

REVIEW

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# Prevalence and sero-epidemiology of bluetongue with special reference to eastern and north-eastern states of India

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## Abstract

**Background:** Bluetongue (BT), an infectious, non-contagious arthropod borne viral disease of domestic and wild ruminants is caused by bluetongue virus (BTV), a type species of *Orbivirus* genus and *Reoviridae* family. Albeit bluetongue in India is endemic to southern and western parts, eastern and north-eastern parts of the country didn't experience any outbreak or reported active disease.

**Main body of abstract:** International, national and regional disease statuses including some un-published data were narrated to give a clear picture. Until 1940s, BT was mainly restricted to African countries, however started spearheading to Asian and European countries subsequently. Extensive epidemiological surveys were conducted in central parts of America and Caribbean countries during late 80's. Massive ingress of BT was observed in northern Europe 2006 onward. In India, although first reported in 1964, BT became endemic in southern states during 1980s and became alarming in late 1990s. A thorough sero-epidemiological study was conducted in different agro-climatic zones of eastern and north-eastern states of India during 2001 to 2017. Sero-epidemiological information of different states had been tabulated. This was the first comprehensive document of BT sero-epidemiology of eastern and north-eastern parts of India. Although BT was not reported officially, sero-epidemiology showed BTV was in circulation in animals and vectors of eastern and north-eastern states of India. Published as also un-published data clearly showed animals and vectors harbor BTV in this part of the country.

**Short conclusion:** All the epidemiological components of BT are present in eastern and north-eastern parts of India that warrants presence of the disease in these parts also. Hence, further surveillance and preparedness are recommended to avoid future BT outbreak, if any.

**Keywords:** Bluetongue, Culicoides, Ruminants, Sero-epidemiology, Vector, Virus

## Background

Bluetongue (BT) is an infectious, non-contagious arthropod borne viral disease of domestic and wild ruminants. The disease has been recognized not only in sheep, goat and cattle but also in other domestic animals like water buffaloes, camels and wild ruminants (white tailed deer, elk, blesbuck and elephants) (Ruiz-Fons et al., 2008). In

sheep the disease is acute and mortality may be high, whereas in goats and cattle the disease is milder. In contrast to sheep, infected bovines experience prolonged viraemia and act as reservoir hosts for BT virus (MacLachlan & Dubovi, 2011). For many decades the disease was believed to be confined to the South African continent. The first outbreak occurred in the island of Cyprus. Although BT was first reported in 1964 in India (Maharashtra state) based on clinical signs and detection of anti-bluetongue antibodies (Sapre, 1964), isolation of virus could be made only during 1972. In India, the disease is reported to be an important emerging disease

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affecting large number of sheep. The disease is now included within the category of multiple species disease as per the World Animal Health Information System-OIE, which prevents the export of animals, their products and germplasm from bluetongue endemic to bluetongue free country, thus disrupting international trade.

The bluetongue virus (BTV), a type species of *Orbivirus* genus and *Reoviridae* family, is the causative agent of bluetongue disease (Attoui et al., 2009). BTV is transmitted by *Culicoides* midges. *Culicoides* midges have been shown to transmit the virus biologically between sheep, from cattle to sheep, between cattle, between domestic ruminants to wild and from wild to domestic ruminants. After infection, bluetongue virus replicates in *Culicoides* and the fly remains infected for life. BT is usually enzootic in areas where *Culicoides* vectors are present for most of the times of the year, thus maintaining a continuous cycle of the virus. The species of *Culicoides* are most active in the temperature range of 18–29 °C and become almost inactive below 10 °C or above 30 °C. Though there are more than 1400 species of *Culicoides* worldwide, less than 20 are known to transmit BTV (Chand et al., 2014). These include *C. imicola*, *C. veripennis*, *C. fulvus*, *C. actoni*, *C. wadi*, *C. nubeculosis* etc. *Culicoides* vectors usually strike sheep flocks during late evening. Only female midges take blood meal from vertebrate hosts prior to egg lay. The disease is prevalent in late summer and early autumn that provide congenial environment for multiplication of vectors.

The disease in sheep is characterized by high fever, depression, catarrhal inflammation and oedema causing swelling of lips, nose, face, sub-mandibulum, eyelids and ears, congestion of buccal and nasal mucous membrane, conjunctivitis, hyperaemia of skin (groin, axilla and perineum) and coronary bands, lameness due to coronitis or degeneration of skeletal muscle. Congested tongue becomes swollen and cyanotic. There is serous nasal discharge that becomes mucopurulent later (MacLachlan & Dubovi, 2011).

Bluetongue in India is endemic to Tamil Nadu, Andhra Pradesh and Karnataka. Sporadic reports are available from Maharashtra, Gujarat, Rajasthan, Haryana, Himachal Pradesh and Jammu & Kashmir. Till date, 29 BTV serotypes have been identified worldwide (Yang et al., 2021). A total of 23 serotypes (except 22, 25–29) have been reported from India by serological assays and/or virus isolation mostly in Maharashtra, Uttar Pradesh, Tamil Nadu, Andhra Pradesh with scanty evidences from Haryana, Himachal Pradesh, Karnataka, Jammu and Kashmir, West Bengal, Rajasthan, Gujarat, Uttarakhand, Madhya Pradesh (Chand et al., 2014; Hemadri et al., 2017). So far, 15 serotypes (BTV-1 through 6, 9, 10, 12, 16 through 18, 21, 23 and 24) were identified by

virus isolation, while 22 serotypes (BTV-1 through 20, 23 and 24) were reported by serological testing (Saminathan et al., 2020). The occurrences of bluetongue have variations in different parts of India depending on time of rainfall. Maximum numbers of outbreaks were recorded during north-east monsoon period (October to December) followed by the south west monsoon period (June to September) in Andhra Pradesh (Anon., 2014). Similarly in Tamil Nadu the outbreaks were more frequent during the north-east monsoon period. In Rajasthan, the outbreaks were mostly recorded during months of September and November.

Eastern and north-eastern parts of the country didn't experience any outbreak or reported active disease. But that doesn't warrant declaring the states as bluetongue free; as always there exists a complex interaction between BTV, *Culicoides* midges (vector), susceptible hosts (sheep, goats, cattle, buffalo and wild animals) and environmental factors that finally give rise to active disease. It is quite expedient to explore this interaction in a holistic manner to reveal the prevalence of sub-clinical bluetongue and circulating BTV in the states, if any. Hence a thorough sero-epidemiological study was conducted in the eastern and north-eastern states of India primarily followed by isolation and characterization of bluetongue virus (BTV) from susceptible hosts and vectors. The study was conducted July, 2001 through March, 2017.

## Main text

### Prevalence of bluetongue: historical perspective

#### International scenario

In South Africa, bluetongue shattered the livestock industry for long, may be from the very beginning of sheep farming. Henning (1949) noted that the Report of the Cattle and Sheep Diseases Commission for 1876 stated: "For many years, if not from the time of the introduction of the Merino sheep into the Colony, there has been prevalent among the flocks; a disease known as "fever". This disease is most prevalent during the summer months, and is very much worse in wet seasons." Hutchison reported bluetongue in 1881, referring to it as "fever or epizootic catarrh" in sheep. Spreull (1905) was the first to study the disease in detail. In 1933, bluetongue was recognized for the first time as a disease of cattle. In these animals it apparently gave rise to a stomatitic infection similar to foot-and-mouth disease.

In 1943, an outbreak occurred in Cyprus and later in Turkey (Gambles, 1949). Bluetongue caused a major epizootic during 1956–57 in Portugal and Spain in which 1,79,000 sheep died resulting in 75% mortality rate (Manso-Ribiero & Noronha, 1957). In Israel, the disease was reported in 1951 (Komorov & Goldsmit, 1951).

BT disease was reported in Japan by many workers (Omori, 1961; Inaba et al., 1966; Ishitani, 1967).

Australia was considered to be free from BT disease till 1977. However, bluetongue virus (BTV) was isolated from insect and healthy cattle in 1978 (St. George et al., 1980).

Bluetongue was first described in South Africa, where it has probably been endemic in wild ruminants since antiquity. It is currently recognized that BT is endemic throughout most of South Africa and 22 of the 26 known serotypes have been detected in the region. Multiple serotypes circulate each vector season with the occurrence of different serotypes depending largely on herd-immunity. Indigenous sheep breeds, cattle and wild ruminants are frequently infected but rarely demonstrate clinical signs, whereas improved European sheep breeds are most susceptible (Coetzee et al., 2012).

The central focus of BTV research was roaming around Africa. However, it became increasingly clear that the being unrecognized BTV was circulating in some countries across a broad endemic range encompassing tropical, subtropical and even some temperate regions, reaching to almost 50°N in North America (Dulac et al., 1989).

Between 1987 and 1992, a regional prospective study was carried out on the epidemiology of bluetongue virus (BTV) serotypes covering 11 countries in Central America and the Caribbean. In this study, over 300 BTV isolations were done from cattle and sheep. Extensive network of sentinel herds in the Central America detected BTV-1 as the predominant serotype besides BTV-3 and BTV-6. In Puerto Rico and the Dominican Republic, BTV-4 became the predominant serotype, without detection of BTV-8 and BTV-17, which were common in one of the studies (Mo et al., 1994).

In 1998, BTV-9 was detected on four Greek islands (Rhodes, Leros, Kos and Samos). It was considered as the most extensive, prolonged and costly period of BTV incursions into Europe in recorded history (Mellor & Wittmann, 2002; Purse et al., 2005).

The involvement of bluetongue virus (BTV-2 and BTV-9) was confirmed serologically and virologically in the outbreaks and mortalities (about 11%) of three sheep holdings communities of Italy at San Gregorio Magno, Laviano and Carpino between July and September 2002 (Savini et al., 2005).

BTV was detected in northern Europe for the first time in 2006. The outbreak of bluetongue, caused by a BTV-8 strain, thought to be of sub-Saharan origin (Maan et al., 2008).

BT was noticed in a sheep flock at the border of Belgium and the Netherlands in 2006 and very soon spread across Belgium, Netherlands, Germany, Luxemburg and

France (Toussaint et al., 2007 and Meroc et al., 2008). The outbreak also reported clinical disease in sheep and cattle (Thiry et al., 2006). The virus responsible for this epidemic was isolated and identified as BTV-8, a serotype not previously encountered in Europe (Toussaint et al., 2007). Besides livestock, red deer (*Cervus elaphus*) was also found to be susceptible for BTV-8 as shown by sero-conversion in 2006 outbreak (Linden et al., 2008).

Re-emerging bluetongue virus serotype 8 (BTV-8) in Germany was detected first in May 2007 in a sentinel cow and in February 2008 in an export heifer (Hoffmann et al., 2008a).

A few new field strains have been isolated from the Caribbean region during 2012 (Legisa et al., 2013). These strains were obtained in French Martinique Island (BTV 2, 10, 11, 13, 17, 18, 22, and 24) and Guadeloupe Island (BTV5 and 17).

Lager et al. (2004) isolated BTV4 from sentinel cattle which sero-converted in 1999–2001 from Argentina's northeast region. Clavijo and co-workers isolated BTV12 from an outbreak reported in Parana state in Brazil (Clavijo et al., 2012).

During January–April 2014 samples from goats were analyzed and the virus showed genome sequence was similar to that of the BTV-25 segment 2. The virus showed highest identity with the BTV-25 and BTV-26 serotypes circulating in Switzerland and Kuwait, respectively (Maan et al., 2011; Hofmann et al., 2008b).

The complete segment 2 sequence of the new virus from Corsica (termed BTV-n) shared 73% nucleotide and 75% amino acid identity with BTV-25 sequences and 65% nucleotide and 60% amino acid identity with BTV-26 sequences (Zientara et al., 2014).

The clinical, pathological and epidemiological aspects of 17 outbreaks of bluetongue (BT) disease in sheep occurring between December 2014 and July 2015 in the central region of Rio Grande do Sul state (RS), southern Brazil was reported. The virus involved in the outbreaks was subsequently isolated and shown to belong to serotype 17, for the first time reported in Brazil (Bianchi et al., 2017).

#### **Indian scenario**

The first outbreak of BT in sheep and goats in the country was recorded in 1964 in Maharashtra by Sapre (Sapre, 1964). In 1973, an outbreak of BT was recorded in Russian Merino sheep, on Kothipura Farm in Himachal Pradesh (Uppal & Vasudevan, 1980). Later, a further outbreak of BT was reported in 1975 in exotic 'Corriedale' sheep in the Central Sheep Breeding Farm (CSBF), Hisar, Haryana.

An outbreak of BT in sheep in Bidar, Gulbarga and nine other districts of Karnataka was reported where

morbidity was 50% (Vasudevan, 1982; Srinivas et al., 1982).

BT has been reported in several states of India, including northern (Haryana, Himachal Pradesh, Jammu and Kashmir, Punjab, Rajasthan and Uttar Pradesh), central (Madhya Pradesh), western (Gujarat and Maharashtra) and southern areas (Andhra Pradesh, Karnataka, and Tamil Nadu). However, there are no reports of BT in the northeastern states of India (Prasad, 2000; Prasad & Srivastava, 1995; Sreenivasulu et al., 1996; Srivastava et al., 1995).

An outbreak of BT occurred in eastern Maharashtra adjoining to the Telangana region of Andhra Pradesh (southern India), which later spread to western Marathwada, affecting sheep in all districts of the region with up to 80% morbidity in some village flocks (Singh et al., 1982).

Harbola et al. (1982) recorded an outbreak of BT in Maharashtra state and suggested that crosses of the native Chokla breed with Merinos were more susceptible than the native breed itself, although Deccani sheep (another native breed) also showed clinical signs during the outbreak. Of 8,980 exotic, crossbred and native sheep, 868 were affected clinically, 100 of which died, giving an overall case fatality rate of 11.5%.

In 1983, an outbreak of BT was recorded in Rajasthan, in which Rambouillet and Merino breeds were found to be more susceptible than native breeds. The morbidity rate in Merino and Rambouillet was 33% and 24%, respectively. Sharma et al., (1985) conducted a systematic epidemiological study in the Central Sheep and Wool Research Institute (CSWRI), Rajasthan and reported a fatality rate of 15%.

An epidemiological study carried out in Central Sheep Breeding Farm (CSBF), Hisar, revealed mortality and case fatality rates of 13.3% and 31%, respectively in 1985, which increased to 16.7% and 43%, respectively in 1986. In 1987 and 1988 the mortality rate fell back to 7% (Mahajan et al., 1991).

A widespread outbreak of BT in the native sheep of Tamil Nadu occurred during November and mid-December 1989 (Mehrotra et al., 1991). A concurrent outbreak of BT and morbillivirus (PPRV) in sheep of Maharashtra was reported by Mehrotra et al. (1996). The morbidity and mortality was observed among indigenous and exotic breeds of sheep and in all outbreaks distinct clinical disease was distinct.

Kulkarni et al. (1992) also reported severe outbreaks of BT in native sheep in rural areas of Maharashtra, with overall morbidity, mortality and case fatality rates of 32%, 8% and 25%, respectively.

All of the southern Indian breeds of sheep were found to be susceptible to disease and developed clinical BT,

although Saravanabava (1992) reported variations in susceptibility between breeds.

Bluetongue outbreak was studied in detail in six western districts of Tamil Nadu namely, Namakkal, Salem, Erode, Coimbatore, Karur and Dindigul during 2003–2006. The classical signs of cyanosis of the tongue and reddening of the coronary band in native sheep, Macheri breed, were observed (Anon, 2006).

During 1995–2007, a total number of 4124 outbreaks were observed in different parts of Andhra Pradesh. During the outbreak, 103658 numbers of animals died and the case fatality rate was 20.86 (Anon, 2008a).

During the year 2007–2008, there were 142 outbreaks of the disease reported in seven districts covering the Eastern and Central parts of Karnataka. Totally 2685 animals were affected resulting in 410 deaths among the small ruminants (Anon, 2008b).

During the year 2013–2014, 114 outbreaks were reported from Karnataka. Out of those outbreaks 452 blood samples were collected and tested. Total 77 samples were positive for bluetongue virus by RT-PCR (Anon, 2014).

## Sero-epidemiology of bluetongue

### *International status*

Seroprevalence study on BTV during 1982 through 1984 employing 2550 cattle serum samples randomly selected from 274 herds revealed that 39% of the serum samples were positive in Louisiana State as assessed by Enzyme Linked Immunosorbent Assay (ELISA) (Hugh-Jones et al., 1989).

In an epidemiological survey on sero-incidence of BTV in Jiangsu province of China, 68 (1.5%) out of 3853 cattle sera and 63 (2.2%) of 2426 sheep and goat sera were detected as positive, respectively as assessed by ELISA (Chang et al., 1995).

To estimate sero-prevalence of bluetongue virus (BTV) and the geographic distribution of sero-positive cattle herds in Illinois and western Indiana, serum samples (10,585) obtained from cattle was tested using competitive ELISA. Overall, BTV antibodies were detected in 156 (1.5%) samples. In the southernmost zone, annual seroprevalence ranged from 8.65% to 11.00%. Adult cattle were 2.35 times as likely as juvenile cattle to be seropositive (Boyer et al., 2007).

A total of 204 goat samples (10.2%) from 38 locations with goats (46.9%) were seropositive. A total of 1,627 sheep samples (38.3%) from 149 locations with sheep (69.6%) were seropositive in Netherlands during 2006 (Elbers et al., 2008).

The presence of antibodies to BTV in wild ruminants was studied in Belgium and Germany. In 2006, BTV



seroprevalence was 0.58% in Belgian cervids (Linden et al., 2008). The survey in Germany showed BTV seroprevalence rates of 0.09% in red deer, 5.7% in roe deer, and 4.9% in mouflon (Francisco et al., 2008).

Out of 1898 serum samples (439 roe deer, 480 red deer, 473 chamois, 506 ibex) screened for BTV antibodies by ELISA, 118 showed an optical density percentage (ODP, inversely proportional to the optical density OD) value greater than 30%, which was the cut-off determined for this study. Ten of the 118 samples with an ODP > 30% was observed in Switzerland. Overall, estimated seroprevalence was 0.5% (95% confidence interval [CI] 0.3–1%) (Casaubon et al., 2013).

A total of 756 serum samples were collected from west and northwest provinces of Iran during 2007–2008. Sera were tested with competitive enzyme-linked immunosorbent assay (c-ELISA). The seroprevalence rate in sheep was 40.87% (Khezri & Azimi, 2013a).

A total of 996 sheep sera were collected from 8 provinces in Iran and tested for Bluetongue virus specific antibody using c-ELISA. The results showed that the seroprevalence of BT in sheep was 34.93%, with the highest and lowest prevalence seen in West-Azerbaijan (64.86%) and Qom (12.1%) areas, respectively (Khezri & Azimi, 2013b).

A total number of 1293 animal serum samples (sheep-1028 and goats-265) were collected during the vector breeding time from September to November, 2010 in Egypt. A substantial number of sheep (17.5%) and goat (14.7%) were found positive when tested by Agar Gel Immuno-precipitation Test (AGPT) (Mahmoud & Khafagi, 2014).

A sero-epidemiological study was conducted to determine the seroprevalence and associated risk factors of bluetongue in small ruminants of South Western Ethiopia. A total of

422 serum samples were screened for the presence of bluetongue virus (BTV) specific antibodies using c-ELISA and 30.6% of the sheep and goat serum samples were found positive (Abera et al., 2018).

### **Indian status**

While assessing serum samples for anti-BT antibodies from seven states (Andhra Pradesh, Karnataka, Gujarat, Punjab, Orissa, Himachal Pradesh and West Bengal) during early 80's, 18% of cattle sera were found positive for BTV antibodies (Mehrotra & Shukla, 1984).

Oberoi et al. (1988) demonstrated the presence of BTV specific antibodies in 70% of cattle sera in Punjab state. In Haryana, 4.2% cattle sera were found positive for BT antibodies (Jain et al., 1992).

One c-ELISA directed against VP7 of BTV was used with 133 sheep, 134 goats, 147 cattle and 43 buffalo

serum samples for BTV precipitating antibody. Twenty five sheep sera (18.7%) from Haryana, Himachal Pradesh and Punjab were found positive. Out of 134 goat serum samples screened from Haryana, HP and Rajasthan, 102 (76%) were found positive for BTV antibody while of 137 cattle serum samples, 83 (60.5%) were found positive for BTV antibody. Of the 43 buffalo serum samples collected from Punjab and Haryana, 26 (16.5%) were found positive for BTV antibody (Naresh & Prasad, 1995).

When c-ELISA was employed to detect incidence of BTV with 26 cattle sera, the incidence varied from 17 to 89% (Singer et al., 1997).

While conducting serological survey on BTV in cattle at selected sites in Andhra Pradesh, Karnataka and Tamilnadu by c-ELISA using polyclonal antibody it was recorded seroprevalence rate as 65.91%, 79.51% and 80.95% in Andhra Pradesh, Karnataka and Tamilnadu, respectively (Dayakar et al., 2001).

Kakker et al. (2002) carried out extensive serological survey in northern India (Haryana, Himachal Pradesh, Punjab, Jammu & Kashmir and Rajasthan) and detected BTV specific antibodies in 23.4% of the cattle tested.

A comparative study was done to assess the performance of the standard AGID test and the c-ELISA for detection of antibody against BTV in clinically healthy and diseased camels in Gujarat. Out of 176 sera tested, 22 (12.55%) and 34 (19.3%) were positive for group specific bluetongue antibodies by AGID and cELISA, respectively. Maximum seropositivities of 18% by AGID and 25.8% by c-ELISA were recorded in Kutchhi breed, and of 6.9% and 12.65%, respectively, in Marwari breed (Chandel et al., 2003).

A total of 1,010 sera collected from the 14 districts of Kerala were screened for the presence of group-specific BTV antibodies by dot enzyme-linked immunosorbent assay (dot ELISA). The overall BTV antibody prevalence was  $5.1 \pm 1.9\%$  (Ravishankar et al., 2005).

In Maharashtra State, a total of 453 sera comprising 218 sheep, 148 goats, 52 cattle and 35 buffaloes were collected and screened by c-ELISA for qualitative analysis of the BT antibodies. A high percentage of animals showed BT antibodies consisting of 40.36% sheep, 64.86% goats, 30.76% cattle and 37.14% buffaloes. The overall percentage of BT positive animals was 47.01% (Waghmare & Deshmukh, 2006).

While conducting serological survey on BTV in goat in Nagpur district of Vidarbha region by c-ELISA it was found, out of 250, 129 (51.60%) serum samples were positive. The highest prevalence was observed in Bhivapur tehsil (68.29%) followed by Kalmeshwar (64.51%) and in Nagpur Rural (57.57%) (Potey et al., 2006).

In West Bengal one serological assessment was conducted for BT infection using different species of animals

(322 sheep, 104 goats and 74 cattle). Out of 500 serum samples screened by c-ELISA, 111 (34.47%) sheep, 25 (24.03%) goats and 12 (16.21%) cattle were found to be positive (Chakrabarti et al., 2007).

Serological surveillance of bluetongue virus (BTV) group-specific antibody in goats of the coastal saline (Sunder ban) area of West Bengal was conducted. Field-collected goat sera ( $n=1202$ ) were tested and a serological prevalence rate of 47% was observed in the study area (De et al., 2008).

When c-ELISA was performed to assess anti-bluetongue antibodies in sheep, goat and cattle of different agro-climatic zones (Old alluvial, New alluvial, Red laterite and Coastal saline) of West Bengal, it was found that 66.97% of goat, 59.68% of sheep and 33.33% of cattle were positive. The overall sero-positivity was 60.44% (Biswas et al., 2009).

The sero-epidemiological investigation on bluetongue was carried out from November 2007 to March 2008 in Jammu province. A total of 200 sera (61 from sheep and 139 from goat) were screened using indirect-ELISA (i-ELISA) for detection of bluetongue virus group-specific antibody. An overall sero-prevalence of 29.5% was found, which was significantly higher ( $P<0.05$ ) in goats (37.4%) as compared to sheep (11.5%). The prevalence in goats was highest in district Kathua (59.1%) followed by Udhampur (45.0%), Samba (32.4%) and Jammu (30.2%). In sheep, it was highest in Samba (25.0%) followed by Kathua (22.2%) and Udhampur (6.8%) districts (Singh et al., 2009).

When 302 numbers of animals comprising 137 sheep, 115 goat and 50 cattle, covering five agro-climatic zones (Old alluvial, New alluvial, Red laterite, Coastal saline and Terai) and seven districts (Jalpaiguri, Dakshin Dinajpur, Malda, Nadia, Burdwan and South 24 Parganas) of West Bengal were screened by i-ELISA, 66.95% goat, 57.66% sheep and 52% cattle were found to have anti-bluetongue antibodies (Panda et al., 2011).

During 2009 when sheep serum samples of West Bengal were assessed by i-ELISA, it was found that 47.72% serum samples possessed anti-BT antibody. In case of goat and cattle the percentages were 55.66 and 56.82, respectively (Halder, 2011).

When 160 sheep sera of West Bengal were collected and screened by both c-ELISA and dipstick ELISA, 54 samples (33.75%) and 51 samples (31.87%) were found positive, respectively (Mandal et al., 2012).

A sero-surveillance conducted in the Pithoragarh hills of Uttarakhand in India during the autumn of 2011. Of the 51 collected samples, 18 (35%) were positive to an indirect ELISA and 33 (64%) resulted positive to a BTV ELISA antigen (Mondal et al., 2013). The goat serum samples were found to contain high titer of neutralizing

antibodies against BTV-23, nonetheless the virus could not be isolated.

Out of 313 animal serum samples screened (sheep-68, goat-195 and cattle-50), 58.82% of sheep, 31.79% of goat and 70.00% of cattle samples were found positive in Assam. The overall seropositivity against anti-bluetongue antibodies ranged from 31–50% in different agro-climatic zones of Assam (Joardar et al., 2013).

A total of 91 serum and 26 whole blood samples were obtained from sheep and goat from Uttar Pradesh to conduct a study between September and November, 2012. Of the 91 small samples examined, 26 (28.6%) were found to harbor BTV antibody by c-ELISA. Similarly, species level prevalence of BTV antibody was 8 (13.8%) and 18 (54.5%) in sheep and goats, respectively (Bitew et al., 2013).

When total 364 animal sera were screened by i-ELISA, 32 of sheep (26.66%), 35 of goat (31.25%) and 69 (52.27%) of cattle samples were found positive in Orissa (Joardar et al., 2014).

During the year 2013–14, 865 serum samples were screened by c-ELISA for studies on seroprevalence of bluetongue in Hyderabad. The samples screened gave a positive result of 82% in sheep, 86.2% in bovine and 62.7% in goat (Anon, 2014).

#### Sero-epidemiology of eastern and north-eastern states of India

Blood samples were collected from susceptible animals, viz. sheep, goat and cattle of different eastern and North Eastern (NE) states, viz. Jharkhand, West Bengal, Meghalaya, Assam, Manipur, Nagaland and Mizoram. Sera were separated and stored at  $-20^{\circ}\text{C}$ . Serum samples were screened by i-ELISA using one laboratory developed i-ELISA based kit. This kit is developed and standardized by Mukteswar centre of All India Network Programme on Bluetongue (AINP-BT) scheme of Indian Council of Agricultural Research- Indian Veterinary Research Institute (ICAR-IVRI), Mukteswar campus, Nainital (Uttarakhand). The standardized procedure was described elsewhere (Joardar et al., 2013; Panda et al., 2011). Diluted VP-7 based recombinant BTV group specific antigen was used for coating the plates. Anti-species,

**Table 1** Seroprevalence of anti-BTV antibodies among West Bengal animals

Species	Number of samples collected & tested	Number of positive samples	Percent (%) positivity
Sheep	1064	440	41.35
Goat	1307	613	46.90
Cattle	377	137	36.33
Overall	2748	1190	43.30

**Table 2** Seroprevalence of anti-BTV antibodies among Bihar animals

Animal species	Name of the district	Number of sample collected and tested	Number of positive samples	% positivity
Sheep	Begusarai	41	28	68.29
Goat	Bhagalpur	99	31	31.93
	Gaya	80	19	23.75
Cattle	Begusarai	65	53	81.54
Buffalo	Purnia	30	14	46.66
Total		315	145	46.03

viz. anti-sheep, anti-goat, anti-cattle horse radish peroxidase coupled secondary immunoconjugates were used for detecting the polyclonal antibodies of the animal sera.

A total of 7346 animal serum samples (sheep-2147, goat- 3290, cattle- 1844 and mithun-35, buffalo-30) were tested for sero-monitoring to detect anti-BTV antibodies. The test sera were collected from different parts of West Bengal, Bihar, Orissa, Jharkhand, Sikkim, Assam, Meghalaya, Nagaland, Manipur, Mizoram and Tripura. Out of the total samples tested, 3065 samples (sheep- 797, goat-1369, cattle- 1844, buffalo-14 & mithun- 19) were found positive to anti-BTV antibodies. The percent (%) positivity of animals includes sheep–37.12, goats– 41.61, cattle–46.96, mithun-54.28 and buffalo-46.66.

The state-wise results are described below-

#### West Bengal

Total 2748 serum samples (sheep-1064, goat-1307, cattle-377) were collected from different districts (agro-climatic zones) of West Bengal and tested subsequently. The results showed 41.35% sheep, 46.90% goat and 36.33% serum samples were positive (Table 1). These results are summation of certain previous reports documented from the laboratory of AINP-BT, Kolkata centre (Biswas et al., 2009; Chakrabarti et al., 2007; Halder et al., 2016; Mandal et al., 2011; Panda et al., 2011).

#### Bihar

Out of 315 serum samples (41 sheep, 179 goat, 65 cattle & 30 buffalo) screened for anti-BT antibodies, an overall sero-prevalence of 68.29%, 27.93%, 81.54% and 46.66% was observed in sheep, goats, cattle and buffaloes, respectively (Table 2) [un-published data].

District-wise distribution of goat serum samples along with their assessment of anti-bluetongue antibodies are depicted in the Table 2. Highest seropositivity was observed in cattle followed by sheep, buffaloes and goats.

#### Jharkhand

Out of total 480 animal serum samples (sheep-190, goats-210 and cattle-80) screened in Jharkhand, 43.68%

**Table 3** Seroprevalence of anti-BTV antibodies among Jharkhand animals

Species	Number of samples collected and tested	Number of positive samples	Percent (%) positivity
Sheep	190	83	43.68
Goat	210	91	43.33
Cattle	80	46	57.50
Total	480	220	45.83

**Table 4** Seroprevalence of anti-BTV antibodies among Orissa animals

Species	Number of samples collected and tested	Number of positive samples	Percent (%) positivity
Sheep	120	32	26.66
Goat	112	35	31.25
Cattle	132	69	52.27
Overall	364	136	37.36

of sheep, 43.33% of goat and 57.50% of cattle sera were found positive (Table 3). The % positivity ranged between 41 and 51% in different agro-climatic zones. The results showed slight higher seroprevalence in cattle than sheep and goats in different agro climatic zones of Jharkhand.

The incidence of BT not detected officially, so far, the present seroprevalence status of BT in Jharkhand indicates presence of BT infection in the state for the first time (Tigga et al., 2015a, b).

#### Orissa

Out of total 364 animal serum samples screened (sheep-120, goat-112 and cattle-132) in different districts of Orissa, viz. Cuttack, Mayurbhanj, Khurda and Bhubaneswar, 26.66% of sheep, 31.25% of goat and 52.27% of cattle serum samples were found positive for the presence of anti-BTV antibodies (Table 4). The results showed slight higher seroprevalence in cattle than goats and sheep in different districts of Orissa. Outbreak/incidence of BT in animals not being reported in Orissa, the study indicated

**Table 5** Seroprevalence of anti-BTV antibodies among Sikkim animals

Species	Number of samples collected and tested	Number of positive samples	Percent (%) positivity
Sheep	49	9	18.36
Goat	200	105	52.50
Cattle	81	63	77.77
Total	330	177	53.63

**Table 6** Seroprevalence of anti-BTV antibodies among Assam animals

Species	Number of samples collected and tested	Number of positive samples	Positivity (%)
Sheep	204	120	58.82
Goat	355	94	26.47
Cattle	170	103	60.58
Total	729	317	43.48

presence circulating BTV in the state (Joardar et al., 2014).

#### Sikkim

A total of 330 serum samples (49 sheep, 200 goat and 81 cattle) screened for anti-BTV antibodies showed an overall sero-prevalence of 53.63%.

The species-wise seropositivity for anti-BTV antibodies found was 18.36%, 52.50% and 77.77% in sheep, goat and cattle, respectively. Species-wise distribution of serum samples along with their assessment of anti-bluetongue virus antibodies are depicted in the Table 5.

Earlier, seroprevalence of bluetongue in goats and cattle of Sikkim was reported indicating presence of potent vector midges and circulating bluetongue virus (BTV) in the state (Ramudamu et al., 2017).

#### Assam

Out of total 729 serum samples (sheep-204, goat-355, cattle-170) screened, 58.82% of sheep, 26.47% of goat and 60.58% of cattle serum samples were found positive (Table 6). Presence of anti-bluetongue virus antibodies was found highest in cattle population followed by sheep and goat. The prevalence in different agro climatic zones ranged between 30–50%. Incidence of bluetongue in animals of Assam being not reported so far; the present seroprevalence status of bluetongue in Assam indicates presence of sub-clinical infection in the state for the first time (Joardar et al., 2013).

**Table 7** Seroprevalence of anti-BTV antibodies among Meghalaya animals

Species	Number of samples collected and tested	Number of positive samples	Percent (%) positivity
Sheep	147	43	29.25
Goat	188	114	60.63
Cattle	367	168	45.77
Total	702	325	46.29

**Table 8** Seroprevalence of anti-BTV antibodies among Manipur animals

Species	Number of samples collected and tested	Number of positive samples	Percent (%) positivity
Sheep	332	70	21.08
Goat	603	208	34.49
Cattle	502	196	39.04
Total	1437	474	32.98

#### Meghalaya

A total of 702 (sheep-147, goat-188, cattle-367) serum samples were collected from different districts (agro-climatic zones) of Meghalaya and tested subsequently. Forty three 29.25% sheep, 60.63% goat and 45.77% cattle were found to possess anti-BTV antibodies. Presence of anti-bluetongue antibodies was found highest in goat population followed by cattle and sheep (Table 7).

The study reported first time the sero-prevalence status of bluetongue in Meghalaya which might indicate the presence of circulating bluetongue virus in the state (Nongdhar et al., 2017).

#### Manipur

Out of total 1437 animal serum samples screened (sheep-332, goat-603 cattle-502) in Manipur, 21.08% of sheep, 34.49% of goat and 39.04% of cattle serum samples were found positive. This study revealed seroprevalence of bluetongue in cattle, goats and sheep of Manipur in descending order (Table 8).

Earlier also seroprevalence of sheep was reported lower than cattle and goats (Joardar et al., 2012).

#### Tripura

Out of total 195 samples (goat- 136 and cattle- 59) collected and tested from Tripura, 43.88% goat and 42.37% cattle were found to possess anti-BTV antibodies (Table 9).

This study revealed seroprevalence of bluetongue in goats and cattle of Tripura in descending order. The present study reported sero-prevalence status of bluetongue



**Table 9** Seroprevalence of anti-BTV antibodies among Tripura animals

Species	Number of samples collected & tested	Number of positive samples	Percent (%) positivity
Goat	136	59	43.38
Cattle	59	25	42.37
Total	195	13	43.07

**Table 10** Seroprevalence of anti-BTV antibodies among Mizoram animals

Species	Number of samples collected and tested	Number of positive samples	Percent (%) positivity
Cattle	11	6	54.54
Total	11	6	54.54

**Table 11** Seroprevalence of anti-BTV antibodies among Mithun of Nagaland

Types of animal (Mithun)	Samples screened	No. positive	Positivity (%)
Male	22	8	36.36
Female	28	11	39.28
< 2 years old	24	9	37.50
> 2 years old	26	10	38.46
Total	50	19	38.00

in Tripura, for the first time, indicating presence of BT infection in the state (Joardar et al., 2016).

#### Mizoram

From Mizoram, cattle samples were collected from animal farm of Veterinary College, CAU, Aizawl and subsequently tested by ELISA. Out of 11 samples tested, 6 were found positive (54.54%) (Table 10) [Un-published data].

#### Nagaland

Out of total 50 Mithun (*Bos frontalis*) serum samples of Nagaland screened, 19 serum samples (38%) were found positive for the presence of anti-BTV antibodies. In the present study, % positivity found 36.36% and 39.26% in male and female animals, respectively. Again animals of age < 2 years had % positivity of 37.50, but animals of age > 2 years had 38.46% sero-positivity. The difference in % positivity between male and female or due to age variation was not significant at two degree of freedom (5%) (Table 11).

The study indicates presence of bluetongue virus in Nagaland that warrants further studies on potent vectors

and BTV serotypes circulating in the state (Joardar et al., 2015).

#### Isolation of BTV from eastern part of India

With the aim of exploring the bluetongue epidemiology, attempts were made to isolate bluetongue virus (BTV) from blood collected from sheep and goats of different areas of West Bengal, Jharkhand, Assam.

#### Sheep samples

A total of 13 blood samples were collected from Maidan pasture area of Kolkata, West Bengal. Two samples were confirmed by AINP-BT, Hisar centre as BTV-15 and BTV-21 (Joardar et al., 2009).

Incidentally, BTV-21 has not been previously reported or isolated from anywhere on the Indian subcontinent. BTV isolates in India have previously come from infected sheep in areas affected by outbreaks. However, the two isolates described here were obtained from sheep grazing pasture in Kolkata Maidan, a region of the Indian subcontinent that is not considered to be affected by bluetongue. Sheep grazing in Kolkata Maidan are usually brought from different areas of Rajasthan, Gujarat and Uttar Pradesh states, for selling in the local meat markets of Kolkata or for rearing in villages in various districts of West Bengal. This might be a route of entry for strains of BTV from the northern and western states of India to West Bengal. As the eastern and north-eastern states contain large numbers of sheep that are susceptible to BTV infection, there is a potential for exotic strains of BTV to affect sheep in these areas.

#### Goat samples

The animals (of north 24 pgs district of West Bengal) showing characteristic symptoms of BT during the time of collection were used for BTV isolation. The sample (WB/G/13), generated from goat blood, producing CPE in BHK-21 cell line was considered as viral isolate.

The overall findings in embryonated chicken eggs, cell line and RNA PAGE indicated that the suggestive sample (WB/G/13) was actually a positive sample of BTV.

Then dsRNA extracted from infected BHK-21 cell line was further used for confirmation of BTV isolate using molecular tools viz. RT-PCR using primers of NS1 and NS3 genes. The molecular characterisation of BTV isolate was done targeting the VP2 gene for sequence based serotyping. In this study, the sequence report of the plasmid product of the isolate (WB/G/13) confirmed that the isolated virus from goat was of actually BTV-16 (un-published data).

#### Vector (*Culicoides* midges)

Each batch of non-engorged midges was pooled to prepare individual sample. The samples (n=3) each

**Table 12** Habitat of collected *Culicoides*

Identified species of <i>Culicoides</i>	Sex	Habitat
<i>Culicoides (Oecacta) schultzei</i> (Enderlein)	Male	Animal shed, open drain, cow dung, saline soil by the side of river
<i>Culicoides (Oecacta) schultzei</i> (Enderlein)	Female	Animal shed, manger, cracked walls, cow dung, biogas plant near cattle shed
<i>Culicoides oxystoma</i>	Male	Animal shed, mud, bush, holes of tree trunk
<i>Culicoides oxystoma</i>	Female	Cow dung, byre, manger, cracked walls of animal shed
<i>Culicoides definitus</i>	Male	Cow dung, byre, manger, bush
<i>Culicoides definitus</i>	Female	Animal shed
<i>Culicoides kamrupi</i>	Male	Animal shed, mud, bush
<i>Culicoides kamrupi</i>	Female	Cow dung, byre, manger, cracked walls
<i>Culicoides actoni</i> Male	Male	Water logged ditches and decomposed manure, open drain
<i>Culicoides actoni</i>	Female	Walls of cattle shed (wooden plank, roof thatch)
<i>C. inornatithorax</i>	Male	Water logged ditches and decomposed manure on the back of the animal shed
<i>C. inornatithorax</i>	Female	Floor of animal shed, open drain, manure

numbering up to 200 midges were considered for BTV isolation. This BHK-21 cell lysate sample (CCS-1) was chosen for further processing. Polyacrylamide gel electrophoresis of extracted RNA of the suggestive sample (CCS-1) showed 10-segmented profile, characteristic of BTV. A band corresponding to 274 bp was obtained in agarose gel when RT-PCR was performed using NS1 specific primers. Subsequently, the isolate was serotyped as BTV-16 by Hissar centre of AINP-BT (Halder et al., 2013).

### Vector biology

#### Morphology of *Culicoides*

Haematogenous flies collected upon sweeping (using mechanical fly trappers) from time to time were sent to Diptera section, Zoological Survey of India (ZSI), New Alipore, Kolkata for morphological identification of *Culicoides* midges up to species level. Several species of *Culicoides* were identified in eastern Indian states based on morphological characteristics viz. wing vein, genital structure. Six predominant species were identified from different districts of West Bengal, viz. *Culicoides actoni*, *C. clavipalpis*, *C. oxystoma*, *C. anophelis*, *C. palpifer* and *C. alatus* (Biswas et al., 2011; Halder et al., 2013), *C. innoxius*, *C. peregrines*, *C. fulvus* (Anon, 2016). Two species of *Culicoides* were identified from six districts of Assam, viz. *Culicoides schulzei* and *C. kamrupi* (Barkataki et al., 2012). *C. oxystoma* was considered as predominant species in various districts of Jharkhand (Tigga et al., 2015a, b) and *C. actoni* along with *C. inornatithorax* were found in Meghalaya (Nongdhar, 2015).

#### Habitat of the *Culicoides*

Habitat of *Culicoides* midges was observed at different districts of West Bengal, Assam, Jharkhand, Meghalaya

during 2011–2015. Mostly midges are observed at animal shed, cow dung, nearby wet areas of animal shed etc. (Table 12) (un-published data).

### Conclusions

Since bluetongue disease is an outcome of the interaction of virus, susceptible hosts and competent vectors (*Culicoides* spp.), the complex inter-relation should be studied more vividly to reveal the comprehensive epidemiology of the disease. All the epidemiological components of BT are present in eastern and north-eastern parts of India that warrants presence of the disease in these parts too. Hence, further surveillance and preparedness are recommended to avoid future BT outbreak, if any.

#### Abbreviations

AINP-BT: All India Network Programme on Bluetongue; AGID: Agar Gel Immunodiffusion; AGPT: Agar Gel Immuno-precipitation Test; BT: Bluetongue; BT: Bluetongue virus; cELISA: Competitive Enzyme Linked Immunosorbent Assay; iELISA: Indirect Enzyme Linked Immunosorbent Assay; I.C.A.R.: Indian Council of Agricultural Research O.D.: Optical Density; WBUAFS: West Bengal University of Animal and Fishery Sciences.

#### Acknowledgements

Funds provided by the Indian Council of Agricultural Research (I.C.A.R.), New Delhi to carry out the research work is thankfully acknowledged. Thanks are due to the Vice Chancellor, West Bengal University of Animal and Fishery Sciences, Kolkata, for providing necessary infrastructural facilities to carry out the work. The Author acknowledges the contributions of his colleagues, viz. Prof. Amalendu Chakrabarti and Professor Chandan Lodh & Research Associates and students of the Bluetongue laboratory of WBUAFS.

#### Authors' contributions

The author supervised the work, analysed the results and wrote the manuscript.

#### Funding

Indian Council of Agricultural Research (I.C.A.R.), New Delhi funded [F. No. 3(8)/98 ASR-IV] scheme All India Network Programme on Bluetongue (AINP-BT).

**Availability of data and materials**

All the data and materials used to reach the conclusion of this study are available from the corresponding author on reasonable request.

**Declarations****Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The Author declare that there is no competing interests.

Received: 23 September 2020 Accepted: 12 February 2022

Published online: 03 March 2022

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