International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; 8(3): 72-75

www.veterinarypaper.com
Received: 05-02-2023
Accepted: 11-03-2023

Sonali M
College of Veterinary science, Rajendranagar, PVNRTVU, Hyderabad, Telangana, India

Enumula K
College of Veterinary science, Rajendranagar, PVNRTVU, Hyderabad, Telangana, India

N Karuna Sree
College of Veterinary science, Rajendranagar, PVNRTVU, Hyderabad, Telangana, India

Reddy YN
College of Veterinary science, Rajendranagar, PVNRTVU, Hyderabad, Telangana, India

Dhanalakshmi K
College of Veterinary science, Rajendranagar, PVNRTVU, Hyderabad, Telangana, India

Sukanya N
Assistant Professor, University College of Pharmaceutical, Sciences, Kakatiya University, Warangal, Telangana, India

Corresponding Author:
Sonali M
College of Veterinary science, Rajendranagar, PVNRTVU, Hyderabad, Telangana, India

Molecular characterization of bluetongue virus serotype 2

Sonali M, Enumula K, N Karuna Sree, Reddy YN, Dhanalakshmi K and Sukanya N

DOI: https://doi.org/10.22271/veterinary.2023.v8.i3b.523

Abstract
Bluetongue is an economically major infectious, arthropod vector borne, viral disease of many free ranging and captive wild animals as well as of domestic animals. There are many serotypes circulating in the world. The current study was undertaken having an objective to characterize the BTV 2(M11) isolate that engaged for the vaccine trial. BHK_{21} cell lines were utilized for cultivation of BTV serotype 2 and followed by its RNA was extracted. Further, Reverse Transcriptase-PCR was standardized for detection of BTV serotype 2 by targeting segment 7 (VP7) gene. Characterization of isolate at molecular level was conducted by the sequencing of VP7 gene. By using the available sequences in Genbank database, the sequences were compared. Further analysis denoted that, all sequences of VP7 nucleotide was sequestered into seven phylogenetic clades. The homogeneity of BTV-2 (M11) with Australian and Chinese isolates of BTV-15 and BTV Taiwan isolate was 75%, 66%, and 74% respectively. Further analysis signified that, BTV-2 (M11) was nearly compared to BTV-12 Brazil and BTVTPT.

Keywords: Domestic animals, Genbank database

1. Introduction
Bluetongue (BT) is, one of the World Organization for Animal Health listed diseases of sheep and goats and also endemic to India, causing suggestive financial losses to the small ruminant industry (OIE 2018). The disease is caused by *bluetongue virus* (BTV) that belongs to the genus *Orbivirus* of family *Reoviridae* and subfamily *Sedoreovirinae* (ICTV 2011) [11]. The disease is chiefly transmitted by the bite of a Culicoides midge (Mellor P S *et al*., 1995) [14], occasionally by direct contact (Battern C., 2014 and Breard *et al*., 2018) [1] and also by vertical (transplacently) routes (Saegerman C *et al*., 2011) [20].
Presently there are 29 different serotypes of BTV are circulating worldwide (Thota *et al*., 2021) [24]. This disease is endemic in India with a total of 24 serotypes reported and were predominantly reported from Southern states of Peninsular India (Thota *et al*., 2021 and Reddy Y V *et al*., 2016) [24, 18]. The genome of BTV is consisting of double-stranded RNA (dsRNA) in the form of 10 linear segments. These 10 segments code for both nonstructural and structural proteins. (Bommineni *et al.*, 2008. Rao PP *et al.*, 2012 and Maan NS *et al*., 2012.) [2, 17, 13]. The VP7 protein is encoded by segment 7 of BTV genome and is reasonably conserved. Researchers observed that this VP7 protein is showing serological cross-reactions between different isolates within separate *Orbivirus* serogroups (Huismans and Erasmus, 1981; Gumn and Newman, 1982) [10, 9]. For topotyping or geo-typing purposes, the serogroup specific genes such as VP3 (Gould and Pitchard, 1990) [8] VP7 (Bonneau *et al*., 2000 and Wilson *et al*., 2000) [4, 27] and S10 (Bonneau *et al*., 1999) [3] are considered as good targets. The VP7 protein, which is amenable for cell tropism of BTV in the insect vector, has been progressing with different species of culicoides existing in numerous regions of the world to configure diversified topotypes (Bonneau *et al*., 2000) [4]. Thorough genetic knowledge on segment 7 (VP7) gene may be helpful not only to anticipate variations among the Indian isolates but also to track the origin of BTV and its spread, to characterize the strain, to genotype, to produce vaccine and for development of drugs. The present study was taken up with the objectives of molecular characterization of Bluetongue virus serotype 2 based on VP7 gene.

2. Materials and Methods

In this study, BTV serotype 2 which we used was serotyped at serotyping Centre, Haryana Agricultural University, Hissar under All India Network Project on Bluetongue disease. BHK_{21} cell lines were used for passaging of Bluetongue virus (for five times). Acid Phenol method was used for extraction of RNA from BTV infected cell cultures as described by earlier (Chomczynski and Sacchi, 1987) [6] and later the quality of extracted RNA was assessed by 1% agarose gel electrophoresis. The amplification method standardized by Wade Evans *et al*. (1990) [26] based on segment 7 sequence of BTV-1 South Africa (GenBank Acc. No. X53740) was used for multiplication of segment 7 gene. The primers used were mentioned in the table 1.

Table 1: Primers sequences used for amplification of VP7 gene

Sl. No.	Primers coding for S 7 gene (VP7)	Product size
1	Forward primer - 5'GTTAAAAATCTATAGAG3'(1-17)	1156 bp
2	Reverse primer - 5'GTAAGTGTAATCTAAGAGA3'(1156-1138)	

Further, RNA mix was prepared as mentioned in the table 2. The mix was heated at 68 ºC for 5 min. and snap-cooled on ice. Later cDNA mixture was prepared as mentioned in the table 4.

Table 3: Components of RNA mixture

Sl. No.	Components to be added	Quantity
1	Forward primer (20 pmol/µl)	1µl
2	Reverse primer (20 pmol/µl)	1µl
3	RNA	8µl

Table 4: Components of cDNA mixture

Sl. No	cDNA mix components	Quantity
1	5X RT Buffer	4.0 µl
2	DTT (0.1M)	1.0 µl
3	RNase out (40 U/µl)	0.5 µl
4	AMV RT (15 U/µl)	1.0 µl
5	dNTPs	2.0 µl
6	NFW	1.5 µl

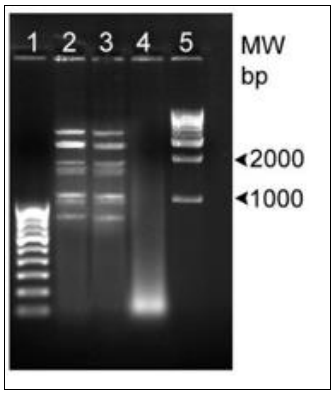
cDNA mixture was added to RNA mixture and incubated for 15min at 25 ºC and then for 45min at 42 ºC, later kept at 4 ºC. Amplification of cDNA was done as described earlier (Sonali *et al*., 2017) [21]. The cycling conditions were mentioned in the table 5.

Initiation	30 cycles			Final extension
	Denaturation	Annealing	Extension	
95 ºC-3 min	95 ºC-20 sec	39 ºC- 60 sec	70 ºC-2 min	70 ºC-7 min.

The amplified product was confirmed by subjecting it to 1% agarose gel electrophoresis. The conformed amplicon was purified as per the method described earlier (Sonali *et al*., 2017) [21] and then sequenced at M/s Bioserve Biotechnologies Ltd, Hyderabad, using PCR sequencing method in Beckman CEQTM 8000 Genetic Analysis System using S7 gene specific primers. The obtained VP 7 gene sequence was aligned with the sequences available in the database by applying NCBI BLAST (www.ncbi.nlm.nih/gov/blast) to confirm their identity. Further, Multiple alignment was done with 75 different BTV segment 7 gene sequences obtained from Genbank and then cladogram was developed by loading the above sequences in the CLUSTAL W (www.ebi.ac.uk/clustalw) software (Thompson *et al*., 1997) [23].

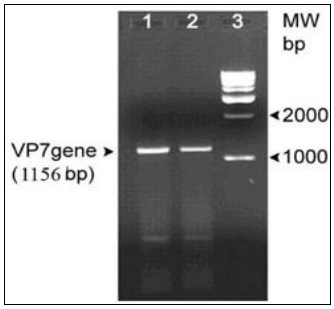
3. Results and Discussion

The dsRNA was extracted from BTV-2isolate infected BHK_{21} cell cultures and subjected to 1% agarose gel electrophoresis procedure, revealed the segmented genome pattern with 10 bands including two inseparable bands (Fig 1) which is a distinctive feature of BTV nucleic acid. Determining RNA bands were not clear for two segments. The RT-PCR was optimized for VP7gene of BTV serotype 2(M11) as a specific band of 1156 bp was identified by 1% agarose gel electrophoresis (Fig. 2). The sequencing of VP7 gene of BTV serotype 2 was performed by using VP7 gene specific forward (F) and reverse (R) primers and the reverse complementary sequence obtained using reverse primer was lined with the forward sequence to attain full length gene sequence. By using the available sequences in Genbank database, the sequences were compared with help of Clustal W software. The percentage of homogeneity was shown in table 1.



Lane: 1 100 bp ladder
Lane: 2 and 3: BTV – 2 (M11) ds RNA
Lane: 4 BHK_{21}Cell RNA
Lane: 5 1kb ladder

Fig 1: Migration patterns of the BTV – 2 (M11)



Lane: 1 & 2 BTV-2 (M11)
Lane: 3 1 kb ladder

Fig 2: VP7 gene PCR product of BTV-2 (M11)

Table 1: Percentage identity between VP7 gene sequences of BTV isolates

BTV serotype	Relation with other BTV isolates	% identity
BTV-2 (M11)	BTV-15 (N12)	73
	BTV-15AU	75
	BTV-15Ch	66
	BTV kmTh	74

Phylogenetic analysis of BTV-2 (M11) isolate in regard to the VP7 gene sequence with other available sequences of BTV

serotypes were. The developed cladogram relegated the BTV -2 (M11) and other available sequences of BTV serotypes into 7 clades (Fig 3) and BTV-2 and BTV-15 isolates are segregated into clade 1(Fig 3) and the same was reported by Sonali *et al*., 2017 [21]. Further analysis revealed that there was 73% homogeneousness between BTV-2 (M11) and BTV-15 (N12) isolates and the similar results were reported by Sonali *et al*., 2017 [21]. BTV-2 (M11) was closely compared to BTV-12 Brazil and BTVTPT. Nucleotide homology of segment 7 (S7) between Indian isolates of BTV-2, 9 and BTV-10 US was 79% (Prasad 2005). A close association was observed between BTV -15 isolate from Indian and BTV-10 and BTV-17 isolates from USA by Dalal *et al*. (2008) [7]. Kowalik *et al*. (1990) [12] reported that there was 7-20% of divergence was observed among the BTV -2, 10, 11, 13 and 17 with BTV -13 and announced it is most divergent serotype after the supervising the cloning and sequencing of VP7 gene of all the 5 US serotypes.

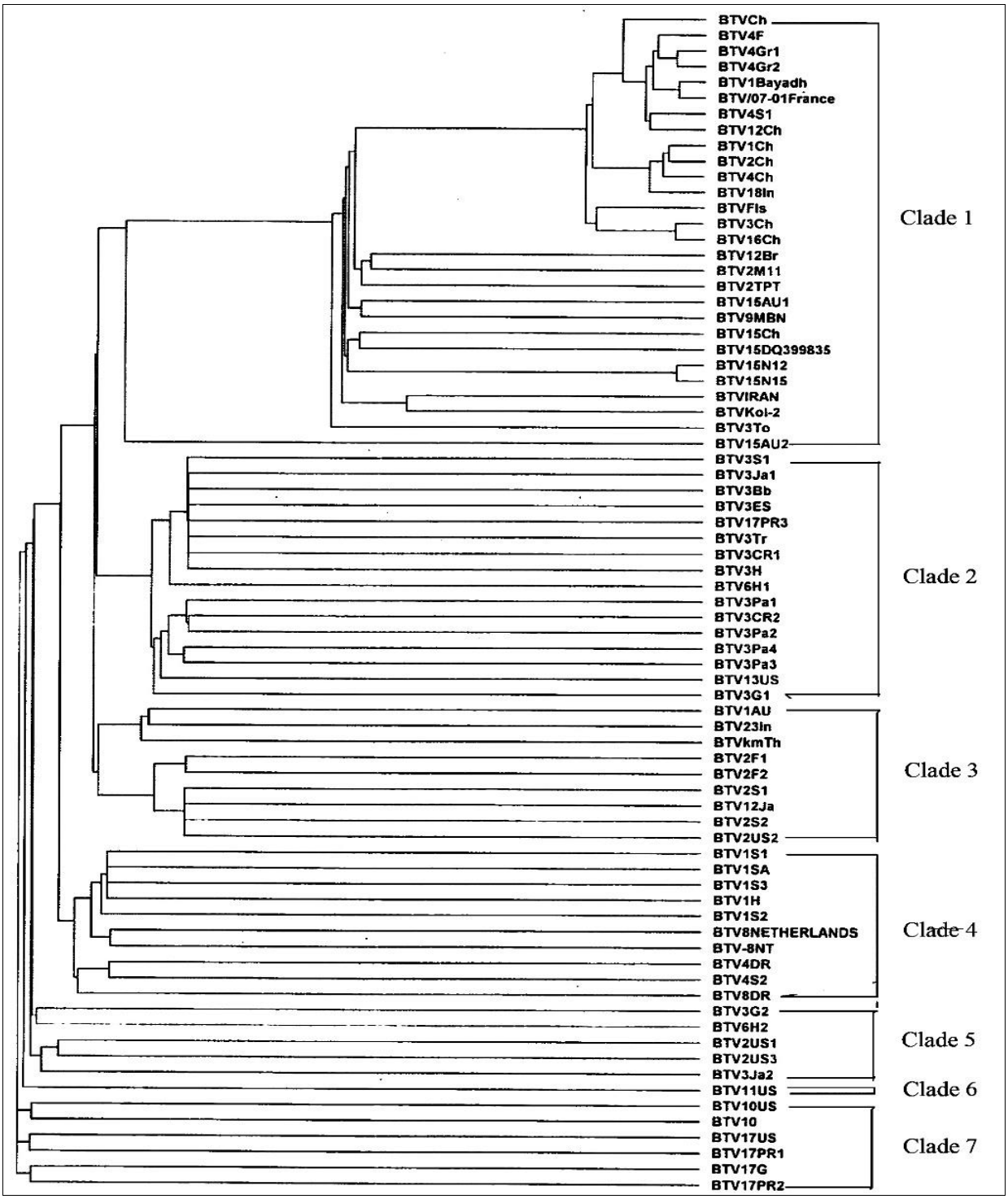


Fig 3: Phylogenetic analysis of VP7gene sequence of various BTV isolates

The results of this study reported that BTV-2 (M11) isolate (Indian) might have been emerged from Brazil or Tirupati strain. Currently, the data on available sequences is not reasonable to provide strong outcomes. Data of other preserved genes of BTV 2 might be more beneficial in to potyping than that of VP7 gene. Further the reassortment and genetic drift may be addressing a significant purpose in emergence of new BTV strains.

4. Reference

1. Batten C, Darpel K, Henstock M, Fay P, Veronesi E, Gubbins S, *et al*. Evidence for transmission of bluetongue virus serotype 26 through direct contact. PLoS One. 2014;9(5):e96049.
2. Bommineni YR, Reddy YN, Rao PP, Sudheer D, Susmitha B, Sarma BJ, *et al*. Isolation of bluetongue virus serotype 2, 9 and 15 from sheep in India. Veterinary Record. 2008;163(18):545-546.
3. Bonneau KR, Zhang N, Zhu J, Zhang F, Li Z, Zhang K, *et al*. Sequence comparison of the L2 and S10 genes of bluetongue viruses from the United states and the people's republic China. Virus Research. 1999;61(2):153-60.
4. Bonneau KR, Zhang NZ, Wilson WC, Zhu JB, Zhang FQ, Li ZH, *et al*. Phylogenetic analysis of the S7 gene does not segregate Chinese strains of bluetongue virus into a single topotype. Archives of Virology. 2000;145(6):1163-71.
5. Bréard E, Schulz C, Sailleau C, Bernelin Cottet C, Viarouge C, Vitour D, *et al*. Bluetongue virus serotype 27: experimental infection of goats, sheep and cattle with three BTV27 variants reveal atypical characteristics and likely direct contact transmission BTV-27 between goats. Transbound. Emerging Infectious Diseases. 2018;65(2):e251-63.
6. Chomczynski, Sacchi N. Single step method of RNA Isolation byAcid Guanidinium Thiocyanate-Phenol-Chloroform Extraction. Analytical Biochemistry. 1987;162(1):156-159.
7. Dalal S, Prasad G, Minakshi Sushila M. VP7 Gene based molecular Characterization of two Indian isolates of bluetongue virus. Haryana Veterinarian. 2008;47:41-45.
8. Gould AR, Pritchard LI. Relationships amongst bluetongue viruses revealed by comparisons of capsid and outer coat protein nucleotide sequences. Virus Research. 1990;17(1):31-52.
9. Gumm ID, Newman JF. The preparation of purified bluetongue virus group antigen for use as a diagnostic reagent. Archives of Virology. 1982;72:83-93.
10. Huismans H, Erasmus BJ. Identification of the serotype specific and group specific antigens of bluetongue virus. Onderstepoort Journal of Veterinary Research. 1981;48(2):51-58.
11. ICTV. Reoviridae – dsRNA Viruses – dsRNA Viruses – International Committee on Taxonomy of Viruses (ICTV) [Internet]; c2019. [cited 2019 Nov 8], 2011. Available from: https: //talk.ictvonline.org/ictv-reports/ictv_9th_report/dsrna-viruses2011/w/dsrnaviruses/188/ reoviridae.
12. Kowalik FT, Yang Y, Li KJ. Molecular cloning and comparative sequence analysis of bluetongue virus S1 segments by selective synthesis of specific full-length DNA of dsRNA genes. Virology. 1990;177(2):820-823.
13. Maan NS, Maan S, Belaganahalli MN, Ostlund EN, Johnson DJ, Nomikou K, *et al*. Identification and differentiation of the twenty-six bluetongue virus serotypes by RT-PCR amplification of the serotype-specific genome segment 2. PLOS ONE. 2012;7(2):e32601.
14. Mellor PS, Boorman J. The transmission and geographical spread of African horse sickness and bluetongue viruses. Annals of Tropical Medicine and Parasitology. 1995;89(1):1-15.
15. OIE. Animal Health in the World, OIE-listed Diseases, Infections and Infestations in Force in OIE, Paris, France; c2018.
16. Prasad PUVS. Phylogenetic analysis and sequencing of part of VP7 gene bluetongue virus. M.V.Sc thesis. Acharya N.G. Ranga Agricultural University, Hyderabad; c2005.
17. Rao PP, Reddy YN, Hedge NR. Complete Genome Sequence of Bluetongue virus Serotype 9: Implications for Serotyping. Journal of Virology. 2012;86(15):8333.
18. Reddy YV, Krishnajyothi Y, Susmitha B, Devi BV, Brundavanam Y, Gollapalli SR, *et al*. Molecular typing of bluetongue viruses isolated over a decade in South India. Transboundary and Emerging Diseases. 2016;63(5):e412-8.
19. Roy P. From genes to complex structure of bluetongue virus and their efficacy as vaccines. Veterinary Microbiology. 1992;33(1-4):155-168.
20. Saegerman C, Bolkaerts B, Baricalla C, Raes M, Wiggers L, de Leeuw I, *et al*. The impact of naturally-occurring, trans-placental bluetongue virus serotype-8 infection on reproductive performance in sheep. Veterinary Journal. 2011;187(1):72-80.
21. Sonali M, Reddy YN, Dhanalakshmi K, Reddy MR. Vp7 gene based phylogenetic analysis of blue tongue virus serotype 15. International Journal of Science, Environment and Technology. 2017;6(3):1660-1665
22. Squire KRE, Chuang RY, Osburn BI, Knydson DL, Doi RH. Rapid methods for comparing the double stranded RNA genome profiles of bluetongue virus. Veterinary Microbiology. 1983;8(6):543-553.
23. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research. 1997;25:4876-4882.
24. Thota R, Ganji VK, Machanagari S, Yella NR, Buddala B, Yadlapati K, *et al*. VP2 Gene-Based Molecular Evolutionary Patterns of Major Circulating Bluetongue Virus Serotypes Isolated during 2014–2018 from Telangana and Andhra Pradesh States of India. Intervirology. 2021;64(1):8-15.
25. Verwoerd DW. Purification and characterization of bluetongue virus. Virol. 1969;38(2):203-212.
26. Wade Evans AM, Mertens PPC, Bostock CJ. Development of the polymerase chain reaction for the detection of bluetongue virus in tissue samples. Journal of Virological Methods. 1990;30(1):15-24.
27. Wilson WC, Ma HC, Venter EH, vanDjik AA, Seal BS Mecham JO. Phylogenetic relationships of bluetongue viruses based on gene S7. Virus Research. 2000;67(2):141-51.