Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Environmental monitoring of viruses of veterinary concern by analyzing farm aggregated samples and slaughterhouse wastewater: a Bluetongue virus outbreak in Catalonia

Ignasi Estarlich Landajo
University of Barcelona: Universitat de Barcelona https://orcid.org/0009-0000-4739-9387
Marta Rusiñol
University of Barcelona https://orcid.org/0000-0002-6546-8857
Diana Ribeiro
University of Porto
Sandra Martínez-Puchol
University of Barcelona https://orcid.org/0000-0002-7549-9614
Marta Itarte
University of Barcelona https://orcid.org/0000-0002-0314-0768
Carlos Sanchez-Martí
Generalitat de Catalunya
Carles Riera
Generalitat de Catalunya
Rosina Girones
University of Barcelona https://orcid.org/0000-0002-1097-6395
Sílvia Bofill-Mas
sbofill@ub.edu

University of Barcelona https://orcid.org/0000-0001-8581-7180
Xavier Fernandez-Cassi
University of Barcelona https://orcid.org/0000-0003-2314-9126

Research Article

Keywords: - Bluetongue virus (BTV), - Passive sampling, - Slaughterhouse wastewater, - Aggregated environmental samples, - Veterinary surveillance.

Posted Date: October 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11320886/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: The authors declare no competing interests.

Abstract

Bluetongue virus (BTV) is a vector-borne Orbivirus causing substantial livestock losses, with emergence increasingly driven by climate change. Current surveillance mainly relies on testing individual animals, which is invasive, resource-intensive and may miss early or subclinical circulation. We evaluated environmental surveillance as a complementary approach using aggregated samples (n = 63) from BTV-confirmed farms and a slaughterhouse during an outbreak. Farm samples included drinking water (n = 4), straw bedding (n = 9) and manure (n = 14), while slaughterhouse wastewater was monitored by grab (n = 15) and passive sampling (PS) (n = 9), complemented by retrospective archived samples (n = 12). BTV RNA was detected across multiple farm matrices despite few confirmed infected animals, with highest detection in drinking water and straw. In wastewater, PS consistently detected BTV, including days with limited slaughter from outbreak-declared municipalities. Retrospective monitoring detected BTV weeks to months before official notifications. Environmental surveillance of aggregate samples provides a sensitive, non-invasive early warning approach supporting BTV surveillance, situational awareness and informed veterinary decision-making.

INTROUCTION

Currently, veterinary surveillance of viral pathogens relies primarily on clinical testing of suspected animals, typically via blood sampling followed by PCR or serology (Rojas et al., 2019). Although considered the gold standard, these approaches are resource-intensive, invasive, and dependent on the presence of clinical signs, limiting their capacity to detect subclinical infections or early viral circulation (Burrough et al., 2024).

Environmental surveillance has recently emerged as a complementary approach for animal disease monitoring (Cronin et al. 2026), leveraging pathogen shedding into shared matrices such as manure, bedding, drinking water, and wastewater (Xiao & Zhang, 2023). Because a single environmental sample can represent hundreds to thousands of animals, it provides a cost-effective, population-level sentinel system for detecting notifiable pathogens such as bluetongue virus (BTV), even in the absence of clinical cases. This approach parallels wastewater-based epidemiology (WBE), which has revolutionized human pathogen surveillance, supporting efforts ranging from poliovirus eradication to SARS-CoV-2 monitoring and virome characterization (Singh et al. 2024). Despite this success, the application of environmental surveillance in veterinary systems remains limited, and its potential for animal viruses is still poorly understood.

Passive sampling (PS) offers an additional advancement by integrating viral signals over time and improving detection sensitivity relative to grab sampling (West et al., 2023). While passive samplers have been validated in wastewater virology (Bivins et al., 2022), their application to slaughterhouse wastewater and animal viral surveillance has not yet been reported.

BTV impose substantial burdens on livestock and wildlife, with global economic impacts estimated at ~ USD 3 billion annually (Rushton & Lyons, 2015), and their emergence is increasingly driven by climate

change (Thomson & Stanberry, 2022).

BTV (genus *Orbivirus*, family *Sedoreoviridae*) is a non-enveloped, segmented dsRNA virus with a multilayered icosahedral capsid. Its genome comprises 10 segments encoding structural and non-structural proteins, enabling frequent reassortment (Matthijnssens et al. 2022). Transmission primarily occurs via *Culicoides* biting midges, whose distribution is highly climate-sensitive (El Moustaid et al. 2021).

Although fecal excretion has not been demonstrated, the systemic nature of BTV infection, characterized by viremia, dissemination to skin, mucosal and lymphoid tissues, and associated lesions (Saminathan et al., 2020), supports the plausibility of multi-route environmental shedding and thus environmental detectability.

Here, we evaluate aggregated environmental sampling as a surveillance tool for BTV in livestock systems by assessing viral detection in farm matrices (drinking water, straw bedding, manure) and slaughterhouse wastewater, and by comparing passive and grab sampling strategies.

2. MATERIALS AND METHODS

2.1. Collection of samples from Slaughterhouse and Farms

2.1.1. Farm samplings

Environmental sampling was conducted at 2 bovine and 1 sheep farms between June-November 2024 in Catalonia during a BTV outbreak (Departament d'Acció Climàtica, Alimentació i Agenda Rural, 2024). Samples were collected from multiple matrices, including drinking water (n = 4), manure (n = 14), straw bedding (n = 9), and surface swabs from drinkers and carcass containers (n = 2).

Farm selection and sampling logistics were coordinated by the Servei de Sanitat Animal from the Departament d'Agricultura, Ramaderia, Pesca i Alimentació of the Generalitat de Catalunya. Following confirmation of BTV infection by the National Reference Laboratory (Algete, Spain), sampling was conducted within approximately 15–30 days.

All samples were transported at 4°C in cold boxes to the laboratory, where they were processed immediately upon arrival under Biosafety Level 2 (BSL-2) containment measures.

2.1.2. Slaughterhouse samplings

Slaughterhouse wastewater sampling was conducted at two facilities in Catalonia, Spain. Slaughterhouse 1, located in the central-eastern Barcelona metropolitan area, processes approximately 2,000 cattle and 2,500 sheep per week, while Slaughterhouse 2, located in the north-eastern Barcelona

metropolitan area, processes around 800 cattle per day. Animals at both facilities originate primarily from farms in Catalonia, with additional livestock sourced from other regions of Spain.

An intensive sampling campaign was conducted at slaughterhouse 1 between October 8th and October 17th, 2024. During this period, three grab samples (200 mL each) of slaughterhouse wastewater were collected on October 8, 9, 15, 16, and 17 (n = 15). In parallel, three passive samplers (PS) were deployed on October 8, 15, and 16 and retrieved after 24 hours (n = 9). Each PS contained one nylon (NY) membrane (0.47 mm, Merck Millipore) and one nitrocellulose (NC) membrane (EZ-Pak flters, 0.4 mm, Merck Millipore).

In addition, monthly grab samples of slaughterhouse wastewater were collected between April and September 2024 as part of the VIRALERT project. These samples were obtained from both slaughterhouse 1 (n = 6) and slaughterhouse 2 (n = 6).

All wastewater samples were transported to the laboratory at 4°C and stored at − 20°C until further processing.

2.2. Sample Processing

A detailed flowchart of the processing steps for each sample type is provided in Supplementary Fig. 1.

2.2.1. Passive Samplers (PS)

PS were processed based on Mejías-Molina et al. 2024. Briefly, 1 NY and 1 NC membranes from the same PS were cut under aseptic conditions and each membrane placed into PowerBead tubes containing 700 µL of 0.25 M glycine buffer (pH 9.5). Samples were homogenized using a FastPrep® instrument (4 m/s, 30 s) to release viral particles. Supernatants (350 µL) from both membrane types were pooled and extracted together as described in Section 2.3.

2.2.2. Liquid matrices

Slaughterhouse wastewater (50 mL) was mixed with an equal volume of 0.25 M glycine buffer (pH 9.5), shaken on ice for 30 min, and centrifuged at 4,750 × g for 30 min at 4°C. The alkaline glycine buffer promotes viral desorption from organic matter and solids, improving elution efficiency (Peinado et al., 2022). Viral particles from the supernatants were concentrated by ultrafiltration using the CP Select™ concentrating pipette (InnovaPrep®) and viruses were eluted with 0.075% Tween-20/Tris using Wet Foam Elution™ cans (InnovaPrep®) adjusting the final volume to 350 µL with PBS 1X. Mean viral recoveries from wastewater concentrated using the CP Select™ system were previously reported by our group for MS2 (27.72 ± 24.46%) and MHV (7.51 ± 6.14%) (Forés et al., 2021).

From open drinkers (cattle Farm 1, sheep Farm 1), 20 L of drinking water were sampled, whereas 2 L were collected from narrow self-filling troughs (cattle Farm 2). Samples were concentrated using a two-step ultrafiltration process with the InnovaPrep® large volume concentration kit followed by CP Select™ concentration (InnovaPrep®), yielding a final volume of 350 µL (Forés et al., 2022).

2.2.3. Solid matrices

Straw bedding (80 g) was suspended in 200 mL of 0.25 M glycine buffer (pH 9.5), shaken on ice for 30 min, and centrifuged at 8,000 × g for 30 min at 4°C. Supernatants were filtered using Stericup filters (0.22 µm) (Millipore®) and concentrated using the CP Select™ system (InnovaPrep®), with viruses eluted in 350 µL.

For manure samples, 10 g of cattle manure were suspended in 90 mL of glycine buffer and shaken on ice for 30 min. 10 g of sheep manure were first suspended in 20 mL PBS and incubated for 24 h at 4°C to facilitate homogenization, followed by addition of 70 mL glycine buffer and shaken on ice for 30 min. All suspensions were centrifuged at 8,000 × g for 30 min at 4°C, supernatants were concentrated using the CP Select™ concentrating pipette (InnovaPrep®) and eluted with Wet Foam Elution™ cans (InnovaPrep®) yielding a final volume of 350 µL.

2.2.4. Drinkers and carcase boxes surface sampling

The surface of drinkers and carcass boxes were screened by swabbing approximately 25 cm² using PBS pre-moistened swabs (Deltalab) with PBS. After the screening the swabs were transported to the laboratory in cold conditions and processed immediately upon arrival to the laboratory.

2.3. Nucleic acid extraction and molecular detection

Nucleic acids (NA) from all samples, except swabs (Section 2.2.4.), were extracted from 280 µL of viral concentrate using the QIAamp Viral RNA Kit (Qiagen) on a QIAcube system and eluted in 80 µL of AVE buffer following the manufacturer instructions. Viral NA from swabs were extracted by using the PureFood GMO kit from Promega (AS1600). In brief, swabs were incubated in 300 µL lysis buffer and 30 µL proteinase K (PureFood GMO kit, Promega) at 56°C for 10 min. After recovery of the liquid phase, nucleic acids were purified using the on the Maxwell® RSC instrument (Promega) and eluted in 80 µL of the Elution Buffer from the kit.

To enable reverse transcription of dsRNA, extracts were denatured at 98°C for 3 min and cooled on ice for 5 min. cDNA synthesis was performed using random primers and SuperScript™ IV Reverse Transcriptase (Thermofisher) (23°C for 10 min, 55°C for 30 min, 85°C for 10 min). Both undiluted and 1:10 diluted NA extracts were processed to mitigate inhibition.

The molecular assays employed in this study are detailed in Supplementary table 1. BTV detection was performed using nested (RT)PCR targeting segment 5 (Katz et al. 1993). Serotype identification was conducted using serotype-specific nested (RT)PCR assays targeting segment 2 (Maan et al. 2012) of circulating serotypes in Catalunya, BTV-4 and BTV-8 (SESC, 2024 & DEFRA, 2024). Nested (RT)PCR reactions used 25 µL of TaqMan® Environmental Master Mix 2.0 (Themofisher) with 10 µL cDNA for the first round and 5 µL of product for the second round (final volume 50 µL).

Bovine and ovine polyomaviruses (BPyV and OPyV) were quantified by qPCR as indicators of fecal/urine contamination in all tested samples (Hundesa et al., 2010; Rusiñol et al., 2013) (Supplementary table 1).

Strict contamination control measures were implemented during nested (RT)PCR and qPCR procedures. Negative extraction controls and non-template controls were included throughout all steps.

2.4. Sequencing, bioinformatic analysis, statistics and data visualization

All BTV positive samples, except 2 drinking water samples, were confirmed by Sanger sequencing. Sequences were curated using Geneious Prime 10.2.6 and queried against the NCBI nucleotide database using BLASTn. Representative sequences generated in this study were deposited in GenBank under accession numbers PX736862–PX736870 (segment 5) and PX830591 (segment 2).

The sensitivity of the different environmental matrices for BTV and BPyV detection on farms, as well as the sensitivity of grab sampling and PS for BTV detection in slaughterhouse wastewater, were compared using Fisher's exact test. For multiple comparisons, Bonferroni correction was applied. Differences were considered statistically significant when $p < 0.05$.

Data visualisations were performed using R 4.1.2 (R Core Team, 2021) and ggplot2 package (Wickham, 2016).

2.5. Gathering outbreak data and animal origin information

To explore the relationship between BTV detection in slaughterhouse wastewater and reported clinical outbreaks, information on the origin of slaughtered animals was obtained from slaughterhouse records. These records included the municipalities of origin and the number of animals processed during the sampling period. Data on officially reported BTV outbreaks were retrieved from the online database of the Spanish Ministry of Agriculture, Fisheries and Food. All analyses were performed at the municipal level; no information at farm level was available.

For the intensive sampling campaign (October 8th-17th, 2024), BTV cases reported between September 1st and October 17th, 2024 were considered. For the retrospective analysis of stored samples, cases reported from 3 weeks before to 3 weeks after each sampling date were included.

Municipalities of origin of slaughtered animals were then cross-referenced with municipalities reporting BTV outbreaks to evaluate the spatial agreement between wastewater detection and notified clinical cases.

3. RESULTS

3.1. Monitoring of BTV in Aggregated Environmental Samples from Farms

BTV detection varied by matrix and farm (Fig. 1, Supplementary table 2). Drinking water (4/4) and swabs (2/2) showed the highest positivity rate, followed by straw bedding (6/9), while manure showed lower detection (3/14). Detection was significantly associated with sample type (Fisher's test, $p = 0.046$), with higher positivity in drinking water than manure ($p = 0.008$). Straw bedding showed a non-significant trend versus manure after correction ($p = 0.042$), while no difference was observed between drinking water and straw bedding ($p = 0.5$).

BTV positivity varied across farms, with the highest detection rate observed in Cattle Farm 2 (71.4%), followed by Cattle Farm 1 and Sheep Farm 1 (45.5% each). In both cattle farms, BTV was consistently detected in drinking water and straw bedding samples, whereas manure yielded lower detection rates (14.3% in Cattle Farm 1 and 50% in Cattle Farm 2). In Sheep Farm 1, BTV was detected in all swab samples (2/2), as well as in straw bedding (2/5) and drinking water (1/1), but was not detected in manure (0/3) (Supplementary Table 2).

All positives were confirmed by Sanger sequencing except two drinking waters. Serotyping targeted BTV-4 and BTV-8, the main circulating strains in the region (SESC, 2024; DEFRA, 2024), but amplification succeeded only in Cattle Farm 2, where BTV-8 was confirmed (Accession no. PX830591).

BPyV and OPyV were used as indicators of fecal/urinary input. In cattle farms, BPyV was most frequent and in higher loads in straw bedding and manure, with no detection in drinking water in Farm 2. In Sheep Farm 1, OPyV was mainly detected in straw bedding, occasionally in manure, and absent in drinking water, indicating higher recovery in fecal-associated matrices (Supplementary Table 2).

3.2. BTV Monitoring in Slaughterhouse Wastewater

A total of 24 wastewater samples were collected in October 2024 using passive sampling (PS, $n = 9$) and grab sampling ($n = 15$). Overall, BTV was detected in 37.5% (9/24) of samples, with higher detection in PS (66.7%, 6/9) than in grab samples (20.0%, 3/15). PS showed consistent detection across sampling events (3/3, 2/3, and 1/3 positive samples over successive events). The difference between methods was significant (Fisher's test, $p = 0.036$), suggesting higher sensitivity of PS (Fig. 2).

Serotyping of BTV-4 and BTV-8 from positive wastewater samples was unsuccessful due to lack of amplification.

BPyV was used as a fecal indicator and detected in wastewater on October 8th and 17th, confirming intermittent fecal contamination.

Retrospective analysis of frozen samples confirmed BTV RNA in both slaughterhouses outside the sampling period. In slaughterhouse 1, detection occurred in September, while in slaughterhouse 2 BTV was detected in July and August. BPyV was intermittently detected in both facilities (slaughterhouse 1: May, August; slaughterhouse 2: July, September) (Fig. 3).

4. DISCUSSION

4.1. Monitoring of BTV in Aggregated Environmental Samples from Farms

Although direct fecal or urinary excretion of BTV has not been demonstrated, viral replication in skin and tonsillar tissues (Darpel et al., 2012), together with lesions in the tongue and gastrointestinal mucosa (Ayuti et al., 2025), supports the plausibility of environmental detection. BTV RNA may therefore reach the environment via saliva, fecal contamination, and skin debris. In addition, as a non-enveloped icosahedral virus, BTV is expected to show environmental stability, similar to other *Sedoreoviridae* members (Matthijnssens et al., 2022).

Detection varied across farms and matrices, with higher positivity in drinking water and straw bedding than manure (Fig. 1). Drinking water showed consistent detection across farms and enabled serotyping, suggesting a key role for BTV environmental surveillance. This aligns with viral replication in tonsils and shedding via saliva and nasal secretions (Darpel et al., 2012). Low BPyV detection in water further supports a salivary origin. Its simpler processing also supports its use as a routine monitoring matrix.

Despite low PCR-confirmed infection rates (0.90%, 0.49%, 0.16%), BTV RNA was consistently detected across farms in environmental samples, suggesting the sensitivity of environmental surveillance at low prevalence. However, the limited number of farms restricts generalizability, and larger studies are needed. Environmental sampling is non-invasive, reduces animal handling, and offers a practical complementary approach to conventional veterinary sampling.

4.2. BTV Monitoring in Slaughterhouse Wastewater

This study evaluated wastewater surveillance for BTV detection in slaughterhouses using grab and PS, being the first application of PS in this context. As a viremic virus (Gasparini et al., 2021), BTV detection in wastewater is likely associated with blood released during slaughter, supported by the low detection of BPyV, suggesting that fecal and urine inputs are minor compared with blood, tissue residues, and cleaning water.

Overall, PS showed higher sensitivity and more consistent detection than grab sampling (Fig. 2), likely due to time-integrated viral accumulation (West et al., 2023). Although direct comparison with composite sampling was not feasible due to operational constraints, passive sampling consistently outperformed grab sampling. On October 8th, when 12.82% of processed animals originated from municipalities with reported BTV cases, all three grab-sample replicates were negative. Conversely, on October 9th, when only 0.12% of animals originated from affected municipalities, BTV was detected in just one of three grab-sample replicates. In contrast, all three passive samplers deployed during this period tested positive for BTV, highlighting the greater sensitivity and temporal integration provided by passive sampling. Although this suggests a link between detected RNA and daily slaughter batches, we cannot totally exclude partial contribution from previous.

Retrospective analysis also detected BTV RNA in both slaughterhouses (Fig. 3) in the absence of officially reported outbreaks in source municipalities, suggesting undetected or underreported circulation, suggesting its potential as an early-warning system.

In summary, PS appears as a sensitive sentinel tool for slaughterhouse surveillance, supporting early detection and reconstruction of transmission dynamics. Further optimization of sampling and molecular methods will improve its applicability for routine monitoring.

4.3. Limitations

Serotyping assays targeting BTV-4 and BTV-8 were selected based on reported circulation in Spain and neighbouring European countries during the study period (Gondard et al., 2024). Serotype identification was successful only in Cattle Farm 2, where BTV-8 was detected. Although BTV-8 was the dominant strain in Catalonia at the time, amplification failed in the remaining farms and slaughterhouses. This may reflect the limited robustness of the nested PCR typing assays used under environmental conditions, where low viral loads and PCR inhibitors are common, as well as the use of identical primer sets in both amplification rounds, reducing assay sensitivity. Additional factors such as RNA degradation or fragmentation may also have impaired target amplification, while the presence of other serotypes not efficiently detected by the assay cannot be excluded. These limitations highlight the need for serotyping tools specifically optimized and validated for environmental surveillance.

A further limitation is that the persistence and environmental stability of BTV RNA in the analysed matrices were not assessed. However, the aim was not to characterize RNA persistence, but to evaluate whether environmental detection could support or trigger clinical surveillance. In this context, even transient detection of BTV RNA may be considered relevant for guiding targeted veterinary actions.

5. CONCLUSIONS

This study demonstrates that aggregated environmental sampling is a sensitive and practical complementary approach for the surveillance of BTV circulation in livestock systems. Despite low apparent prevalence of clinically confirmed infections, BTV RNA was consistently detected in farm-level environmental matrices, particularly drinking water and straw bedding, highlighting their suitability as sentinel samples for early viral circulation.

Importantly, and at a larger scale, slaughterhouse wastewater surveillance provided BTV detection linked to the geographical origin of slaughtered animals, with PS outperforming grab sampling in terms of sensitivity and temporal consistency. Retrospective analysis further suggests possible underreported circulation of BTV weeks to months prior to official outbreak notifications at the municipal level, underscoring the capacity of wastewater-based surveillance to uncover silent or underreported transmission, especially in cattle populations where infections are often subclinical.

Overall, these findings are a proof of concept that support the integration of environmental surveillance at two different levels, farm and slaughterhouses into existing veterinary health frameworks. Such

approaches offer a non-invasive, cost-effective, early warning system that can enhance outbreak detection, improve epidemiological situational awareness, and support more timely and targeted disease control interventions scalable to other emerging veterinary viruses.

Declarations

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding sources

This work was partially funded by the VIRALERT project (PID2021-128014OB-I00) and by the VIRTRACK project (PID2024-159580OB-I00), both supported by MCIN/AEI/10.13039/501100011033/FEDER, UE. I. Estarlich-Landajo was a predoctoral fellowship from AGAUR (2025 STEP 00181). S. Bofill-Mas is a Serra Húnter Fellow at the University of Barcelona. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

We acknowledge the collaboration of Servei de Sanitat Animal, Departament d'Agricultura, Ramaderia, Pesca i Alimentació, Generalitat de Catalunya for their support in the organization and implementation of the sampling campaigns. In addition, the authors would like to thank the farmers and slaughterhouse staff for their support across all sampling campaigns.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process.

During the preparation of this manuscript, the authors used ChatGPT solely for language editing and improvement of clarity. After using this tool, the authors reviewed and edited the content as necessary and take full responsibility for the content of the published article.

Author contributions: CRediT

Conceptualization: Ignasi Estarlich-Landajo (IEL), Sílvia Bofill-Mas (SBM) and Xavier Fernandez-Cassi (XFC)

Sampling: IEL, Marta Rusiñol (MR), SBM, XFC

Data curation: IEL, MR, CSM, CR, XFC

Formal analysis: IEL, Diana Ribeiro (DR), SBM, XFC

Funding acquisition: Rosina Girones (RG), SBM

Investigation: IEL, MR, DR, Sandra Martinez-Puchol (SMP), Marta Itarte (MI), CSM, CR, Carlos Sanchez-Martí (CSM), Carles Riera (CR), RG, SBM, XFC

Methodology: IEL, MR, DR, SMP, MI, CSM, CR, SBM, XFC

Project administration: RG, SBM, XFC

Resources: MR, RG, SBM, XFC

Supervision: MR, SBM, XFC

Visualization: IEL, SBM

Writing – original draft: IEL

Writing – review and editing: IEL, MR, SBM, XFC

References

1. Ayuti SR, Khairullah AR, Lamid M, Warsito SH, Al Arif MA, Kim EJ, Moses IB, Shin S, Wardhani BWK, Wasito W, Khalisa AT, Ahmad RZ (2025) Bluetongue in ruminants: Global epidemiology, pathogenesis, and advances in diagnostic and control strategies within a One Health framework. Vet World 3070–3093. https://doi.org/10.14202/vetworld.2025.3070-3093
2. Bivins A, Kaya D, Ahmed W, Brown J, Butler C, Greaves J, Leal R, Maas K, Rao G, Sherchan S, Sills D, Sinclair R, Wheeler RT, Mansfeldt C (2022) Passive sampling to scale wastewater surveillance of infectious disease: Lessons learned from COVID-19. Sci Total Environ 835:155347. https://doi.org/10.1016/j.scitotenv.2022.155347
3. Burrough ER, Derscheid RJ, Mainenti M, Piñeyro P, Baum DH (2025) The diagnostic process for infectious disease diagnosis in animals. javma 263, S6–S16. https://doi.org/10.2460/javma.24.10.0657
4. Cronin P, Siegers JY, Heang V, Tok S, Sin S, Sievers B, Omondi V, Nuon S, Chhel K, Nouhin J, Chim V, Seng B, Hak M, San S, Tum S, Claes FF, Firth C, Su YCF, Smith GJD, Karlsson EA (2026) Environmental metagenomics enhances detection of circulating viruses from live poultry markets in Cambodia. Nat Commun 17:1525. https://doi.org/10.1038/s41467-025-68245-8
5. Darpel KE, Monaghan P, Simpson J, Anthony SJ, Veronesi E, Brooks HW, Elliott H, Brownlie J, Takamatsu H-H, Mellor PS, Mertens PP (2012) Involvement of the skin during bluetongue virus infection and replication in the ruminant host. Vet Res 43:40. https://doi.org/10.1186/1297-9716-43-40

6. Departament d'Acció Climàtica, Alimentació i Agenda Rural (2024), June 12 RESOLUCIÓ ACC/2115/2024, d'11 de juny, de declaració oficial de la llengua blava a Catalunya i de delegació de competències en la persona titular de la Direcció General d'Agricultura i Ramaderia en matèria de declaració dels focus i adopció de mesures de lluita contra la malaltia. Diari Oficial de la Generalitat de Catalunya, (9182). https://www.gencat.cat/dogc
7. Department for Environment, Food & Rural Affairs (DEFRA) (2024) Updated outbreak assessment for bluetongue virus (BTV) in Europe. United Kingdom Government; Mar 15. Available from: https://www.gov.uk/government/publications/bluetongue-virus-in-europe/15-march-2024-updated-outbreak-assessment-for-bluetongue-virus-in-europe
8. El Moustaid F, Thornton Z, Slamani H, Ryan SJ, Johnson LR (2021) Predicting temperature-dependent transmission suitability of bluetongue virus in livestock. Parasites Vectors 14:382. https://doi.org/10.1186/s13071-021-04826-y
9. Forés E, Bofill-Mas S, Itarte M, Martínez-Puchol S, Hundesa A, Calvo M, Borrego CM, Corominas LL, Girones R, Rusiñol M (2021) Evaluation of two rapid ultrafiltration-based methods for SARS-CoV-2 concentration from wastewater. Sci Total Environ 768:144786. https://doi.org/10.1016/j.scitotenv.2020.144786
10. Forés E, Rusiñol M, Itarte M, Martínez-Puchol S, Calvo M, Bofill-Mas S (2022) Evaluation of a virus concentration method based on ultrafiltration and wet foam elution for studying viruses from large-volume water samples. Sci Total Environ 829:154431. https://doi.org/10.1016/j.scitotenv.2022.154431
11. Gasparini M, Laguardia-Nascimento M, Sales ÉB, Oliveira AGG, Lobato ZIP, Camargos MF, Fonseca Júnior AA (2021) Study of molecular diagnosis and viremia of bluetongue virus in sheep and cattle. Braz J Microbiol 52:1623–1626. https://doi.org/10.1007/s42770-021-00518-y
12. 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 AL, 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 Res 350:199489. https://doi.org/10.1016/j.virusres.2024.199489
13. Hofmann M, Griot C, Chaignat V, Perler L, Thür B (2008) Blauzungenkrankheit erreicht die Schweiz. Schweizer Archiv für Tierheilkunde 150:49–56. https://doi.org/10.1024/0036-7281.150.2.49
14. Hudson AR, McGregor BL, Shults P, England M, Silbernagel C, Mayo C, Carpenter M, Sherman TJ, Cohnstaedt LW (2023) *Culicoides* -borne Orbivirus epidemiology in a changing climate. J Med Entomol 60:1221–1229. https://doi.org/10.1093/jme/tjad098
15. Hundesa A, Bofill-Mas S, De Motes M, Rodriguez-Manzano C, Bach J, Casas A, Girones M, R (2010) Development of a quantitative PCR assay for the quantitation of bovine polyomavirus as a microbial source-tracking tool. J Virol Methods 163:385–389. https://doi.org/10.1016/j.jviromet.2009.10.029
16. Katz JB, Alstad AD, Gustafson GA, Moser KM (1993) Sensitive identification of bluetongue virus serogroup by a colorimetric dual oligonucleotide sorbent assay of amplified viral nucleic acid. J Clin

Microbiol 31:3028–3030. https://doi.org/10.1128/jcm.31.11.3028-3030.1993

17. Maan NS, Maan S, Belaganahalli MN, Ostlund EN, Johnson DJ, Nomikou K, Mertens PPC (2012) Identification and Differentiation of the Twenty Six Bluetongue Virus Serotypes by RT–PCR Amplification of the Serotype-Specific Genome Segment 2. PLoS ONE 7:e32601. https://doi.org/10.1371/journal.pone.0032601
18. Matthijnssens J, Attoui H, Bányai K, Brussaard CPD, Danthi P, Del Vas M, Dermody TS, Duncan R, Fāng (□□) Q, Johne R, Mertens PPC, Mohd Jaafar F, Patton JT, Sasaya (□□□□) T Suzuki (□□□□), N., Wei (□□□), T., 2022. ICTV Virus Taxonomy Profile: Sedoreoviridae 2022: This article is part of the ICTV Virus Taxonomy Profiles collection. J Gen Virol 103. https://doi.org/10.1099/jgv.0.001782
19. Mayo C, McDermott E, Kopanke J, Stenglein M, Lee J, Mathiason C, Carpenter M, Reed K, Perkins TA (2020) Ecological Dynamics Impacting Bluetongue Virus Transmission in North America. Front Vet Sci 7:186. https://doi.org/10.3389/fvets.2020.00186
20. Mejías-Molina C, Estarlich-Landajo I, Martínez-Puchol S, Bofill-Mas S, Rusiñol M (2024) Exploring waterborne viruses in groundwater: Quantification and Virome characterization via passive sampling and targeted enrichment sequencing. Water Res 266:122305. https://doi.org/10.1016/j.watres.2024.122305
21. Napp S, García-Bocanegra I, Pagès N, Allepuz A, Alba A, Casal J (2013) Assessment of the risk of a bluetongue outbreak in Europe caused by Culicoides midges introduced through intracontinental transport and trade networks. Med Vet Entomol 27:19–28. https://doi.org/10.1111/j.1365-2915.2012.01016.x
22. Peinado B, Martínez-García L, Martínez F, Nozal L, Sánchez MB (2022) Improved methods for the detection and quantification of SARS-CoV-2 RNA in wastewater. Sci Rep 12:7201. https://doi.org/10.1038/s41598-022-11187-8
23. Rojas JM, Rodríguez-Martín D, Martín V, Sevilla N (2019) Diagnosing bluetongue virus in domestic ruminants: current perspectives. Vet Med Res Rep 10:17–27. https://doi.org/10.2147/vmrr.s163804
24. Rushton J, Lyons N (2015) Economic impact of Bluetongue: a review of the effects on production. Vet Ital 51:401–406. https://doi.org/10.12834/VetIt.646.3183.1
25. Rusiñol M, Carratalà A, Hundesa A, Bach A, Kern A, Vantarakis A, Girones R, Bofill-Mas S (2013) Description of a novel viral tool to identify and quantify ovine faecal pollution in the environment. Sci Total Environ 458–460:355–360. https://doi.org/10.1016/j.scitotenv.2013.04.028
26. Saminathan M, Singh KP, Khorajiya JH, Dinesh M, Vineetha S, Maity M, Rahman AF, Misri J, Malik YS, Gupta VK, Singh RK, Dhama K (2020) An updated review on bluetongue virus: epidemiology, pathobiology, and advances in diagnosis and control with special reference to India. Veterinary Q 40:258–321. https://doi.org/10.1080/01652176.2020.1831708
27. Servei d'Epidemiologia i Salut Pública de Catalunya (SESC). Bluetongue in Catalonia and its detection in slaughterhouses. SESC, July 26 (2024) Available at: https://sesc.cat/en/bluetongue-in-catalonia-and-its-detection-in-slaughterhouses/

28. Singh S, Ahmed AI, Almansoori S, Alameri S, Adlan A, Odivilas G, Chattaway MA, Salem SB, Brudecki G, Elamin W (2024) A narrative review of wastewater surveillance: pathogens of concern, applications, detection methods, and challenges. Front Public Health 12:1445961. https://doi.org/10.3389/fpubh.2024.1445961
29. Thomson MC, Stanberry LR (2022) Climate Change and Vectorborne Diseases. N Engl J Med 387:1969–1978. https://doi.org/10.1056/NEJMra2200092
30. West NW, Hartrick J, Alamin M, Vasquez AA, Bahmani A, Turner CL, Shuster W, Ram JL (2023) Passive swab versus grab sampling for detection of SARS-CoV-2 markers in wastewater. Sci Total Environ 889:164180. https://doi.org/10.1016/j.scitotenv.2023.164180
31. World Organisation for Animal Health (2021) Bluetongue (Infection with bluetongue virus). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Chap. 3.1.3). World Organisation for Animal Health
32. Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer, New York. https://doi.org/10.1007/978-3-319-24277-4
33. Xiao K, Zhang L (2023) Wastewater pathogen surveillance based on One Health approach. Lancet Microbe 4:e297. https://doi.org/10.1016/S2666-5247(23)00039-3
34. Zeng L, Li J, Lv M, Li Z, Yao L, Gao J, Wu Q, Wang Z, Yang X, Tang G, Qu G, Jiang G (2023) Environmental Stability and Transmissibility of Enveloped Viruses at Varied Animate and Inanimate Interfaces. Environ Health 1:15–31. https://doi.org/10.1021/envhealth.3c00005

Figures

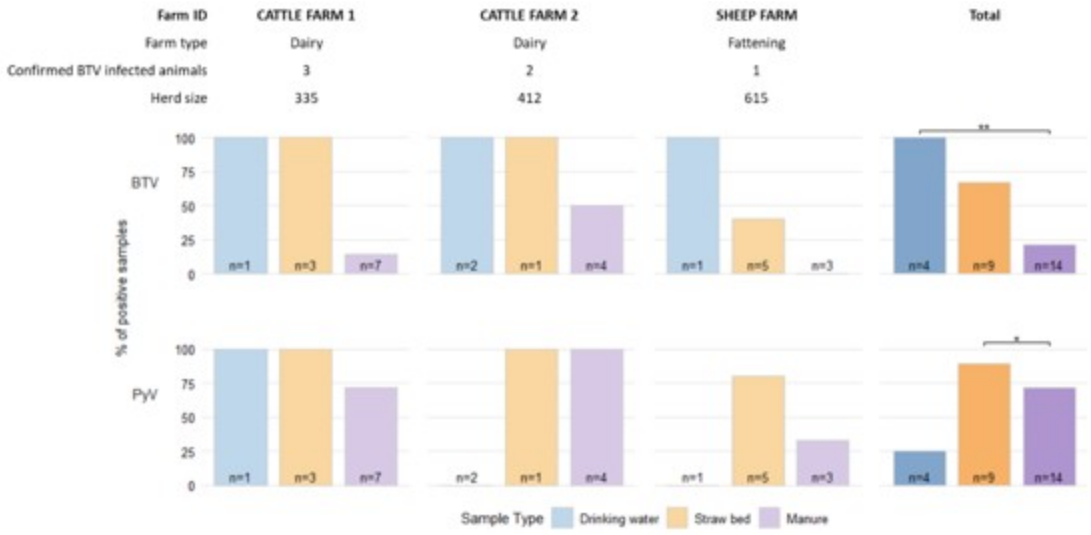


Figure 1

Detection of bluetongue virus (BTV) and polyomaviruses (bovine polyomavirus (BPyV) in cattle farms; ovine polyomavirus (OPyV) in the sheep farm) in aggregate environmental samples collected on farms. Bars show the percentage of positive samples by matrix (drinking water, straw bedding, and manure) for

each farm; the number of samples is indicated (n). The table above the graphs summarizes key farm characteristics, including farm type, herd size, and number of confirmed BTV-infected animals by veterinarian authorities. Fisher test: * $p < 0.05$; ** $p < 0.01$

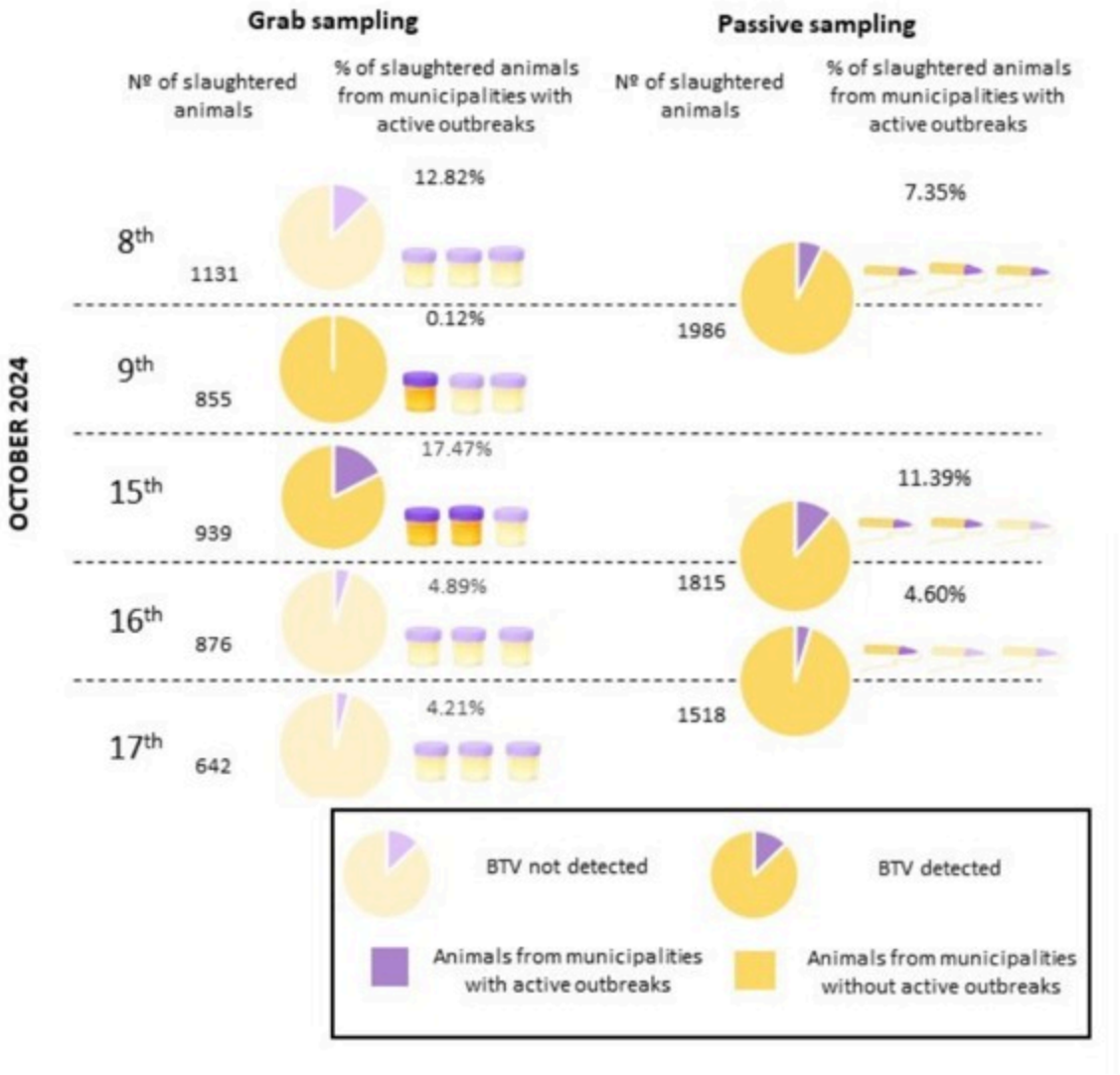


Figure 2

Number of animals slaughtered between 8-17 October 2024 and BTV detection in slaughterhouse wastewater using grab and passive sampling. Pie charts show the proportion of animals originating from municipalities with (purple) and without (orange) officially declared BTV cases during the active outbreak period (1 September–17 October 2024). Dark-coloured pie charts indicate BTV detection on the corresponding sampling day and method, whereas light colours indicate no detection. Individual icons represent replicates (grab samples or torpedo-style passive samplers), shown in dark colour when positive and light when negative.

	April	May	June	July	August	September
BTV officially declared	No	No	Yes	Yes	Yes	Yes
Slaughterhouse 1	-	-	-	-	-	+
Slaughterhouse 2	-	-	-	+	+	-

Figure 3

Detection of bluetongue virus (BTV) in monthly grab samples collected from slaughterhouse 1 and slaughterhouse 2 between April and September 2024. Results are reported as positive (+) or negative (−). BTV positive samples are shown in dark blue, while negative samples are shown in light blue.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- BTVsupplementary.pdf