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Detection of Shamonda Virus EU1 Variant in Diseased Cattle, Greece, 2026

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Short Report

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Abstract

Shamonda virus (SHAV) is an emerging orthobunyavirus associated with ruminants. Recent detection in Europe indicates an expanding geographical distribution. This work reports the first detection of SHAV from cattle in Greece. The dairy cattle farm exhibited clinical signs compatible with an infectious disease; diarrhea, fever, a marked reduction in milk production and reproductive problems, including return to estrus and abortions. Six days after the onset of symptoms, whole blood samples were collected from 14 lactating cows with diarrhea and fever. In eight of them, SHAV RNA was detected (Ct range 25–35) by a commercial RT-qPCR kit (ID Gene™ SHLV Duplex, Innovative Diagnostics, Grabels, France). Further investigation by conventional RT-PCR, sequencing and phylogenetic analysis of partial sequences of the S, M and L viral segments revealed that the strain belongs to the newly detected in various European countries SHAV-EU1 variant. Further investigations are needed to evaluate the distribution and impact of this emerging vector-borne pathogen in livestock production.

Introduction

Shamonda virus (SHAV) is an arthropod-borne virus belonging to the genus *Orthobunyavirus*, family *Peribunyaviridae*, and the Simbu serogroup. SHAV possesses a tripartite, negative-sense, single-stranded RNA genome comprising large (L), medium (M), and small (S) segments, encoding the viral RNA-dependent RNA polymerase, envelope glycoproteins, and nucleocapsid protein, respectively. The segmented genome facilitates genetic reassortment among related orthobunyaviruses, potentially contributing to viral diversity and emergence (Coupeau et al. 2019; O'Connor et al. 2024).

SHAV was first isolated from cattle in Nigeria in 1965 and was subsequently identified in cattle and *Culicoides* biting midges in Japan. The veterinary significance of SHAV is primarily associated with infection in ruminants, particularly cattle. *Culicoides* biting midges are considered the most likely vectors (Cappa et al. 2026; Yanase et al. 2005; Molini et al. 2026a).

In June 2026, a Shamonda-like virus was identified in dairy cattle in Europe, constituting the first recognized emergence of this virus in the region. Affected herds showed acute clinical signs, such as fever, diarrhea, lethargy, and reduction in milk production. Molecular investigation and genomic sequencing confirmed the circulation of SHAV-related viruses in several European countries, including Germany, France, Switzerland, and Italy (Molini et al. 2026b; Kelleci et al. 2026; Ceglie et al. 2026; Wernike, Hoffmann, et al. 2026). Subsequent genomic investigations identified two distinct European variants, designated SHAV-EU1 and SHAV-EU2, highlighting the importance of continued molecular surveillance (Molini et al. 2026b; Wernike, Pfaff, et al. 2026). More recently, in October 2026, the first documented human case of SHAV-associated disease was reported in France (Bonnet et al. 2026) in a patient with a pre-existing severe illness. However, the implications for human health remain uncertain. In this context, we report the first detection of SHAV from cattle in Greece, providing additional evidence of its circulation in European livestock populations and contributing to the growing knowledge of this emerging veterinary pathogen.

Materials and Methods

In a livestock-intensive area of Central Macedonia, Northern Greece, characterized predominantly by dairy cattle and beef-fattening operations, local veterinarians and farmers began reporting cattle exhibiting fever, diarrhea, and a marked reduction in milk production. Given the current epidemiological situation in Greece, particularly the circulation of bluetongue virus (BTV) serotype 8, BTV was initially considered the primary differential diagnosis. Nutritional issues were also considered, especially as the onset of clinical signs coincided with the seasonal introduction of

newly harvested corn silage into cattle diets. Samples collected from cattle on affected farms in the area had been submitted to private diagnostic laboratories, with negative results for BTV. Following discussions between local veterinary practitioners and members of the study team, the symptomatology raised a strong clinical and epidemiological suspicion of SHAV circulation in the area. This prompted further investigation into the potential involvement of SHAV in the observed cases.

In the specific cattle farm, animals exhibited clinical signs compatible with an infectious disease. The farm experienced a pronounced clinical episode, with diarrhea affecting multiple age groups, a marked reduction in milk production and reproductive disturbances, including abortions in adult cows. Detailed clinical and epidemiological data for the farm is provided in Table 1. Samples submitted to a private diagnostic laboratory were negative for BTV. A total of 14 whole blood samples in EDTA tubes were collected from the same number of lactating cattle exhibiting clinical signs (diarrhea and fever). The samples were sent immediately to the laboratory in cold chain for investigation.

Table 1
Clinical and epidemiological characteristics of cattle farm with SHAV-positive samples in Greece, 2026

Characteristic	Description
Location	Central Macedonia Region, Greece
Herd size	Approximately 250 cattle, including 110 lactating cows
Estimated date of symptom onset at herd level	7 October 2026
Clinical presentation	Diarrhoea, fever, anorexia, marked reduction in milk production, and reproductive disturbances (return to estrus, abortions)
Affected animals and spread	Approximately all lactated cows became affected over a period of 15 days. 20–25% of the rest of the animals (young calves, and heifers) became affected
Milk production impact	Milk production decreased by approximately 30–40% during symptoms
Reproductive effects	Increased returns to oestrus (suggestive of early embryonic losses, mainly in heifers) and eight abortions over 15 days at different gestational stages (approximately 60–230 days) in adult cows
Sampling date	13 October 2026
Sampled animals	Fourteen lactating cows with diarrhea and fever
Herd clinical status at sampling	Sampling was conducted during the clinical episode from animals with clinical signs
Recovery / resolution	Twenty days after the onset at herd level, milk production recovered to approximately the previous level
Recent animal introductions	The last purchase of animals from Germany was approximately one year before the outbreak; the area was described as one where animal imports from abroad are common

On arrival, nucleic acids were extracted from 100 µl of whole blood with the KingFisher Flex Automated System and MagMAX CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions for the Whole Blood Workflow. Extracts were immediately investigated using the commercial ID Gene™ SHLV Duplex real-time reverse transcription PCR (RT-qPCR) assay (Innovative Diagnostics, Grabels, France), which detects SHAV-EU1 and SHAV-EU2 variants, on a QuantStudio 5 qPCR system (Thermo Fisher

Scientific, Waltham, MA, USA). The assay detects the SHAV target in the FAM channel and an endogenous non-target control (NTPCen) in the Cy5 channel.

In a second step of investigation, viral RNA was extracted from 140µl whole blood of the RT-qPCR positive samples with the highest yield according to Ct value, using the QIAmp Viral RNA Mini kit (Qiagen, Hilden, Germany) following the manufacturers' instructions; the extracts were used in conventional reverse transcription polymerase chain reaction (RT-PCR) assays, with previously published primers specific for the S, M, and L segments of the Simbu serogroup which were already available to the laboratory (Walt et al. 2023). All materials used and conditions applied followed the exact authors instructions (see Table S1 of supplementary file by Walt et al.). PCR products were obtained only for the hemi-nested M segment. The partial M segment amplicons were purified with the PureLink® Quick Gel Extraction Kit (Thermo Fisher Scientific, Waltham, USA) and sequenced as referred below in this section; as sequencing results demonstrated the suspected presence of SHAV EU variant, specific primers for the S and L segments were designed using Primer-BLAST and alignments of representative EU1 and EU2 variant sequences available at NCBI GenBank (PZ797893.1, OZ516536.1, PZ831165.1, OZ541483.1, OZ541493.1, OZ541433.1). The primers used for obtaining the partial sequences of the S, M and L segments in this investigation are shown in Table 2. Regarding the S segment, two overlapping primer sets were designed to cover a larger genomic area.

Table 2
Primers used to obtain PCR products for sequencing in current study

Segment	PCR round	Oligo name	Product size	Oligo sequence (5'-3')	Position	Reference
S1	-	S1-F19	503	GAGGTCGGGTATGTGGCATT	19–521	This study
		S1-R521		ATTGTTCCATAGCGTTGGCG		
S2	-	S2-F344	450	ACATGCAAGGCTAGTGTCCT	344–793	This study
		S2-R793		TGCAGTATAATGGTGTGCAATACA		
M	1st	Simbu M2671+	807	CATCMAGATATWGAAWMHTWYATAKCAG	2671–3476	Walt. 2023
		Simbu M3476-		GCATGRCAWAYATAATCAAATYKNGG		
	hemi-nested	Simbu M2671+	421	CATCMAGATATWGAAWMHTWYATAKCAG	2671–3110	
		Simbu M3110-		CCCCATTCTTCRCARCCC		
L	-	L-F411	741	TGCTGACCCTTTGCGAGATAC	411–1151	This study
		L-R1151		ATTCTCTTTGATGTCTGTCTTGCC		

For the S and L segment RT-PCR assays, Superscript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Thermo Fisher Scientific, Waltham, USA) was used following manufacturer instructions; 25 µL of 2X Reaction mix, 1 µL of the primers (10 µM), 16 µL of nuclease-free water, 2 µL of the SuperScript III/Platinum Taq mix, and 5 µL of RNA to a final volume of 50 µL. RT-PCR reaction conditions were as follows: 55°C for 30 min, 94°C for 2 min, 40 cycles of 94°C for 15 s, 54°C for 30 s, and 68°C for 60 s followed by a final extension of 68°C for 5 min and hold step at 4°C. The PCR products were visualized in a 1.5% agarose gel at 90 V for 70 min using GelRed® Nucleic Acid Gel Stain. All

S and L segments PCR products were purified using the PureLink PCR Purification Kit (Thermo Fisher Scientific / Invitrogen, Waltham, USA).

All obtained PCR products for the three viral segments were bidirectionally sequenced using the fluorescent BigDye Terminator Cycle sequencing kit v3.1 (Thermo Fisher Scientific / Applied Biosystems, Waltham, USA), followed by fragment separation with a 3730xl DNA Analyzer (Thermo Fisher Scientific / Applied Biosystems, Waltham, USA).

To identify homologous sequences, a BLASTN search was performed against the NCBI nucleotide database using default parameters (Camacho et al. 2009). Alignments, assembly of the overlapping S segment sequences and Maximum-likelihood phylogenetic analyses using the Tamura-Nei model were performed separately for the three partial segments using MEGA12 (Kumar et al. 2024). Branch support was assessed using 1,000 slow bootstrap replicates. All sequences were downloaded from NCBI GenBank.

Results

A total of eight out of the 14 samples were found positive with the commercial RT-qPCR kit for the presence of SHAV (Ct range 25–35). All positive, negative, and internal controls included in the commercial kit were used and performed as expected according to the manufacturers' protocols and guidelines. The three RT-qPCR positive samples with the highest yield (Ct values of 25, 28 and 30) were further investigated by RT-PCR as referred in the Materials and Methods section. All obtained partial sequences of S, M and L segments were identical in the three investigated samples and submitted on GenBank under Accession No QB336137, QB336138 and QB336139 for the partial S, M and L segments respectively. The obtained S and M partial sequences were of 708 bp and 415 bp size respectively, presenting 99.9–100% and similarity with recently SHAV-EU1 variants detected in Europe in cattle; the partial S segment had the similar to other cattle SHAV-EU1 strains mutation (S67G) compared to the isolate reported in a fatal encephalitis human case in France recently (Bonnet et al. 2026). The partial sequence of the L segment, 718 bp in size, showed 99.6–99.7% similarity with the recently detected EU1 variants, with two to three single nt polymorphisms (SNPs). Phylogenetic analysis of the partial sequences of all three segments demonstrate that the genome of the SHAV-GRE2026 strain belongs to SHAV-EU1 clade with no evidence of reassortment compared to the strains isolated from animals and the human case (Fig. 1).

Discussion

The detection of SHAV-EU1 variant from dairy cattle with characteristic clinical signs in Greece provides growing evidence of extended SHAV circulation in Europe. This finding highlights the need for further investigation to define the virus's geographical distribution and genetic diversity, as well as continued surveillance of cattle herds to better understand its epidemiology and significance for animal health.

The infected cattle farm was sampled (whole blood) six days after the reported onset of clinical signs from animals showing fever and diarrhea. Similar investigations by whole blood from cattle farms in the area following reports of farmers for similar clinical signs delivered negative results; in these cases samples were collected approximately 15–20 days after the onset of the symptoms. This may be consistent with declining viral RNA levels during the later stages of infection. Previous studies of the closely related Schmallenberg virus (SBV) have demonstrated a relatively short period of detectable viraemia in cattle, highlighting the importance of sampling during the acute phase (Wernike et al. 2012; Hoffmann et al. 2012).

The route of the virus introduction into the country remains unclear, as no recent animal introductions were reported in the farm. However, the widespread distribution of *Culicoides* biting midges and the prolonged period of climatic

suitability for vector activity may facilitate viral spread (Molini et al. 2026a) from other, potentially unidentified sites. Furthermore, limited awareness of SHAV and its non-specific clinical symptoms may have contributed to previously unrecognized infections in cattle herds.

The findings of this study should be interpreted with caution, in the context of the limited number of samples examined, which does not allow conclusions to be drawn regarding the prevalence or wider distribution of SHAV in Greece. Apart from BTV, animals were not investigated for the presence of other pathogens, thus clinical signs should not be attributed only to SHAV, even though the symptomatology is similar to the one reported from other SHAV infected cattle farms in Europe.

In conclusion, the detection of SHAV from cattle in Greece provides further evidence of its presence in Europe and emerges concerns for a widespread distribution in cattle herds. In this regard, awareness of SHAV infections should be increased among veterinarians, and the virus should be considered in the differential diagnosis of compatible clinical symptoms in cattle, particularly in areas where its circulation has been documented. Continued surveillance will be important to improve recognition of the infection, understand its significance for animal health and support preparedness for emerging diseases within a One Health framework.

Declarations

Ethical statement

Samples were collected as part of routine veterinary diagnostic investigations. Therefore, an ethical permit was not needed.

Conflict of Interest Statement:

The authors declare that they have no relevant financial or non-financial interests to disclose.

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Author Contribution

G.V., I.G., E.M. and V.M. conceptualized this work. G.V. and A.V. defined the methodology. A.V., A.M., K.K., D.K., A.M. and G.A. performed the formal analysis and investigation. M.L. and I.G. performed the original draft preparation. Writing - review and editing: G.V., V.M. and E.M. performed the review and editing. G.V. supervised the work.

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This manuscript has been submitted for pre-print to bioRxiv.

Data Availability

Sequences data were submitted to NCBI Genbank, as reported in the manuscript. All data supporting the findings of this study are available within the paper .

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Figures

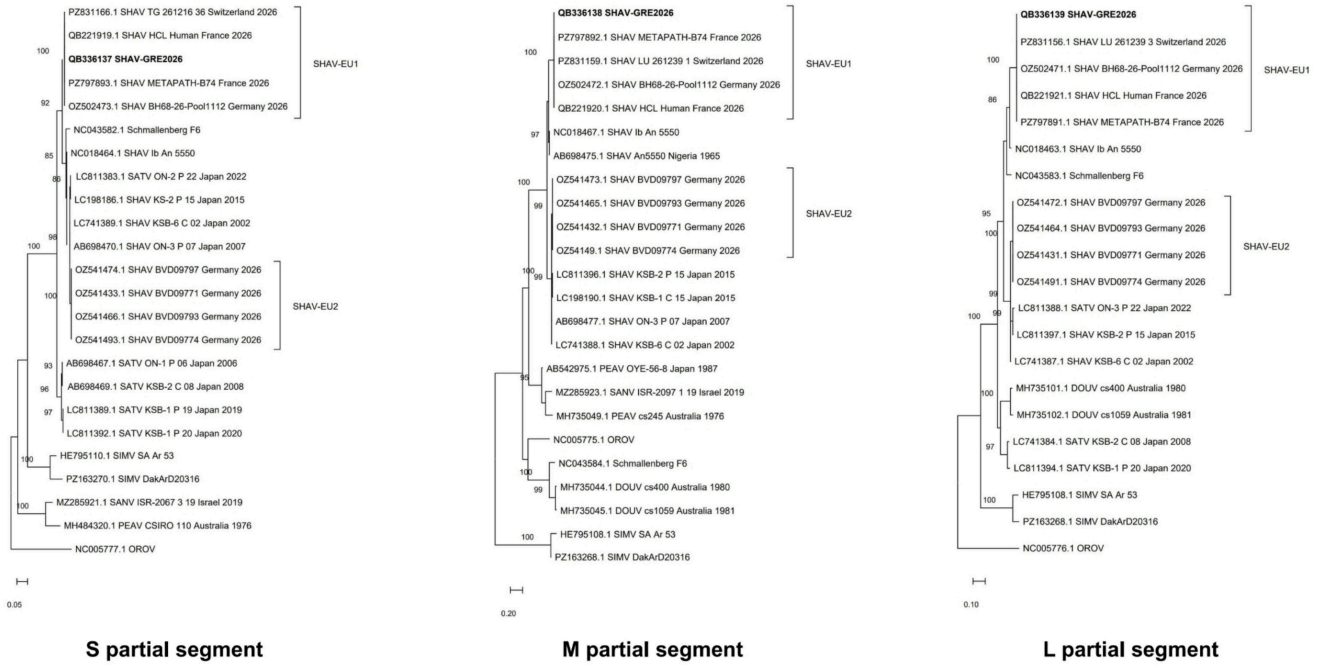


Figure 1

Maximum Likelihood Phylogenetic Trees of the L, M, and S partial genome segments of Shamonda virus EU1 variant detected in a cattle farm, Greece, 2026 and representative Orthobunyaviruses. Only bootstrap values ≥ 80% are shown. Scale bars are in substitutions per site, one per panel. SHAV strain of this study is in bold.