Type-specific seroprevalence of bluetongue in Andhra Pradesh, India, during 2005–2009

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Abstract Bluetongue (BT) is an infectious, arthropod-borne viral disease of domestic and wild ruminants caused by bluetongue virus (BTV), which is a double-stranded segmented RNA virus. Of the 26 confirmed BTV serotypes, 23 were reported in India based on the detection of antibodies or virus. In order to assess the prevalence of different serotypes in Andhra Pradesh, serum samples which were positive for BTV by group-specific antibody ELISA were subjected to type-specific neutralization of BTV serotypes 1, 2, 9, 10, 21 and 23. Of the 52 samples tested, 50.0, 44.23, 21.15, 26.92, 0, and 15.38 % neutralized BTV serotypes 1, 2, 9, 10, 21 and 23, respectively. However, 32.69 % of the ELISA positive sera could not neutralize any of these serotypes, indicating that there could be other serotype viruses (e.g., BTV-3 and -16) circulating in the State. This method can be used for surveillance of the circulating serotypes as well as for assessing the level of herd immunity, and assist in determining the vaccine strains to be used in multivalent vaccines.

Keywords Bluetongue virus · Serotype · Surveillance · Virus neutralization assay · Epidemiology

Bluetongue (BT) is one of the major economically important livestock diseases in India. The disease is caused by bluetongue virus (BTV), a double-stranded segmented RNA virus belonging to genus *Orbivirus* of family *Reoviridae*. Clinical BT is observed only in sheep, and not in other domestic or wild animals. However, antibodies against BTV are frequently observed in cattle, buffaloes, goats and some wild ruminants, indicating asymptomatic infection in these species [21, 23, 25, 27, 36]. Twenty-six different serotypes of BTV are recognized worldwide and distinct topotypes defined by closely related sequences of each genome segment have been proposed [19]. In India, 23 serotypes of BTV have been reported, identified either by serology or virus isolation, and eight serotypes (BTV-1, -2, -3, -9, -10, -16, -21, and -23) have been isolated from different regions during the last decade [4, 5, 8, 12, 17, 22, 26, 28, 30, 33, 36, 37].

BT is endemic in India. Multiple serotypes of BTV commonly circulate in the same geographical area, and can be isolated from the same flock or even from the same animals. Further, recent reports indicate the incursion of Western topotypes of BTV into India [12, 17, 18, 20, 29, 31]. Therefore, the identification of circulating serotypes of BTV is important. However, virus isolation followed by serotyping is time consuming. In addition, by the time the viruses are isolated and the serotype is determined, new serotypes may appear, resurface or dominate, making it difficult to select the serotypes for incorporation in vaccines. An inactivated polyvalent vaccine has been developed in India using five serotypes of BTV (BTV-1, -2, -10,

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-16, -23) [16]. Recently three serotypes (BTV-3, -9, and -21) have been isolated from India [5, 17, 30, 31, 37] (<http://www.ainpbt.com/achievement.html>). To select different serotypes of BTV for inclusion in polyvalent vaccines, a comprehensive and continuous type-specific sero-prevalence is needed. Attempts to carry out serotype-based surveillance were made earlier by typing samples at the World Reference Laboratory, Onderstepoort Veterinary Laboratory, but sero-surveillance of BT in India is mostly limited to detection of group-specific antibodies in different species of wild and domestic animals [3, 6, 7, 15, 21, 23, 26, 27, 32]. Thus, rapid detection of BTV serotypes is vital for understanding the epidemiology as well as to design prevention strategies.

Monitoring of sentinel herds has been used as a method of choice for identifying the circulating serotypes. Because apparently healthy sheep as well as other animal species can be infected with BTV without producing overt clinical disease, herds of such animals can serve as sentinels for routine surveillance of BTV [1, 9–11, 13, 14, 24, 34, 38]. To develop a platform for sero-surveillance, we collected sera from different parts of Andhra Pradesh and used six available Indian BTV serotypes (BTV-1, -2, -9, -10, -21, -23) in

neutralization assays to retrospectively determine serotypes circulating in Andhra Pradesh.

BTV-1 and -23 [16], BTV-2 [35], BTV-9 [31], BTV-10 [12], and BTV-21 [37] were used. The TCID₅₀ of the viruses was determined in Vero cells. In total, 1299 unvaccinated sheep serum samples are collected from different parts of Andhra Pradesh during 2005–2009 from sheep aged 0.5–3 years (see Supplementary Table 1 and 2 for details). Seropositivity was determined by competitive enzyme-linked immunosorbent assay (c-ELISA) to detect antibodies against BTV protein VP7 using a commercial kit (Veterinary Diagnostic Technology, Inc. USA). Considering percentage inhibition (PI) >50 as seropositive, of the 1299 sera tested, 788 were positive. As this was a retrospective study of stored samples, sufficient quantities of only 52 sera were available for further analysis to detect serotype-specific antibodies by neutralization assay using all the six BTV serotypes. For neutralization, 100 TCID₅₀/50 µl of virus was incubated with 50 µl of 1:20 diluted sheep serum at 37 °C for 1 h and then inoculated onto confluent monolayers of Vero cells in 96-well tissue culture plates. The cells were observed for BTV-specific cytopathology for 5 days. The ability of sera to neutralize

Fig. 1 Detection of BTV serotype-specific antibodies in sheep in Andhra Pradesh: Sera collected from sheep were subjected to group-specific type-specific assays as described in materials and methods. The map shows the presence of the antibodies to BTV-1, -2, -9, -10, -21, and -23 serotypes in different districts of the State

Fig. 2 Prevalence of BTV serotype specific antibodies in sheep in Andhra Pradesh, India: BTV positive sheep sera (52 No.) were subjected to neutralization assay with BTV-1, -2, -9, -10, -21 and -23. The graph shows number of animals positive for antibodies against individual serotypes of BTV tested

viruses was determined based on the appearance or absence of cytopathology when compared to controls as well as the back-titrated viruses. The results are shown in Figs. 1 and 2 and Supplementary Table.

Among the 52 samples, highest numbers were positive for BTV-1 (50 %) followed by BTV-2 (44.23 %), BTV-10 (26.92 %), BTV-9 (21.15 %) and BTV-23 (15.38 %). BTV-1, -2, -9, and -10 have been isolated from outbreaks during this period, corroborating these results. On the other hand, we could not detect neutralizing antibodies to BTV-21 even though it was isolated in 2007, and BTV-23 was detected only serologically, although the virus was isolated from the neighbouring States of Karnataka and Tamil Nadu. Importantly (a) some sera (32 %) did not neutralize any of the tested serotypes, suggesting the existence of other serotypes, and (b) majority of the sera neutralized more than one serotype, indicating superinfection or circulation of multiple serotypes in the sampled flocks. It is noteworthy that BTV-16 was not tested in this study but has been isolated during the study period [2, 22], and it is possible that BTV-16 may be one of the additional serotypes, although the presence of other serotypes cannot be ruled out.

Only five serotypes of BTV isolated from India were used in this study as they were the only available viruses at the time of the study and inclusion of other serotypes of BTV by importing them from outside India is risky and not acceptable. Further, large quantity of serum sample is required for testing them in neutralization using all 26 serotypes. We therefore tested against available serotypes,

and sera which are unable to neutralize any of these serotypes can be tested against all 26 serotypes in a reference laboratory.

Protective immunity against homologous serotype of BTV following infection in the field is about 3 years. This duration roughly coincides with waning immunity as well as replacement of stocks, leading to significant reduction in herd immunity, rendering the flocks more susceptible for particular serotype(s) of BTV. Based on the status of circulating serotypes in different states/regions and the status of herd immunity against these serotypes, one may be able to predict the serotype(s) which may dominate in the next season. Predicting the dominating serotypes for a specific period will limit the number of serotypes to be included in the vaccine for that season, resulting in reduction of the cost of vaccine production.

Our study was limited by the number of samples collected but encompassed most of the districts densely populated with sheep in Andhra Pradesh. Extending this pilot study further and evolving a programme for routine monitoring should contribute immensely in controlling BT in India.

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