprevalence of 5.1% was observed in sheep and goats. An overall BT seroprevalence of 33.16% was observed among sheep, goats, cattle and buffaloes of Madhya Pradesh (Sikrodia *et al*., 2012). In contrast, a high BT seroprevalence (73.8%) was reported in Tamil Nadu by Malmarugan *et al*. (2008). This variation can be due to high number of BT outbreaks in Tamil Nadu in the past.

In Kerala, the sheep population being relatively small, there have been no previous reports of BT outbreaks. Limited isolation trials carried out on samples collected from suspected BT outbreaks could not isolate the virus.

Bluetongue virus is transmitted by certain species of biting midges, *Culicoides* spp. (Schwartz-Cornil *et al*., 2008). Of the 1400 species of *Culicoides* worldwide, 39 have been found in India (Sreenivasulu *et al*., 2004). Though these midges have been trapped in Northern Kerala but its role in disseminating the disease has not been studied yet (Ravishankar *et al*., 2005). But it is assumed that *Culicoides* being the predominant biological vector of BTV, it may be the same scenario in Kerala also.

The seroprevalence (9.3%) observed in the present study is an indication of possible outbreaks in future. In Kerala goats and cattle encompass the majority of ruminant population in which BT is inapparent. However the virus has evolved globally with the emergence of BTV serotype-25 which is more pathogenic to goats (Hofmann *et al*., 2008). There are also 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 period in goat and cattle may cause

disease in these species. Moreover in India BTV has been isolated from goats suffering from Peste des Petits Ruminants (Biswas *et al*., 2010).

Restricted movement of animals from the neighboring states might help in pre-empting any incidences of the disease. Proper surveillance based on seromonitoring can forecast the possible future outbreaks. The study was intended to observe the change in the prevalence pattern of the disease from the earlier work conducted in Kerala and it was found that there is increase in the prevalence percentage. Future studies may be aimed at isolation and serotyping of the virus from the domestic ruminants of the state.

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