

Brief Report

Unbiased Sequencing Identifies Batai Virus as an Emerging Orthobunyavirus in Hungarian Cattle

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Abstract

This case report describes the unexpected identification and genomic characterization of Batai virus (BATV), an Orthobunyavirus, during routine bovine viral diarrhea virus (BVDV) surveillance on a cattle farm in Hungary. The discovery was facilitated by next-generation sequencing (NGS) technologies and highlights the importance of unbiased sequencing in pathogen surveillance. Although BATV currently exhibits limited zoonotic impact, its detection in livestock underscore the critical need for integrated surveillance at the human–animal–environment interface and the adoption of proper diagnostic tools to identify potential threats before they escalate.

Keywords: BVDV; Batai virus; NGS

1. Introduction

Bovine viral diarrhea virus (BVDV) is an economically important pathogen of cattle worldwide. The causative agent belongs to the *Pestivirus* genus within the *Flaviviridae* family and has a positive sense single-stranded RNA genome of nearly 12.3 kb length [1]. BVDV is divided into three species (BVDV-1, BVDV-2, and BVDV-3), which contain multiple subgenotype viruses, with BVDV-1 demonstrating the highest diversity [1]. The virus exists as two biotypes based on their cytopathic effect (CPE) on cell culture: cytopathic (cp) and non-cytopathic (ncp). The virus primarily targets the immune and reproductive systems, leading to a wide spectrum of clinical outcomes ranging from subclinical infections to severe disease manifestations such as respiratory illness, diarrhea, reproductive failure, and mucosal disease. Transmission of the virus occurs through direct contact with infected animals, particularly persistently infected (PI) individuals, which serve as the main reservoir [2]. Control strategies typically involve biosecurity measures, testing and removal of PI animals, and vaccination programs.

Batai virus (BATV) is a mosquito-borne virus belonging to the genus *Orthobunyavirus* within the family *Peribunyaviridae* [3]. BATV has a tri-segmented, single-stranded, negative-sense RNA genome, consisting of S segment, which encodes nucleocapsid (N) and non-structural protein (NSs); M segment, which encodes glycoproteins (Gn, Gc) and NSm; and L segment, encoding the viral RNA-dependent RNA polymerase [3]. BATV is transmitted by mosquitoes (e.g., *Culex*, *Anopheles*) and possibly ticks and biting midges. Its host range includes a wide variety of vertebrates including ruminants, pigs, horses, birds, and occasionally humans [4]. In humans, BATV typically causes mild febrile illness, but can occasionally lead to encephalitis or neurologic symptoms [5]. In animals, BATV infections are associated with reproductive disorders in cattle and sheep (e.g., abortions,



Academic Editors: Estelle Venter and Antoinette Van Schalkwyk

Received: 3 December 2025

Revised: 21 January 2026

Accepted: 28 January 2026

Published: 1 February 2026

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birth defects) and encephalitis in harbor seals [4,6,7]. BATV has a wide geographic distribution, including Asia, Africa, and Europe, with increasing detection in Central and Northern Europe [8]. In Hungary, there has been no systematic investigation on BATV prevalence. According to Hubálek et al. [9], it has been sporadically found in mosquitos and seropositivity was observed in livestock. Neither confirmed human or veterinary cases nor virus isolate or nucleic acid sequences have been reported from Hungary. Currently, no vaccine or specific antiviral treatment is available against BATV. Reassortment with related viruses (e.g., Bunyamwera virus) can increase virulence, raising concerns for emerging zoonoses [8,10,11].

A voluntary BVDV eradication program in Hungary yielded BVDV isolates of different subgenotypes and biotypes [12–14], which prompted us to characterize representative isolates from functional and molecular aspects, including whole genome sequencing (manuscript in preparation). One BVDV isolate yielded more BATV reads than BVDV and allowed the assembly of the whole genome of both viruses. The detection of the virus prompted us to reveal its prevalence in the herd.

2. Materials and Methods

As part of a voluntary BVDV eradication program, a Hungarian dairy farm of 618 heifers (younger than 6 month) and 618 milk-producing cows (1205 animals altogether) at the time of sample collection (July 2024), conducted an investigation to reveal persistently infected immunotolerant animals to BVDV in the herd. Initially, serum samples as pools of 30 individuals at maximum were tested by a commercial qPCR kit (EXOone BVDV-BDV EXOPORUM 100, EXOPOL, Zaragoza, Spain); the positive pools were split until individual virus positive status could be determined.

The BVDV positive samples were submitted to virus isolation on MDBK cells from 5× diluted serum samples in 1:1 mixture of MEM-H and MEM-E medium on Falcon T25 cell culture flasks (Corning Inc., Corning, NY, USA) at 37 °C in a 5% CO₂ atmosphere. Cell cultures were examined daily by both bright-field and immunofluorescence microscopy for evidence of cytopathic effect (CPE), and observations were concluded four to five days post-inoculation. The isolates were passaged three times, and each passage was tested for BVDV.

To improve our sequence inventory, the third passages (3p) of the CPE positive isolates were submitted to whole genome NGS by using the Oxford Nanopore Technologies (ONT) platform upon extracting the RNA by ZymoBIOMICS DNA/RNA Miniprep kit (Zymo Research, Irvine, CA, USA) with DNase treatment. The host rRNA was depleted with Invitrogen RiboMinus Eukaryote System v2 (Thermo Fisher Scientific, Waltham, MA, USA). REPLI-g WTA Single Cell Kit was used for reverse transcription and cDNA amplification. The library was prepared with Rapid Barcoding Kit (Oxford Nanopore Technologies, Oxford, UK) and was sequenced on R10.4.1 flowcell in MinION Mk1B sequencing device.

ONT data were subjected to quality control prior to downstream analysis by MinKNOW v24.06.16 software, a proprietary of Oxford Nanopore Technologies. An automated BLAST v2.16.0 search of the quality-controlled reads identified more *Orthobunyavirus*-origin than BVDV-origin reads in the case of isolate D7809/760/24. Complete genome sequence of the *Orthobunyavirus* was assembled with mapping to reference KJ542635, KJ542634, and KJ542633 GenBank sequences using the Minimap2 plugin within Geneious Prime® v.2022.2.2 (Biomatters, Auckland, New Zealand, <https://www.geneious.com>, accessed on 27 January 2026). The consensus sequences were called using the 0%—majority threshold. Consensus was annotated based on sequence homology with references and edited manually within Geneious Prime.

Codon-based nucleic acid alignments of the coding region from each segment (L: RNA-dependent RNA polymerase—RdRp; M: glycoprotein; S: nucleoprotein gene) were generated using the Muscle algorithm within Geneious Prime software. Phylogenetic analysis was performed using the MEGAX package [15]. Gene-specific substitution models were evaluated, and the best-fit models were selected based on the Bayesian information criterion (RdRp, Glycoprotein: General Time Reversible; Nucleoprotein: Tamura-Nei). Maximum-likelihood trees were generated, and tree topologies were validated by bootstrap analysis (100) as implemented in MEGAX.

A BATV specific RT-qPCR [16] along with EXOone BVDV-BDV qPCR kit was used for estimating the BATV:BVDV ratio of the D7809/760/24 mixed isolate and its change during passages after nucleic acid extraction from the three passages with the method described above. The same BATV specific qPCR was used for the determination of the prevalence of the virus in the collected serum samples from the concerned farm, utilizing the same pools used for the search for BVDV PI individuals.

3. Results

The isolation attempt resulted in a CP effect of the isolate D7809/760/24 after five days of incubation, characterized by rounding and detachment of the cells. The sequencing approach by ONT and the BLAST-assisted characterization of the obtained sequences indicated the simultaneous presence of both pathogens in the sample with the apparent overrepresentation of the *Orthobunyavirus* compared to that of the *Pestivirus*. The former was identified as *Orthobunyavirus bataiense* (BATV) according to the most recent virus taxonomy [17]. Bioinformatic analysis yielded the whole genome of both pathogens. Since the original aim was to isolate BVDV from the sample, the mixed nature of the D7809/760/24 isolate prevented its further cultivation.

Sequencing resulted in 908000 reads of which 8144 were identified as *Pestivirus* in origin and 12173 as *Orthobunyavirus* in origin. The L, M, and S segment sequences of the BATV were determined in lengths of 6870 bp, 4440 bp, and 943 bp, with mean coverage of 313x, 727x, and 1711x, respectively, and uploaded to NCBI Genbank with Accession Numbers PV956151-3. The phylogenetic analysis of all protein coding genes of BATV revealed the close relationship of the D7809/760/24 isolate with other European strains isolated to date (Figure 1).

The relative quantification of the two viruses by specific RT-qPCR assays showed that BATV overgrew BVDV during the three consecutive passages. BVDV-specific Ct values increased slightly (24.4, 24.7, and 26.7, respectively), whereas BATV-specific Ct values decreased markedly (18.9, 11.3, and 11.9, respectively), indicating approximately a two-log increase in BATV viral load.

The prevalence investigation revealed that 10 of the 42 serum sample pools tested positive using BATV-specific RT-qPCR. After disaggregating these pools, 23 of the 1205 individual samples were confirmed positive, corresponding to an overall prevalence of 1.9%. Notably, the two age cohorts differed markedly: only 1 of the 587 sera from milk-producing cows (0.2%) was positive, whereas 22 of the 618 sera from heifers were positive, corresponding to a 3.6% prevalence in this group.

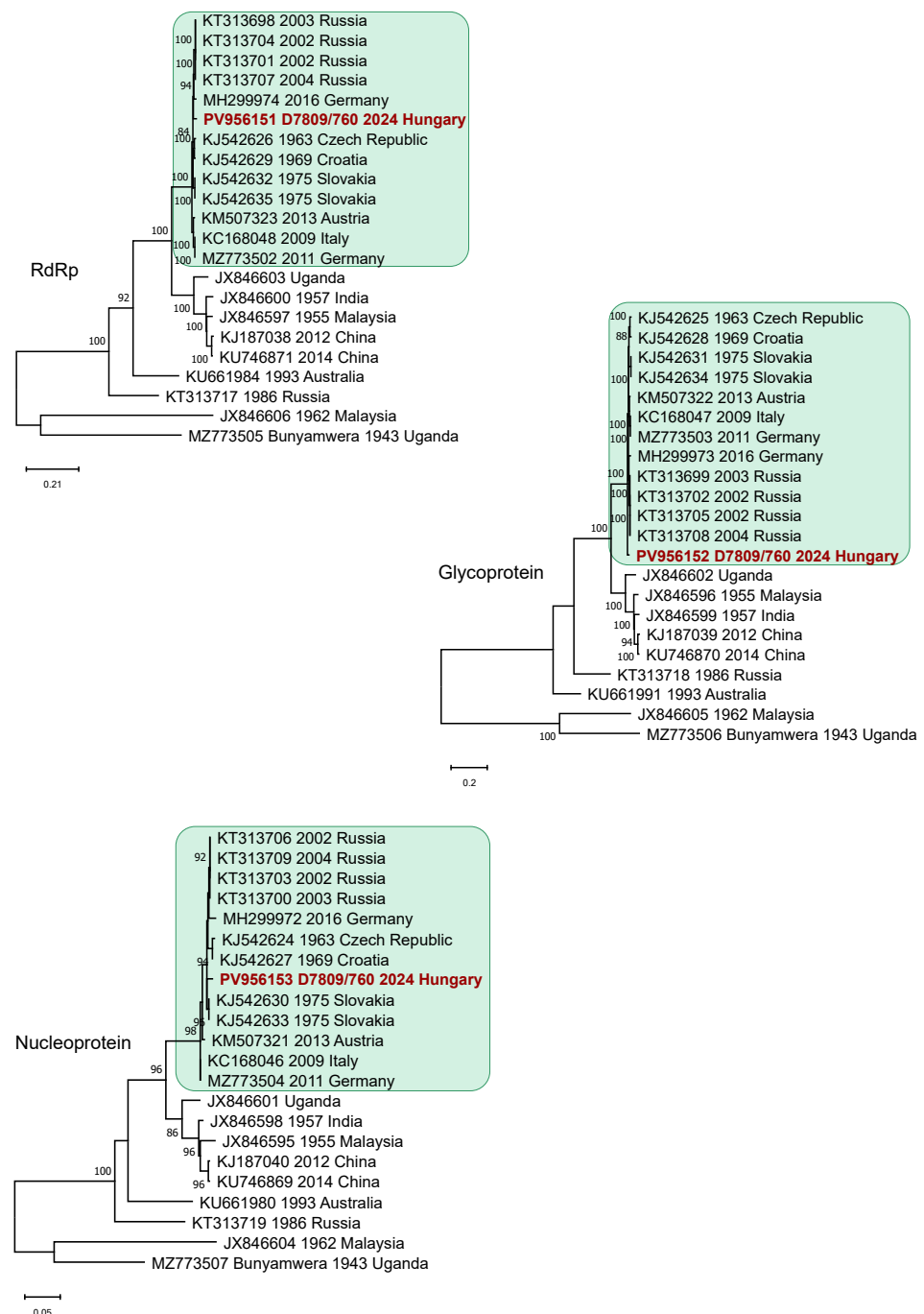


Figure 1. Phylogenetic trees generated from the codon-based nucleic acid alignments of the coding region from each segment. Isolate analyzed in the recent work was colored with burgundy. Green rectangle indicates the distinct clade of BATV isolates described in Europe thus far. Phylogenetic trees were calculated by the maximum likelihood method based on the best-fit model selected for each gene. Bootstrap values higher than 70% are shown.

4. Discussion

The targeted diagnostic workflow identified a cytopathogenic (cp) BVDV in a virus isolate originating from a BVDV-infected herd. However, only after applying NGS did a whole-genome analysis reveal an additional, unexpected finding: the presence of BATV. This result highlights a key limitation of specific (q)PCR-based assays and underscores the diagnostic strength of unbiased sequencing approaches. Because the observed CPE

likely reflected the combined effects of both BATV and BVDV, the isolate was excluded from further BVDV-focused investigations.

Although previous reports have described sporadic human seropositivity to BATV in Hungary [9], this is the first confirmed detection of the virus in the country. Furthermore, the whole-genome sequence generated in this study represents the first BATV genome published from Hungary. Our phylogenetic analysis showed that the detected virus clusters within the distinct “European” clade, supporting the notion that BATV is endemic in this region as well.

BATV has been sporadically detected across Europe since the 1960s [3], but in the absence of systematic monitoring its true prevalence is likely underestimated. Nevertheless, when investigated rigorously, surprisingly high seroprevalence rates have been reported—ranging from 13 to 50% in game mammals and 16 to 45% in ruminant livestock [3,7]. In a study of ruminants in the German state of Saxony-Anhalt, 27.7% of animals were seropositive: sheep and goats showed moderate prevalences (16.5% and 18.3%, respectively), whereas cattle exhibited the highest antibody rate (41.4%) [7]. Although serological datasets are not directly comparable to our RT-qPCR-based positivity rate, the overall 1.9% prevalence observed in the Hungarian dairy farm appears relatively low. The pronounced difference between heifers (3.6%) and cows (0.2%) is particularly intriguing; however, in the absence of comparable datasets, its interpretation remains speculative and warrants further targeted investigations.

Human infections with BATV are rare and generally mild, typically presenting as a flu-like illness in Europe [8]. Consequently, BATV infections are seldom laboratory-confirmed and may even be misdiagnosed; for example, BATV-infected patients in Sudan were initially diagnosed with fever of unknown origin or with malaria [18]. Although BATV is not widely recognized as a human pathogen, its zoonotic potential—particularly among vulnerable groups such as children and immunocompromised individuals—highlights the importance of continued attention.

Several mosquito genera (i.e., *Aedes*, *Anopheles*, *Culex*, *Coquillettidia*) are known vectors of Batai virus [3,9]. These genera are also characteristic components of the Hungarian mosquito fauna. One of the principal vector species, *Coquillettidia richiardii*, typically prefers vegetated, often rural aquatic habitats rather than warm urban environments [19], increasing the likelihood of contact with livestock. Moreover, the high abundance of *Cq. richiardii* reported from the Vienna region and its documented presence in Transylvania [20,21] suggests an elevated potential for BATV spread by mosquitoes across Central Europe.

The detection of BATV in a Hungarian cattle herd is therefore noteworthy, indicating that the virus may be more widespread than previously recognized and potentially underdiagnosed due to limitations in routine surveillance.

Laboratory diagnostic investigations are inherently complex, with each step influencing the accuracy and the reliability of the final results. No method is entirely free from some limitations. Quantitative PCR (qPCR) remains the cornerstone for sensitive detection and identification of specific viral strains; however, it is typically designed for known targets and may fail to detect unexpected or novel pathogens. Viral isolation, while labor-intensive, is indispensable for comprehensive characterization and becomes essential when downstream applications, i.e., kinetic studies or vaccine development, are anticipated. Identifying emerging pathogens remains a formidable challenge in both human and veterinary medicine. Their rarity often coincides with the absence of specific diagnostic tools, delaying early detection and containment. Conventional methods, serology, and PCR are optimized for known pathogens and may fail to detect novel or low-abundance agents [22].

Although many viruses exhibit characteristic CPE in cell culture, these features are not always distinct; for example, porcine circovirus 2 and porcine parvoviruses can produce similar CPE [23]. The similar CPE of the different viruses may “camouflage” the presence of the unexpected one, like BATV in this case. The incidental discovery of BATV through NGS demonstrates its utility in uncovering hidden viral threats and highlights the contribution of non-targeted NGS methods to the alertness/preparedness to emerging pathogens in routinely monitored sample matrices. To avoid downstream complications, target-independent diagnostic platforms, such as NGS, are useful for integration into standard veterinary and public health workflows. These approaches enable detection of both known and novel pathogens, even in complex or low-titer samples [22,24].

Our results raise important questions regarding the prevalence of BATV in livestock and its potential spillovers to humans. The apparent underestimation of BATV prevalence is likely driven by the scarcity of systematic monitoring efforts and the limited etiological investigation of mild and rapidly resolving cases in both veterinary and human settings. Given the ecological overlap among cattle, mosquitoes, and humans—particularly in rural agroecosystems—enhanced surveillance efforts and integrated One Health approaches will be essential for assessing and mitigating future risks.

5. Conclusions

The unexpected detection of BATV in a Hungarian cattle herd, along with the accompanying genome sequence, provides new insights into the geographic distribution and genetic diversity of this emerging virus in Europe. The differing prevalence between age cohorts further raises that warrant additional investigation.

These findings should prompt veterinary and public health stakeholders to carry out the following:

- Increase awareness of BATV and related arboviruses.
- Expand surveillance programs in livestock and mosquito populations.
- Investigate potential human infections, particularly among vulnerable groups.
- Integrate NGS-based workflows into routine diagnostics to enhance early detection and response capacity.

Moreover, this study highlights certain limitations of commonly used laboratory techniques, demonstrating that targeted characterization can yield unexpected yet important discoveries beyond the original diagnostic objectives. Overall, our results reinforce the need for improved diagnostic vigilance and strengthened interdisciplinary collaboration within a One Health framework.

Author Contributions: Conceptualization, Z.G.H. and I.K.; methodology, Z.G.H. and R.V.-K.; data curation, Z.G.H. and R.V.-K.; writing—original draft preparation, I.K.; writing—review and editing, I.K., Z.G.H. and R.V.-K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The L, M and S segment sequences of the BATV were determined in lengths of 6870 bp, 4440 bp, and 943 bp, respectively, and uploaded to NCBI Genbank with Accession Numbers PV956151-3.

Acknowledgments: The authors are grateful to Attila Dobos, Viktória Sebestyén, Edit Fodor and Eszter Vida, and Dóra Vargáné-Terebes and Andrea Katona, for organizing sample collection, RT-qPCR, virus isolation, and NGS, respectively. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of Interest: The authors were employed by Ceva-Phylaxia Ltd. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

BVDV	Bovine viral diarrhea virus
BATV	Batai virus
NGS	Next-generation sequencing
CPE	Cytopathic effect

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