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Prevalence and molecular study of FMD SAT2 serotype in Northern and Central Iraq

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ABSTRACT

In Iraq, foot-and-mouth disease (FMD) remains a significant challenge to most livestock production. Building upon our previous investigation, which reported overall FMDV prevalence and the first successful isolation of the SAT2 serotype in Iraqi cattle, the present study examines the prevalence of FMDV and provides molecular characterization of selected isolates. A total of 100 clinical samples collected from cattle across five provinces during the July 2023– October 2024 outbreak were analyzed. Using RT-qPCR, 85% (85/100) was positive for the 3D gene, and 35.3% (30/85) of these were positive for the SAT2-specific 1D gene, and there was no significant provincial differences observed in the detection of either gene. Vesicular fluid exhibited a 100% positivity rate for the 1D gene and was significantly higher than the 27.6% positivity in tongue epithelial tissue ($P < 0.0001$). Logistic regression analysis confirmed lower odds of detecting the 1D gene compared with the 3D gene across all provinces ($P < 0.05$). Three isolates were sequenced: one 3D-positive sample and two 1D-positive samples. Phylogenetic analysis of the 3D and 1D regions demonstrated clustering within SAT2, showing close relationships to strains circulating in Western Asia and Eastern Africa. This study provides the province-level overview of SAT2 distribution in Iraq and molecular evidence supporting the regional evolution of the circulating strain, emphasizing the need for continued surveillance and updated vaccination strategies.

Keywords: FMD, SAT2, 1D, sequencing, Iraq

INTRODUCTION

Foot-and-mouth disease (FMD) remains one of the most economically significant transboundary animal diseases affecting cloven-hoofed livestock worldwide (**1**). Its causative agent, foot-and-mouth disease virus (FMDV), is a highly contagious Aphthovirus of the family Picornaviridae, encompassing seven antigenically distinct serotypes (O, A, C, Asia1, SAT1, SAT2, and SAT3) (**2**). The virus possesses a positive-sense, single-stranded RNA genome of approximately 8.5 kb enclosed within an icosahedral capsid composed of the structural proteins VP1, VP2, VP3, and VP4. The capsid-coding region, particularly VP1, is of special epidemiological importance because it contains major antigenic determinants and exhibits substantial sequence variability, making it useful for serotype discrimination, phylogenetic analysis, and vaccine-related evaluation (**3**). The absence of cross-protection between serotypes complicates control programs, particularly in endemic regions where multiple serotypes co-circulate (**4**).

For decades, Iraq has had recurring FMD outbreaks and the serotypes O, A, and Asia1 were and still the dominating the epidemiological landscape. Several investigations in

different area of Iraq investigated different animal species, cattle, buffaloes, and even camels (**5**), using different diagnostic techniques have shown FMDV transmission throughout regions. A major epidemiological survey in Nineveh found a high frequency of FMDV and many risk factors for its spread (**6**). A systematic assessment of the national illness history revealed periodic large-scale outbreaks caused by unregulated animal movement, permeable borders, and low vaccine coverage (**7,8**). Molecular investigations in central and southern Iraq have confirmed the transmission of numerous serotypes and the appearance of new genetic variations in recent years (**9-12**).

Some studies from northern Iraq have also provided important epidemiological data related to FMD spreading. Studies from Sulaymaniyah revealed genetic variation among Asia virus 1 isolates, indicating significant differences from vaccine strains such as As1/Shamir/89, raising concerns about vaccine efficacy (**13**). Subsequent analyses identified additional viral strains and the introduction of new genotypes into the Kurdistan Region (**14-16**). Investigations in Duhok confirmed the presence of circulating serotypes O, A, and Asia1, highlighting the ongoing evolution of local strains (**15**). Meanwhile, serological studies in camels revealed continued transmission of serotypes A and O to non-cattle hosts, suggesting a broader environmental role in the spread of the virus throughout central Iraq (**5**).

Within this context the emergence of the serotype SAT2 in Iraq represents an important epidemiological development. While historically confined to sub-Saharan Africa, the serotype has been reported in several Middle Eastern countries recently, including Iraq (**17**). Local molecular evidence shows that there is an increasing detection of the SAT2 strain in cattle and buffalo, and a high degree of genetic similarity to regional isolates prevalent of the Middle East and East Africa (**10,11,18**). This may reflect a combination of cross-border animal movement, informal livestock trade in the region, and weak border controls, and insufficient vaccination coverage (**19,20**). The first successful isolation of the SAT2 strain from Iraqi cattle was recently documented, confirming the prevalence of this serotype in local herds (**16**), furthermore, an outbreak of the SAT2 strain has been reported in Muthanna, with further molecular analyses confirming ongoing viral evolution (**12**).

Given the recent emergence of the isolate ofSAT2 in Iraq and its spread across many regions, a molecular and epidemiological characterization is essential to understand its distribution and assess the potential impact of vaccination programs. This study constitutes the second part of two-part study conducted during the 2023–2024 outbreak. The first part documented the general spread, the detection of *3D* and *1D* genes of the FMD, in addition to the successful isolation of the FMD virus. This study expands on this effort by analyzing the spatial spread across five regions in Iraq and doing partial sequencing and phylogenetic analysis of selected strain to determine their genetic relationships with regional SAT2 strains.

MATERIALS AND METHODS

Ethical Approval

The experimental design and protocols used in this study were reviewed and approved by the local Animal Care and Use Committee of the College of Veterinary Medicine, University of Baghdad, Iraq, under Animal Utilization Protocol Certificate P.G./1322, dated July 11, 2024.

Sample Collection and RNA Extraction

Animal selection, sample collection (tongue epithelial tissue and vesicular fluid) from five Iraqi provinces (Diyala, Baghdad, Kirkuk, Babylon, and Salahaddin), sample preparation, and RNA extraction and quantification were performed as previously described (21). Briefly, a total of 100 samples were collected from suspected FMD cases between July 2023 and October 2024. RNA was extracted and quantified, yielding high-quality material suitable for downstream molecular analysis.

Detection of FMDV and SAT2 Serotype by Real-Time RT-PCR

The detection of general FMDV presence (targeting the *3D* gene) and the specific identification of the SAT2 serotype (targeting the *1D* gene) were carried out using one-step real-time RT-PCR (RT-qPCR) as detailed in our previous publication. The primer and probe sequences, reaction components, and thermal cycling conditions for the RT-qPCR assay were identical to those previously reported (**21**). This assay identified 85 samples positive for the general FMDV 3D gene, of which 30 were positive for the SAT2-specific *1D* gene.

Conventional RT-PCR for Sequencing

To generate amplicons for sequencing, conventional RT-PCR was conducted on all SAT2 (1D) RT-qPCR positive samples (n = 30) derived from the 3D-positive subset (n = 85).

Table 1. Primer sequences designed in the current study for conventional RT-PCR amplification of the FMDV *3D* and SAT2 *1D* regions

Gene Name	Primer	Sequence (5' → 3')
3D	Forward	CGT AGACAC TAT GAG GGA GT
	Reverse	CAA CGC AGG TAA AGT GAT CTG
1D	Forward	CCA CAC CAA CAA GAC CAC CT
	Reverse	CTG TCG TGC ATG GCC GCT GT

Two primer sets (**Table 1**) were created in current study with Primer3Plus, based on multiple sequence alignments of reference sequences obtained from the National Center for Biotechnology Information (NCBI) GenBank database. Sequences corresponding to serotypes SAT2, O, A, and Asia1 from various geographic origins (SAT2 [Ethiopia: MT602090.1, Uganda: HM067705.1], O [Pakistan: MH784405.1], A [Iran: MZ493234.1], and Asia1 [Turkey: KM268898.1]) were aligned to identify conserved primer-binding regions, and primers were designed to amplify a 428 bp fragment appropriate for sequencing. For

the SAT2 *1D* gene, representative SAT2 sequences from various countries (Ethiopia 22: OQ557397.1, Jordan: PP093362.1, Ethiopia-1991: AY343938.1, and Iraq: LC801596.1) were aligned to determine conserved primer-binding sites surrounding a variable region, and primers were designed to amplify a 798 bp fragment. All nucleotide sequences were provided by IDT DNA (USA).

The reactions were conducted using a one-step Reverse Transcriptase Enzyme Kit and RT-2x PCR Master Mix (abm, Canada). Each 20 µL reaction contained 4 µL RNA template (200 ng/µL), 10 µL RT-PCR Master Mix (1×), 0.4 µL RT-PCR Enzyme Mix (1×), 1 µL of each forward and reverse primer (50 picomoles), and 3.6 µL nuclease-free DEPC-treated water. Each conventional RT-PCR run included RNA from samples previously confirmed as positive by RT-qPCR for the corresponding target gene, in addition to nuclease-free water as a no-template negative control.

The thermal cycling conditions were as follows: cDNA synthesis at 42°C for 30 min; heat inactivation at 95°C for 5 min; 32 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 35 sec, and extension at 72°C for 35 sec; a final extension at 72°C for 7 min; and a final hold at 4°C.

The PCR products were analyzed on a 2% agarose gel stained with ethidium bromide (5 µL) aliquot of each product and was loaded alongside a 100 bp DNA ladder. Electrophoresis was run at 120V for 50 min in TAE buffer. DNA bands were visualized under UV light and documented.

Sequencing and Phylogenetic Analysis

Amplified products of the expected size for the 3D gene (one sample) and the 1D gene (two samples) were sent for sequencing to Macrogen Inc. (South Korea) using the Sanger method.

The obtained sequences were compared with the NCBI GenBank database using the BLASTn tool to confirm the identity of the isolates and the sequences were deposited in the GenBank database.

For phylogenetic analysis, related sequences were retrieved from GenBank. Multiple sequence alignments were performed using ClustalW within MEGA 12 software (22,23). Phylogenetic trees for 3D and 1D genes were constructed using the Neighbor-Joining method based on the p-distance model. The constructed trees included reference sequences representing the most prevalent FMDV serotypes (O, A, SAT 1, SAT2, SAT 3 and Asia 1) to evaluate the evolutionary discriminative capacity of each amplified region. The robustness of the tree topology was assessed by bootstrap analysis with 1000 replicates. The resulting trees were visualized and annotated using the Interactive Tree of Life (iTOL) platform (24).

Statistical Analysis

Statistical analyses were performed using JMP Pro 16.00 software (SAS Institute Inc., USA). Chi-square (χ^2) tests of independence were used to assess differences in gene detection prevalence across the five provinces and between the two tissue types. Fisher's exact test was employed when cell counts were small. Analysis of logistic regression was conducted to compare the odds of detecting the *1D* gene relative to the *3D* gene in each region, with odds ratios (ORs) and 95% confidence intervals (CIs) calculated. For all tests, A *P*-value of ≤ 0.05 was considered statistically significant.

RESULTS

Prevalence of FMDV and SAT2 Serotype

As mentioned in our initial study, screening of 100 samples via RT-qPCR detected the general FMDV *3D* gene in 85 samples (85.0%), and the SAT2-specific *1D* gene in 30 of these positive samples (35.3%). In this study, the detection of the 3D gene, confirming general FMDV infection, was consistently high across all regions, with no significant difference in prevalence (χ^2 = 0.825, *P* = 0.934). Prevalence ranged from 80% in Salahaddin to 90.0% in Babylon (**Table 2**). Similarly, there was no significant difference in 3D gene detection between tongue epithelial tissue (84.4%) and vesicular fluid (90%).

Table 2. Prevalence of the *3D* gene (general indicator of FMD) and*1D* gene (SAT2 serotype) by area and tissue type, with 95% confidence intervals (CIs) and chi-square (χ^2) test results

3D		Total samples	Positive count (%)	95% CIs	χ^2	*P*-value
Area	Babylon	10	9 (90.0)	59.6-98.2	0.825	0.934
	Baghdad	15	13 (86.7)	62.1-96.3		
	Diyala	35	29 (82.9)	67.3-91.9		
	Kirkuk	25	22 (88.0)	70.0-95.8		
	Salahaddin	15	12 (80.0)	54.8-92.9		
Tissue	Tongue Epithelial	90	76 (84.4)	75.6-90.5	0.218	0.6407
	Vesicular fluid	10	9 (90.0)	59.6-98.2		
1D						
Area	Babylon	9	2 (22.2)	6.32-54.7	1.202	0.878
	Baghdad	13	4 (30.8)	12.7-57.6		
	Diyala	29	11 (37.9)	22.7-55.9		
	Kirkuk	22	9 (40.9)	23.3-61.3		
	Salahaddin	12	4 (33.3)	13.8-60.9		
Tissue	Tongue Epithelial	76	21 (27.6)	18.8-38.6	18.45	<0.0001
	Vesicular fluid	9	9 (100)	70.1-100		

In contrast, the detection of the SAT2 serotype (*1D* gene) showed significant variation depending on the sample type. While there was no significant difference in SAT2 prevalence across the different provinces, a

significant difference (P<0.0001) was observed between tissue types. The *1D* gene was detected in 100% of the vesicular fluid samples (9/9), compared to only 27.6% of the tongue epithelial tissue samples (**Table 2**).

Table 3. Logistic Regression Analysis of *1D* Gene Detection Relative to *3D* Gene Across Regions, with Odds Ratios and 95% Confidence Intervals

Region	Odd Ratio	95% CIs	*P*-value
Babylon	0.0317	0.0024 – 0.4256	0.0092
Baghdad	0.0684	0.0102 – 0.4564	0.0056
Diyala	0.1264	0.0398 – 0.4015	0.0005
Kirkuk	0.0877	0.0202 – 0.3807	0.0012
Salahaddin	0.1250	0.0219 – 0.7149	0.0194

Logistic regression analysis showed that the odd of detecting the SAT2-specific 1D gene was significantly lower than the odd of detecting the general FMDV 3D gene across all five provinces (**Table 3).**

Confirmation by Conventional RT-PCR

To facilitate sequencing, conventional RT-PCR was successfully performed on selected samples, generating amplicons of the expected sizes: 428 bp for the *3D* gene and 798 bp for the *1D* gene. The gel electrophoresis results confirmed the specificity of the reaction, with clear bands in positive samples and the absence of bands in negative controls (**Figures 1 and 2**).

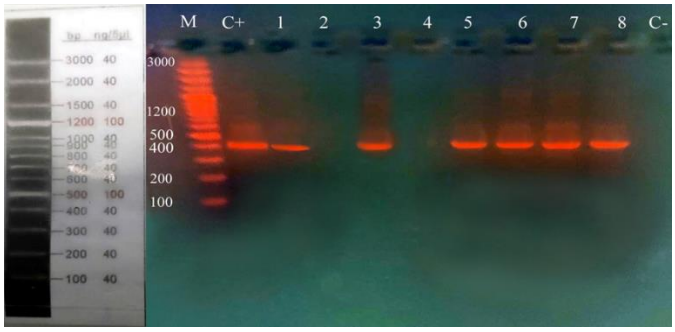

Figure 1. Agarose gel electrophoresis of conventional RT-PCR products for the FMDV *3D* gene (428 bp). Lane M: 3kb DNA ladder. Lane C-: Negative control. Lane C+: RNA from a previously RT-qPCR-confirmed positive field sample used as a positive amplification control. Lanes 1, 3, 5, 6, 7, 8: RT-PCR positive samples. Lanes 2 and 4: RT-PCR negative samples. All the samples were electrophoresed in 2% agarose in TAE buffer under 120V power supply for 50 min

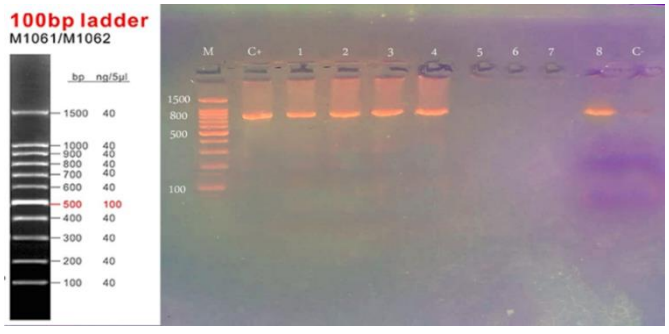

Figure 2. Agarose gel electrophoresis of conventional RT-PCR products for the SAT2-specific *1D* gene (798 bp). Lane M: 1500bp DNA ladder. Lane C-: Negative control. Lane C+: Positive control. Lanes 1, 2, 3, 4, 8: RT-PCR positive samples. Lanes 5, 6, 7: RT-PCR negative samples. All the samples were electrophoresed in 2% agarose in TAE buffer under 120V power supply for 50 min

Sequencing and phylogenetic analysis

The three FMDV isolates sequenced in this study were deposited in the NCBI GenBank database under accession numbers PQ037258.1 (*3D* gene), PQ337353.1 (*1D* gene), and PQ337354.1 (*1D* gene).

The phylogenetic tree constructed from the *3D* nucleotide sequence (PQ037258.1) placed the investigated Iraqi isolate within the SAT2 serotype clade (**Figure 3**). Of the 344 aligned nucleotide positions, 275 were conserved and 69 were variable, including 58 parsimony-informative sites and 11 singleton sites, indicating that most of the observed variation contributed to phylogenetic resolution. The Iraqi isolate demonstrated a close genetic relationship with sequences from Western Asia (Turkey, Iraq, Bahrain, Jordan) (**Table 4**). By contrast, Ethiopian SAT2 sequences clustered more distantly within the clade, reflecting their East African origin. Bootstrap analysis supported these relationships (SAT2 bootstrap = 72.6%). Unlike the 1D tree, the 3D phylogeny indicated that SAT1 was the closest lineage to SAT 2 (bootstrap = 71.7%), whereas SAT3 was the most distant (bootstrap = 99.9%).

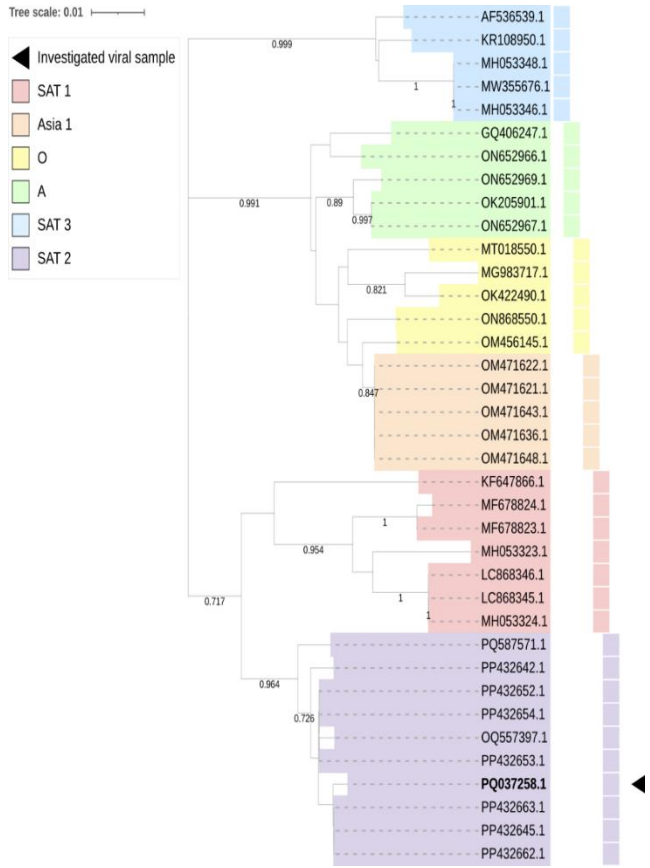

Figure 3. Neighbor-joining phylogenetic tree based on the 3D nucleotide sequence of FMDV. The tree was generated from 344 aligned sites using the p-distance model with 1000 bootstrap replicates. The investigated Iraqi isolate (PQ037258.1, indicated by a black arrow) grouped within the SAT2 serotype clade (purple), showing close relationships with reference strains from Turkey and Iraq (bootstrap = 72.6%). SAT1 appeared as the closest lineage to SAT2, whereas SAT3 was the most divergent. Each clade is represented by a distinct color. The scale bar indicates the number of substitutions per site

Table 4. List corresponding of reference viruses for *3D* gen for SAT2 serotype obtained from GeneBank used for phylogenetic analysis

GenBank Acc. no.	Serotype	Host	Country
PQ587571.1	SAT2	cattle	Ethiopia
PP432642.1	SAT2	cattle	Bahrain
PP432652.1	SAT2	cattle	Jordan
PP432654.1	SAT2	cattle	Jordan
OQ557397.1	SAT2	cattle	Ethiopia
PP432653.1	SAT2	cattle	Jordan
PQ037258.1	SAT2	cattle	Iraq
PP432663.1	SAT2	cattle	Turkey
PP432645.1	SAT2	water buffalo	Iraq
PP432662.1	SAT2	cattle	Turkey
MF678823.1	SAT1	cattle	Nigeria
MF678824.1	SAT1	cattle	Nigeria
KF647866.1	SAT1	cattle	Uganda
MH053324.1	SAT1	cattle	Uganda
LC868345.1	SAT1	cattle	Iraq
LC868346.1	SAT1	cattle	Iraq
OM471622.1	Asia1	buffalo	Pakistan
OM471621.1	Asia1	buffalo	Pakistan
OM471643.1	Asia1	buffalo	Pakistan
OM471636.1	Asia1	buffalo	Pakistan
MT018550.1	O	cattle	Palestine
MG983717.1	O	cattle	Nepal
OK422490.1	O	buffalo	India
ON868550.1	O	cattle	Viet Nam
OM456145.1	O	buffalo	Pakistan
GQ406247.1	A	cattle	Viet Nam
ON652966.1	A	cattle	Viet Nam
ON652969.1	A	cattle	Viet Nam
OK205901.1	A	buffalo	Viet Nam
ON652967.1	A	cattle	Viet Nam
AF536539.1	SAT3	-	-
KR108950.1	SAT3	African buffalo	South Africa
MH053348.1	SAT3	cattle	Zimbabwe
MW355676.1	SAT3	cattle	Zimbabwe
MH053346.1	SAT3	cattle	Zimbabwe

The phylogenetic tree based on the *1D* nucleotide sequences revealed that the investigated Iraqi isolates clustered within the SAT2 serotype clade with bootstrap at 100% (**Figure 4**). Both isolates formed a single monophyletic group together with reference sequences predominantly originating from Western Asia (Iraq, Turkey, Bahrain, Jordan) and East Africa (Ethiop) (**Table 5**). Alignment of 654 nucleotide positions identified 157 conserved sites, while 497 were variable, including 483 parsimony-informative sites and 14 singleton sites. This distribution indicates a strong phylogenetic signal, contributing to a reliable tree topology. The SAT3 serotype was identified as the closest lineage to SAT2 (bootstrap = 98.8%), whereas SAT1 was the most divergent (bootstrap = 100%.

DISCUSSION

The present study provides updated insight into the prevalence and molecular characteristics of FMDV circulating in Iraq during the outbreak period from July 2023 to October 2024, complementing the earlier phase of this investigation.

The consistent detection of the 3D gene across all five provinces indicates widespread viral circulation, supporting earlier reports that describe FMD as endemic in Iraq with recurrent outbreaks (**8**). Similar epidemiological

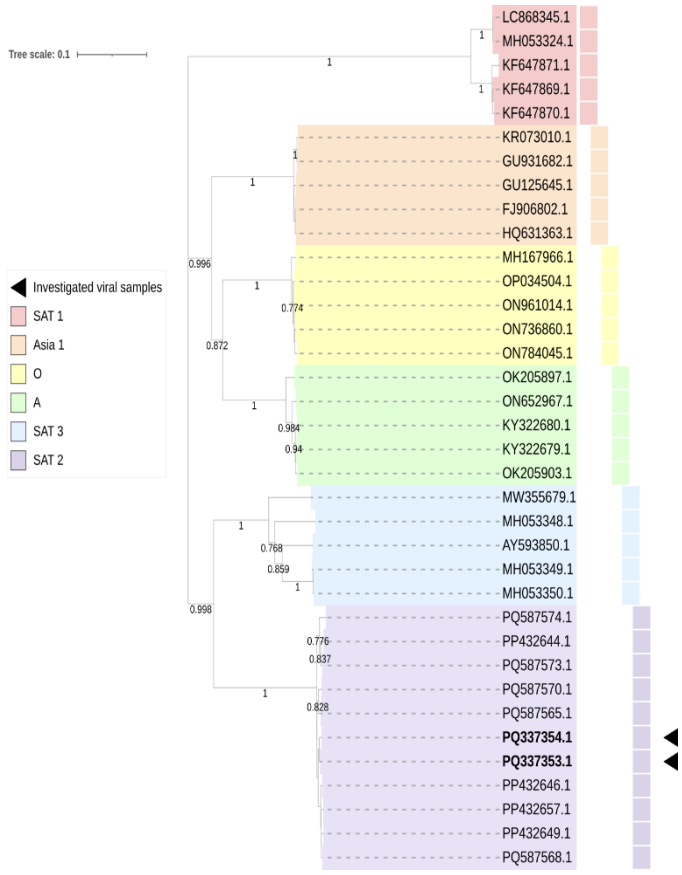

Figure 4. Neighbor-joining phylogenetic tree based on *1D* nucleotide sequences of FMDV. The tree was constructed from 654 aligned sites using the p-distance model with 1000 bootstrap replicates. The investigated Iraqi isolates (PQ337353.1 and PQ337354.1, indicated by black arrows) clustered within the SAT2 serotype clade (purple) with high bootstrap support (100%), alongside reference sequences from Western Asia. SAT3 was the closest lineage to SAT2, while SAT1 represented the most divergent clade. Each clade is represented by a distinct color. The scale bar indicates the number of substitutions per site

Table 6. List corresponding of reference viruses for *1D* gen for SAT2 serotype obtained from GeneBank used for phylogenetic analysis

GenBank Acc. no.	Serotype	Host	Country
PQ337353.1	SAT2	cow	Iraq
PQ337354.1	SAT2	cow	Iraq
PP432646.1	SAT2	water buffalo	Iraq
PQ587568.1	SAT2	cattle	Ethiopia
PP432649.1	SAT2	cattle	Iraq
PP432657.1	SAT2	cattle	Turkey
PQ587565.1	SAT2	cattle	Ethiopia
PQ587570.1	SAT2	cattle	Ethiopia
PQ587573.1	SAT2	cattle	Ethiopia
PP432644.1	SAT2	cattle	Bahrain
PQ587574.1	SAT2	cattle	Ethiopia
KF647870.1	SAT1	cattle	Nigeria
KF647869.1	SAT1	cattle	Nigeria
KF647871.1	SAT1	cattle	Nigeria
MH053324.1	SAT1	cattle	Uganda
LC868345.1	SAT1	cattle	Iraq
OK205897.1	A	pig	Vietnam
OK205903.1	A	pig	Vietnam
KY322680.1	A	cattle	Vietnam
KY322679.1	A	cattle	Thailand
ON652967.1	A	cattle	Vietnam
OP034504.1	O	cattle	Uganda
ON961014.1	O	cattle	Uganda
MH167966.1	O	cattle	Uganda
ON784045.1	O	cattle	Uganda

ON736860.1	O	cattle	Uganda
GU125645.1	Asia1	cattle	Vietnam
HQ631363.1	Asia1	pig	China
FJ906802.1	Asia1	pig	China
GU931682.1	Asia1	cattle	China
KR073010.1	Asia1	pig	China
MW355679.1	SAT3	African buffalo	Zimbabwe
AY593850.1	SAT3	guinea pigs	South Africa
MH053350.1	SAT3	African buffalo	Zimbabwe
MH053349.1	SAT3	African buffalo	Zimbabwe
MH053348.1	SAT3	cattle	Zimbabwe

patterns have been documented in Nineveh province, where high infection rates were associated with factors such as high herd density, uncontrolled livestock movement, and insufficient biosecurity measures (**6**). In addition, previous molecular studies have confirmed the circulation of multiple serotypes, including O, A, and Asia1, in different Iraqi regions such as Al-Qadisiyah (**9**), Basra (**25**), Duhok (**15**), and Sulaimani (**13,16**), underscoring the persistent and evolving nature of the virus.

Although the SAT2 serotype was detected at a lower frequency than the general FMDV marker, its presence across all examined provinces is epidemiologically important. This finding indicates that SAT2 is no longer limited to localized outbreaks but is now more widely distributed. Recent reports from southern Iraq have documented SAT2 infections in cattle and buffalo populations (**10-12,18**), supporting this observation. The widespread detection of SAT2 across provinces likely reflects ongoing livestock movement, which has been consistently identified as a major driver of FMD transmission in Iraq (**26**).

Differences in detection rates between sample types were also observed. Vesicular fluid demonstrated complete positivity for the 1D gene, whereas tongue epithelium samples showed lower detection rates. This can be explained by the pathogenesis of FMDV infection. The virus exhibits a strong affinity for epithelial tissues, where replication leads to vesicle formation and accumulation of high viral loads. During the acute phase, vesicular fluid therefore represents a highly reliable diagnostic specimen compared with epithelial tissue remnants (**19,27**). Similar findings have been reported in previous Iraqi studies, where vesicular samples showed higher diagnostic sensitivity than epithelial tissues for detecting serotypes such as O and Asia1 (**9,13**).

Sequencing analysis provided molecular confirmation of the RT-qPCR findings and enabled phylogenetic characterization of the SAT2 isolates. The obtained *1D* sequences clustered within the SAT2 group with strong bootstrap support and showed close genetic similarity to isolates previously reported in Iraq and neighboring countries, as well as East Africa. These findings are consistent with regional analyses demonstrating that recent SAT2 outbreaks in Western Asia originated from East African lineages and subsequently spread across the Middle East (**20**). In particular, the isolates collected during 2023–2024 were shown to form a distinct clade closely related to East African strains, with multiple countries, including Iraq, sharing highly similar sequences (**20**).

The relationship between circulating strains and the vaccine used in Iraq is of particular importance. The quadrivalent vaccine administered during the study period includes the strains A/TUR/06-20 IRAN 05, O/TUR/5/2009 PANASIA-2, ASIA 1/PAK/08 SINDH-8, and SAT2/ERITREA 98. Previous vaccine-matching studies demonstrated strong antigenic compatibility between SAT2 isolates from Iraq and neighboring countries and the SAT2/ERITREA 98 vaccine strain, with additional compatibility reported for the ZIM 83 strain (**20**). Given that the SAT2 isolates identified in the present study cluster within the same phylogenetic lineage as those evaluated previously, it is reasonable to infer likely genetic compatibility with the vaccine strain used in Iraq. However, this remains an indirect conclusion, as antigenic matching was not performed in the current study. Direct vaccine-matching assays are therefore required to confirm vaccine effectiveness against circulating strains.

The observed nucleotide variability within the *1D* gene further supports the concept of ongoing viral evolution. Similar genetic diversity has been reported in northern Iraq, where heterogeneity among serotypes, including Asia1, suggested continuous viral evolution and repeated introduction of new lineages (**14**). Such variability may contribute to antigenic drift and could potentially affect vaccine performance over time. This highlights the importance of continuous molecular surveillance and periodic reassessment of vaccine suitability (**15,16**).

This study increases current understanding of SAT2 epidemiology in Iraq by integrating prevalence data with molecular characterization. The detection of SAT2 across multiple provinces, together with its close genetic relationship to regional and East African strains, emphasizes the transboundary nature of FMDV transmission. While existing evidence suggests that the SAT2/ERITREA 98 vaccine strain remains appropriate, direct antigenic evaluation remains essential to validate this assumption and to guide future vaccination strategies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The authors contributed equally to this work.

ARTIFICIAL INTELLIGENT DECLARATION

The authors declare that they are responsible for the accuracy and integrity of all content of the manuscript, including part generated by AI, and it is not used as a co-author.

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الانتشار والدراسة الجزيئية للنمط المصلي SAT2 لمرض الحمى القلاعية في العراق

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الخلاصة

لا يزال فيروس الحمى القلاعية (FMDV) يُشكل تحديًا كبيرًا لإنتاج الثروة الحيوانية في العراق. بناءً على بحثنا السابق، الذي أفاد بانتشار فيروس الحمى القلاعية بشكل عام وأول عزل ناجح للنمط المصلي SAT2 في الأبقار العراقية، تدرس هذه الدراسة انتشار فيروس الحمى القلاعية وتُقدم توصيفًا جزيئيًا لعزلات مختارة. تم تحليل 100 عينة سريرية جُمعت من الأبقار في خمس محافظات خلال تفشي المرض في الفترة من يوليو 2023 إلى أكتوبر 2024. أظهرت 85 عينة (85.0%) نتائج إيجابية لجين D3، و30 عينة (35.3%) نتائج إيجابية لجين D1 الخاص بـ SAT2. لم تُلاحظ أي اختلافات جوهرية بين المحافظات في الكشف عن أيّ من الجينين. أظهر السائل الحويصلي نسبة إيجابية 100% لجين D1، وكانت أعلى بكثير من نسبة الإيجابية البالغة 27.6% في الأنسجة الظهارية للسان ($P < 0.0001$). أكد تحليل الانحدار اللوجستي انخفاض احتمالات اكتشاف الجين D1 مقارنةً بالجين D3 في جميع المحافظات (قيمة الاحتمال < 0.05). تم تسلسل ثلاث عزلات: عينة واحدة إيجابية للجين D3 وعينتان إيجابيتان للجين D1. أظهر التحليل التطوري لمنطقتي D3 و D1تكتلًا داخل SAT2، مما يُظهر علاقات وثيقة مع السلالات المنتشرة في غرب آسيا وشرق أفريقيا. تقدم هذه الدراسة نظرة عامة على مستوى المحافظات حول توزيع SAT2 في العراق، بالإضافة إلى أدلة جزيئية تدعم التطور الإقليمي للسلالة المنتشرة، مع التأكيد على ضرورة استمرار المراقبة وتحديث استراتيجيات التطعيم.

الكلمات المفاحية: مرض الحمى القلاعية، النمط المصلي الافريقي 2، D1 جين، التتابع الجيني، العراق