VETERINARIA ITALIANA
RIVISTA DI SANITÀ PUBBLICA VETERINARIA

Short communication

Emergence of Bluetongue virus serotype 5 in Sardinia- Italy, 2025

Maurilia Marcacci[1], Stefano Cappai[2], Andrea Palombieri[1], Giantonella Puggioni[2], Gloria Plebani[3], Roberta Irelli[1], Nicandro Rodi[1], Angela Maria Rocchigiani[2], Gardenia Gatta[1], Liana Teodori[1], Alessandra Leone[1], Daniela Manunta[2], Ottavio Portanti[1], Claudia Casaccia[1], Valentina Curini[1], Daria Di Sabatino[1], Stacey L.P. Scroggs[4], Massimo Spedicato[1], Alessio Lorusso[1*]

[1]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy - IT*
[2]*Istituto Zooprofilattico Sperimentale della Sardegna - IT*
[3]*Università degli Studi di Teramo, Teramo, Italy; Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy - IT*
[4]*Arthropod-Borne Animal Diseases Research Unit, Agricultural Research Service, United States Department of Agriculture, Manhattan, KS, 66502, USA - US*

[*]*Corresponding author at: Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy - IT*
E-mail: a.lorusso@izs.it

Veterinaria Italiana, Vol. 62 No. 1 (2026) DOI: 10.12834/VetIt.3925.39028.1

Abstract

Southern Europe, and Italy in particular, has historically been exposed to repeated incursions of orbiviruses, including multiple bluetongue virus (BTV) serotypes and epizootic haemorrhagic disease virus serotype 8. Sardinia, Italy represents a key sentinel location due to its geographic position in the Mediterranean basin. In August 2025, fatal bluetongue cases in sheep were reported in Sardinia during a period of intense BTV circulation in Italy, characterised by widespread outbreaks associated with BTV-3 and BTV-8. These clinical samples tested positive for BTV but could not be serotyped using the available real-time RT-PCR assays. Whole-genome sequencing identified the causative agent as BTV serotype 5 (BTV-5), representing the first detection of this serotype in Europe. The virus was also successfully isolated in cell culture. Segments 2 and 6 showed the highest nucleotide identity with a BTV-5 strain identified in Nigeria in 1982, whereas the rest of the genome seems to be composed by gene segments originating from multiple BTV serotypes circulating in Africa and Europe. At the time of writing, no official reports of BTV-5 circulation were available from Northern Africa; however, during the editorial process, BTV-5 was reported in Tunisia, further supporting the Mediterranean basin as a single interconnected epidemiological ecosystem for orbivirus emergence and spread.

Keywords

Bluetongue, BTV-5, Genomic characterization, Italy

Short Communication

Bluetongue virus (BTV) is the aetiological agent of bluetongue (BT), a non-contagious, vector-borne disease primarily affecting domestic and wild ruminants (Mellor et al., 2000; MacLachlan, 2010). BTV belongs to the genus *Orbivirus* within the family *Sedoreoviridae* and possesses a double-stranded RNA genome composed of ten segments that encode seven structural (VP1–VP7) and five non-structural proteins (NS1–NS5) (Schwartz-Cornil et al., 2008; Ratinier et al., 2011; Roy, 2017). BTV strains are classified into distinct serotypes based on antigenic and genetic variation in segment 2 (Seg-2), which encodes the outer capsid protein VP2, the major determinant of serotype specificity and the primary target for molecular serotyping and genotyping assays (Maan et al., 2007; Roy, 2017). Beyond serotype classification, BTV strains are also grouped into major phylogeographic lineages, commonly referred to as western and eastern topotypes, reflecting their historical geographical origins and evolutionary trajectories (Mertens et al., 2005; Cappai et al., 2019; White et al., 2019). These topotypes differ substantially at the nucleotide level across multiple genome segments and can influence reassortment dynamics and emergence patterns (Carpi et al., 2010;

How to cite: Marcacci et al. Emergence of Bluetongue virus serotype 5 in Sardinia- Italy, 2025. Veterinaria Italiana, Vol. 62 No. 1 (2026) DOI: 10.12834/VetIt.3925.39028.1

Nomikou et al., 2015; White et al., 2019).

Transmission of BTV occurs through the bite of infected *Culicoides* spp. midges, which act as biological vectors (Verwoerd and Erasmus, 2004; MacLachlan, 2010). Sheep, the most susceptible domestic species, often develop overt clinical disease characterized by fever, facial oedema, oral ulcerations, lameness and, in severe cases, cyanosis of the tongue and death. In contrast, BTV infection in cattle is generally subclinical, although notable exceptions exist; nevertheless, cattle play a pivotal epidemiological role as reservoirs that sustain viral circulation and facilitate geographical spread (MacLachlan, 2010; Coetzee et al., 2012).

To date, up to 36 BTV serotypes have been described, including 24 classical and 12 atypical serotypes (Ries et al., 2020; Ries et al., 2021). Classical serotypes are those historically associated with clinical BT and official control measures, and they largely fail to confer cross-protective immunity (Jimenez-Clavero et al., 2012; Martinelle et al., 2018; Fay et al., 2021). The simultaneous circulation of multiple serotypes, as currently observed in Italy and other European countries, complicates vaccination strategies and diagnostic workflows, particularly in the absence of multivalent vaccines and comprehensive diagnostic reagents.

In Sardinia, BTV-3, BTV-8 and BTV-4 have co-circulated in recent years, with BTV-3 and BTV-8 accounting for most reported cases (Plebani et al., 2025). Between late August and early September 2025, two sheep from two different farms located in the Sulcis-Iglesiente province of Sardinia died after exhibiting clinical signs consistent with BT. Initial diagnostic testing was performed at the Istituto Zooprofilattico Sperimentale della Sardegna (IZS Sardegna) using a real-time RT-PCR targeting Seg-10 (NS3; real-time RT-PCR_NS3), which yielded positive results for BTV.

Subsequent serotype identification was attempted using a panel of real-time RT-PCR assays targeting Seg-2 of BTV serotypes known to circulate in the Mediterranean basin (real-time RT-PCR_BTV-EUR, Lorusso et al., 2018; Portanti et al., 2023). All samples tested negative by these assays, suggesting infection by a BTV strain not covered by the available serotyping/genotyping panel.

To further characterize the virus, samples were shipped to the National Reference Laboratory for Bluetongue (NRL-BT) at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM). BTV positivity was confirmed by real-time RT-PCR targeting Seg-10 (NS3), with Ct values of 21.2 and 30.4 for the two samples, respectively. Consistent with IZS Sardegna's results, all samples tested negative in the BTV-EUR real-time RT-PCR assays and were therefore classified as BTV-positive but non-typable using the currently available molecular typing tools.

The sample with the lowest Ct value (internal ID: 2025.TE.22547.1.1) was selected for whole-genome sequencing (WGS). Following DNase treatment, RNA was purified using the RNA Clean & Concentrator kit (Zymo Research, Irvine, CA, USA) and processed using a SISPA-orbivirus protocol based on random-tagged primers (FR26RV-N: GCCGGAGCTCTGCAGATATCNNNNNNN) and orbivirus-specific tagged primers targeting conserved segment termini (FR-BT_F: GCCGGAGCTCTGCAGATATCGTTAAAN; FR-BT_R: GCCGGAGCTCTGCAGATATCGTAAGTN) (Marcacci et al., 2016; Sghaier et al., 2022). Reverse transcription was performed using the SuperScript™ IV kit (Life Technologies, Carlsbad, CA, USA) with primers FR26RV-N and FR-BT_R, followed by second-strand cDNA synthesis using FR-BT_F and Klenow Fragment (3′→5′ exo-) (New England Biolabs, Ipswich, MA, USA). PCR amplification was carried out using primer FR20_Rv (GCCGGAGCTCTGCAGATATC) and Q5® Hot Start High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA, USA). Amplified products were purified using Expin™ PCR SV (GeneAll Biotechnology, Seoul, Korea), quantified with the Qubit® DNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA), and 100 ng of DNA was used for library preparation with the Illumina DNA Prep kit. Sequencing was performed on a NextSeq 1000 platform (Illumina, San Diego, CA, USA) using the NextSeq™ 1000/2000 P2 XLEAP-SBS™ Reagent Kit (300 cycles), generating paired-end reads of 150 bp.

Sequencing yielded 6,537,922 raw reads with an average Phred quality score of 37.73. Bioinformatic analysis was performed using the GENPAT platform (https://genpat.izs.it/). Host reads (*Ovis aries*, NCBI GCF_002742125) were removed using the "Host depletion and de novo" pipeline, followed by de novo assembly with SPAdes (Bankevich et al., 2012). Contigs were screened using the "3TX_Species" tool against BTV reference sequences (taxid: 40051) in the NCBI database. The best-matching reference for each segment was imported into GENPAT, and consensus sequences were generated using the "Mapping segmented virus" tool (iVar v1.4.4).

Complete consensus sequences (horizontal coverage, Hcov, 100%) were obtained for Seg-1, -3, -4, -5, and -6, near-complete sequences for Seg-2 (Hcov 90%) and Seg-8 (Hcov 96.6%), and partial sequences for Seg-7 (Hcov 45%), Seg-9 (Hcov 67.9%) and Seg-10 (Hcov 59.2%). BLAST analysis of Seg-2 and Seg-6 revealed the highest nucleotide identity with BTV-5 Nigeria 1982, showing 96.56% identity for VP2 and 98.09% for VP5, enabling serotype identification as BTV-5. The remaining genome segments displayed a likely reassortant constellation involving

How to cite: Marcacci et al. Emergence of Bluetongue virus serotype 5 in Sardinia- Italy, 2025. Veterinaria Italiana, Vol. 62 No. 1 (2026) DOI: 10.12834/VetIt.3925.39028.1

segments related to BTV-2, -3, -4, and -8 strains reported in Africa and Europe over recent decades, consistent with the pervasive reassortment potential of segmented orbiviruses (Carpi et al., 2010; Nomikou et al., 2015; Plebani et al., 2025).

Following confirmation, the outbreak was officially reported to the Italian Ministry of Health, which subsequently informed the World Organisation for Animal Health (WOAH) and the European Commission. As a precautionary measure, from 30 September 2025 onward, movements of susceptible animals out of Sardinia and from southern to northern areas of the island were prohibited. Due to the absence of specific diagnostic assays, a real-time RT-PCR targeting Seg-2 of the Italian BTV-5 strain (real-time RT-PCR_BTV-5) was developed. Primer and probe sequences were: BTV-5_ITA2025_Fw (GCCATTTCATACAGATATATATCGATAG); BTV-5_ITA2025_Rv (GCTCCACGTTATACATTCCACA); BTV-5_ITA2025_Probe ([HEX]TGAATCCAAAGGATGTGACCATACACAG[BHQ2]).

The assay was optimized using the TaqMan™ Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), with final concentrations of 400 nM for primers and 200 nM for the probe. Cycling conditions included RNA denaturation at 95°C for 3 min, followed by reverse transcription at 45°C for 10 min, polymerase activation at 95°C for 10 min, and 40 amplification cycles of 95°C for 15 s and 60°C for 1 min. Testing of 84 previously non-typable Sardinian samples collected between August and September 2025, primarily sheep blood samples, confirmed that all were positive for BTV-5 (data not shown).

Virus isolation was successfully achieved by propagation of blood samples on KC cells (1 passage) followed by two passages on Vero cells. RNA extracted from culture supernatants was sequenced using the same SISPA-orbivirus and Illumina workflow (Marcacci et al., 2016; Sghaier et al., 2022). The resulting isolate, designated BTV-5 ITA2025, generated 8,871,708 raw reads with an average quality score of 37.81. Complete consensus sequences (Hcov 100%) were obtained for all ten segments, with vertical coverage ranging from 70.97× to 2712.63×. Sequences were shared with the GenBank database under the following accession numbers (PX460302-PX460311) (Table I). For Seg-2, the closest match was BTV-5 Nigeria 1982 (96.61% nt identity), followed by two BTV-5 strains from the USA - identified in 2003 and 2022- with lower nucleotide identities (85.68% and 84.69%). Seg-6 showed a similar pattern, with the highest identity to the Nigerian strain and lower identities to South African BTV-5 strains isolated in 1953 and 2004. Overall, the genome constellation of BTV-5 ITA2025 reflects a likely reassortant virus composed of segments related to BTV serotype 2, -3, -4, -8, and -18 circulating in Africa, consistent with documented reassortment processes in BTV evolution (Nomikou et al., 2015; White et al., 2019). Phylogenetic analysis of Seg-2 was performed using the Auspice toolkit (v2.53.0) integrated into GENPAT. Maximum-likelihood trees generated with IQ-TREE and based on 38 BTV-5 Seg-2 sequences available in NCBI revealed a close genetic relationship between BTV-5 ITA2025 and BTV-5 Nigeria 1982 (AJ585182.1), while BTV-5 strains reported in India, Israel, China, Australia, and the USA clustered into distinct clades (Figure 1).

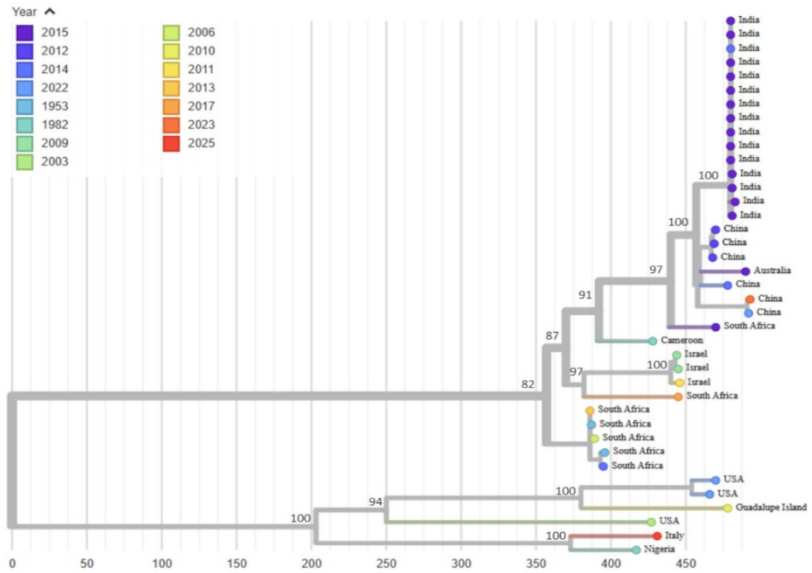

Figure 1. Maximum Likelihood tree obtained by IQ-TREE of 38 Seg-2 sequences of BTV-5 strains available in NCBI database. Confidence values higher than 70% are displayed.

How to cite: Marcacci et al. Emergence of Bluetongue virus serotype 5 in Sardinia- Italy, 2025. Veterinaria Italiana, Vol. 62 No. 1 (2026) DOI: 10.12834/VetIt.3925.39028.1

BTV-5 ITA2025	Best Match	Country	Year	Acc NO NCBI	% ID nt
Seg-1	BTV-8	South Africa	1937	MT078269.1	96.32
	BTV-2	South Africa	1959	MT119863.1	96.15
	BTV-24	South Africa	2013	JX272369.1	95.82
Seg-2	BTV-5	Nigeria	1982	AJ585182.1	96.61
	BTV-5	Florida, USA	2003	KX164060.1	85.68
	BTV-5	USA	2022	PQ625403.1	84.69
Seg-3	BTV-4	Morocco	2014	PV926246.1	97.55
	BTV-4	Greece	2014	MT879213.1	97.37
	BTV-4	Hungheria	2014	KP268816.1	97.33
Seg-4	BTV-2	Tunisia	2000	KP821279.1	96.57
	BTV-3	Tunisia	2016	KY432372.1	96.57
	BTV-3	South Africa	2017	MG255622.1	96.57
Seg-5	BTV-4	France	2020	OK018214.1	98.25
	BTV-4	Greece	2014	MT879215.1	98.20
	BTV-4	Hungheria	2014	KP268818.1	98.14
Seg-6	BTV-5	Nigeria	1982	AJ586702.1	97.98
	BTV-5	South Africa	2004	AJ586700.1	90.71
	BTV-5	South Africa	1953	MT070944.1	90.65
Seg-7	BTV-3	Tunisia	2016	MF124298.1	97.49
	BTV-16	South Africa	1971	MT078355.1	95.85
	BTV-3	South Africa	2016	MT028405.1	95.76
Seg-8	BTV-2	Tunisia	2000	KP821761.1	98.49
	BTV-2	France	2001	KP821749.1	98.40
	BTV-3	South Africa	2016	MT028406.1	98.31
Seg-9	BTV-8	France	2008	KP821826.1	96.76
	BTV-1	Italy	2006	KJ736009.1	96.66
	BTV	Germany	2008	MN839218.1	96.66
Seg-10	BTV-18	South Africa	2013	JX272448.1	97.81
	BTV-18	South Africa	2020	MT078378.1	97.69
	BTV-18	USA	2009	FJ713329.1	97.69

Table I. Results of BLAST analysis of the ten genomic segments of BTV-5 ITA2025. The first three sequences with the highest % of nt identity were reported.

Since 1998, Southern Europe has been repeatedly affected by incursions of multiple BTV strains belonging to different serotypes and topotypes, facilitated by vector habitat suitability and climatic connectivity across the Mediterranean basin (Calistri et al., 2004; Hammami, 2004; MacLachlan, 2010). Within this epidemiological framework, the emergence of BTV-5 in Sardinia appears compatible with previously documented introduction pathways, including wind-borne dispersal of infected *Culicoides* from North Africa. A comparable hypothesis was proposed for the incursion of epizootic haemorrhagic disease virus serotype 8 (EHDV-8) into Italy, which affected areas overlapping those later involved in the BTV-5 outbreak and was also plausibly introduced via infected vectors originating from North Africa (Martinez et al., 2025; Portanti et al., 2025). Therefore, a Northern African origin for the Sardinian BTV-5 strain is probable. However, unlike the situations documented for BTV-3 and EHDV-8 — for which sustained circulation had been demonstrated in Tunisia through long-term collaboration and genomic surveillance (Sghaier et al., 2017; Lorusso et al., 2018; Sghaier et al., 2022; Lorusso et al., 2023) — at the time of initial investigation no official reports of BTV-5 circulation were available from Northern African countries. During the editorial process of the present report, BTV-5 was subsequently officially notified in Tunisia and reported through the WOAH WAHIS platform (https://wahis.woah.org/#/in-review/7169), further supporting the hypothesis that the Mediterranean basin represents a single, highly connected epidemiological ecosystem for orbivirus emergence and spread. Notably, the Tunisian detection was supported by laboratory confirmation performed in Teramo, reinforcing the value of cross-Mediterranean diagnostic and genomic capacity-building initiatives. Sequencing analysis on the Tunisian samples is currently ongoing.

The present report is intended as a preliminary description of the emergence of this novel serotype in Italy. Its immediate value lies in supporting rapid development, validation, and sharing of diagnostic assays, thereby strengthening preparedness and response capacity. A comprehensive epidemiological characterization of BTV-5 in Sardinia will follow and will address spread, host range, clinical impact, and the potential role of domestic and wild ruminants in maintaining transmission cycles. Furthermore, the involvement of reassortant strains with BTV strains already circulating in Sardinia cannot be excluded and warrants careful investigation, particularly in a setting where multiple serotypes co-circulate and reassortment can occur (Carpi et al., 2010; Nomikou et al., 2015; Plebani et al.,

How to cite: Marcacci et al. Emergence of Bluetongue virus serotype 5 in Sardinia- Italy, 2025. Veterinaria Italiana, Vol. 62 No. 1 (2026) DOI: 10.12834/VetIt.3925.39028.1

2025). Importantly, even if the number of BTV-5 outbreaks remains limited in the short term, the development of vaccines targeting this serotype should be considered. Historical evidence from BTV-3 supports this precautionary approach: after its initial detection in Italy, BTV-3 circulated at low levels and later re-emerged with increased outbreak numbers, in parallel with reassortant forms involving endemic serotypes (Nomikou et al., 2015; Sghaier et al., 2017; Lorusso et al., 2018). While reassortment does not inherently imply increased virulence, these observations reinforce that VP2-defined serotypes may persist and re-emerge under favorable ecological and epidemiological conditions (MacLachlan, 2010; Nomikou et al., 2015).

To date, active outbreaks caused by BTV serotypes 3, 4, 5, and 8 have been reported in Sardinia. The co-circulation of multiple serotypes in a restricted geographical area underscores the importance of integrated genomic surveillance within BT control programmes, including whole-genome sequencing to rapidly identify novel serotypes and monitor reassortment (Nomikou et al., 2015; White et al., 2019). Vaccination campaigns, movement restrictions and vector surveillance remain essential pillars of disease control (MacLachlan, 2010). The recent emergence of BTV-3 and other serotypes in Northern Europe and BTV-5 in Sardinia illustrates an increasingly complex and dynamic BT epidemiological landscape in Europe, reinforcing the need for sustained genomic surveillance and cross-Mediterranean capacity-building.

Conclusion

At present, the geographical distribution of BTV-5 in Sardinia remains only partially defined. A total of 104 outbreaks have been reported, including 50 outbreaks attributed solely to BTV-5 and 54 outbreaks involving co-infections with BTV-3 and/or BTV-8, which have been mainly detected in the southern part of the island. However, the true extent of virus circulation may be underestimated, as asymptomatic infections — particularly in cattle and goats — can remain undetected. A dedicated study combining serological and molecular surveys to better define the spread of BTV-5 across Sardinia is currently underway and will provide essential data to refine risk assessment and control strategies. These investigations will also be crucial to better characterise BTV-5-associated morbidity and mortality, including potential species-specific clinical outcomes and the impact of co-infections on disease severity.

Acknowledgments

The authors wish to thank all colleagues working in the field who are involved in sample collection, first-line diagnosis, and shipment of samples to IZSAM.

Ethical approval

No ethical authorization was required. All included samples were collected for routine testing and surveillance programmes for BTV

Conflict of interest

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

Author Contributions

Conceptualization: Alessio Lorusso, Maurilia Marcacci, Daria Di Sabatino, Massimo Spedicato; Methodology: Gloria Plebani, Andrea Palombieri, Valentina Curini, Ottavio Portanti; Formal analysis: Maurilia Marcacci, Andrea Palombieri, Gardenia Gatta, Daria Di Sabatino, Stacey L.P. Scroggs; Investigation: Stefano Cappai, Andrea Palombieri, Giantonella Puggioni, Roberta Irelli, Nicandro Rodi, Angela Maria Rocchigiani, Gardenia Gatta, Liana Teodori, Alessandra Leone, Daniela Manunta, Ottavio Portanti, Claudia Casaccia, Valentina Curini; Writing original draft preparation: Alessio Lorusso; Writing, review and editing: Alessio Lorusso, Maurilia Marcacci, Gloria Plebani, Daria Di Sabatino, Massimo Spedicato, Stacey L.P. Scroggs; Visualization: Stacey L.P. Scroggs; Supervision: Alessio Lorusso; Project administration: Alessio Lorusso; Funding acquisition: Alessio Lorusso. All authors have read and agreed to the published version of the manuscript.

How to cite: Marcacci et al. Emergence of Bluetongue virus serotype 5 in Sardinia- Italy, 2025. Veterinaria Italiana, Vol. 62 No. 1 (2026) DOI: 10.12834/VetIt.3925.39028.1

Data availability

The sequences were submitted to the NCBI database with the following acc. No. PX460302-PX460311

Fundings

Funding for this work was provided by the by the Italian Ministry of Health through the projetc "*CARBO - Caratterizzazione biologica e fattori di virulenza di nuovi e vecchi arbovirus animali ",* grant code MSRCTE0223. Dr. Scroggs is funded by the U.S. Department of Agriculture, Agricultural Research Service, NP-103 Animal Health National Program, Project #3020-32000-019-00D and #3020-32000-020-00D

References

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. Journal of computational biology: a journal of computational molecular cell biology, 19(5), 455–477. https://doi.org/10.1089/cmb.2012.0021.

Calistri, P., Giovannini, A., Conte, A., Nannini, D., Santucci, U., Patta, C., Rolesu, S., & Caporale, V. (2004). Bluetongue in Italy: Part I. Veterinaria italiana, 40(3), 243–251.

Cappai, S., Rolesu, S., Loi, F., Liciardi, M., Leone, A., Marcacci, M., Teodori, L., Mangone, I., Sghaier, S., Portanti, O., Savini, G., & Lorusso, A. (2019). Western Bluetongue virus serotype 3 in Sardinia, diagnosis and characterization. Transboundary and emerging diseases, 66(3), 1426–1431. https://doi.org/10.1111/tbed.13156.

Carpi, G., Holmes, E. C., & Kitchen, A. (2010). The evolutionary dynamics of bluetongue virus. Journal of molecular evolution, 70(6), 583–592. https://doi.org/10.1007/s00239-010-9354-y.

Coetzee, P., Stokstad, M., Venter, E. H., Myrmel, M., & Van Vuuren, M. (2012). Bluetongue: a historical and epidemiological perspective with the emphasis on South Africa. Virology journal, 9, 198. https://doi.org/10.1186/1743-422X-9-198.

Fay, P. C., Mohd Jaafar, F., Batten, C., Attoui, H., Saunders, K., Lomonossoff, G. P., Reid, E., Horton, D., Maan, S., Haig, D., Daly, J. M., & Mertens, P. P. C. (2021). Serological Cross-Reactions between Expressed VP2 Proteins from Different Bluetongue Virus Serotypes. Viruses, 13(8), 1455. https://doi.org/10.3390/v13081455.

Hammami S. (2004). North Africa: a regional overview of bluetongue virus, vectors, surveillance and unique features. Veterinaria italiana, 40(3), 43–46.

Jiménez-Clavero M. Á. (2012). Animal viral diseases and global change: bluetongue and West Nile fever as paradigms. Frontiers in genetics, 3, 105. https://doi.org/10.3389/fgene.2012.00105.

Lorusso, A., Cappai, S., Loi, F., Pinna, L., Ruiu, A., Puggioni, G., Guercio, A., Purpari, G., Vicari, D., Sghaier, S., Zientara, S., Spedicato, M., Hammami, S., Ben Hassine, T., Portanti, O., Breard, E., Sailleu, C., Ancora, M., Di Sabatino, D., Morelli, D., … Savini, G. (2023). Epizootic Hemorrhagic Disease Virus Serotype 8, Italy, 2022. Emerging infectious diseases, 29(5), 1063–1065. https://doi.org/10.3201/eid2905.221773.

Lorusso, A., Sghaier, S., Di Domenico, M., Barbria, M. E., Zaccaria, G., Megdich, A., Portanti, O., Seliman, I. B., Spedicato, M., Pizzurro, F., Carmine, I., Teodori, L., Mahjoub, M., Mangone, I., Leone, A., Hammami, S., Marcacci, M., & Savini, G. (2018). Analysis of bluetongue serotype 3 spread in Tunisia and discovery of a novel strain related to the bluetongue virus isolated from a commercial sheep pox vaccine. Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases, 59, 63–71. https://doi.org/10.1016/j.meegid.2018.01.025.

Maan, S., Maan, N. S., Samuel, A. R., Rao, S., Attoui, H., & Mertens, P. P. C. (2007). Analysis and phylogenetic comparisons of full-length VP2 genes of the 24 bluetongue virus serotypes. The Journal of general virology, 88(Pt 2), 621–630. https://doi.org/10.1099/vir.0.82456-0.

Maclachlan, N. J., & Guthrie, A. J. (2010). Re-emergence of bluetongue, African horse sickness, and other orbivirus diseases. Veterinary research, 41(6), 35. https://doi.org/10.1051/vetres/2010007.

Marcacci, M., De Luca, E., Zaccaria, G., Di Tommaso, M., Mangone, I., Aste, G., Savini, G., Boari, A., & Lorusso, A. (2016). Genome characterization of feline morbillivirus from Italy. Journal of virological methods, 234, 160–163. https://doi.org/10.1016/j.jviromet.2016.05.002.

Martinelle, L., Dal Pozzo, F., Thys, C., De Leeuw, I., Van Campe, W., De Clercq, K., Thiry, E., & Saegerman, C. (2018). Assessment of cross-protection induced by a bluetongue virus (BTV) serotype 8 vaccine towards other BTV serotypes in experimental conditions. Veterinary research, 49(1), 63. https://doi.org/10.1186/s13567-018-0556-4.

Martínez, R., De Los Ángeles Risalde, M., Cano-Terriza, D., Lorusso, A., & Spedicato, M. (2025). From Africa to Europe: the rise of epizootic haemorrhagic disease virus serotype 8. Veterinaria italiana, 61(4), 10.12834/VetIt.3793.35560.1. https://doi.org/10.12834/VetIt.3793.35560.1.

Mellor, P. S., Boorman, J., & Baylis, M. (2000). Culicoides biting midges: their role as arbovirus vectors. Annual review of entomology, 45, 307–340. https://doi.org/10.1146/annurev.ento.45.1.307.

Mertens, P.P.C., Maan, S., Samuel, A., Attoui, H. (2005). Genus Orbivirus, p 466–483. In Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (ed), Virus taxonomy: eighth report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, London, United Kingdom.

Nomikou, K., Hughes, J., Wash, R., Kellam, P., Breard, E., Zientara, S., Palmarini, M., Biek, R., & Mertens, P. (2015). Widespread Reassortment Shapes the Evolution and Epidemiology of Bluetongue Virus following European Invasion. PLoS pathogens, 11(8), e1005056. https://doi.org/10.1371/journal.ppat.1005056.

Plebani, G., Palombieri, A., Sghaier, S., Gatta, G., Ben Hassine, T., Curini, V., Thabet, S., Parolini, F., Hammami, S., Ancora, M., Spedicato, M., Di Sabatino, D., Marcacci, M., Scroggs, S.L.P., & Lorusso, A. (2025). Evolutionary Dynamics of Bluetongue virus serotypes 3, 4, and 8 circulating in Italy, 2024-2025. Veterinaria Italiana, Vol. 61 No. 4 (2025); DOI: 10.12834/VetIt.3915.38031.1.

Portanti, O., Ciarrocchi, E., Irelli, R., Palombieri, A., Salini, R., Melegari, I., Pisciella, M., Pulsoni, S., Di Sabatino, D., Spedicato, M., Savini, G., & Lorusso, A. (2025). Validation of a molecular multiplex assay for the simultaneous detection and differentiation of bluetongue virus and epizootic haemorrhagic disease virus in biological samples. Journal of virological methods, 332, 115064. https://doi.org/10.1016/j.jviromet.2024.115064.

Portanti, O., Thabet, S., Abenza, E., Ciarrocchi, E., Pisciella, M., Irelli, R., Savini, G., Hammami, S., Pulsoni, S., Casaccia, C., Coetzee, L., Marcacci, M., Di Domenico, M., & Lorusso, A. (2023). Development and validation of an RT-qPCR for detection and quantitation of emerging epizootic hemorrhagic disease virus serotype 8 RNA from field samples. Journal of virological methods, 321, 114808. https://doi.org/10.1016/j.jviromet.2023.114808.

Ratinier, M., Caporale, M., Golder, M., Franzoni, G., Allan, K., Nunes, S. F., Armezzani, A., Bayoumy, A., Rixon, F., Shaw, A., & Palmarini, M. (2011). Identification and characterization of a novel non-structural protein of bluetongue virus. PLoS pathogens, 7(12), e1002477. https://doi.org/10.1371/journal.ppat.1002477.

Ries, C., Sharav, T., Tseren-Ochir, E. O., Beer, M., & 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), 42. https://doi.org/10.3390/v13010042.

Ries, C., Vögtlin, A., Hüssy, D., Jandt, T., Gobet, H., Hilbe, M., Burgener, C., Schweizer, L., Häfliger-Speiser, S., Beer, M., & Hoffmann, B. (2021). Putative Novel Atypical BTV Serotype '36' Identified in Small Ruminants in Switzerland. Viruses, 13(5), 721. https://doi.org/10.3390/v13050721.

Roy P. (2017). Bluetongue virus structure and assembly. Current opinion in virology, 24, 115–123.

https://doi.org/10.1016/j.coviro.2017.05.003.

Schwartz-Cornil, I., Mertens, P. P., Contreras, V., Hemati, B., Pascale, F., Bréard, E., Mellor, P. S., MacLachlan, N. J., & Zientara, S. (2008). Bluetongue virus: virology, pathogenesis and immunity. Veterinary research, 39(5), 46. https://doi.org/10.1051/vetres:2008023.

Sghaier, S., Lorusso, A., Portanti, O., Marcacci, M., Orsini, M., Barbria, M. E., Mahmoud, A. S., Hammami, S., Petrini, A., & Savini, G. (2017). A novel Bluetongue virus serotype 3 strain in Tunisia, November 2016. Transboundary and emerging diseases, 64(3), 709–715. https://doi.org/10.1111/tbed.12640.

Sghaier, S., Sailleau, C., Marcacci, M., Thabet, S., Curini, V., Ben Hassine, T., Teodori, L., Portanti, O., Hammami, S., Jurisic, L., Spedicato, M., Postic, L., Gazani, I., Ben Osman, R., Zientara, S., Bréard, E., Calistri, P., Richt, J. A., Holmes, E. C., Savini, G., … Lorusso, A. (2022). Epizootic Haemorrhagic Disease Virus Serotype 8 in Tunisia, 2021. Viruses, 15(1), 16. https://doi.org/10.3390/v15010016.

Verwoerd, D.W. & Erasmus, B.J. (2004). Bluetongue, in: Coetzer J.A.W., Tustin R.C. (Eds.), Infectious diseases of livestock, 2nd ed., Oxford University Press Southern Africa, Cape Town, pp. 1201–1220.

White, J. R., Williams, D. T., Wang, J., Chen, H., Melville, L. F., Davis, S. S., Weir, R. P., Certoma, A., Di Rubbo, A., Harvey, G., Lunt, R. A., & Eagles, D. (2019). Identification and genomic characterization of the first isolate of bluetongue virus serotype 5 detected in Australia. Veterinary medicine and science, 5(2), 129–145. https://doi.org/10.1002/vms3.156.

How to cite: Marcacci et al. Emergence of Bluetongue virus serotype 5 in Sardinia- Italy, 2025. Veterinaria Italiana, Vol. 62 No. 1 (2026) DOI: 10.12834/VetIt.3925.39028.1