Bluetongue and footrot outbreaks in migratory Sheep due to unseasonal rains/ floods: Special reference to BTV Serotype 12

B M CHANDRANAIK, MANJUNATHA MAYACHARI, K NAGARAJA, AMITHA REENA GOMES, APSANA RIZWAN, M S ALAMELU and S M BYREGOWDA

Karnataka Veterinary Animal and Fisheries Sciences University, Bengaluru, Karnataka 560 024 India

Received: 7 November 2021; Accepted: 11 April 2023

ABSTRACT

Following heavy rains and floods during October-November of 2019, outbreaks of Bluetongue (BT) disease was witnessed in migratory sheep in flood affected areas. The disease was investigated in fifteen migratory flocks in Karnataka state, involving a total of 3575 sheep with over 1480 ailing and 428 deaths. Samples collected from 208 ailing and 79 dead animals were initially subjected for NS1 genome based group specific Bluetongue virus (BTV) detection by Reverse Transcription-PCR (RT-PCR) and later for segment 2 genome based serotype specific RT-PCR. The RT-PCR and phylogenetic analysis confirmed the virus involved in the current outbreak as BTV serotype 12. This data gives further insights into BT epidemiology and recommends inclusion of locally circulating BTV serotype/s in vaccines in endemic regions for effective control of BT. Since these migratory sheep were forced to stand in water stagnated agricultural fields/lands for days to weeks due to continuous rains, they were concurrently affected with acute footrot caused by *Fusobacterium necrophorum* and *Staphylococcus aureus*. Foot-tanks and higher antibiotics were very effective in treatment of footrot in the current outbreak. Present study is an evidence of how unforeseen natural calamities can disrupt animal health with profound socio-economic consequences eventually affecting the food-chain and here a few scientific measures have been attempted to mitigate such animal health crisis.

Keywords: Bluetongue, Climate change, Epidemiology, Floods, Footrot, Serotype 12, Vector borne disease

Climate change in recent times, has significantly affected the rain patterns resulting in extreme weather conditions like droughts, unseasonal rains and frequent floods especially in tropical countries (Wenju et al. 2014). Further, these environmental changes have steered the expansion of many diseases in humans and animals (Jones et al. 2019). Sheep and Goat husbandry is a major source of livelihood to millions of poor and marginal farmers in tropical countries (Mamatha et al. 2017). Migratory shepherds move with their animals in search of availability of feed and water to their animals, wherein they shelter their sheep in large, open, agricultural lands. Bluetongue (BT) is an economically important, arthropod-borne, non-contagious viral disease of ruminants. The disease is caused by *Bluetongue virus* (BTV), belonging to the genus *Orbivirus* of the family Reoviridae (Reddy et al. 2016). The virus has 10-segmented, double stranded RNA genome (Roy et al. 1990). The genome codes for seven structural proteins (VP1-VP7) and four to five non-structural proteins (NS1, NS2, NS3/NS3a, NS4 and NS5). The sequences on the outer-capsid protein VP2 encoded by segment 2 (Seg-2) of the BTV genome determines the serotype, topotype, and geographical origin of BTV (Mertens et al. 2007). BTV-25 (Toggenburg virus) of goats in Switzerland (Hofmann et al. 2008, Chaignt et al. 2009); BTV-26 of sheep in Kuwait (Maan et al. 2011); BTV-27 of goats in France (Jenckel et al. 2015, Savni et al. 2017); BTV-28 of sheep in Middle East (Bumbrov et al. 2020), and BTV-29 of an Alpaca in South Africa (Wright. 2013) have been added to the existing list of twenty four distinct serotypes of BTV. Recent surveillance studies suggest circulation of more novel/atypical serotypes namely, BTV-30, 31, 32, 33, 34 and 35 (Ries et al. 2020).

Footrot is a contagious, debilitating and economically important, mixed bacterial disease of small ruminants, caused by the synergistic action of highly fastidious anaerobes to easily cultivable aerobe (Stewart et al. 1984, Tadich and Hernandez 2000). The disease is prevalent worldwide and has severe economic impact in countries with heavy rainfall (Anto et al. 2014).

In this study we describe massive outbreaks of bluetongue and footrot in migratory sheep, following unseasonal rain and flooding causing huge economic loss to the farmers. This study exemplifies the vulnerability, and economic struggles of the migratory shepherds in developing countries. Further we describe genome Segment 2 based characterization of the BTV associated with the outbreak.

Present address: 1Institute of Animal Health and Veterinary Biologicales, Hebbal, Bengaluru. 2Department of Animal Husbandry, Government of Karnataka, Hirekerur. *Corresponding author email: drbmchandanaik@gmail.com*
MATERIALS AND METHODS

History of outbreak, investigation of disease and sample collection: The monsoon usually accounts for around 70% of India's annual rainfall. India’s 2019 monsoon season was one of the most unusual in recent decades. From June to September 2019, India received the highest amount of monsoon rain in the past 25 years with a national average of 97 cm (Indian Meteorological Department Report, 2019). The monsoon receded by mid-September but unseasonal heavy rain lashed the country in October and November causing floods (Indian Meteorological Department Report 2019). Following heavy rain, large scale mortality of sheep in many villages was reported with symptoms of high fever, limping, facial swelling and footrot. We investigated the disease problem in fifteen migratory sheep flocks at Koppal, Haveri and Davanagere districts of Karnataka state, India. The details of the flock size, number of ailing animals, deaths due to the disease and details of the samples collected are enlisted in Table 1. The samples were transported to laboratory under strict cold chain conditions.

Confirmation of Bluetongue virus by group specific Reverse Transcription PCR: RNA was extracted from whole blood collected in EDTA from ailing sheep and from spleen samples collected at post-mortem using Trizol, Chloroform and Isoproponol following standard procedures and the cDNA was synthesised as per standard procedures described earlier (Chandranaik et al. 2019). The group specific Reverse Transcription PCR (RT-PCR) was carried out on each sample to confirm the presence of BT viral genome using group specific (NS1) primers, viz. Forward- 5’ GTTCTCTAGTTGGCAACCACC3’ and Reverse- 5’ AAGC CAGACTGTTTCCCGA 3’ to generate an amplicon of 274 bp (Prasad et al. 2013).

Confirmation of Bluetongue serotype by serotype specific RT-PCR: The cDNA samples that were positive by group specific RT-PCR were subjected to species specific RT-PCR using the protocols and Segment-2 genome specific primers of eight most common BTV serotypes prevailing in Southern India described by Reddy et al. (2016). The details of the serotype specific primers used in this study are enlisted in Table 2.

Phylogenetic analysis: Gel purified amplicons were subjected to nucleotide sequencing by Sangers method (M/s Bioserve Ltd, Hyderabad, India). The deduced

<table>
<thead>
<tr>
<th>Village Name Where the migratory flock stationed</th>
<th>Number of migratory flocks</th>
<th>Total flock size</th>
<th>Total Ailing animals</th>
<th>Death due to disease</th>
<th>Total samples collected</th>
<th>EDTA Blood</th>
<th>No of carcasses on which post-mortem conducted</th>
<th>Footrot swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hire Edachi</td>
<td>2</td>
<td>375</td>
<td>120</td>
<td>30</td>
<td>25</td>
<td>13</td>
<td>-</td>
<td>79</td>
</tr>
<tr>
<td>Hirekerur</td>
<td>3</td>
<td>750</td>
<td>350</td>
<td>80</td>
<td>59</td>
<td>15</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Shiragambi</td>
<td>3</td>
<td>800</td>
<td>&gt;400</td>
<td>88</td>
<td>50</td>
<td>14</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>Rattihalli</td>
<td>4</td>
<td>1250</td>
<td>&gt;400</td>
<td>137</td>
<td>44</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kurubara Dhodihalli</td>
<td>3</td>
<td>400</td>
<td>210</td>
<td>93</td>
<td>30</td>
<td>27</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>3575</td>
<td>&gt;1480</td>
<td>428</td>
<td>208</td>
<td>79</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bluetongue virus (BTV) Serotype</th>
<th>Primers (5’ to 3’)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTV-1E</td>
<td>F-TGTCGAGCGGATTTGGAAGATCCGTC R-ATCGTATTCCGCTGGTGTCGG</td>
<td>1180</td>
</tr>
<tr>
<td>BTV-2 W</td>
<td>F-CGTCCGGCTAATGGGTGCG R-GCCCTCTCCGATATCGTTGT</td>
<td>773</td>
</tr>
<tr>
<td>BTV-2 E</td>
<td>F-TACGCACCTCCTGAGAGAGA R-GTTGGAGGAAACCAACTTTCA</td>
<td>1167</td>
</tr>
<tr>
<td>BTV-9 E</td>
<td>F-GATGGAACGGGCTAAACCCA A R-TGGATATTGTAGACGAGCGA</td>
<td>1224</td>
</tr>
<tr>
<td>BTV-10 E</td>
<td>F-TGTATCCTGAAGGGCAGGTCAGCA R-TGTCTTCTAAGGCGCTCTACGC</td>
<td>805</td>
</tr>
<tr>
<td>BTV-16</td>
<td>F-TGGAGAAAGGCGGATACACGT R-CGTTCGCCCTAATCTCGACTTGC</td>
<td>1197</td>
</tr>
<tr>
<td>BTV 12</td>
<td>F-TTTAGGTGACCTGTTGGAGACG R-CAACGGACCTTTGCCA</td>
<td>752</td>
</tr>
<tr>
<td>BTV-21</td>
<td>F-CCAGATCTGCTAACAACGCCAGCC R-TTGGGATTTGCGGCGGCGA</td>
<td>1388</td>
</tr>
<tr>
<td>BTV-23</td>
<td>F-GCGTGTGCGATGGATGAGTATGCA R-GTTGTCATCTTCATCTTCGCGG</td>
<td>1370</td>
</tr>
</tbody>
</table>

E, Eastern Topotype; W, Western Topotype; F, Forward; R, Reverse.
nucleotide sequences were analysed (BLAST) and aligned with the published sequences from GenBank database and the phylogenetic tree was constructed by MEGA 6.2 software using Neighbor-Joining method (Tamura et al. 2013, Chandranaiik et al. 2017).

Bacteriological examination of swab samples from footrot lesions: The swab samples collected from footrot lesions (Table 2) were subjected to aerobic and anaerobic bacterial culture examination as per previously described standard protocols (Quinn et al. 2011).

RESULTS AND DISCUSSION

In the livestock sector, sheep being a valuable and renewable resource occupy an important position. They are raised either under stall fed or grazing system. Shepherds who don’t own agricultural lands and/or can’t afford to purchase/arrange feed to their animals, migrate with their flocks in search of feed and water for sustenance.

In this study, the shepherds had started migration with their sheep by the end of September 2019 when the monsoon rains had receded. However, they were caught amidst by the sudden unseasonal rains in October-November. The ailing animals appeared dull and had pyrexia ranging from 104°F to 106°F. The oral/buccal mucosa was highly congested with swollen head. The tongue was congested in most animals with characteristically bluish discoloration in a few animals (Fig. 1). There was respiratory distress in all ailing animals with nasal discharge and cough. The ailing animals were limping with characteristic congestion around the coronary band of hooves. Ulcerations on the upper palate were prominent in most ailing animals (Fig. 2). Breaks in the wool were distinctly evident. The shepherds stated that many recovered animals had died due to their inability in swallowing with severe cough during regurgitations. On postmortem (PM), the dead animals had lesions of cyanotic tongue, oedematous lips, congested buccal cavity, ulcerations on the upper palate, haemorrhagic gastro-enteritis with ulcerations in rumen and reticulum. A few animals had regurgitated feed materials in the trachea.

In six flocks at Hirekerur and Shiragambi villages apart from the above symptoms and lesions, more than 80% animals had severe footrot lesions of varied degrees with suppurative inflammation in and around the hoof cavity (Fig. 3). Many animals were unable to walk on their hooves and were crawling on their knuckled legs.

Out of 208 EDTA blood and 79 spleen samples subjected for NS1 genome based group specific RT-PCR, 183 blood and 58 spleen samples were positive for BTV. When each of these BTV positive samples were subjected for serotype specific RT-PCR for eight most commonly prevailing BTV serotypes in south India (primer details in Table 2), yielded specific amplicon of 752 bp indicating presence of BTV serotype 12 (Fig. 4). The amplicon (752 bp) obtained upon serotype specific RT-PCR were sequenced and the deduced nucleotide sequences were BLAST analysed on NCBI website and were compared with the nucleotide sequences of BTV serotypes available in GenBank. Phylogenetic analysis showed that sequences of the BTV serotype 12 involved in the current outbreak were homologous within themselves and showed more than 99.99% genetic identity with previously described Indian BTV serotype 12 (Fig. 5).
Further, the sequences showed more than 99% sequence identity with reference strain of the BTV-12 from South Africa and with BTV serotype 12 isolates from Kenya, Middle East and USA (Fig 5).

The lesions noticed in ailing animals and at PM were characteristic and typical of haemorrhagic pathogenesis of BTV (OIE. 2018). The evidence of regurgitated feed material in the trachea of seventeen dead animals could be attributed to rare pathogenesis of BTV causing necrosis of oesophageal musculature causing inability to swallow (Mahrt and Osburn 1986, Lima et al. 2014, OIE 2018). The same was also explained by some animal owners as cause of death in recovered animals.

Recently, BTV strains have been grouped into eastern and western topotypes based on their geographical distribution, phylogeny and evolutionary distance; BTV serotype 12 is classified under western topotype (Rao et al. 2015, Reddy et al. 2016).

Presence of BTV-12 antibodies in sheep and cattle has been documented in Gujarat, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra and Haryana states of India (Prasad et al. 2009). Rao et al. (2015) reported the first isolation of BTV-12 in India from Andhra Pradesh state. Present study records a large-scale outbreak in sheep causing huge mortalities due to Bluetongue serotype 12 in India. Sero-surveillance data shows circulation of 22 BTV serotypes in India and at least 15 BTV serotypes (BTV-1, 2, 3, 4, 5, 8, 9, 10, 12, 16, 17, 18, 21, 23 and 24) have been isolated to date (Maan et al. 2015, Hemadri et al. 2017, Reddy et al. 2018). In spite of these many isolations the currently used Bluetongue vaccine in India has only five serotypes, viz. BTV serotypes 1, 2, 10, 16 and 23. Through this data, we recommend addition of endemic BTV strains in BT vaccines so that devastating economic losses incurred to the poor farmers can be avoided in future. Since presence of segmented genome in BTV may limit use of live vaccines, actions have to be initiated for the development of vaccines with better adjuvant systems (Chandranaik et al. 2020) for providing longer immunity with the currently available inactivated vaccines.

The massive BT outbreak was brought to control by local veterinarians who effectively adopted a combination of conventional and modern therapeutic approaches like; creating temporary animals shelters, burning neem leaves around these shelters (to produce thick smoke to avoid...
vectors), using mosquito nets, avoiding grazing near water stagnant areas especially at dawn and dusk, supportive therapy with antibiotics, antihistamines and intravenous fluids for extremely weak animals.

The swab samples from foot-rot lesions yielded growth *Fusobacterium necrophorum* (*F. necrophorum*), on anaerobic media and *Staphylococcus aureus* (*S. aureus*) on aerobic selective media. The infection appeared chronic with necrotizing disease of the epidermis of the interdigital skin and hoof matrix extending to involve large areas of the hoof matrix. The sensitive lamina and its network of capillaries had been destroyed by the infection. We could infer that, in absence of housing/shelters, animals were made to stand in water stagnated agricultural fields/lands continuously for days to weeks, predisposing them to foot-rot by *F. necrophorum* an ubiquitous pathogen present in soil and faeces and *S. aureus* an opportunistic pathogen present on animal skin. The ailing animals were given systemic antibiotics and were made to walk through small foot tanks containing Potassium Permanganate solution for dipping the affected hooves. Over a period of time, these measures worked effectively in recovery from footrot lesions.

Like in any other vector borne disease, climate plays an important role in the distribution and spread of BTV (Jones et al. 2019). Rainy, wet conditions are congenial for Culicoides propagation and are probably one of the major reasons for higher incidence of BT during heavy rains in endemic areas (Reddy et al. 2016). Climate change due to greenhouse warming has severely disrupted weather patterns and is having profound socio-economic consequences, globally. The present study provides an evidence of how unforeseen natural calamities can potentially disrupt animal health, eventually affecting the food-chain and we attempt to provide glimpses of measures to mitigate such animal health crisis. This paper also emphasises the uphill attempt to provide glimpses of measures to mitigate such animal health crisis, eventually affecting the food-chain and we attempt to provide glimpses of measures to mitigate such animal health crisis.

This study reports bluetongue and footrot outbreaks in migratory sheep in India following unseasonal heavy rain and floods. Phylogenetic analysis characterized the BT virus involved in the current outbreak as BTV serotype 12. The swab samples from foot rot lesions yielded *F. necrophorum* and *S. aureus*. This data recommends inclusion of circulating BTV serotype/s in BT vaccine in endemic regions for its effective control.

ACKNOWLEDGEMENTS

We thank the Director, Animal Husbandry, Karnataka, for the support and extending facilities to conduct this work. We thank all the veterinarians who helped us in sample collection and for effective implementation of measures to control the outbreaks.

REFERENCES


