

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

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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, lineage and geographical origin of BTV (Mertens *et al.* 2007). BTV-25 (Toggenburg virus) of goats in Switzerland (Hofmann *et al.* 2008, Chagnat *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.

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## 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 Isopropanol following standard procedures and the cDNA was synthesised as per standard procedures described earlier (Chandranaiik *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 1. Details of place of disease outbreak, flock size, ailing, dead animals, and samples collected for laboratory examinations

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

Table 2. Details of serotype specific primers used in this study

| Bluetongue virus (BTV) Serotype | Primers (5' to 3')                                        | Amplicon size (bp) |
|---------------------------------|-----------------------------------------------------------|--------------------|
| BTV-1E                          | F- TGTCGAGCCGATTGAAGATCCGTC<br>R- ATCGTCATTCCGTCGTTGTGCG  | 1180               |
| BTV-2 W                         | F- CGTCCTGGACTAATGGGTGCG<br>R- GCCCCTTCCGATATCGTTGT       | 773                |
| BTV-2 E                         | F-TACGCACCTCGTGAGAGAGA<br>R- GTTGGAGGAACCAACTTCCA         | 1167               |
| BTV-9 E                         | F GATGGAACGGCTAAACCAAA<br>R TGGATATTTGACACGAGCGA          | 1224               |
| BTV-10 E                        | F-TGTATCGTTAAGGCGAGGTCAGCA<br>R-TGTCTTCTAACGGCCTCTCACG    | 805                |
| BTV-16                          | F-TCGAGGAAAGCGGATACCACGT<br>R- CGTTGCGCTAACTCGACTTCGC     | 1197               |
| BTV 12                          | F-TTTAGGTGACCATGTGGAGACG<br>R-CAACGCACTTTCGCAAAACC        | 752                |
| BTV-21                          | F-GCAGATTTCGTACAACCAACGGCC<br>R-TTGGGATTTGCGAGGCGCGA      | 1388               |
| BTV-23                          | F-GCGTTGCGATGGATGAGTTAGCA<br>R -GGTGGTCATCTCTTCATCTTCGGGG | 1370               |

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 Neighbour-Joining method (Tamura *et al.* 2013, Chandranaik *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 discolouration 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



Fig. 1. Congestion and bluish discolouration of tongue in a Sheep ailing with Bluetongue.



Fig. 2. Ulcerations on upper palate observed in a Bluetongue affected Sheep.

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.



Fig. 3. Footrot lesions with suppurative inflammation in and around the hoof cavity observed during this study.

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).

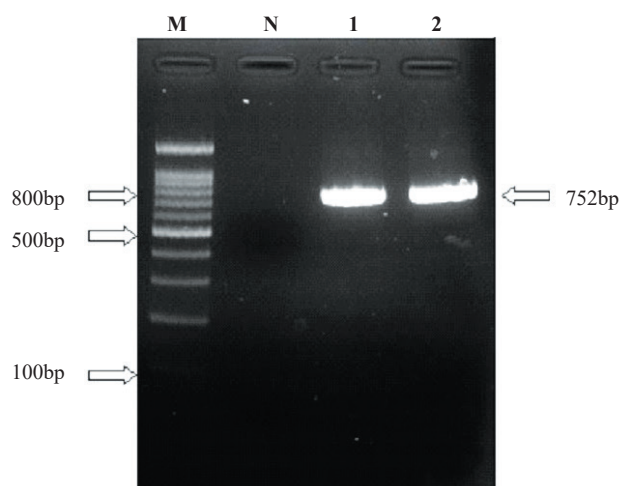


Fig. 4. Gel showing specific amplification of Segment 2 gene of Bluetongue virus serotype 12 at 752 bp. Lane M: 100 bp ladder; Lane N: Negative control; Lane 1: Spleen sample collected from Sheep died with Bluetongue symptoms; Lane 2: Spleen sample collected from a Sheep died with Bluetongue symptoms.

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 (Chandranaiak *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

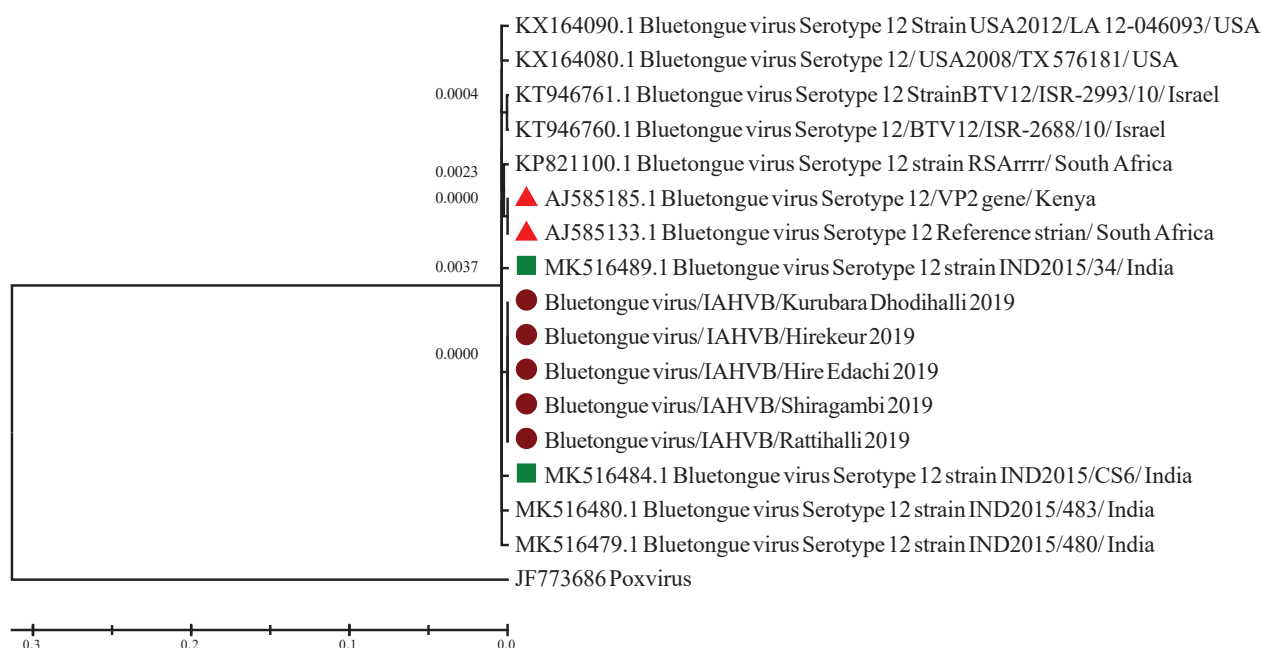


Fig. 5. Segment 2 genome based phylogenetic analysis illustrated that the Bluetongue virus involved in current outbreak was BTV serotype 12 (Red dots) were 100% homologous between themselves and shared highest nucleotide sequence identity with BTV serotypes 12 from Africa, Israel and USA.

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 problems faced by migratory shepherds during migration, their extreme helplessness in catastrophic situations and most importantly, their economic vulnerability due to disease outbreaks.

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

- Anto L, Joseph S, Mini M and Pellissery A J. 2014. Identification of anaerobic and concomitant aerobic bacterial etiologies in caprine footrot. *International Journal of Current Microbiology and Applied Sciences* **3**(4): 197–206.
- Bumbarov V, Natalia Golender Maria Jenckel, Kerstin Wernike, Martin Beer, Evgeny Khinich, Olga alesky and Oran Erster. 2020. Characterization of bluetongue virus serotype 28. *Transboundary and Emerging Diseases* **67**(1): 71–182. doi: 10.1111/tbed.13338.
- Chaignat V, Worwa G, Scherrer N, Hilbe M, Ehrensperger F, Batten C and Thuer B. 2009. Toggenburg Orbivirus, a new bluetongue virus: Initial detection, first observations in field and experimental infection of goats and sheep. *Veterinary Microbiology* **138**(1-2): 11–19. doi: 10.1016/j.vetmic.2009.02.003
- Chandranaiik B M, Kingstad-Bakke B, Lee W, Hatta M, Sonsalla M, Larsen A, Kawoaka Y and Suresh M. 2020. Programming multifaceted pulmonary T cell immunity by combination adjuvants. *Cell Reports Medicine* **1**(6): 100095.
- Chandranaiik B M, Shetty V, Giridhar P, Reddy P, Meghana P and Byregowda S M, 2019. Molecular epidemiology of porcine reproductive and respiratory syndrome virus causing outbreaks in Karnataka. *Indian Journal of Animal Sciences* **89**(10): 1069–72.
- Chandranaiik B M, Shivashankar B P, Umashankar K S, Nandini P, Giridhar P and Byregowda S M. 2017. Mycobacterium tuberculosis infection in free-roaming wild Asian elephant. *Emerging Infectious Diseases* **23**(3): 555–57.
- Hemadri D, Maan S, Chanda M M, Rao P P, Putty K, Krishnajyothi Y and Mertens P. 2017. Dual Infection with Bluetongue virus serotypes and first-time isolation of Serotype 5 in India. *Transboundary Emerging Diseases* **64**(6): 1912–17.
- Hofmann M A, Renzullo S, Mader M, Chaignat V, Worwa G and Thuer B. 2008. Genetic characterization of Toggenburg orbivirus, a new bluetongue virus, from goats, Switzerland. *Emerging Infectious Diseases* **14**(12): 1855–61. doi: 10.3201/eid1412.080818
- Jenckel M, Breard E, Schulz C, Sailleau C, Viarouge C, Hoffmann B and Zientara S. 2015. Complete coding genome sequence of putative novel bluetongue virus serotype 27. *Genome Announcements* **3**(2). doi: 10.1128/genomeA.00016-15.
- Jones A E, Turner J and Caminade C. 2019. Bluetongue risk under future climates. *Nature Climate Change* **9**: 153–57.
- Lima P A, Utiumi K U, Nakagaki K Y R, Bihrer D A, Albuquerque A S, Souza F R, Matos A C, Lobato Z P, Driemeier D, Peconick A P, Varaschin M S and Raymundo D L 2016:. Diagnoses of ovine infection by the Serotype-4 bluetongue virus on Minas Gerais, Brazil. *Acta Scientiae Veterinariae* **44**: 1–4.
- Maan S, Maan N S, Belaganahalli M N, Kumar A, Rao P P and Mertens P P. 2015. Genome sequence of Bluetongue virus type 2 from India: Evidence for reassortment between outer capsid protein genes. *Genome. Announceent* **3**(2). doi: 10.1128/genomeA.00045-15
- Maan S, Maan N S, Nomikou K, Veronesi E, Bachanek-Bankowska K, Belaganahalli M N and Mertens P P. 2011. Complete genome characterisation of a novel 26<sup>th</sup> bluetongue virus serotype from Kuwait. *PLoS One* **6**(10): e26147. doi: 10.1371/journal.pone.0026147.

- Mahrt C R and Osburn B I. 1986. Experimental bluetongue virus infection of Sheep; effect of previous vaccination: Clinical and immunological studies. *American Journal of Veterinary Research* **47**(6): 1191–97.
- Mamatha G S, Shruthi R, Chandranaik B M, Placid E. D'Souza, Thimmareddy P M, Shivashankar B P and Puttalakshamma G C. 2017. Molecular epidemiology and phylogenetic characterisation of Theileria luwenshuni in India: A first report. *Small Ruminant Research* **154**: 52–57.
- Mertens P P, Maan N S, Prasad G, Samuel A R, Shaw A E, Potgieter A C and Maan S S. 2007. Design of primers and use of RT-PCR assays for typing European bluetongue virus isolates: differentiation of field and vaccine strains. *Journal of General Virology* **88**(10): 2811–23. doi: 10.1099/vir.0.83023-0
- OIE. 2018: Bluetongue (infection with bluetongue virus) *Manual of diagnostic tests and vaccines for terrestrial animals*. Paris, France
- Prasad G, Sreenivasulu D, Singh K P, Mertens P P and Maan S. 2009. *Bluetongue in the Indian subcontinent. Bluetongue*. (Eds.) Mellor P S, Baylis M and Mertens P P C. London: Elsevier Academic Press.
- Prasad M, Ranjan K, Kumar P and Prasad G. 2013. Segment 2 based characterization of a novel Indian bluetongue virus isolate. *Veterinary World* **6**(5): 244–48. doi:10.5455/vetworld.2013.244-248
- Quinn P J, Markey B K, Leonard F C, Fitzpatrick E S, Fanning S and Hartigan P J. 2011. *Veterinary Microbiology and Microbial Diseases*, 2<sup>nd</sup> edn. Wiley-Blackwell publications, United Kingdom.
- Rao P P, Reddy Y V and Hegde N R. 2015. Isolation and complete genome sequencing of bluetongue virus serotype 12 from India. *Transboundary Emerging Diseases* **62**(5): e52–59. doi: 10.1111/tbed.12199
- Reddy Y V, Krishnajyothi Y, Susmitha B, Devi B V, Brundavanam Y, Gollapalli S R and Rao P P. 2016. Molecular typing of bluetongue viruses isolated over a decade in South India. *Transboundary Emerging Diseases* **63**(5): e412–418. doi: 10.1111/tbed.12320
- Reddy Y V, Susmitha S, Patil Y, Krishnajyothi K, Putty K V, Ramakrishna and Rao P P. 2018: Isolation and evolutionary analysis of Australasian topotype of bluetongue virus serotype 4 from India. *Transboundary Emerging Diseases* **65**(2): 547–56. doi: 10.1111/tbed.1273
- Report of the Indian Meteorological Department and NASA earth observatory. Current weather status and outlook for next two weeks, October, 2019. Indian Meteorological Department, New Delhi, India.
- Ries C, Sharav T, Tseren-Ochir E O, Beer M and Hoffmann B. 2020. Putative novel serotypes '33' and '35' in clinically healthy small ruminants in Mongolia expand the group of Atypical BTV. *Viruses*. **13**(1): DOI: 10.3390/v13010042.
- Roy P, Marshall J J and French T J. 1990. Structure of the bluetongue virus genome and its encoded proteins. *Current Topics in Microbiology and Immunology* **162**: 43–87.
- Savini G, Puggioni G, Meloni G, Marcacci M, Di Domenico M, Maria Rocchigiani A and Lorusso A. 2017. Novel putative Bluetongue virus in healthy goats from Sardinia, Italy. *Infection Genetics Immunity* **51**: 108–17.
- Stewart D J, Clark B L and Jarrett R G. 1984. Difference between strains of *Bacteroides nodosus* in their effects on the severity of footrot, body weight and wool growth in Merino sheep. *Australian Veterinary Journal* **61**: 348–52.
- Tamura K, Stecher G, Peterson D, Filipski A and Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725–29.
- Tadich N and Hernandez M. 2000. A survey on the foot lesions in sheep from 25 small holdings in the province of Valdivia, Chile. *Archives Medicine Veterinaria* **32**: 63–74.
- Wenju C, Borlace B, Matthieu L, Peter V R, Mat C, Gabriel V, Axel T, Agus S and Michael J M. 2014. Increasing frequency of extreme El Niño events due to greenhouse warming. *Nature Climate Change* **4**: 111–16.
- Wright I M. 2013: Serological and genetic characterisation of putative new serotypes of bluetongue virus and epizootic haemorrhagic disease virus isolated from an Alpaca. South Africa: North-West University, Potchefstroom Campus.