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Short communication

Bluetongue virus serotype 8 (BTV-8) in Serbia, 2025

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Abstract

Bluetongue (BT) is a vector-borne disease of ruminants caused by bluetongue virus (BTV). After more than a decade without reported cases, BT re-emerged in Serbia in 2025. In August 2025, clinical signs consistent with BT were observed in a sheep flock in western Serbia, including fever, submandibular and head oedema, oral haemorrhages, and cyanosis of the tongue. Real-time RT-PCR targeting segment 10 confirmed BTV infection, and serotyping identified BTV-8. Virus isolation in embryonated chicken eggs yielded multiple BTV-8 isolates, which underwent whole-genome sequencing using Oxford Nanopore and Illumina platforms. Complete or near-complete sequences of all ten genomic segments were obtained. Phylogenetic analysis of segment 2 showed close clustering with recent BTV-8 strains from France, Italy, and Spain (2023–2025), with no evidence of reassortment. The data indicate a likely transboundary introduction of BTV-8 from Western Europe and its rapid spread in Serbia.

Keywords

BTV-8, bluetongue, genomic characterisation, Serbia

Case report

Bluetongue (BT) is a vector-borne viral disease affecting domestic and wild ruminants, caused by the bluetongue virus (BTV), a member of the genus *Orbivirus* (family *Sedoreoviridae*) (Mellor et al., 2000; MacLachlan, 2004; MacLachlan & Dubovi, 2017). The virus is primarily transmitted by biting midges of the genus *Culicoides* spp. (Mellor et al., 2000). Bluetongue represents a major veterinary concern due to its significant impact on animal health, livestock productivity, and international trade (MacLachlan, 2004).

In Serbia, BT was first reported in 2000 when BTV serotype 9 (BTV-9) was detected in clinical samples (Djuričić et al., 2004). Following this initial detection, the country remained largely free from reported cases for more than a decade (World Organisation for Animal Health, (WOAH)). A major epidemic occurred in 2014 when BTV-4 was detected for the first time in the country and rapidly spread across multiple regions, causing numerous outbreaks, particularly in small ruminants (Djurić et al., 2017). Subsequent notifications of BTV-4 were reported to the World Animal Health Information System (WAHIS; WOAH) in 2016 and 2020.

After several years without official cases, BT re-emerged in Serbia in July 2025, when two outbreaks caused by BTV-8 were confirmed by the Veterinary Specialised Institute Niš in the administrative division of Bosilegrad.

On 15 August 2025, veterinary services reported clinical signs compatible with BT in a sheep flock located in Rajetiće (Novi Pazar, Serbia). During the field inspection, a five-year-old sheep was found isolated from the flock, presenting with hyperthermia, marked subcutaneous oedema of the submandibular region, head and neck, haemorrhages affecting the gums, upper lip and dental pad, and a thickened, dark-red tongue. Biological samples, including whole

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blood, nasal and oral swabs, were collected and submitted to the Veterinary Specialised Institute Kraljevo (VSIKV) for diagnostic investigation. Total RNA extraction was performed using BioExtract® SuperBall® (Biosellal, France) and IndiMag® Pathogen Kit (Indical Bioscience, Germany), according to the manufacturers' instructions, on the KingFisher™ mL and KingFisher™ Flex automated extraction platforms (Thermo Fisher Scientific, MA, USA), respectively.

Detection of BTV RNA was carried out using a real-time RT-PCR assay targeting segment 10 (Seg-10; NS3), as previously described (Hofmann et al., 2008), on a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, MA, USA). Serotype identification was subsequently performed using a panel of real-time RT-PCR assays targeting segment 2 (Seg-2) (Maan et al., 2016), and all samples tested positive for BTV-8. Additional molecular assays for peste des petits ruminants virus (PPRV) and contagious ecthyma virus (Orf virus) yielded negative results (Kottaridi et al., 2006; Batten et al., 2011).

Between August and November 2025, a total of 132 outbreaks caused by BTV-8 were reported in farms located in the western and southern regions of Serbia (Figure 1).

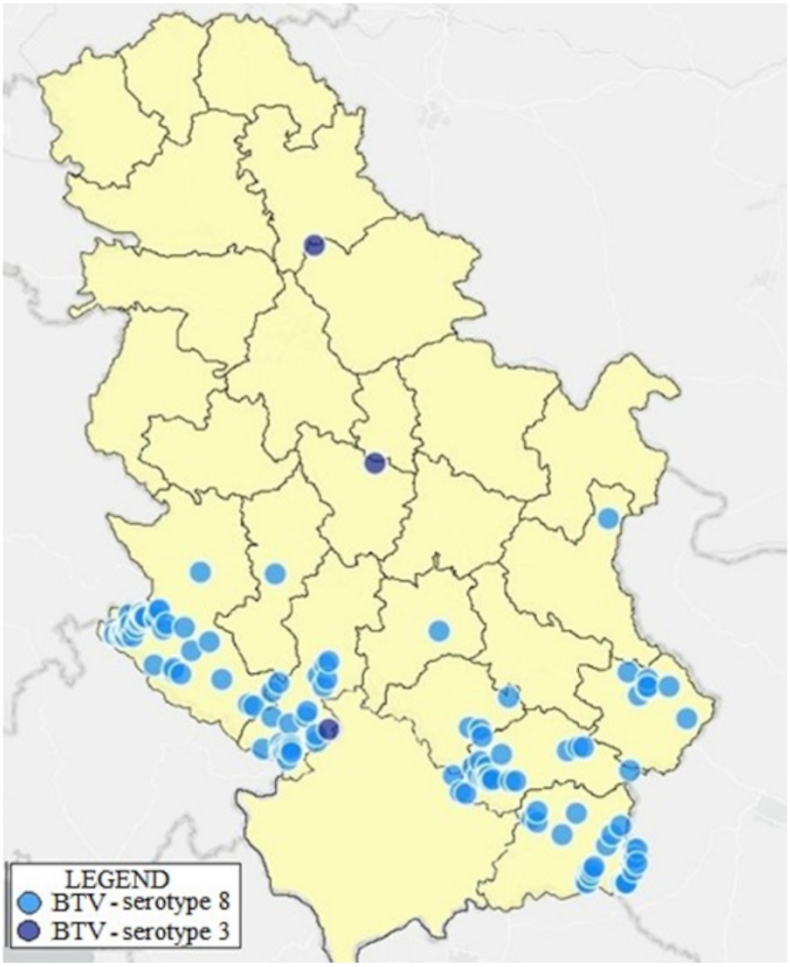

Figure 1. BTV outbreaks in Serbia. The map shows the geographical distribution of BTV-3 and BTV-8 outbreaks occurring in Serbia in 2025.

Virus isolation was performed in embryonated chicken eggs according to the protocol described in the WOAH Terrestrial Manual (Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 3.1.3). Three BTV isolates named BTV-8/SRB/2025/Tutin1, BTV-8/SRB/2025/NoviPazar1, and BTV-8/SRB/2025/Tutin2 were obtained and selected for whole-genome sequencing (WGS) at VSIKV, following the sample preparation protocol described by Marcacci et al. (2026).

Library preparation was carried out using the Native Barcoding Kit SQK-NBD114.24 (Oxford Nanopore Technologies, UK), and sequencing was performed on a MinION device with FLO-MIN114 flow cells (Oxford Nanopore Technologies, UK). Raw read quality was assessed using NanoPlot (v1.44.1) (De Coster & Rademakers, 2023). The isolate BTV-8/SRB/2025/Tutin1 yielded 16,272,980 reads with a mean quality score (Q-score) of 14.7; BTV-8/SRB/2025/NoviPazar1 yielded 1,591,886 reads with a mean Q-score of 15.2; and BTV-8/SRB/2025/Tutin2 produced 596,715 reads with a mean Q-score of 14.7.

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In addition, three further BTV-8 isolates, named BTV-8/SRB/2025/NoviPazar2, BTV-8/SRB/2025/Tutin3, and BTV-8/SRB/2025/Tutin4, were submitted to the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM, Teramo, Italy) within the VETLAB Network, supported by the International Atomic Energy Agency (IAEA, Vienna, Austria), for WGS. Library preparation was performed using the Illumina DNA Prep (M) Tagmentation kit (96 samples) (Illumina Inc., CA, USA). Sequencing was conducted on a NextSeq 1000 platform (Illumina Inc., CA, USA) using the NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (300 cycles), generating 3,680,402, 3,439,864, and 2,248,466 paired-end reads (150 bp), with mean Q-scores of 35.75, 36.83, and 36.72, respectively.

Bioinformatic analyses were performed using the GenPat-VETLAB platform of the Animal Production and Health Section of the Joint FAO/IAEA Centre (https://genpat.izs.it/). The best-matching reference sequence for each genomic segment was identified using BLAST v2.15.0 and subsequently used for mapping with minimap2 v2.26 (Li, 2018) for Nanopore reads and Snippy v4.6 (https://github.com/tseemann/snippy) for Illumina reads. Consensus sequences were generated using iVar v1.4.4 (Grubaugh et al., 2019).

Complete or near-complete consensus sequences were obtained for all ten genomic segments, with horizontal coverage (Hcov) ranging from 85% to 100% and vertical coverage (Vcov) ranging from 5× to 2,017×. The sequences were deposited in GenBank under accession numbers PZ320985-PZ321014 and PZ333767-PZ333786. As the sequences from the two strains, BTV-8/SRB/2025/Tutin3 and BTV-8/SRB/2025/Tutin4, were identical, a single representative sequence was submitted to GenBank.

Phylogenetic analysis of segment 2 (Seg-2) was performed using the Auspice toolkit (v2.53.0) (Hadfield et al., 2018) integrated into the GenPat platform (https://genpat.izs.it/). Maximum-likelihood trees generated with IQ-TREE and based on 52 BTV-8 Seg-2 sequences available in NCBI GenBank (Supplementary File 1) revealed a close genetic relationship between the Serbian BTV-8 strains and homologous sequences from strains circulating in France, Italy, and Spain between 2023 and 2025 (Hadfield et al., 2018; Grubaugh et al., 2019) (Figure 2).

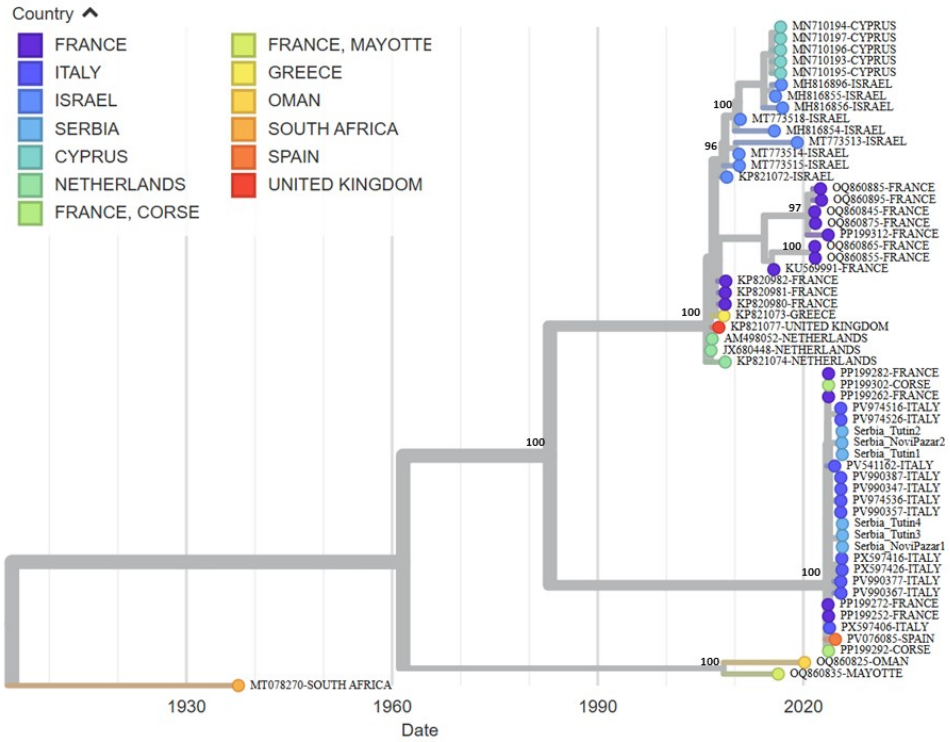

Figure 2. Maximum Likelihood tree obtained using IQ-TREE based on 5 Seg-2 sequences of BTV-8 strains from Serbia and 52 Seg-2 sequences of BTV-8 strains available in the NCBI GenBank database. Confidence values higher than 70% were shown.

For the remaining genomic segments, BLAST analysis showed the highest nucleotide identity (99.57–100%) with corresponding segments of the same French, Italian, and Spanish BTV-8 strains, indicating the absence of reassortment events in the Serbian isolates (data not shown).

The epidemiological situation in the Balkan region is particularly complex due to the concurrent circulation of multiple transboundary and endemic viral diseases affecting small ruminants (Gondard et al., 2024; Plebani et al., 2025). In addition to BTV-8 but with a lesser extent, outbreaks of BTV-3 were reported in three administrative divisions of

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Serbia between October 2025 and January 2026 (Figure 1). Moreover, other pathogens, including sheeppox virus, goatpox virus, PPRV, Orf virus, and foot-and-mouth disease virus, have been reported or are considered potential threats in the region.

Several of these infections may present with overlapping clinical manifestations in the early stages, including fever, mucosal lesions, oedema, and generalised skin lesions. This clinical similarity can complicate field diagnosis and underscores the importance of laboratory confirmation.

In response to this complex epidemiological scenario, the competent veterinary authorities implemented targeted training activities for veterinary professionals operating in affected areas, focusing on early recognition of clinical signs, differential diagnosis among transboundary animal diseases, and appropriate response measures.

Conclusion

This study reports the emergence of BTV-8 in Serbia in 2025. Molecular and phylogenetic analyses demonstrated a close genetic relationship with recent Western European strains, suggesting a transboundary introduction through the international trade of animals.

The concurrent circulation of multiple BTV serotypes increases the risk of reassortment events, potentially leading to the emergence of novel viral variants with unpredictable epidemiological and pathogenic characteristics. In this context, the co-circulation of other small ruminant pathogens further highlights the complexity of the regional epidemiological scenario and the challenges for clinical diagnosis.

These findings underline the critical importance of robust and continuous surveillance systems, supported by rapid laboratory confirmation and coordinated control measures, to limit further spread and better understand bluetongue virus dynamics in the Balkans. Vaccination campaigns, movement restrictions, and vector monitoring remain essential components of disease control strategies.

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Ethical approval

No ethical authorisation was required. All samples included in this study were collected for routine testing 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 VSIKV and IZSAM.

Genbank Accession Numbers

BTV-8/SRB/2025/Tutin1 PZ320985-PZ320994

BTV-8/SRB/2025/Tutin2 PZ321005-PZ321014

BTV-8/SRB/2025/Tutin3 PZ333777-PZ333786

BTV-8/SRB/2025/NoviPazar1 PZ320995-PZ321004

BTV-8/SRB/2025/NoviPazar2 PZ333767-PZ333776

Author Contributions

Dejan Vidanović: Conceptualisation, Project administration, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing, Validation, Data curation; Milovan Stojanović: Investigation, Data curation; Zoran Debeljak: Investigation, Data curation; Nikola Vasković: Investigation, Data curation. Milanko Šekler: Investigation, Data curation; Bojana Tešović: Investigation, Data curation, Formal analysis. Mihailo Debeljak: Investigation, Data curation; Gloria Plebani: Methodology, Formal analysis, Data curation; Iolanda Mangone: Software, Formal analysis; Maurilia Marcacci: Conceptualisation, Software, Formal analysis, Data curation, Validation, Writing – review & editing; Alessio Lorusso: Conceptualisation, Project administration, Supervision, Writing – original draft, Writing – review & editing, Validation, Data curation.

All authors have read and agreed to the published version of the manuscript.

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References

Batten, C. A., Banyard, A. C., King, D. P., Henstock, M. R., Edwards, L., Sanders, A., Buczkowski, H., Oura, C. C., & Barrett, T. (2011). A real time RT-PCR assay for the specific detection of Peste des petits ruminants virus. Journal of virological methods, 171(2), 401–404. https://doi.org/10.1016/j.jviromet.2010.11.022.

De Coster, W., & Rademakers, R. (2023). NanoPack2: population-scale evaluation of long-read sequencing data. Bioinformatics (Oxford, England), 39(5), btad311. https://doi.org/10.1093/bioinformatics/btad311.

Djurić, S., Simeunović, P., Mirilović, M., Stevanović, J., Glavinić, U., Vejnović, B., & Stanimirović, Z. (2017). Retrospective analysis of the bluetongue outbreak in Serbia. Macedonian veterinary review, 40(1), 21-27.

Djuricić, B., Jermolenko, G., Milosević, B., Radojicić, S., Debeljak, Z., & Tomić, A. (2004). The first bluetongue virus isolation in Yugoslavia. Veterinaria italiana, 40(4), 581–582.

Gondard, M., Postic, L., Garin, E., Turpaud, M., Vorimore, F., Ngwa-Mbot, D., Tran, M. L., Hoffmann, B., Warembourg, C., Savini, G., Lorusso, A., Marcacci, M., Felten, A., Roux, A. L., Blanchard, Y., Zientara, S., Vitour, D., Sailleau, C., & Bréard, E. (2024). Exceptional Bluetongue virus (BTV) and Epizootic hemorrhagic disease virus (EHDV) circulation in France in 2023. Virus research, 350, 199489. https://doi.org/10.1016/j.virusres.2024.199489.

Grubaugh, N. D., Gangavarapu, K., Quick, J., Matteson, N. L., De Jesus, J. G., Main, B. J., Tan, A. L., Paul, L. M., Brackney, D. E., Grewal, S., Gurfield, N., Van Rompay, K. K. A., Isern, S., Michael, S. F., Coffey, L. L., Loman, N. J., & Andersen, K. G. (2019). An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. Genome biology, 20(1), 8. https://doi.org/10.1186/s13059-018-1618-7.

Hadfield, J., Megill, C., Bell, S. M., Huddleston, J., Potter, B., Callender, C., Sagulenko, P., Bedford, T., & Neher, R. A. (2018). Nextstrain: real-time tracking of pathogen evolution. Bioinformatics (Oxford, England), 34(23), 4121–4123. https://doi.org/10.1093/bioinformatics/bty407.

Hofmann, M. A., Renzullo, S., Mader, M., Chaignat, V., Worwa, G., & Thuer, B. (2008). Genetic characterization of toggenburg orbivirus, a new bluetongue virus, from goats, Switzerland. Emerging infectious diseases, 14(12), 1855–1861. https://doi.org/10.3201/eid1412.080818.

Kottaridi, C., Nomikou, K., Lelli, R., Markoulatos, P., & Mangana, O. (2006). Laboratory diagnosis of contagious ecthyma: comparison of different PCR protocols with virus isolation in cell culture. Journal of virological methods, 134(1-2), 119–124. https://doi.org/10.1016/j.jviromet.2005.12.005.

Li H. (2018). Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics (Oxford, England), 34(18), 3094–3100. https://doi.org/10.1093/bioinformatics/bty191.

Maan, S., Maan, N. S., Belaganahalli, M. N., Potgieter, A. C., Kumar, V., Batra, K., Wright, I. M., Kirkland, P. D., & Mertens, P. P. (2016). Development and Evaluation of Real Time RT-PCR Assays for Detection and Typing of Bluetongue Virus. PloS one, 11(9), e0163014. https://doi.org/10.1371/journal.pone.0163014.

MacLachlan N. J. (2004). Bluetongue: pathogenesis and duration of viraemia. Veterinaria italiana, 40(4), 462–467.

MacLachlan, N. J., & Dubovi, E. J. (2017). Fenners veterinary virology (5th ed., pp. 269–297). Academic Press.

Marcacci, M., Cappai, S., Palombieri, A., Puggioni, G., Plebani, G., Irelli, R., Rodi, N., Rocchigiani, A. M., Gatta, G., Teodori, L., Leone, A., Manunta, D., Portanti, O., Casaccia, C., Curini, V., Di Sabatino, D., Scroggs, S. L. P., Spedicato, M., & Lorusso, A. (2026). Emergence of Bluetongue virus serotype 5 in Sardinia- Italy, 2025. Veterinaria italiana, 62(1), 10.12834/VetIt.3925.39028.1. https://doi.org/10.12834/VetIt.3925.39028.1.

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.

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, 61(4), 10.12834/VetIt.3915.38031.1. https://doi.org/10.12834/VetIt.3915.38031.1.

World Organisation for Animal Health (WOAH). Bluetongue (infection with bluetongue virus). In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 3.1.3. Paris: WOAH; latest edition.

World Organisation for Animal Health (WOAH). World Animal Health Information System (WAHIS) https://wahis.woah.org/#/event-management.