

# Occurrence of Bluetongue in ruminants in Tamil Nadu, South India

Y. Krishnamohan Reddy<sup>1\*</sup>, K. Brindha<sup>1</sup>, P.I. Ganesan<sup>1</sup>, K. Srinivas<sup>2</sup>, G.S. Reddy<sup>2</sup> and P. Minakshi<sup>3</sup>

<sup>1</sup> All India Network Programme on Bluetongue, Vaccine Research Centre - Viral Vaccines, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, 600051, India.

<sup>2</sup> Indian Immunologicals Limited, Gachibowli, Hyderabad, Telengana State, India.

<sup>3</sup> All India Network Programme on Bluetongue, Department of Animal Biotechnology, College of Veterinary Science, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, 125004, India.

\*Corresponding author at: Vaccine Research Centre - Viral Vaccines, Centre for Animal Health Studies, Madhavaram Milk Colony, Chennai, 600051, India. Tel.: 0091 9962754861, e-mail: drykmreddy@hotmail.com.

*Veterinaria Italiana* 2016, **52** (3-4), 293-297. doi: 10.12834/VetIt.502.2421.2

Accepted: 17.07.2016 | Available on line: 30.09.2016

IV International Conference on *Bluetongue and Related Orbiviruses*. November 5-7, 2014 - Rome, Italy - Selected papers

## Keywords

Bluetongue, India, Mortality, Ruminants, Sequence homology, Serotypes 1, 2, 16, 23, Tamil Nadu.

## Summary

Tamil Nadu is located in the South-Eastern part of Indian peninsula, between 8.087° and 13.09°N and 76.50° and 80.27°E. Bluetongue (BT) was first reported in this region in sheep during 1982 with regular occurrence thereafter. In 1989-1990, 1997-1998 and 2005-2006, there was wide spread occurrence of BT resulting in huge mortality of sheep. The present study had the goal of isolating the BTV from outbreaks in sheep occurred in Tamil Nadu between 2003-2011 and comparing the VP2 gene sequences of the BTV isolates involved in such outbreaks. Serotypes 1, 2, 16, and 23 of the Bluetongue virus (BTV) have been isolated from sheep during BT outbreaks. BTV-16 has also been isolated in goats and cattle in the region; BTV-2 isolated in Tamil Nadu has homology with BTV-2 isolated in Africa; whereas the BTV-23 isolated in this area has homology with BTV-23 from South East Asia, indicating that both Eastern and Western topotypes of BTV are circulating in ruminant population in Tamil Nadu.

## Presenza di Bluetongue nella regione di Tamil Nadu in India

## Parole chiave

Bluetongue, India, Mortalità, Omologia di sequenza, Ruminanti, Tamil Nadu, Sierotipi 1, 2, 16, 23 del virus della Bluetongue.

## Riassunto

Tamil Nadu (coordinate: 8.087° e 13.09°N - 76.50° e 80.27°E) è una regione del Sud-Est dell'India. La Bluetongue (BT) è stata segnalata per la prima volta negli ovini di questa regione nel 1982, per manifestarsi regolarmente negli anni a seguire. Nel 1989-1990, 1997-1998 e 2005-2006, la BT si è diffusa ampiamente causando un'elevata mortalità negli ovini. Lo scopo di questo studio è quello di isolare i ceppi di BTV responsabili dei focolai di BTV verificatisi nella regione tra il 2003 e 2011 e di confrontare le sequenze del gene che codifica per la VP2 con quelle di altri ceppi di BTV depositate in GeneBank. Sono stati isolati i sierotipi 1, 2, 16 e 23 del virus della Bluetongue (BTV). Di questi, il sierotipo 16 è stato isolato in ovini e bovini, il sierotipo 2 è risultato simile al sierotipo 2 isolato in Africa e il sierotipo 23 simile al sierotipo 23 isolato nell'Asia sud orientale. Questi dati indicano che i topotipi occidentale e orientale del BTV circolano tra le specie ruminanti presenti nella regione di Tamil Nadu.

## Introduction

Bluetongue (BT) was first reported in India in 1964. The disease mainly affects sheep. Antibodies against the Bluetongue virus (BTV) have been detected in cattle, buffaloes, goats, camel, and deer. The occurrence of BT in sheep is more severe in Tamil Nadu, Karnataka, Andhra Pradesh and Telengana states of South India, followed by the onset of rains during North-East monsoon and to some extent due to South-West monsoon. The disease is less severe in Gujarat, Haryana and other states of Northern India (Koteeswaran *et al.* 2005). Twenty-two serotypes have been reported in India either on the basis of virus isolation or serology (Ranjan *et al.* 2015, Rao *et al.* 2016). To date, in India BTV serotypes 1, 2, 9, 10, 16, and 23 have been identified by virus isolation<sup>1</sup>.

Of the various diseases affecting sheep BT is the most significant one in Tamil Nadu.

The details of occurrence of BT in Tamil Nadu from 1982 (date of the first report of BT in the region) until 2005-2006 are provided in Table I. Since 2006, BT has occurred regularly in different districts of Tamil Nadu. Sheep of all the breeds and of any age were all affected, although the disease was not common in young lambs. A severe form of disease has been observed every 3 or 4 years (Koteeswaran *et al.* 2005).

Affected sheep showed typical BT symptoms, such as raise of the body temperature (40-40.5°C), excess salivation, wry neck, swollen lips, swollen face, ulcers on mouth, haemorrhages on gums, necrotic areas on the dental pad, hyperaemia of tongue, stiffness of muscles, enteritis, and lameness. Morbidity and mortality were highly variable. Congestion and haemorrhages of internal organs, consolidation of lungs, petechial in intestinal mucosa, and frothy

exudate in the bronchi and trachea were observed on post-mortem examinations. Cyanotic tongue was not observed in the affected sheep. Autopsies revealed inter-alveolar hyperaemia, severe alveolar oedema, and distinctive haemorrhage near the base of the pulmonary artery (Prasad *et al.* 1982, Martinelle *et al.* 2013)<sup>2</sup>.

The disease was widespread and has been reported in all the areas of Tamil Nadu where sheep are being bred, severe manifestations of BT with high mortality were observed mainly in Southern districts, between October and December, due to heavy rainfall during North-East monsoon. A late onset of disease has been reported in 2005 in the Erode district starting early November and ending early January, due to the frost and general chillness. Between June and July of the same year, mild to moderate forms of the disease were observed in areas such as Salem, Dhamapuri, and Erode due to South-West monsoon. The mortality due to BT was nil during this period. Bluetongue was not reported in the Nilgiris district due to high altitude of 900 to 2,636 meters above sea level till 2005 (Koteeswaran *et al.* 2005) However mild disease incidence involving BTV-23 was reported by Venkataramanan and colleagues (Venkataramanan *et al.* (2010).

The present study had the goal of isolating the BTV from outbreaks in sheep occurred between 2003-2011 and comparing the VP2 sequences of the BTV isolates involved in such outbreaks.

## Materials and methods

### Sample collection isolation of BTV

Blood samples in heparinized vacutainer tubes from sick animals were collected. In case of dead sheep, spleen, liver, lung, and heart tissues were collected during BT outbreaks. Blood samples from the sheep exhibiting clinical signs such as limping, mouth lesions, and wry neck were not preferred for the virus isolations, since on most of the occasions these samples were positive for BTV antibody.

### Isolation of BTV

Isolation of BTV was conducted following the method described by Clavijo and colleagues (Clavijo *et al.* 2000). The samples were first processed in embryonated chicken eggs (by intravenous route) and then passaged into BHK<sub>21</sub> cells.

Total RNA was extracted from BTV infected BHK-21

**Table I.** Occurrence of Bluetongue in Tamil Nadu.

1982	First reported in sheep in Dindugal
1986	Reported in sheep in Pasumpan Thevar and Ramanathapuram districts of Tamil Nadu
1989-1990	There was widespread occurrence of Bluetongue in several districts of Tamil Nadu
1991-1996	Virus isolations were made but serotype determination was not done
1997-1998	Occurred in severe form resulting in the death of more than half a million sheep
2000	BTV serotype 23 was confirmed
2003	BTV serotypes 1, 2, and 23 were confirmed (Koteeswaran <i>et al.</i> 2005)
2005-2006	Bluetongue occurred in severe form

<sup>1</sup> AINPBT. 2010. All India Network Programme on Bluetongue, Indian Council of Agricultural Research, New Delhi.

<sup>2</sup> Wildpro - the electronic encyclopaedia and library for wildlife, wildpro.twocrosszoo.org

**Table II.** Primers employed for amplification of NS1 gene and VP2 gene of the serotypes 1, 2, 16, and 23 of Bluetongue virus.

S.No.	Name	5'<----Sequence---->3'	Length	Product base pair length
1	BTV1VP2-S3	AATCCACGCGGTGCAAGAT	21	604
2	BTV1VP2-S4	ATGGTCGAGTTAACCTGTTGATTATGTC	29	
3	BTV2VP2-S9	GTTAAAACAGGATCGCGATGGATG	24	401
4	BTV2VP2-S10	CCGGCTATGTTCCCTCATCAATCGA	24	
5	BTV16VP2-S2 (973-992)	CCG ATC CRG AGA GAA ACG TC	20	768
6	BTV16VP2-S2 (1740-1718)	CTC TCA CAT CCA TGT AAT GCG	21	
7	BTV23VP2-S7	TTGTCCACGCGAGCGCGCA	20	658
8	BTV23VP2-S8	ATCGAACAGGTTCACTCGGC	20	
9	BTVNS1-P1	GTTGGCAACCACCAACATGG	21	366
10	BTVNS1-P2	TCCCACTTTTGGCGCTAATCCTCAA	24	

monolayers using TRI Reagent® in accordance with the protocol of Sigma-Aldrich, St Louis, USA. Then, dsRNA was purified (Attoui *et al.* 2000).

First, reverse transcription-polymerase chain reaction (RT-PCR) of NS1 gene was carried out for the detection of BTV isolates.

The serotype of virus was determined by RT-PCR coupled with sequencing of a fragment of genome segment 2 (Seg 2) coding for VP2. A fragment size was visualized on agarose gel electrophoresis as per the protocol developed at the Hisar centre of AINPBT and Indian Immunologicals Limited, Hyderabad, India.

The primers employed for amplification of NS1 gene and VP2 gene of BTV-1, BTV-2, BTV-16, and BTV-23 are specified in Table II.

The PCR product was sequenced. Basic Local Alignment Search Tool (BLAST)<sup>3</sup> analysis of the sequence of PCR product was carried out for subjecting to Clustal W – multiple sequences alignment.

## Results

Between 2003 and 2011, 32 BTV isolates were confirmed by NS1 gene RT-PCR (Figure 1). The isolates were serotyped by VP2 gene specific RT-PCR as reported in Figure 2 and Table III.

## Discussion

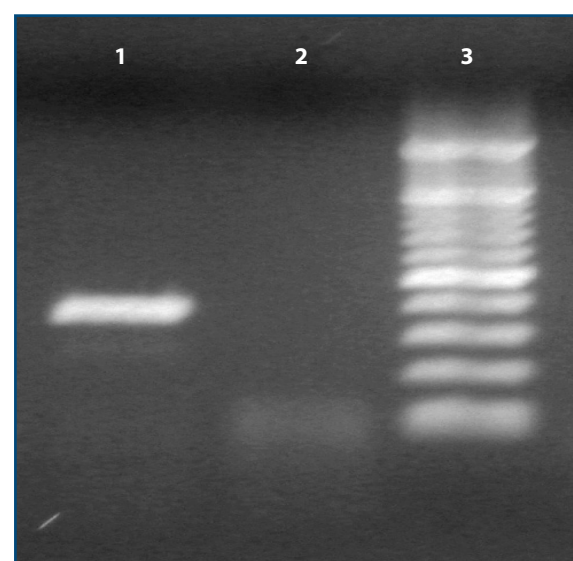
Out of 32 BTV isolates, 6 isolates belonged to BTV-1; 11 isolates belonged to BTV-2; 4 isolates belonged to BTV-16; and 11 isolates belonged to BTV-23. They were isolated from cattle, sheep, and goats.

Recently, BTV-1 was isolated and presence of BTV-23 neutralizing antibodies were identified in goats on the Pithoragarh hills of the region Uttarakhand,

India (Mondal *et al.* 2013). In several occasions, BTV has been isolated from sheep or goats naturally infected with Peste des petits ruminants virus (PPRV). In cases of mixed infection, the PPR symptoms become prominent (Mondal *et al.* 2009). Antibodies to or against BTV were often detected in goats and cattle, although the clinical disease was not reported in Tamil Nadu and Kerala states (Arun *et al.* 2014, Reddy *et al.* 2008).

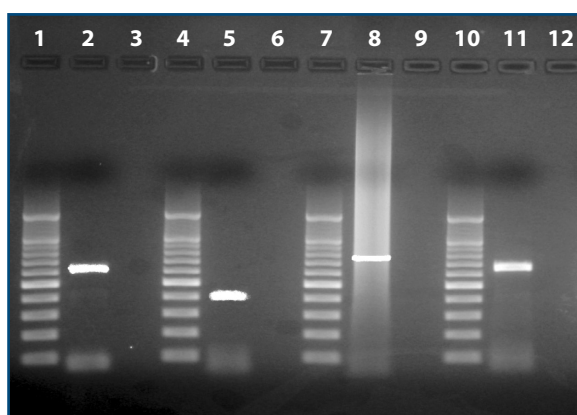
Mann and colleagues (Mann *et al.* 2004) reported the full-length sequence analysis of genome segment 2/VP2 from at least 1 representative isolate of each BTV serotype, which has enabled the construction of phylogenetic trees showing the genetic correlation of different BTV serotypes.

The result of Clustal W – multiple sequences alignment of the BTV-1 sequences has revealed that BTV-1 isolates from Tamil Nadu were identical with BTV-1 sequences from other parts of India.



**Figure 1.** Reverse transcription-polymerase chain reaction of NS1 gene of Bluetongue virus. 1 = BTV NS1 (366 bp); 2 = Negative control; 3 = 100 bp ladder.

<sup>3</sup> AINPBT. 2010. All India Network Programme on Bluetongue, Indian Council of Agricultural Research, New Delhi.



**Figure 2.** Reverse transcription-polymerase chain reaction of VP2 gene of serotypes 1, 2, 16, and 23 of the Bluetongue virus. 1 = 100 bp ladder; 2 = BTV-1 (604 bp); 3 = blank; 4 = 100 bp ladder; 5 = BTV-2 (401 bp); 6 = blank; 7 = 100 bp ladder; 8 = BTV-16 (768 bp); 9 = blank; 10 = 100 bp ladder; 11 = BTV-23 (658 bp); 12 = blank.

The result of Clustal W – multiple sequences alignment of the BTV-2 sequences has revealed that BTV-2 isolates from Tamil Nadu were identical with BTV-2 sequences from Africa and USA. Isolates of BTV-2 identical to the isolates from South-East Asia were absent in Tamil Nadu, whereas these isolates have been reported from other parts of India on few occasions (Balumahendran *et al.* 2009).

The result of Clustal W – multiple sequences alignment of the BTV-23 sequences has revealed that BTV-23 isolates from Tamil Nadu were identical with BTV-23 sequences from other parts of India and South-East Asia.

Sequence analysis of the BTV-1, BTV-2, BTV-16,

**Table III.** Isolation of Bluetongue virus serotypes between 2003 and 2011.

BTV serotype	Year of Isolation	No of BTV isolates
BTV-1	2003 (S), 2005 (S – Two isolates), 2009 (S), 2010 (S), 2011(S)	6
BTV-2	2003 (S), 2004 (S), 2005 (S), 2007 (S), 2008 (S), 2009 (S), 2010 (S – Two isolates), 2011 (S – Three isolates)	11
BTV-16	2010 (C), 2010 (S – Two isolates), 2010 (G)	4
BTV-23	2000 (S), 2003 (S – Two isolates), 2004 (S), 2005 (S), 2007 (S), 2008(S), 2009 (S), 2011 (G), 2011 (S – Two isolates)	11

S = Sheep; G = Goat; C = Cattle.

BTV-23 isolated from Tamil Nadu between 2003 and 2011 has revealed that there is no variation in the VP2 gene sequence of the BTV strains isolated from Tamil Nadu.

Isolates of BTV-2 in Tamil Nadu have homology with BTV-2 from Africa, while BTV-23 isolates have homology with BTV-23 from South-East Asia, indicating that both Eastern and Western topotypes of BTV are circulating in ruminant population in Tamil Nadu. This is possibly due to the increased human traffic and also the cargos arrival from African and South-East Asia to the South Indian Peninsula.

### Acknowledgement

This work was funded by Indian Council of Agricultural Research through 'All India Network Programme on Bluetongue'.

## References

- Arun S., John K., Ravishankar C., Mini M., Ravindran R. & Prejit N. 2014. Seroprevalence of Bluetongue among domestic ruminants in Northern Kerala, India. *Tropical Biomedicine*, **31** (1), 26-30.
- Attoui H., Billoir F., Cantaloube J.F., Biagini P., de Micco P. & de Lamballerie X. 2000. Strategies for the sequence determination of viral dsRNA genomes. *J Virol Methods*, **89**, 147-158.
- Balumahendran K., Srinivasulu D., Chockalingam A. & Byregowda S. 2009. Characterization of VP2 gene of an Indian bluetongue virus serotype 2 and its close phylogenetic relationship to Taiwan isolate. *Res Vet Sci*, **82**, 332-338.
- Koteeswaran A., Y. Krishnamohan Reddy Y., Prabhakar T.G., Wilson A., Chandran N.D.J., Saravanabhava K., Govindarajan R. & Meenambigai T.V. 2005. Project Report: Part II scheme on development of bluetongue vaccine for sheep, Government of Tamil Nadu.
- Mann S., Mann N.S., Samuel A.R., O'Hara R., Meyer A.J., Rao S. & Mertens P.P.C. 2004. Completion of the sequence analysis and comparisons of genome segment 2 (encoding outer capsid protein VP2) from representative isolates of the 24 bluetongue virus serotypes. *Vet Ital*, **40**, 484-488.
- Martnelle M., Sarradin P., De Leeuw P., De Klerca K., Thys C., Thiry E. & Saegerman C. 2013. Pulmonary artery haemorrhage in newborn calves following bluetongue virus serotype 8 experimental infections of pregnant heifers. *Vet Microbiol*, **167**, 250-259.
- Mondal B., Bisht B., Biswas S.K. & Chand K. 2014. Bluetongue virus serotype-1 in goats in the Pithoragarh area of Uttarakhand, India. *Vet Ital*, **49**, 375-380.
- Mondal B., Sen A., Chand K., Biswas S.K., De A., Rajak K.K. & Chakravarti S. 2009. Evidence of mixed infection of peste des petits ruminants virus and bluetongue virus in a flock of goats as confirmed by detection of antigen, antibody and nucleic acid of both the viruses. *Trop Anim Health Prod*, **41**, 1661-1667.
- Prasad G., Jain N.C. & Gupta Y. 1992. Bluetongue virus infection in India. A review. *Rev Sci Tech*, **11**, 699-711.
- Rajan K., Minakshi P. & Prasad G. 2015. Bluetongue: Indian perspective. *Acta Virol*, **59**, 317-337.
- Rao P.P., Hegde N.R., Reddy Y.N., Krishnajyothi Y., Reddy Y.V., Sushmitha B., Gollapalli S.R., Putty K. & Reddy G.H. 2016. Epidemiology of bluetongue in India. *Transbound Emerg Dis*, **63**, 151-164.
- Reddy Y.K., Balachandran S. & Koteeswaran A. 2008. Serological studies of bluetongue virus infection in goats and cattle. *Indian Vet J*, **85**, 680-682.
- Venkataramanan R., Reddy Y.K., Krishnakumar S. & Manohar B.M. 2010. Occurrence of bluetongue in the Nilgiri Hills of Tamil Nadu. *Indian Vet J*, **87**, 724-725.