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Brief Report

Detection of Shamonda Virus RNA in Blood and Milk from Water Buffaloes in Southern Italy

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Simple Summary

Shamonda virus, a virus transmitted mainly by biting midges, was detected in European livestock in 2026. Its effects in water buffaloes are not yet well understood. We investigated four dairy buffalo herds in southern Italy after reports of decreased milk production. Blood was collected from 15 buffaloes on two farms and milk from eight different buffaloes on two other farms. Viral genetic material was found in 10 blood samples and all eight milk samples. Three viruses analyzed genetically belonged to the European group known as SHAV-EU1, which was also reported in cattle elsewhere in Europe. The detection of viral genetic material in milk does not establish that infectious virus was present or that infection can be transmitted through milk. The animals were selected because they showed clinical problems, and the study cannot demonstrate that the virus caused the reported decrease in milk production. Further studies are needed to understand the disease in buffaloes and the significance of the findings in milk.

Abstract

Shamonda virus (SHAV), an emerging orthobunyavirus detected in Europe in 2026, was investigated in dairy water buffaloes (*Bubalus bubalis*) following reports of reduced milk production in Campania, southern Italy. Twenty-three clinically affected lactating buffaloes from four farms were sampled in September 2026. EDTA blood samples were collected from 15 animals on two farms and individual milk samples from eight different animals on two other farms. Samples underwent SHAV RT-qPCR testing, together with molecular testing for Schmallenberg, bluetongue and epizootic hemorrhagic disease viruses. SHAV RNA was detected in 10/15 blood samples and 8/8 milk samples; the other viruses were not detected. Three blood samples from one farm were selected for genomic analysis. Phylogenies of the L, M, and S segments placed the resulting sequences within the SHAV-EU1 clade, alongside European cattle strains detected in 2026. Detection of viral RNA in milk establishes neither infectious virus nor mammary replication and does not demonstrate milk-borne transmission. The small, clinically selected sample and unpaired matrices preclude prevalence estimates, direct matrix comparisons, and causal attribution of reduced milk yield. These observations support targeted SHAV surveillance in water buffaloes.

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1. Introduction

Shamonda virus (SHAV) belongs to the Simbu serogroup of the genus *Orthobunyavirus* (family *Peribunyaviridae*). Like several related orthobunyaviruses, it is considered to be transmitted mainly by *Culicoides* biting midges. SHAV was originally isolated from cattle in Nigeria in 1965 and was subsequently recognized in other African settings and in Japan. Reports of congenital abnormalities associated with infection in Japanese calves and molecular detection in an aborted goat fetus in South Africa drew attention to its possible significance for animal health [1–3].

During the summer of 2026, genetically related SHAV variants were recognized in European cattle, initially in association with fever, diarrhea and reductions in milk production [4]. Subsequent investigations distinguished two genetic groups, designated SHAV Europe 1 (SHAV-EU1) and SHAV Europe 2 (SHAV-EU2), with differences documented across the three genome segments [5,6]. The existence of more than one circulating genetic group is relevant to diagnostic assay selection and to interpretation of sequence data. The European observations also renewed interest in the range of domestic hosts susceptible to SHAV and in the extent to which clinical manifestations differ between species and husbandry systems.

A recent German preprint reported SHAV infection in sheep and goats [7]. In northern Italy, molecular investigation of dairy cattle farms in Trento and Bolzano detected SHAV-EU1 in blood or serum samples from 12 of 28 investigated holdings [8].

Water buffalo husbandry is central to dairy production in Campania. The production system supports the Mozzarella di Bufala Campana protected designation of origin supply chain, making even short periods of poor production potentially important for farm management and veterinary follow-up [9]. Nevertheless, milk yield can decline for many infectious, nutritional, metabolic, environmental and management-related reasons. The observation of reduced production in herds in which SHAV is detected therefore requires cautious interpretation, particularly when quantitative individual records or systematic differential diagnostic investigations are not available.

The objective of this Brief Report is to describe SHAV RNA detection in blood and individual milk specimens obtained during a targeted field investigation in southern Italy, provide sample- and farm-level findings, and characterize the available viral genomes using separate phylogenetic analyses of the three segments.

2. Materials and Methods

2.1. Field Investigation and Sampling

In September 2026, official veterinarians investigated reports of decreased milk yield in dairy water buffalo herds located in Capaccio and Eboli, Salerno province, Campania. Diarrhea and nasal discharge had also been observed in some animals. Between 21 and 23 September 2026, 23 lactating buffaloes from four herds were sampled following a targeted, clinically prompted selection rather than a random survey. According to the field information supplied for this revision, all sampled animals had reduced milk production and inappetence at the time of sampling. No apparently healthy animals were included in the analyzed sample set.

Five EDTA blood samples were collected at CAPACCIO-A and ten at CAPACCIO-C. Individual milk samples were collected from five different animals at CAPACCIO-B

and three at EBOLI-A. No animal contributed both blood and milk: the two matrices were collected from distinct groups on separate farms according to the field sampling arrangements. Farm identifiers have been anonymized. Sampling was carried out for diagnostic investigation; it was not prospectively designed to compare specimen types, and no paired collection was performed.

Approximate farm-level milk-production information was supplied retrospectively for three herds. EBOLI-A reportedly experienced a decrease of 10–20% over approximately ten days; CAPACCIO-C reportedly showed a reduction of around 19% over 7–10 days; and CAPACCIO-B reported an accumulated loss of approximately 700 kg over about ten days. These are farm-level estimates rather than individual-animal production measurements; they were not collected against a uniform baseline. Comparable quantitative data were unavailable for CAPACCIO-A.

The available field records did not permit a consistent assessment of the onset or duration of illness for each buffalo, time to recovery, within-herd morbidity, or mortality. The selection of symptomatic lactating animals was useful for a focused diagnostic investigation but cannot support representative prevalence estimates or an analysis of differences between clinically affected and unaffected animals.

2.2. Nucleic Acid Extraction and RT-qPCR

Nucleic acids were extracted with the KingFisher Flex Purification System and MagMAX CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions. Raw milk was subjected directly to nucleic acid isolation without pre-treatment. Blood specimens were processed as EDTA-anticoagulated samples. SHAV RNA was investigated using the commercial ID Gene SHLV Duplex real-time reverse transcription PCR (RT-qPCR) assay (Innovative Diagnostics, Grabels, France; catalogue no. IDSHLV-100), designed to detect SHAV-EU1 and SHAV-EU2 RNA. [Manufacturer catalogue numbers to be inserted following verification of laboratory records.]

The duplex assay includes a SHAV target detected in the FAM channel and an endogenous non-target positive control (NTPCen), detected in the Cy5 channel. The endogenous signal serves as a sample-quality, extraction-process, and potential amplification-inhibition control. A run was regarded as valid only when the positive amplification control (PAC-SHLV) produced the expected signals, and the negative extraction (NEC-process) and negative amplification (NAC) controls remained negative in both channels. In a tested specimen, a typical FAM amplification signal indicated SHAV RNA detection, irrespective of whether the Cy5 signal was present. FAM-negative samples were interpretable as SHAV not detected only when the Cy5 endogenous control was positive. Double-negative specimens required repeat extraction and/or amplification because of possible poor sample quality or inhibition.

For the sample-level table, an undetected reaction recorded at the assay reporting limit was presented as "Not detected" rather than as a positive Cq. A Cq threshold of 40 was used for categorization in the supplied laboratory table, and the 40.0 entries were classified as negative. RT-qPCR results for Schmallenberg virus [10], bluetongue virus and epizootic hemorrhagic disease virus [11] were also available for the collected specimens. The latter testing covered the listed viral targets only; bacterial, parasitic, metabolic and other viral causes of the reported clinical abnormalities were not systematically investigated in this sample set.

2.3. Sequencing and Consensus Generation

Three EDTA blood samples with the lowest SHAV Cq values (21.2, 22.6 and 24.7), all from CAPACCIO-C, were selected for sequencing. Libraries were prepared using a

sequence-independent single-primer amplification (SISPA) approach in combination with the Illumina DNA Prep Kit (Illumina Inc., San Diego, CA, USA) [12]. Sequencing was performed with an Illumina NextSeq 1000 instrument and NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (300 cycles) to generate 150-bp paired-end reads. Analyses were performed within the GENPAT workflow (https://genpat.izs.it/accessed on 8 October 2026).

Raw reads were subjected to adapter trimming and quality filtering using fastp (reported as v0.23.4) under default settings [13], and read quality was inspected with FastQC (reported as v0.11.5) [14]. The draft laboratory documentation also specifies host-read removal before downstream mapping, although the exact depletion reference and settings are not supplied. De novo assembly was carried out with SPAdes v3.13 [15]. The revised bioinformatic account reports reference mapping with Snippy 4.6.0, ivar 1.4.4, generation of nucleotide pileups with SAMtools v1.10 (maximum depth 1000 reads per position) [16,17], and extraction of a segment-specific consensus with iVar [18]. [Confirm software versions against run logs: Snippy v4.5.1 or v4.6.0; iVar v1.3 or v1.4.4.]

According to the laboratory response, consensus generation used a minimum base quality of Q20, a minimum depth of 1×, and a consensus-frequency setting of zero, under which the highest-frequency nucleotide was selected at each covered site. IUPAC ambiguity symbols were assigned if alternative nucleotides had equal support. Internal zero-coverage positions were represented by N, whereas uncovered ends were excluded from the reported sequence. Because a minimum depth of 1× is permissive, coverage statistics are provided for each segment, and the resulting partial consensus sequences should not be interpreted as complete genomes without gaps.

2.4. Segment-Specific Phylogenetic Analysis

The three SHAV genome segments were analyzed independently using the GENPAT/Nextstrain Augur workflow [19]. Available GenBank SHAV reference sequences were screened for at least 80% coverage relative to the corresponding RefSeq segment (L: NC_018463.1; M: NC_018467.1; S: NC_018464.1). The final analyses included three buffalo-derived sequences and 21, 26, and 31 external references for the L, M, and S segments, respectively, corresponding to 24, 29, and 34 sequences in the three datasets.

Multiple alignments were generated with MAFFT v7.526 using the FFT-NS-2 algorithm against the segment-specific RefSeq references, with columns absent from the reference removed [20]. The reported alignment lengths were 6863 nt (L), 4314 nt (M), and 927 nt (S). No additional sites were masked, and no sequences were excluded after the stated reference-selection step. Maximum-likelihood phylogenetic trees were generated with IQ-TREE (reported as v3.1.2) using the ModelFinder Plus procedure and Bayesian Information Criterion for model selection [21,22]. The selected models were TN + F + G4 for L and M, and HKY + I for S. Branch support was assessed using 1000 ultrafast bootstrap replicates with NNI optimization of bootstrap trees (–bnni) [23]. Trees were visualized in Auspice v2.53.0.

Independent segment trees were preferred over a concatenated "whole-genome" phylogeny because SHAV has a segmented genome and reassortment can lead to discordant evolutionary histories between segments [4,5]. Segment-by-segment inference also makes differences in sequence completeness and phylogenetic resolution explicit. The three segment trees were therefore examined for the consistency of clade assignment, without inferring the geographical origin, precise transmission chains, or absence of reassortment from the limited sequences available here.

3. Results

3.1. Detection by Sample Type and Farm

SHAV RNA was detected in 10 of the 15 blood samples (66.7%) and in all eight individual milk samples (100%) examined. In the blood-positive subset, Cq values ranged from 21.2 to 37.4; in milk, they ranged from 26.7 to 36.0. The blood samples from CAPACCIO-A comprised three positive and two negative reactions; those from CAPACCIO-C comprised seven positive and three negative reactions. Milk samples from CAPACCIO-B (5/5) and EBOLI-A (3/3) were all positive (Table 1). These fractions describe targeted submissions and are not estimates of the prevalence in any farm, municipality, or region.

Table 1. Anonymized farm, specimen type and sample-level RT-qPCR results for SHAV RNA in 23 water buffaloes in Campania, Italy.

Row No.	Farm ID	Material	SHAV Cq/Interpretation
01	CAPACCIO-A	EDTA blood	Not detected
02	CAPACCIO-A	EDTA blood	31.1
03	CAPACCIO-A	EDTA blood	35.1
04	CAPACCIO-A	EDTA blood	Not detected
05	CAPACCIO-A	EDTA blood	36.1
06	CAPACCIO-B	Individual milk	29.9
07	CAPACCIO-B	Individual milk	30.7
08	CAPACCIO-B	Individual milk	30.5
09	CAPACCIO-B	Individual milk	30.6
10	CAPACCIO-B	Individual milk	30.5
11	EBOLI-A	Individual milk	32.3
12	EBOLI-A	Individual milk	26.7
13	EBOLI-A	Individual milk	36.0
14	CAPACCIO-C	EDTA blood	37.4
15	CAPACCIO-C	EDTA blood	21.2
16	CAPACCIO-C	EDTA blood	24.7
17	CAPACCIO-C	EDTA blood	37.1
18	CAPACCIO-C	EDTA blood	22.6
19	CAPACCIO-C	EDTA blood	26.1
20	CAPACCIO-C	EDTA blood	Not detected
21	CAPACCIO-C	EDTA blood	Not detected
22	CAPACCIO-C	EDTA blood	37.3
23	CAPACCIO-C	EDTA blood	Not detected

Values are rounded to one decimal place from the supplied individual results. Entries recorded as Cq 40.0 were designated not detected at the laboratory cut-off of 40. Sequential row numbers are for presentation only and are not original animal IDs. Each row represents a different animal; no blood/milk pairs were available.

At the individual-sample level, a wide range of Cq values was observed in blood, including specimens close to the detection threshold. The eight positive milk Cq values were all within the reported positive range. Because no paired milk and blood specimens were collected from the same buffaloes and matrices originated in different herds, these

observations do not establish whether one specimen type is more frequently positive or more diagnostically sensitive than the other.

SHAV testing was undertaken alongside molecular testing for Schmallenberg virus, bluetongue virus and epizootic hemorrhagic disease virus. None of the three additional viral targets were detected among the analyzed samples. No systematic screening data for bacterial, parasitic, metabolic or other viral differential diagnoses were available for this series. The observed production decline and concurrent clinical signs therefore cannot be specifically attributed to SHAV.

3.2. Genome Recovery and Phylogenetic Placement

The three blood specimens selected for sequencing came from CAPACCIO-C and had Cq values of 21.2, 22.6, and 24.7. Sequencing produced between 15,721,980 and 17,204,648 raw reads per specimen, with a reported mean Phred score of 38.16. Consensus sequences were recovered for L (horizontal coverage, 93.47–98.95%; reported average vertical coverage, 69.87–608×), M (horizontal coverage, 83.96–98.49%; average vertical coverage, 16.12–156.96×), and S (horizontal coverage, 70.65–89.10%; average vertical coverage, 4.62–41.96×). These partial-segment recovery figures should be considered when comparing sequence relationships.

Nine GenBank sequence accession identifiers are now reported for the three viruses and three segments: QB168409–QB168411, QB168412–QB168414 and QB168415–QB168417.

The L, M, and S segment phylogenies consistently placed the three buffalo-derived sequences within SHAV-EU1, alongside sequences obtained in cattle in France, Germany, and Switzerland during the 2026 European emergence (Figure 1A–C). The available data support clade assignment and broad genetic relatedness, but do not permit identification of a direct source herd, a specific introduction event, or farm-to-farm spread.

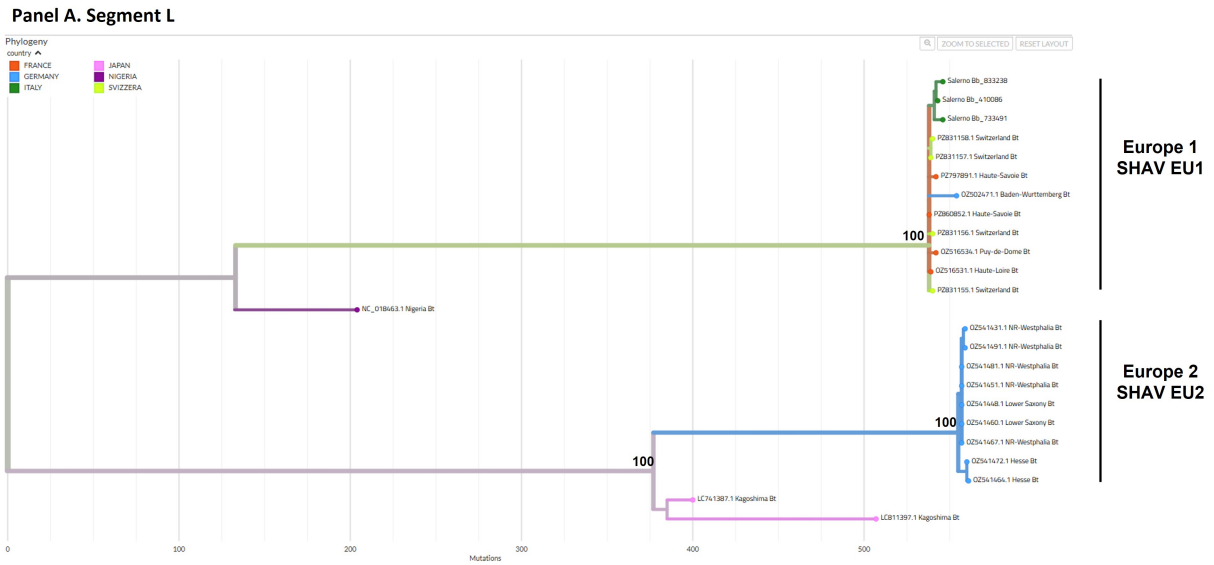


Figure 1. *Cont.*

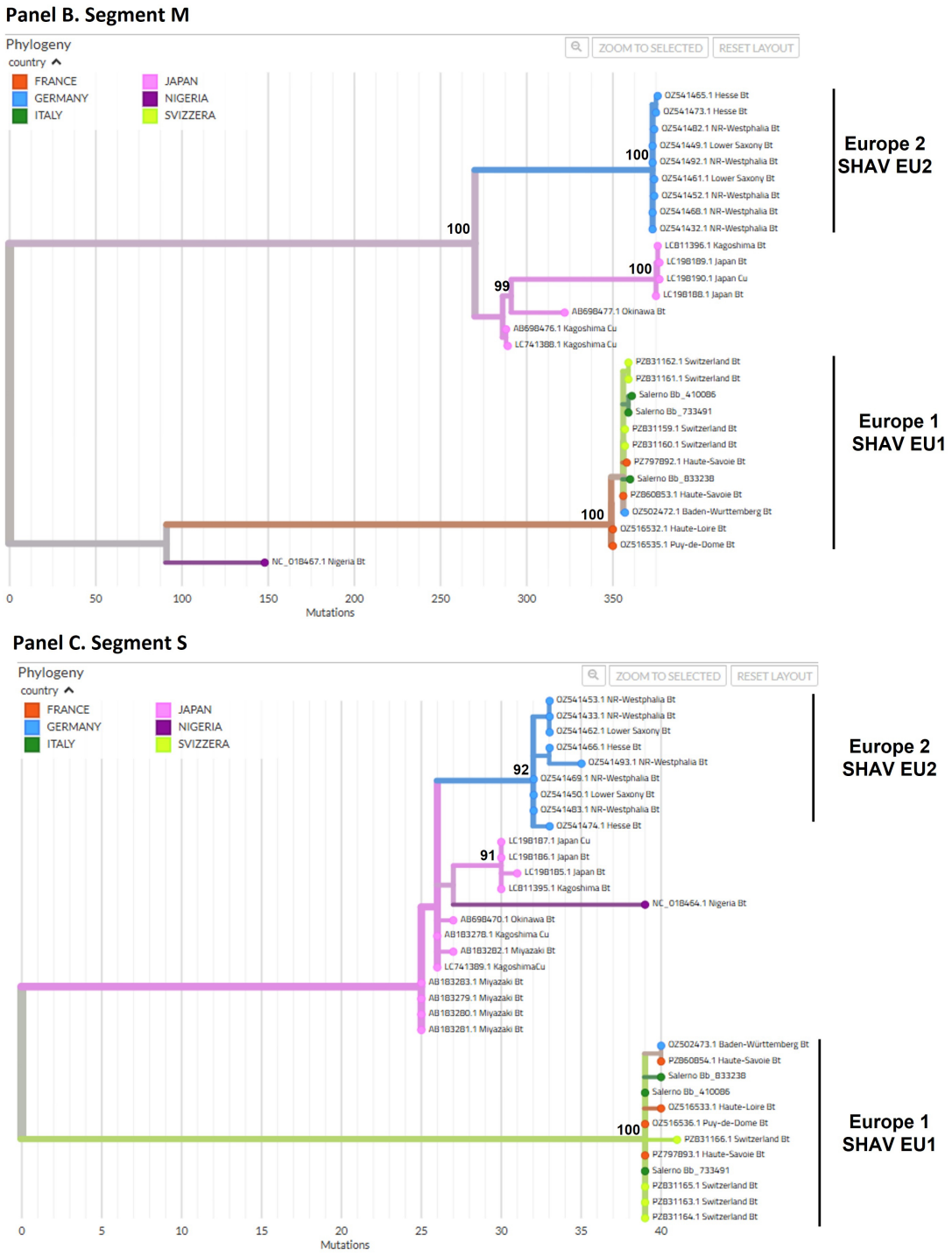


Figure 1. Maximum-likelihood phylogenetic analysis of the L (**A**), M (**B**), and S (**C**) genome segments of SHAV. The three SHAV sequences obtained from water buffaloes in Capaccio clustered within the SHAV-EU1 clade, alongside sequences previously detected in European cattle. Branch support is shown in the original trees. Bb, *Bubalus bubalis*; Bt, *Bos taurus*; Cu, *Culicoides*.

4. Discussion

This field investigation adds individual buffalo blood and milk data to the rapidly developing account of SHAV circulation in European domestic ruminants. The results are consistent with molecular detection of SHAV in the investigated animals and with SHAV-EU1 circulation in the region. The findings are of particular veterinary interest because

buffalo milk supports a specialized dairy production system in Campania, and information on the consequences of SHAV exposure in this species remains limited.

The Campania regional authorities subsequently reported SHAV detection in water buffaloes in Salerno province on 30 September 2026 [24]. These reports indicate that infections were being detected in different ruminant populations within a short period, although the available surveillance designs do not permit a direct comparison of prevalence between locations or species.

The high observed proportion of SHAV-positive specimens should not be interpreted without considering the sampling design. Animals were selected during an investigation of clinical concerns rather than at random; there were only four farms, all within a limited geographic area; and the sample matrices were distributed by farm. Consequently, the data cannot establish how widespread infection was within the investigated herds, identify whether the same patterns would be observed in asymptomatic buffaloes, or quantify population-level disease burden. Establishing these features would require systematic sampling of affected and unaffected animals across a wider selection of holdings.

Molecular identification of virus in a biological matrix should also be distinguished from evidence of infectious virus in that matrix. This distinction is particularly important when samples are milk, because the mere presence of viral RNA does not establish replication in the mammary gland, excretion of viable particles, or transmission to another host. Furthermore, comparison of positivity between milk and blood requires paired sampling and matrix-specific performance data. In their absence, findings from separately selected animals describe the investigated sample sets rather than the relative diagnostic sensitivity of the two matrices.

In this investigation, all eight RNA-positive milk samples originated from two selected herds, whereas the blood samples came from two other herds. Neither virus isolation nor mammary-tissue testing was performed, and the findings cannot be interpreted as evidence of a food-safety hazard. Prospective follow-up involving paired blood and milk collection, serial sampling, matrix-specific assay validation, and isolation attempts would help clarify the kinetics and biological significance of SHAV RNA detection in milk.

The genotyping results place the investigated buffalo-derived strains within the SHAV-EU1 clade in each of the three genomic segments. This is consistent with reports of SHAV-EU1 in cattle in several European countries and in northern Italy [4,5,8]. The recognition of a distinct SHAV-EU2 group in Europe underscores the value of genomic surveillance and interpretation at the segment level [5]. Because a segmented RNA-virus genome can undergo reassortment, phylogenetic relationships should be evaluated for each segment rather than inferred only from a concatenated alignment. The present segment trees did not identify an obvious change in clade assignment for the three buffalo samples, although they cannot rule out older reassortment events or fully resolve relationships with unsampled lineages.

The investigation was prompted by a reported decline in milk yield and accompanying clinical signs. Retrospective herd-level estimates indicate losses of different apparent magnitudes and durations, but they were not gathered systematically, could not be linked to the sampled animals, and lack a common comparator. A decline of a specified proportion over several days and an absolute quantity of milk lost over a similar interval are not directly comparable unless initial production and herd size are known. These figures should therefore be interpreted as background field observations rather than estimates of losses attributable to infection.

The clinical presentation was also nonspecific. Diarrhea, nasal discharge, inappetence and decreased production may accompany other infectious diseases and non-infectious conditions. Although RT-qPCR results for Schmallenberg, bluetongue and epizootic hemorrhagic disease viruses were negative, the investigated pathogen panel was limited. Other

relevant bacterial, parasitic, viral and metabolic causes were not investigated systematically. The detection of SHAV RNA therefore establishes molecular evidence in the submitted samples but cannot, on its own, prove that SHAV caused the observed clinical signs or production changes.

Future surveillance in buffalo herds could incorporate prospective individual clinical records, daily milk production before and after clinical onset, relevant laboratory differential diagnoses and concurrent entomological sampling. Paired blood and milk collection during the same visit, followed by repeated sampling at defined intervals, would allow more meaningful assessment of the detection window in each matrix. If sufficient material becomes available, isolation and infectious-virus assays would be necessary to move beyond RNA detection and address questions about virus viability. Wider genomic sampling could also clarify whether the same clade predominates across regions and production systems.

Study Limitations

This study comprised 23 clinically selected lactating buffaloes from four herds and did not include an unaffected comparator group or random sampling. Blood and milk were obtained from different animals on different farms; therefore, paired-matrix analyses and relative diagnostic-performance comparisons were not possible. Individual quantitative milk-production records, standardized severity scores, consistent follow-up and complete differential diagnostic investigations were unavailable. No virus isolation or mammary-tissue testing was performed. Genomic comparisons were based on three selected blood samples with incomplete segment coverage. Accordingly, neither the causal role of SHAV in clinical disease nor the infectivity or transmission potential of RNA-positive milk can be determined.

5. Conclusions

SHAV RNA was detected in individual blood and milk samples from dairy water buffaloes sampled in Campania in September 2026. Three buffalo-derived strains were assigned to SHAV-EU1 by independent analyses of the L, M and S genome segments. The presence of viral RNA in milk does not demonstrate infectious virus or milk-borne transmission. Targeted prospective studies incorporating paired specimens, comprehensive differential diagnoses, milk-production measurements and virus isolation are required to establish the clinical and biological significance of these findings.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study, including the sample-level data, are included in the article. The nucleotide sequences generated in this study have been submitted to NCBI GenBank under submission number QB168409–QB168411, QB168412–QB168414 and QB168415–QB168417. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

Cq	quantification cycle
EDTA	ethylenediaminetetraacetic acid
Hcov	horizontal coverage
NTPCen	endogenous non-target positive control
RT-qPCR	reverse transcription quantitative PCR
SHAV	Shamonda virus
Vcov	vertical coverage
WGS	whole-genome sequencing

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