Bluetongue virus infection in India: a review

G. PRASAD, N.C. JAIN and Y. GUPTA *

Summary: The history and epizootiology of bluetongue (BT) in India are reviewed. BT has become endemic in India. The first outbreak of BT in sheep and goats in the country was recorded in 1964 in Maharashtra State. Since then, several outbreaks of BT have been reported in sheep. Exotic sheep are more susceptible than indigenous and cross-bred sheep. A serological survey has indicated the presence of bluetongue virus (BTV) antibodies in cattle and buffalo in several states in India. However, clinical BT has not been observed in cattle or buffalo to date. Of the 24 known serotypes of BTV, 18 have been reported in India. Although BTV has been isolated from Culicoides midges, the particular species responsible for transmission has not yet been identified.

KEYWORDS: Bluetongue virus Buffalo - Cattle - Culicoides Disease control Epidemiological surveys - Epizootiology - Goat India - Sheep.

INTRODUCTION

Bluetongue (BT), an arthropod-transmitted viral infection of domestic and wild ruminants, was first reported in Africa by Hutcheon (18) in 1881 under the name of "epizootic catarrh". The disease apparently remained confined to the African continent for nearly half a century, probably due to a lack of susceptible sentinel animals, insect vectors or movement of affected animals from Africa to other parts of the world. In 1949, Gambles (10) described a severe epizootic of BT in Cyprus. The disease was later recorded in Palestine and Syria (23). BT virus (BTV) appears to have spread to Pakistan through the countries of the Middle East, as reported in 1959 (16). It is logical to assume that BTV infection reached India via Pakistan, either by movement of infected animals or due to the insect vector, since India has a very long border with Pakistan. However, the possibility of BTV having entered India through the importing of sheep and cattle from other countries where BT is endemic cannot be ruled out.

The first outbreak of BT in India was recorded in 1964 among sheep and goats in Maharashtra State, on the basis of clinical signs and detection of BTV antibodies in the sera of animals which had recovered (36). However, BTV could not be isolated due to a lack of the necessary infrastructure. It is interesting to note that the disease was also observed in indigenous sheep and goats, which are said to be comparatively resistant to severe BTV infection. Subsequent to the report by Sapre (36), Bhambani and Singh (5) recorded an outbreak of BT in sheep on a government livestock farm in Uttar Pradesh and claimed to have isolated BTV by successfully transmitting the disease to experimental sheep. However, they did not describe the breed of sheep...
affected with the disease. Serum samples from both naturally- and experimentally infected sheep were tested by Dr J.G. Bowne at the Arthropod-borne Animal Diseases Research Laboratory, United States Department of Agriculture, Denver, Colorado, United States of America (USA). Dr Bowne confirmed the presence of BTV antibodies in the sera. Since the initial detection of BTV in sheep and goats in Maharashtra in 1964, serological evidence of the infection has been reported from almost all regions of India, in different kinds of ruminant livestock. The epizootiology, diagnostic methods, insect vector, BTV serotypes and species distribution of BTV antibodies in ruminant livestock are reviewed (20).

Sheep

There are approximately 48.79 million sheep in India (2). Over the past three decades, several exotic breeds of sheep have been introduced into the country for cross breeding and improvement of indigenous stock. Thus, more susceptible sentinel animals for BTV are present now than in the early 1960s when the first outbreak of BT was noticed in Maharashtra State. Between 1961 and 1963, several outbreaks of BT occurred in indigenous as well as exotic sheep and goats in India. The sera from recovered animals were tested for BTV antibodies which confirmed the presence of BTV. Since the initial report in 1964, several outbreaks of BT in indigenous, cross bred and exotic sheep have been reported on the basis of clinical signs and/or serology (5, 14, 15, 19, 24, 25, 27, 31, 38, 39, 43, 44, 46) (Fig. 1).

Dr R.C. Mogha and colleagues observed BT between 1967 and 1970 on the Tathawada Exotic Breed Farm, Poona, Maharashtra. Virus serotypes 1 and 16 were isolated in cell culture, while serotypes 2, 7, 9 and 10 were detected on the basis of serum neutralising antibodies. The breeds of sheep affected were Southdown, Rambouillet and Southdown × Bannur cross (47). In 1973, an outbreak of BT was recorded in Russian Merino on Kothipura Farm, Himachal Pradesh, by Dr G.C. Calley and Dr P.K. Uppal (47). The clinical material from the affected sheep sent to the BTV Reference Laboratory in Onderstepoort, South Africa, revealed the presence of BTV serotypes 3, 9, 16 and 17. From another outbreak of BT in Russian Merino on Dhulia Farm, Maharashtra in 1973, Dr Calley collected clinical material and sent it to Onderstepoort, where serotypes 2, 3, 4 and 16 were isolated (47). In 1975, Vasudevan and Calley recorded a BTV outbreak on the Central Sheep Breeding Farm (CSBF), an Indo-Australian Project in Hisar, Haryana, India. The Animal Virus Research Institute in Pirbright, United Kingdom, was able to isolate BTV serotypes 1 and 4 from the clinical material sent by these workers (47). The breed of sheep affected in this outbreak was Corriedale. Previously, Uppal and Vasudevan (46) had also described an outbreak of BT in imported Russian Merino sheep on Kothipura Farm in Himachal Pradesh. The disease was characterised by excessive salivation, swollen lips and tongue, petechial haemorrhages in the mucous membrane of the mouth and coronitis.

In a serological survey conducted by Sodhi and colleagues (40) in Punjab State, 6.64% sheep were positive for BTV antibodies by the immunodiffusion test. The prevalence of BTV antibodies was observed to be higher in exotic breeds than in indigenous animals. Srinivas and colleagues (43) reported an outbreak of BT in sheep and goats in Bidar, Gulbarga and nine other districts of Karnataka State, where about 50% of the population of these animals was affected. The mortality ranged between 2% and 50%. A very severe outbreak occurred in eastern Maharashtra adjoining the Telangana region of Andhra Pradesh, which later spread to western Marathawada,
Prevalence of bluetongue virus antibodies in sheep and goats in various states in India
affecting sheep in all districts of the region. The morbidity was as high as 80% in village flocks (39). In 1982, Harbola and colleagues (14) attended an outbreak of BT in Maharashtra, where high temperature (ranging from 105 to 107°F) was recorded, followed by hyperaemia of buccal and oral mucosa, and later erosion of the lips, dental pads and tongue. Swelling of the face and coronitis were invariably observed in the affected sheep. Post mortem examination showed haemorrhages near the cardiac end of the pulmonary artery and aorta. Attempts to isolate the virus were unsuccessful. The authors reported that crosses of indigenous Chokla breed with Merino were more susceptible than pure Chokla breed. However, Deccani sheep (indigenous breed) showed clinical signs during the outbreak. Of 8,980 exotic, cross-bred and indigenous sheep, 868 were affected, 100 of which died, giving an overall case fatality rate of 11.52%. Lonkar and colleagues (25) observed an outbreak of BT in sheep at the Central Sheep and Wool Research Institute (CSWRI) in Avikanagar, Rajasthan, and reported that Rambouillet and Merino were more susceptible than indigenous breeds. The morbidity rate in Merino and Rambouillet was 33.3% and 23.5% respectively. Mortality in adult Merino was 35.3%. Bandyopadhyay and Mullick (3) reported BTV antibodies in sheep sera obtained from Haryana, Uttar Pradesh, Rajasthan and Andhra Pradesh. The incidence of BTV antibodies was higher in exotic sheep than in indigenous and cross-bred animals. Mehrotra and Shukla (28) detected BTV antibodies in sheep sera obtained from Maharashtra, Andhra Pradesh, Karnataka, Rajasthan, Jammu and Kashmir, and Himachal Pradesh. The prevalence of BTV antibodies ranged between 16.4% and 61.1% in different states.

Kulkarni and Kulkarni (24), working in the Department of Veterinary Microbiology at the College of Veterinary Sciences, Parbhani, Maharashtra, claimed to have isolated BTV serotypes 8 and 18 from sheep affected with BT in developing chicken embryos. Haribabu (15) conducted haematological studies on sheep naturally-infected with BTV and reported that there was a marked decrease in the total leukocyte count in the affected sheep. More recently, similar observations have been made in sheep experimentally-inoculated with BTV serotype 1 (6).

Sharma and colleagues (37) conducted a systematic epizootiological study of BT at the CSWRI. They reported 15.3% fatality due to this infection. They also monitored the presence of BTV antibodies in exotic sheep of various breeds including Merino, Rambouillet, Dorset, Suffolk, Karakul, cross-breds and indigenous sheep breeds. They concluded that exotic breeds of sheep were more susceptible to BTV infection than indigenous breeds.

Jain and colleagues (19), at the CSBF, reported a severe outbreak of BT in Rambouillet sheep three months after their arrival from the USA. The characteristic clinical signs included high temperature, excessive salivation, oedema of the head and muzzle, erosions in the oral cavity, tongue and lips, and coronitis leading to lameness. BTV serotype 1 was isolated in ten- to twelve-day-old chicken embryos and BHK-21 cell cultures from the blood of affected sheep. The disease was transmitted to the cross breds sheep experimentally, using infective material from sheep affected in this outbreak.

Subsequently, in 1986, a serological survey was conducted of BTV antibodies in the sheep, goat, cattle, buffalo, camel and horse populations in and around Hisar, Haryana, using an immunodiffusion (ID) test. The results of this survey indicated the presence of BTV antibodies in 82.2% of exotic ewes which had aborted, 36.6% of apparently healthy exotic ewes and 14.3% of exotic rams. The prevalence of BTV antibodies was lower in indigenous sheep than in exotic breeds. However, the authors
failed to detect BTV antibodies in camels, horses, goats and buffalo (35). In 1987, Dubey and colleagues (9) reported the presence of BTV antibodies among sheep at the CSWRI in Rajasthan. Besides these two northern and western states, another serological survey of BTV infection was conducted in four organised sheep farms of Andhra Pradesh in the south of India. Here, overall morbidity was reported as 21.5%; morbidity was higher in cross-bred sheep than in exotic and indigenous breeds (31).

Between 1985 and 1988, another epizootiological study of BT was carried out in the CSBF in Haryana (27). The study revealed a case fatality rate of 31.2% in 1985, which increased to 42.7% in 1986. In 1987 and 1988 the mortality rate fell to 7.0% and 7.4% respectively.

In a retrospective study at the CSWRI between 1980 and 1987, Srivastava and colleagues (44) correlated congenital defects in lambs with BTV infection. Among the rare deformities recorded in various breeds of sheep were suicephaly, presence of two lateral oral openings, bifid tongue, absence of various parts of the body, such as the pelvic girdle, rectum, sex organs, hind legs, abdominal muscles and skin. Since the incidence of BT in the flocks at the CSWRI coincides with the breeding cycle and the presence of this disease has been confirmed on the farm, the study indicated the possible role of BTV in congenital lamb defects.

**Goats**

There are approximately 95.2 million goats in India. Besides being used for meat and hides, milk from goats is also consumed in India. The first evidence of BT in goats was recorded by Sapre (36) in Maharashtra. Although typical BT has not been reported, there are several reports of the presence of BTV antibodies in goats in a number of states (Fig. 1).

Sodhi and colleagues (40) reported a 1.4% incidence of BTV antibodies in goats in Punjab State, while Bandyopadhyay and Mullick (3) reported a 3.0% prevalence of BTV antibodies in goats in Uttar Pradesh. In a recent serological survey carried out at the Western Regional Station of the Central Goat Research Institute in Avikanagar, Rajasthan, 33.3% of goat sera were positive for BTV antibodies by the ID test (Jain and Prasad, unpublished data). This could be due to the presence nearby of a sheep farm with endemic BTV infection (the CSWRI in Avikanagar). In another recent serological survey conducted in Hisar, Haryana, forty goat serum samples were tested, of which fourteen (35%) were found positive for BTV antibodies. However, clinical BT disease was not observed in these goats. The role of goat populations in the maintenance of BTV in nature is not known.

**Cattle and buffalo**

The earliest evidence of BTV infection in bovines in India is documented in the Annual Report of the Indian Veterinary Research Institute (1). According to this report, 3.7% of cattle sera were positive for BTV antibodies as detected by the agar gel immunodiffusion (AGID) test. In 1981, Sharma and colleagues (38) conducted a serological survey in cattle and buffalo in Punjab State. They recorded the presence of BTV antibodies in 6.8% of cattle sera. However, 40.6% of Sahiwal cattle sera were positive, indicating the presence of BTV infection in indigenous cattle. In this study, none of the buffalo sera tested were positive for BTV antibodies. Subsequently, Oberoi and colleagues (33) demonstrated the presence of BTV antibodies in 38.5%
of buffalo sera in Punjab State. They also recorded 70% of cattle sera positive for BTV antibodies. In a study conducted by Tongaonkar and colleagues (45) in Gujarat State, 13.4% of buffalo and 15.6% of cattle were positive for BTV antibodies. Bandyopadhyay and Mullick (3) reported 3.7% of cattle sera positive for BTV antibodies.

Tongaonkar and colleagues (45) tested sera from aborted and apparently healthy buffalo in Gujarat and reported that 15.5% of samples were positive for BTV antibodies. However, they could not correlate BTV infection with abortion. The positive sera revealed presence of antibodies against serotypes 1, 15 and 17. This study was one of the earliest studies on bluetongue in buffalo (Bubalus bubalis). Subsequently, Mehrotra and Shukla (28) tested serum samples from seven states (Andhra Pradesh, Karnataka, Gujarat, Punjab, Orissa, Himachal Pradesh and West Bengal). A total of 154 cattle sera were tested, of which 28 (18%) were positive for BTV antibodies.

These reports established that BTV antibodies are widely present in cattle and buffalo herds in India (Fig. 2). Prasad and colleagues (35) failed to detect BTV antibodies in buffalo, while only 4% of cross bred cattle sera were positive in the study conducted in Hisar, Haryana State. In this serosurvey, none of the indigenous cattle sera were found to be positive for BTV antibodies. Similarly, another serological survey was conducted by Oberoi and colleagues (33) on a cattle farm in Ropar and a buffalo farm in Ludhiana, both in Punjab State. The presence of BTV antibodies was detected in 70% of cattle sera and 37.5% of buffalo sera by the AGID test. In 1990, an extensive survey of BTV antibodies in cattle and buffalo was conducted in Haryana State. Serum samples were collected from a total of 549 cattle and 498 buffalo, of which 4.2% and 10.6% respectively were positive. In this study, 6.9% and 13.6% respectively of sera taken from males were also positive (12). However, no attempts have been made to date to isolate BTV from semen. No studies have been conducted on the semen quality of bulls with BTV or BTV antibodies. No clinical BT has been observed in cattle and buffalo, but these animals may play an important role in the maintenance of the virus in nature. The presence of BTV antibodies in cattle and buffalo has become a serious impediment to the export of the germplasm of these animals.

Other animals

The presence of BTV antibodies has been reported in several wild ruminants from other countries of the world. However, no systematic survey has yet been conducted to assess the status of BTV infection in Indian wildlife. Only one elephant serum sample tested has been found positive for BTV antibodies (29). One serum sample from black buck has been found negative for BTV antibodies. In a separate test, none of 128 camel sera samples were found positive for BTV antibodies (35), although the presence of BTV antibodies in camel has been demonstrated in some other countries (4).

Bluetongue virus serotypes reported from India

The double stranded and segmented RNA genome of BTV makes it prone to frequent mutations and genetic reassortments leading to the emergence of a new serotype or antigenic variant of the same serotype. To date, 24 serotypes of BTV have been recorded world-wide, 18 of which have been reported in India (Table I).
Prevalence of bluetongue virus antibodies in buffalo and cattle in different states in India
TABLE I

Bluetongue virus serotypes detected on the basis of neutralising antibodies and isolation of the virus from various states in India

<table>
<thead>
<tr>
<th>State</th>
<th>On the basis of serum neutralising antibodies</th>
<th>On the basis of virus isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gujarat</td>
<td>1, 2, 3, 5, 8, 9, 10, 11, 12, 13, 15, 16, 17, 20</td>
<td>6</td>
</tr>
<tr>
<td>Haryana</td>
<td>1, 2, 8, 12, 16</td>
<td>1, 4</td>
</tr>
<tr>
<td>Himachal Pradesh</td>
<td>4</td>
<td>3, 9, 16, 17</td>
</tr>
<tr>
<td>Karnataka</td>
<td>1, 4, 16</td>
<td></td>
</tr>
<tr>
<td>Maharashtra</td>
<td>1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 16, 17</td>
<td>1, 9, 16, 18</td>
</tr>
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Uppal and Vasudevan (46) reported the presence of BTV serotypes 3, 4, 9, 16 and 17 in Russian Merino sheep affected in two outbreaks. The material collected from Kothipura Farm in Himachal Pradesh was sent to the Veterinary Research Institute in Onderstepoort, South Africa, for analysis. BTV serotypes 1 and 4 were incriminated in a BT outbreak in Australian Corriedale Sheep at the CSBF after tests by the Animal Virus Research Institute in Pirbright, United Kingdom (46). Subsequently, serum samples sent to Onderstepoort by Sriguipi (42) and Chaudhary (8) from Maharashtra State showed the presence of antibodies against BTV serotypes 1, 2, 3, 4, 7, 9, 10, 16 and 17.

Kulkarni and Kulkarni (24) reported the isolation of BTV serotypes 9 and 18 from two outbreaks (in 1981 and 1983) in Maharashtra. Serotyping of these isolates was made by the Foreign Animal Disease Research Laboratory of the United States Department of Agriculture in New York, USA.

In 1985, BTV serotype 1 was isolated from Rambouillet sheep affected with BTV at the CSBF (19). Subsequently, Jain and colleagues (22) isolated BTV from a Culicoides sp. of midge collected from the same farm. However, the serotype of this isolate could not be ascertained.

Vector

Several species of Culicoides midges have been demonstrated to be vectors of BTV (30). The presence of a number of Culicoides species has been reported in India, including C. albipennis, C. anapheles, C. bimaculicosta, C. brevimanus, C. chortu, C. deleki, C. detoni, C. fiontivagus, C. fulvithorax, C. glavipulpis, C. himalayee, C. macrostoma, C. molestus, C. nitichelus, C. odiosus, C. opacus, C. oxystoma, C. paivoi, C. perigrumun, C. pictivatris, C. poecilopteris, C. quadrilobatus, C. setiger, C. smiles, C. speapularis, C. tattoni, C. varipulpis and C. xanthocoma (32).
Jain and colleagues (21) isolated BTV from Culicoides midges. However, the authors could not identify the species of Culicoides involved. The above seems to be the only report on the isolation of BTV from any insect vector in India. There is a complete lack of information on the BTV vector in India. Any one of the several Culicoides spp. reported in India may be a vector for bluetongue, while the presence of multiple vectors cannot be ruled out. Therefore, an extensive survey for identification of the insect vectors of BTV is imperative in order to understand the dynamics of the BTV maintenance cycle in nature.

**Laboratory methods for the isolation of bluetongue virus and detection of antibodies in serum**

Inoculation of susceptible sheep with blood collected from BT-affected animals has been used to isolate BTV (5, 19, 24). As well as animal inoculation, BHK 21 clone 13 cell cultures and developing chicken embryos have also been used for initial isolation of BTV. The authors have found BHK-21 to be as effective as chicken embryo for the isolation of BTV from infected sheep blood and insect vectors (19, 21). Other cell lines, such as Vero, L-cells, MDBK, MVPK and HeLa, have been successfully used for adaptation and propagation of BTV in other countries (17, 26).

Diagnosis of BT in India has been based on clinical signs and serology. The first recorded outbreak of BTV in sheep and goats was suspected on the basis of clinical signs. Although several diagnostic tests have been developed for the diagnosis of BTV infection in domestic and wild ruminants (41), the agar gel precipitation test (AGPT) remains one of the most widely-used serological tools for surveys of BTV antibodies (34). In India, most of the serological studies have been conducted using AGPT.

In 1988, Gupta (11) standardised the process of counter current immunoelectrophoresis (CCIE) for detection of BTV antibodies in sheep sera and reported that CCIE was more sensitive than AGPT. Recently, Gupta and colleagues (12) published the results of a comparative evaluation of different serological tests for the detection of BTV antibodies in field sheep serum samples, which indicated that dot immunobinding assay (DIA), a modified enzyme-linked immunosorbent assay (ELISA) on nitrocellulose paper strip, was as sensitive as ELISA and more sensitive than AGPT and CCIE. To further evaluate the efficacy of DIA for detection of BTV antibodies, a comparative evaluation was conducted on different tests in experimentally inoculated sheep (6). The experimental evaluation corroborated findings on the field sera, and suggested that DIA is more sensitive than serum neutralisation and AGPT, and equally as sensitive as ELISA (7). Since ELISA requires a sophisticated ELISA reader, it may not find wide application in developing countries. However, because of its simple procedure, economy and sensitivity, DIA could replace ELISA for field serological surveys (7, 12, 13). Recently, DIA has been used for detection of BTV antibodies in cattle and buffalo sera. It has been able to detect BTV antibodies in animals declared negative by AGPT (22). The viral nucleic acid hybridisation tests which have been used in technologically-advanced countries have not been adapted for the detection of the BTV genome in India.

**Geoclimatic conditions and seasonality of bluetongue**

The climate in India varies quite widely between different parts of the country. The monsoon season, which lasts from June to October, is the most favourable period for multiplication of Culicoides spp. and is therefore propitious for the occurrence of BT in sheep. Most of the outbreaks of BT in India have been recorded during
this period. In one of the sheep-breeding farms located near Hisar, meteorological data were gathered during the months of BT occurrence over a four-year period (from 1985 to 1988). Bluetongue outbreaks occurred between April and October. During this period, the maximum temperature ranged between 26°C and 40.3°C, relative humidity between 61% and 89% and monthly rainfall between 5.3 and 204 mm (27). The number of sheep affected during 1987 was low compared to the previous year. This could be due to the severe drought in 1987 which may have affected the activity of Culicoides midges (27). Most BT outbreaks have been found by the authors to occur during the rainy season, when the humidity is very high and the temperature is favourable for breeding of the insect vector. BT outbreaks have not been recorded in the winter season in India.

Control

In India there is no well-defined control strategy for BT as in other countries. Vaccination is not performed against BTV in India, mainly due to non-availability of the vaccine. Importing the vaccine from South Africa is expensive and politically unfeasible. Moreover, the exact number of serotypes circulating in India is still not known. With regard to vector control, no attempt at control has been made on any farm in this country. There is no restriction on the movement of animals from one region to another within the country. Thus, outbreaks may also occur due to transportation of animals.

CONCLUSION

Bluetongue virus infection is widely distributed in domestic ruminants. Evidence for the existence in India of 18 of the 24 known serotypes of BTV has been reported on the basis of presence of serum neutralising antibodies from different states in the country. Although BTV has been isolated from Culicoides, the species has not been identified. Outbreaks of BT occur in sheep and cause heavy mortality and morbidity. Clinical BT has not been observed in cattle and buffalo. BT vaccine is not available in the country, hence vaccination against this disease is not practised. No study has been conducted to date to study the dynamics of the virus infection in wild ruminants and the role of these animals in transmission of the virus among domestic ruminants. In order to combat the impact of this disease on the Indian livestock industry, implementation of a clearcut national policy is imperative.

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REFERENCES


