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Epidemiology and Diagnosis of Bluetongue Virus in India

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Abstract

Bluetongue (BT) is an economically important, vector-borne, non-contagious, viral disease of ruminant animals. It is caused by the Bluetongue virus (BTV) and belongs to the genus *Orbivirus* under the family *Reoviridae*. The clinical form of the disease is reported in sheep, white-tailed deer, bighorn sheep, etc. The subclinical infections are reported from camelids, cattle, and goats. Due to its high morbidity, mortality, and huge economic loss to the livestock sector, BT is listed as a multispecies disease by World Organization for Animal Health (OIE). The diagnosis of BTV is required for the identification of etiological agents and control of disease which is essential for the international trade of livestock and its products. Some of the diagnostics techniques such as reverse transcription-polymerase chain reaction (RT-PCR), competitive enzyme-linked immunosorbent assay, agar gel immunodiffusion assay are OIE recommended tests for BTV diagnosis at the international level. For BTV control, serological and vector surveillance, vector control, mass vaccination of susceptible animals, etc. are used.

Keywords: Bluetongue virus; Vector; Culicoides; Serotype.

Introduction

Bluetongue (BT) is a viral disease of ruminants caused by the Bluetongue virus (BTV) of the genus *Orbivirus* under the family *Reoviridae*. The genome of BTV consists of 10 segments of dsRNA surrounded by icosahedral capsid. BTV encodes 7 structural (VP1-VP7) as well as 5 non-structural (NS1-NS5) proteins (Stewart et al. 2015). BTV infection led to huge economic losses which are associated with high morbidity, mortality, abortions, fetal abnormalities, stillbirths, reduced milk yield, etc. Due to severe economic loss, trade restrictions are imposed on live ruminant animals, their products, and germplasm from BT-infested to BT-free countries (Gethmann et al. 2020). BT is also listed under the multispecies disease section by World Organisation for Animal Health (OIE) (OIE, 2008). The clinical form of the disease is mostly reported from pronghorn antelope, white-tailed deer, llamas, alpacas, and sheep. However, goats, cattle, and camelids usually remain asymptomatic or may exhibit the sub clinical form of the disease (Schulz et al. 2012). The major route of transmission of BTV to the susceptible host is by the biting of *Culicoides* spp. (Benelli et al. 2017). However, BTV transmission may also take place via alternative routes such as oral, venereal, and transplacental transmission (Saminathan et al., 2020). Based on differences in the segment-2 genome sequence, there are 28 different BTV serotypes have been identified globally (Bumbarov et al. 2020). The BTV-27 was identified in France in 2014 in goat (Schulz et al. 2016) and BTV-28 from live-attenuated lumpy skin disease and sheep pox vaccines in Israel (Bumbarov et al., 2020). Being India a vast country with a tropical climate, huge ruminant animal population,

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and presence of *Culicoides* vector, several BTV serotypes have been reported (Rao et al. 2016). In a current review paper, the epidemiology of BTV along with its diagnosis and control has been discussed especially in the Indian scenario.

BTV Epidemiology in India

BT is endemic in the Indian subcontinent and causes huge economic losses to the livestock sector. Most of the BTV outbreaks in India were reported in cross-breed and exotic breeds of sheep. However, native breeds of sheep were also found susceptible to BTV in south India. Due to the endemic nature of BT in India, several BTV serotypes have been reported. Out of 28 serotypes of BTV, 23 serotypes (except serotype 22 and 25-28) have already been reported from different regions of India by virus isolation and identification of BTV specific neutralizing antibodies (Ranjan et al. 2015; Krishnajyothi et al. 2016; Hemadri et al. 2017). Previous study suggested the seropositivity of cattle, buffalo, goat, camel, and Mithun for BTV specific antibodies without exhibition of clinical form of the disease (Karam et al. 2018). Studies also suggested that most of the BTV serotypes were isolated from the southern states of India (Krishnajyothi et al. 2016; Hemadri et al. 2017).

Isolation of BTV Serotypes in India

Since the first reported case of BT in India in the 1960s, several BTV outbreaks have been recorded throughout the country. Bhambani and Singh (1968) successfully isolated the BTV in sheep in Uttar Pradesh without confirming the virus serotype. Subsequently, BTV-2 was isolated from Tamil Nadu in 1982 (Maan et al. 2012). Later on, several other serotypes were also reported in India such as BTV-2 (Ranjan et al., 2012), BTV-9 (Ranjan et al., 2013), BTV-10 (Prasad et al., 2013), BTV-16 (Dadawala et al., 2013; Ranjan et al., 2014) and BTV-23 (Ranjan et al., 2017), etc.

With the advancement in molecular techniques, other BTV serotypes such as BTV-1 from aborted and stillbirth foetuses of goats from Gujarat (Chauhan et al., 2014), BTV-12 from sheep flocks in Andhra Pradesh (Rao et al., 2015), BTV16 from goats in Tamil Nadu (Minakshi et al., 2012), BTV-16 from sheep in Karnataka (Ranjan et al., 2016), etc. were isolated. In 2010, BTV-24 was identified from sheep in Telangana during BT outbreaks. The vp2 gene sequence analysis revealed its close similarity with western BTV-24 isolates indicating the entry of exotic serotype in the Indian subcontinent (Krishnajyothi et al. 2016). Similarly, BTV-5 was isolated in Karnataka in sheep during 2010-2011 (Hemadri et al. 2017).

The first generation capillary sequencer can also be used for complete gene sequencing of BTV serotypes. In one of the studies, a capillary sequencer was used for complete gene (vp2, vp5, and ns1) sequencing of the Indian isolate of BTV-16. The result revealed that vp2 and vp5 gene was of eastern topotype origin whereas ns1 gene was belonging to western topotype, indicating the reassortment in BTV-16 in India (Kumar et al., 2013). Next-generation sequencing (NGS) or deep sequencing techniques has revolutionized molecular research. The NGS-based machines are much faster than first-generation capillary sequencing machines (Minakshi et al., 2014). The complete genome sequencing and subsequent phylogenetic analysis have assisted in the identification of several reassortant BT viruses having both eastern wells as western topotype genome segments. Full genome sequencing of BTV-2 revealed the presence of nine genome segments of the eastern topotype and one segment (Segment-5) of the western topotype in origin, indicating the genomic reassortment (Maan et al., 2012). Similarly, a reassortant strain of BTV-2 was isolated in India which showed segment-5 and segment-9 of western topotype origin and segment-2 of eastern topotype origin from other BTV-2 strains. The segment-6 of this virus was found closer to the eastern topotype strain of BTV-1 which indicated the reassortment in outer-capsid proteins (VP2 and VP5) (Maan et al. 2015). The study also revealed that the reassortment between genome segments of eastern and western origin may result in the origin of BT viruses with enhanced virulence which may cause a subsequent outbreak in Indian sheep breeds (Maan et al., 2012).

Diagnosis of Bluetongue

Diagnosis of animal viral diseases is essential for a successful control, eradication of disease, and reduction in economic losses (Hamblin, 2004; OIE 2008). BT can be tentatively identified by its clinical sign. Laboratory diagnosis of BTV depends mainly on the identification of antigens and antibodies. The antigen identification assays include virus isolation either in cell lines or embryonated chicken eggs (ECEs), immunofluorescence test, virus neutralization test, reverse transcription polymerase chain reaction (RT-PCR), real-time RT-PCR, sandwich enzyme linked immunosorbent assay (s-ELISA) (OIE 2008; Rojas et al. 2019), etc.

The RNA-Polyacrylamide gel electrophoresis

(RNA-PAGE) is a sensitive and low cost technique for the detection of genome segments of BTV. Several modifications in RNA-PAGE and silver staining for the identification of the BTV genome have been done by researchers. One such modification was carried out as the development of a novel staining method for RNA-PAGE where ultrasensitive eriochrome black t-silver staining (EBT-SS) was used to stain BTV RNA-PAGE. The novel staining protocol was found eight times more sensitive than routine silver staining of BTV RNA-PAGE (Minakshi et al., 2013). Several other studies have also highlighted the importance of RNA-PAGE in the identification of BTV genome segments (Ranjan et al. 2015; Rojas et al. 2019).

The virus neutralization test (VNT) is commonly used for serotyping of newly isolated BTV serotypes. VNT is the gold standard assay for serotyping of BTV isolates (OIE, 2008). The major disadvantage of this assay is the requirement of standard reference sera of known BTV serotypes which may be difficult especially during the first time outbreak of newer serotypes (OIE, 2008).

The sandwich ELISA (s-ELISA) or antigen-capture ELISA (Ag-ELISA) is a very sensitive assay and is commonly used in the laboratory for the detection of antigen. The MAb-based s-ELISA of a detection limit of 104 TCID50/ml was developed for direct detection of BTV in infected serum samples (Ten Haaf et al., 2017).

The immunofluorescence assay is a reliable assay for the detection of BTV serotypes. In immunofluorescence assay, MAbs or primary antibodies are subjected to bind with a secondary antibody which is tagged with specific fluorochromes viz., fluorescein isothiocyanate (FITC) to give detectable fluorescence (Rojas et al., 2019). For the identification of BTV, MAbs against group-specific VP7 protein have been widely used.

The segment 2 (vp2 gene) based primers in RT-PCR are commonly used for the identification of BTV serotype. The RT-PCR is a highly sensitive technique and can detect up to ten infectious BTV particles in cell culture grown virus (Prasad et al., 1999). Similarly, nested PCR is even more sensitive and may detect up to five BTV particles (Ayanur et al. 2016).

With the advancement in first generation capillary sequencing and next generation sequencing (NGS) technologies, a paradigm shift occurred in the detection of serotype and topotype of BTV (Maan et al., 2012). Genome sequencing generates a huge amount of sequencing data which can be used for various purposes such as designing serotype-specific primers which can be used for screening of newer BTV serotypes. The only disadvantage of sequencing technology is that it is not a cost-effective technique and is also not available in all the research institute/laboratories.

Real-time PCR is much sensitive than conventional PCR assay. It can identify the very low level of BTV specific RNA from cell culture-grown viruses, viraemic animals, a semen sample, etc. (Saminathan et al. 2020). Segment 1, segment 2, segment 5, and segment 10 based real-time PCR assay have been successfully used for diagnosis of BTV (Toussaint et al. 2007; van Rijn et al. 2012). Segment 5 (ns1 gene) of BTV is the highly conserved genome segment. Therefore, segment-5 based real-time PCR is commonly used for the identification of BTV serotypes (Vishwaradhya et al. 2013).

Apart from a direct antigen or nucleic acid detection, BTV can also be diagnosed in the animal sample by detection of BTV specific antibodies by agar gel immunodiffusion (AGID), competitive ELISA (c-ELISA), indirect ELISA (i-ELISA), serum neutralization test (SNT), etc (Rojas et al. 2019). The c-ELISA, RT-PCR, and AGID are sensitive assays and recommended by OIE for the diagnosis of BTV in international trade (Rojas et al. 2019).

The AGID is a serological assay where soluble BTV antigen is precipitated by a specific antibody in a medium made up of transparent agarose gel. AGID assay is usually used for diagnosis of group specificity of BTV using antibodies against the conserved VP7 protein of BTV (Chandel et al. 2003).

For BTV specific antibody detection, ELISA is a reliable technique. For antibody detection, different formats of ELISA are used. The c-ELISA is commonly used to detect the BTV specific antibodies in ruminant animal sera (Rojas et al. 2019) because it is a more specific, sensitive, and rapid method than other serological assays such as AGID, plaque neutralization assays, and CFT (Kramps et al. 2008). It is also extensively used for monitoring BTV during national and international trade (Rojas et al. 2019). In one of the experiments, VP7 protein based antigen capture c-ELISA was described to detect antibodies against EHDV and BTV where VP7 antigens were expressed in the baculovirus expression system (Mecham and Wilson 2004).

The other ELISA format commonly used for BTV specific antibody detection is i-ELISA. The i-ELISA is a rapid technique for the detection and quantification of antibodies in serum samples (Chand et al. 2019; Rojas et al. 2019). The NS3 antigen-based i-ELISA has been employed for the

differentiation of BTV vaccinated animals to the BTV infected ones. In infected animals, higher levels of NS3 specific antibodies are detected in comparison to vaccinated animals. Thus, i-ELISA can be used for the DIVA strategy whereas c-ELISA can't be used for DIVA (Rojas et al. 2019). The major disadvantage of i-ELISA is that it requires species-specific secondary antibody conjugates, which may create a practical problem for routine sero-diagnosis of BT like multi species disease (Chand et al. 2017).

Control and Prevention of BTV

BTV outbreaks can be controlled by mass vaccination of susceptible ruminant animals, vector control by use of insecticides, vector repellents and attractants (decoy host), and larviciding agents, serological as well as vector surveillance and sentinel program (Mayo et al. 2017; van Rijn, 2019; Ranjan et al., 2019). In India, a pentavalent inactivated adjuvant vaccine having serotypes 1, 2, 10, 16, and 23 has been developed and licensed for the BT control program (Reddy et al. 2010).

Conclusions and Future Perspectives

BT is an endemic disease in India due to the hot and humid climate which favors Culicoides breeding. Most of the BTV serotypes (23 out of 28 reported) have been identified from India probably due to the reassortment of BTV genome segments, a large number of ruminant animals, and the Culicoides vector population. BTV study in India suggested that regular sero surveillance programs should be maintained to monitor the emerging, re-emerging, and endemic nature of BTV serotypes. The data from such surveillance programs are a gold mine for the development of an area specific multivalent vaccine that can be used as a tool for the control of BTV in endemic regions. Moreover, for effective BT control, vector control programs need to be executed in endemic regions. Simultaneously, the role of wild ruminants and vectors in wildlife habitats should also be monitored from an epidemiological point of view for successful BTV control because wild ruminants may act as a reservoir host for BTV along with vector maintenance. Moreover, the interface of domestic and wild ruminants near the forest areas should also be monitored to control the spread of BTV to domestic ruminants.

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