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Molecular Detection and Phylogenetic Characterization of PPRV in Goats: Evidence of Lineage IV Circulation and Province-Associated Genetic Clustering

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Abstract | Peste des petits ruminants (PPR) remains endemic in Iraq, but the molecular epidemiology and phylogenetic features of circulating strains in central provinces are poorly understood. The research aimed to investigate the active circulation of PPR virus (PPRV) in goats in the regions of Wasit, Thi-Qar, and Maysan, and to characterize local strains using N-gene sequencing and phylogenetic analysis. A cluster sampling design was used, with blood samples collected from 240 goats across 18 herds. Taqman qPCR was performed on the N gene to detect viral RNA, and traditional PCR was used to verify the qPCR-positive samples. The 15 representative amplicons were sequenced using the Sanger sequencing method and compared phylogenetically with regional and global lineage IV strains. PPRV RNA was also detected in 27.5 percent of goats, with a high variance among provinces ($p < 0.05$), and Wasit had the highest prevalence (43.8 percent). Although there is a high prevalence of silent infection, 34.2 per cent of animals displayed clinical symptoms of PPR. Partial N-gene fragment sequencing revealed little intraprovincial diversity, and all isolates belonged to the same lineage IV. Wasit isolates were also a well-supported monophyletic group with high bootstrap support, and Iraqi isolates showed the greatest nucleotide identity (99.33–99.90) to a Turkish lineage IV isolate. In conclusion, this research presents initial molecular and phylogenetic data on active PPRV circulation in central Iraq and shows silent infection, province-specific clustering, and a close relationship with viruses in the region's lineage IV. These results demonstrate the need for increased molecular surveillance and for targeted vaccination strategies.

Keywords | Goat, Morbillivirus, N gene, Peste des petits ruminants (PPR), qPCR

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INTRODUCTION

Peste des petits ruminants (PPR) is a prevalent viral disease of small ruminants that continues to pose a significant economic and livelihood burden in Africa, the Middle East, and Asia (Ugochukwu *et al.*, 2019; Ahaduzzaman, 2020). Peste des petits ruminants virus (PPRV) is a causative

agent classified in the genus Morbillivirus of the family Paramyxoviridae and is closely related to measles virus, canine distemper virus, and the eradicated rinderpest virus (Mansour and Hasso, 2021). Even though PPRV is regarded as antigenically conserved, molecular studies have identified four lineages (I-IV), with lineage IV found in the Middle East and Asia (Kumar *et al.*, 2014; Cêtre-Sossah *et al.*, 2016).

PPRV is most commonly spread through respiratory secretions and direct contact between infected and vulnerable animals. The classical disease manifestations are fever, ocular and nasal discharge, stomatitis, diarrhea, and respiratory distress; however, clinical diagnosis is not reliable in most cases, as it is similar to that of other small ruminant diseases (Santhamani *et al.*, 2016). Subsequently, molecular diagnostic techniques, particularly quantitative real-time PCR, have been needed to enable accurate infection detection and timely detection of active infection.

In Iraq, PPR is endemic and has been reported over several decades. The vast majority of molecular research has been conducted in the northern provinces, particularly Erbil and Al-Sulaymaniyah, where both domestic and wild goats have been reported to be infected with the disease (Hoffmann *et al.*, 2012; Candlan *et al.*, 2017; Khoran *et al.*, 2021). In comparison, central and southern localities, such as Wasit, Thi Qar, and Maysan, have been assessed mainly by serological survey, which has put wide gaps in comprehending the active viral circulation, molecular epidemiology, and phylogenetic features of PPRV in these regions (Muhsen, 2013; Jarad *et al.*, 2022). These provinces are significant livestock areas where goats are highly populated, and the networks of animal movements are dense, which contribute to the preservation of the virus and its transmission.

Although the epidemiological importance of PPRV is significant, there is no molecular or phylogenetic data to evaluate the virus's circulation in these central provinces. Therefore, the genetic identity, lineage affiliation, transmission mechanisms, and relationships within the country are not documented for the viral strains circulating in this area. To enhance national surveillance programmes and promote the global agenda for PPR eradication, these knowledge gaps should be addressed.

Based on this, the following objectives were used in the current research: (i) to identify active PPRV infection using the quantitative real-time PCR in goats in the regions of Wasit, Thi Qar, and Maysan; (ii) to validate the presence of qPCR-positive cases of PPRV infection through conventional PCR; (iii) to determine the genetic diversity and phylogeny of the existing PPRV strains by partial N-gene sequencing. These data are the initial molecular and phylogenetic data from central Iraq and serve as a baseline for enhancing control and prevention measures in the country.

MATERIALS AND METHODS

STUDY AREA AND ANIMAL SAMPLING

The research took place in the central Iraqi provinces of Wasit, Thi Qar, and Maysan, one of the largest regions of the country with goat production and an active network

of animal movement supporting the spread of PPRV (Jarad *et al.*, 2022). A cluster-based sampling strategy was necessary to achieve sufficient herd-level coverage and minimize sampling bias, in line with guidelines for conducting field epidemiological surveys of transboundary diseases (Santhamani *et al.*, 2016).

A mixed-age population of 240 domestic goats (*Capra hircus*) of both sexes was sampled (18 herds, 6 in each province). A total of 12 goats were randomly chosen from every herd. In herds with an inadequate number, other goats were sampled from herds belonging to the same cluster. Herds were all under traditional husbandry systems that were used in rural central Iraq.

CLINICAL EXAMINATION AND BLOOD COLLECTION

A close clinical observation of each goat was conducted, focusing on clinical signs likely to be typical of PPR, such as fever, mucopurulent nasal and ocular secretions, stomatitis, respiratory distress, diarrhea, and lymphadenopathy, as outlined in standardized clinical diagnostic guidelines (Ahaduzzaman, 2020). Aseptic samples of about 10 mL of jugular venous blood were taken from every animal. 2.5 mL of this volume was placed into EDTA tubes for molecular detection, and 7.5 mL into plain tubes for possible serological detection. Sample EDTA was stored at -20 °C until RNA extraction.

RNA EXTRACTION AND cDNA SYNTHESIS

Viral RNA was isolated from 200 µL of whole blood using TRIzol Reagent (Bioneer, Korea) according to the manufacturer's instructions. RNA purity and concentration values were determined by using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), and samples with an A260/280 of about 1.8-2.1 were said to be acceptable. DNase treatment was performed to eliminate any residual genomic DNA, followed by synthesis of complementary DNA (cDNA) using the M-MLV Reverse Transcriptase Kit (Promega, USA) and a random hexamer primer (previously tested for PPRV detection).

QUANTITATIVE REAL-TIME PCR (qPCR)

A Taqman-based qPCR assay targeting a conserved region of the nucleocapsid (N) gene was used to detect PPRV RNA, a popular method for sensitive molecular diagnosis of the virus (Kumar *et al.*, 2014). Reactions were performed with GoTaq Probe qPCR Master Mix (Promega, USA) in a final volume of 20 µL, containing 10 µL master mix, 4 µL cDNA, and 1 µL each of forward and reverse primers and probe at optimal concentrations. Primers and probe used were the following (Santhamani *et al.*, 2016): Forward: 5'AGA GTT CAA TAT GTT RTT AGC CAT '3, Reverse: 5' TTCCCC ART CAC TCT YCT TTG T'3, and Probe: FAM-CAC CGG AYA CKG CAG CTG

The thermal cycling program consisted of a preliminary denaturation at 95 °C for 2 min and 40 cycles of 95 °C for 15 s and 60 °C for 30 s. The samples that exhibited typical sigmoidal amplification curves with a cycle threshold (Ct) value of 37 or lower were considered positive according to the accepted diagnostic criteria (Centre-Sossah *et al.*, 2016). Each run included no template control (NTC), a positive PPRV RNA control, and an extraction control to assess the assay's performance.

CONVENTIONAL PCR CONFIRMATION

All positive qPCR samples were forwarded to conventional PCR of the N gene to generate amplicons for sequencing, as done in other regional molecular epidemiology studies (Hoffmann *et al.*, 2012; Candlan *et al.*, 2017). Amplification was done with GoTaq G2 Green Master Mix (Promega, USA): Forward: 5'-GTC TCG GAA ATC GCC TCA CAG ACT-3' and Reverse: 5'-CCT CCT CGT CCT CCA GAA TCT-3'.

PCR was conducted in reaction mixtures (25 µL total volume) with an initial denaturation period of 95 °C (3 min), then 35 cycles at 95 °C (30 s), 56 °C (30 s), and 72 °C (45 s) of reaction with a final extension of 72 °C (5 min). PCR products were stained according to the protocols described in Agar stains.

SEQUENCING AND PHYLOGENETIC ANALYSIS

Sanger sequencing was performed randomly on 15 PCR-positive samples (7 per province). Chromatograms were manually checked, and bases with Phred scores < 20 were clipped. The 372 bp fragments that were obtained were aligned with the help of ClustalW, and the phylogenetic trees were created with the assistance of the Neighbor-Joining technique with the Maximum Composite Likelihood model and 1,000 bootstrap replicates in accordance with the established standards of molecular epidemiology (Kumar *et al.*, 2014; Centre-Sossah *et al.*, 2016). A comparison of local sequences with reference lineage IV strains from Turkey, India, Ethiopia, China, and Egypt, all accessed from GenBank.

STATISTICAL ANALYSIS

All statistical analyses were performed using GraphPad Prism (version 10). Clinical and molecular findings were summarized using descriptive statistics. The prevalence of PPRV was determined as 95% confidence intervals (CI) per province. Epidemiological analysis methods used to identify differences in prevalence between the provinces were evaluated using the Chi-square test ($p < 0.05$ considered significant), as suggested by epidemiologists conducting PPRV research (Truong *et al.*, 2014).

CLINICAL FINDINGS

Clinical observation of 240 goats sampled found that 82 (34.2 percent) showed evidence of PPR, and 158 were clinically normal. Table 1 describes the distribution of clinical signs, including fever, mucous membrane congestion, tachypnea, and diarrhea. The fact that most animals were asymptomatic ($p = 0.0229$) indicates silent or subclinical PPRV circulation, which is also consistent with results from endemic areas. Table 1 highlights clinical examination (Total = 240 goats) of goats studied in this research.

Table 1: Clinical examination of study goats (Total number = 240).

Symptom	Total animals (No: 240)
Fever	61 (25.42%)
Tachypnea	34 (14.17%)
Tachycardia	19 (7.92%)
Congestion of mucous membranes	47 (19.58%)
Diarrhea	28 (11.67%)
Constipation	6 (2.5%)
Abortion	19 (7.92%)
Enlargement of the lymph node	17 (7.08%)
Apparently healthy	158 (65.83%) *
p-value	0.0229
Discrepancy	18.01
95%CI	3.218 to 32.80

MOLECULAR DETECTION BY qPCR

The PPRV RNA was detected in 66/240 goats by quantitative real-time PCR, yielding a total prevalence of 27.5 (95% CI: 22.0, 33.6). Table 2 presents provincial prevalence values. Wasit was found to be the most positive in qPCR, with 43.8 percent, followed by Thi Qar (25.0 percent) and Maysan (13.8 percent). These were statistically significant differences ($p < 0.05$). Figure 1 shows representative amplification curves for each province, with Ct values ranging from 16 to 37. This indicates that the Wasit samples were more likely to have low Ct values, implying higher viral loads.

Table 2: Prevalence of PPRV (PPRV) among study goats using qPCR.

Province	Total No.	Positive samples	
		No.	%
Wasit	80	35	43.75
Maysan	80	11	13.75
Thi-Qar	80	20	25
Total sample	240	66	27.5

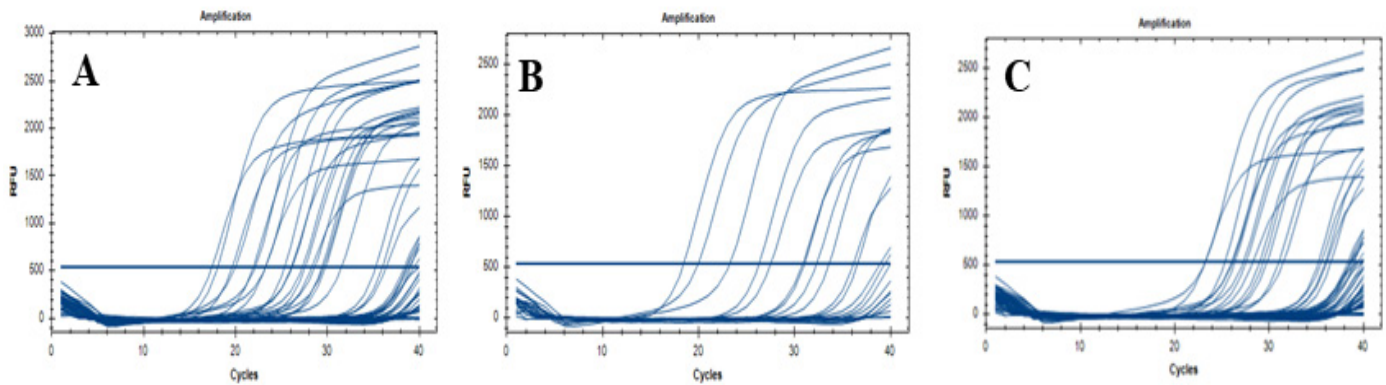


Figure 1: (A) PPRV was found in the goat blood samples by positive qPCR amplification. The threshold cycle of the positive samples ranged from 16 to 35. (B) PPRV was detected by positive qPCR amplification of PPRV in goat blood samples from Maysan. The positive sample threshold cycle ranged from 18 to 37. (C) The positive qPCR amplification of the PPRV of the Thi-qar goat blood samples. The positive samples had threshold cycles between 21 and 36.

CONVENTIONAL PCR

The 66 qPCR-positive samples were confirmed by conventional PCR (80.3% overall). Table 3 demonstrates the province-specific confirmation rates, with the highest ones being Wasit (82.9%). Probably, not all samples were amenable to amplification because of low viral loads or degraded RNA, as previous molecular research has verified (Hoffmann *et al.*, 2012; Candlan *et al.*, 2017). The results of gel electrophoresis of representative PCR amplicons are presented in Figure 2.

Table 3: Positive results of conventional PCR among the positive study samples by qPCR.

Province	Total No.	Positive samples	
		No.	%
Wasit	35	29	82.86 *
Maysan	11	8	72.73
Thi-Qar	20	16	80 *
Total sample	66	53	80.3

MULTIPLE SEQUENCE ALIGNMENT AND SEQUENCE ANALYSIS

A total of 15 partial N-gene sequences (five per province) were obtained and deposited in GenBank under accession numbers PQ287405-PQ287419. The length of each sequence was 372 bp, and the chromatograms were inspected; high-quality peaks were observed after trimming bases with Phred scores below 20. ClustalW multiple sequence comparison showed that Iraqi isolates had high nucleotide identity in some conserved regions, interspersed with scattered point mutations, indicating that genetic variation in the area is low. Congruent with closely related international lineage IV reference strains, which showed the same trends of conservation and variation, in agreement with the world diversity of PPRV (Figure 2).

PHYLOGENETIC, COMPARATIVE HOMOLGY AND REGIONAL, GLOBAL PPRV SEQUENCES

Neighbor-Joining phylogenetic analysis of the 15 partial N-gene sequences placed all the Iraqi PPRV isolates in lineage IV, in agreement with those that circulate in the Middle East and Asia (Figure 3). The Wasit isolates were closely clustered in a monophyletic pattern, with high bootstrap values (96%), and there was evidence of a local pattern of viral circulation with low levels of external introductions. Isolates from Thi Qar and Maysan, on the other hand, were more genetically heterogeneous, indicative of more complex transmission patterns or multiple events of viral introduction. Comparative analysis of the nucleotide identity with the international GenBank reference sequences indicated that the Iraqi isolates had the highest homology (99.33-99.90) with a Turkish strain of lineage IV (Supplementary Table S1). A little fewer identities were observed between Indian and Ethiopian isolates (97.64-98.32) (Supplementary Tables S2 and S3), and then between Chinese isolates (97.31-97.98) (Supplementary Table S4). The Egyptian isolates had the lowest level of similarity (96.97-97.64) (Supplementary Table S5). Genetic variation compared to the Turkish isolate ranged from 0.10 to 0.67 per cent., whereas variation relative to Egyptian isolates ranged from 2.36 to 3.03 per cent., confirming that Iraqi PPRV isolates were most closely related to viruses found in Turkey and adjacent areas. The findings indicate that continuous regional viral spread and the highly probable cross-border spread of lineage IV PPRV are highly probable.

DISCUSSION

Goats are a key part of small-ruminant production systems in Iraq, which provide meat, milk, income, and socio-economic stability for households (Awad *et al.*, 2021; Abdulkareem *et al.*, 2023; Mahmoud and Yassein, 2024). The current research shows that there is a significant active

DNA Sequences	Translated Protein Sequences
Species/Abbrev	Δ *
1. JN647694.1:PPR virus isolate India	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T C
2. KT006588.1: PPR virus isolate Egypt	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
3. KX421388.1PPR virus isolate China	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T C C
4. MN657232.1:PPR virus isolate Turkey	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
5. ON110960.1: PPR virus isolate Ethiopia	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
6. PQ287405.1 PPR virus IQG-No.1 wasit isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
7. PQ287406.1 PPR virus IQG-No.2 wasit isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
8. PQ287407.1 PPR virus IQG-No.3 wasit isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
9. PQ287408.1 PPR virus IQG-No.4 wasit isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
10. PQ287409.1 PPR virus IQG-No.5 wasit isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
11. PQ287410.1 PPR virus IQG-No.1 maysan isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
12. PQ287411.1 PPR virus IQG-No.2 maysan isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
13. PQ287412.1 PPR virus IQG-No.3 maysan isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
14. PQ287413.1 PPR virus IQG-No.4 maysan isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
15. PQ287414.1 PPR virus IQG-No.5 maysan isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
16. PQ287415.1 PPR virus IQG-No.1 Thi-qar isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
17. PQ287416.1 PPR virus IQG-No.2 Thi-qar isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
18. PQ287417.1 PPR virus IQG-No.3 Thi-qar isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
19. PQ287418.1 PPR virus IQG-No.4 Thi-qar isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C
20. PQ287419.1 PPR virus IQG-No.5 Thi-qar isolate	C G C A G T C C G C A A C A G A A T T G C A G A A G A T C T A T C A C T T C

DNA Sequences	Translated Protein Sequences
Species/Abbrev	Δ *
1. JN647694.1:PPR virus isolate India	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
2. KT006588.1: PPR virus isolate Egypt	G G C G G T T C A T G G T G T C T C T C A T A C T T G A C A T C A A G A G G A
3. KX421388.1PPR virus isolate China	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
4. MN657232.1:PPR virus isolate Turkey	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
5. ON110960.1: PPR virus isolate Ethiopia	G G C G G T T C A T G G T G T C T C T C A T A C T T G A C A T C A A G A G G A
6. PQ287405.1 PPR virus IQG-No.1 wasit isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
7. PQ287406.1 PPR virus IQG-No.2 wasit isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
8. PQ287407.1 PPR virus IQG-No.3 wasit isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
9. PQ287408.1 PPR virus IQG-No.4 wasit isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
10. PQ287409.1 PPR virus IQG-No.5 wasit isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
11. PQ287410.1 PPR virus IQG-No.1 maysan isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
12. PQ287411.1 PPR virus IQG-No.2 maysan isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
13. PQ287412.1 PPR virus IQG-No.3 maysan isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
14. PQ287413.1 PPR virus IQG-No.4 maysan isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
15. PQ287414.1 PPR virus IQG-No.5 maysan isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
16. PQ287415.1 PPR virus IQG-No.1 Thi-qar isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
17. PQ287416.1 PPR virus IQG-No.2 Thi-qar isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
18. PQ287417.1 PPR virus IQG-No.3 Thi-qar isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
19. PQ287418.1 PPR virus IQG-No.4 Thi-qar isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A
20. PQ287419.1 PPR virus IQG-No.5 Thi-qar isolate	G G C G G T T C A T G G T A T C T C T C A T A C T T G A C A T C A A G A G G A

Figure 2: Multiple sequence alignment of nucleocapsid protein (N) gene partial sequence in PPRV goat 15 isolates and NCBI-Genbank PPRV isolates, associated isolates. The ClustalW alignment tool was used to construct the multiple alignment analysis (Online). The similarity of nucleotide alignment (marked by the sign of (*)) and mutation substitution in the nucleocapsid protein (N) gene between isolates was found in the alignment analysis.

circulation of peste des petits ruminants virus (PPRV) in central Iraq, with an overall qPCR prevalence of 27.5% and significant variance across provinces (Table 2). The observation that a significant percentage of the animals were clinically healthy (65.8% Table 1) despite the presence of viral RNA is consistent with other studies done in endemic areas where the prevalence of subclinical or mild infection is widespread due to the constant exposure of animals to the virus, partial immunity, or unsynchronized outbreaks (Abdollahpour *et al.*, 2006; Hanna *et al.*, 2013). This difference between clinical and molecular diagnoses supports the low validity of clinical diagnosis in endemic areas (Santhamani *et al.*, 2016).

The much greater prevalence in Wasit (43.83) than in Thi Qar (25.03) and Maysan (13.83) indicates that the dynamics of PPRV transmission in the area are spatially heterogeneous. Possible factors include variation in herd density, animal movement patterns, marketing networks, and non-formal cross-provincial trade routes, all of which are established risk factors for PPRV persistence (Jarad *et al.*, 2022; Parida *et al.*, 2016). The lower Ct in Wasit (Figure 1) indicates higher viral loads, which might reflect more vigorous or recent viral circulation, consistent with the phylogenetic clustering identified.

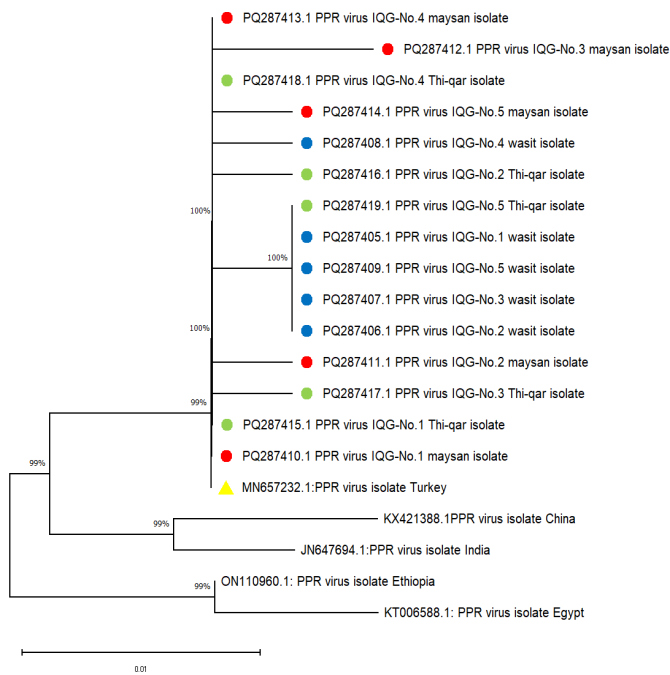


Figure 3: Phylogenetic tree of PPRV isolates of the partial N-gene sequences of the Neighbor-Joining method. The 15 Iraqi isolates (Wasit, Maysan, and Thi Qar) belong to two major clades, further subdivided into regional subgroups, reflecting significant intra-country genetic diversity. Closest relationships are observed between the isolates and regional ones, especially those in Turkey and India, with more distant relationships between them and Ethiopian, Chinese, and Egyptian reference strains.

The conventional PCR confirmation rate (80.3%, Table 3) was high, suggesting that most qPCR-positive samples had sufficient viral load to undergo downstream molecular analysis. Samples that did not amplify could have contained viral loads close to the qPCR detection limit, spoiled RNA, or sequence differences that could affect primer binding, as observed in previous regional studies (Hoffmann *et al.*, 2012; Candlan *et al.*, 2017).

The sequence analysis of 15 partial N-gene fragments showed limited intra-provincial diversity, with the most significant genetic homogeneity observed in the Wasit isolates (Figure 2). High bootstrap values (96%) indicated that this tight clustering formed a solid monophyletic group on the phylogenetic tree (Figure 3). This monophyly would indicate localized viral diversification in relatively closed or stable networks of animal-to-animal movements, a trend previously observed with lineage IV PPRV in the Middle East (Cêtre-Sossah *et al.*, 2016). Conversely, the greater heterogeneity in clustering of Thi Qar and Maysan isolates could also be due to multiple viral introductions or more extensive trade.

The clustering of all Iraqi isolates in lineage IV, which was the dominant lineage in the Middle East and Asia

(Kumar *et al.*, 2014), and the highest nucleotide identity (99.33–99.90) was to a Turkish isolate of lineage IV. Such a high level of similarity supports the cross-regional viral connectivity hypothesis, which is most likely enabled by body-to-body contact between small ruminants in Iraq and other nations (Ahaduzzaman, 2020; Cêtre-Sossah *et al.*, 2016). Ethiopian, Chinese, and Egyptian strains exhibit low levels of identity, consistent with well-recognized global phylogeographic patterns of PPRV (Parida *et al.*, 2016).

All of these findings provide strong evidence for the active, mostly silent circulation of PPRV lineage IV in central Iraq. The region's geographic arrangement, especially the monophyletic Wasit cluster, emphasizes the role of local epidemiological variables in the dynamics of viral transmission. These findings highlight the urgent need for more thorough molecular monitoring, the introduction of additional sample types with higher viral load (e.g., ocular and nasal swabs), and the reinforcement of vaccination efforts in regions of high prevalence, such as Wasit. In addition, since Iraq is a prospective location in regional livestock trade networks, cross-border control initiatives will be critical to mitigating the spread of the virus and promoting the eradication of PPR worldwide.

LIMITATIONS

Several limitations should be recognized. To start with, the blood samples were analyzed even though mucosal swabs usually have higher viral loads and could be more effective at detecting the virus. Second, the interpretation of risk factors was limited, as detailed data on vaccination history, herd management, and animal movement were unavailable. Third, the study used partial N-gene sequencing, which has moderate resolution for genetic data; whole-genome sequencing would be a better source of information about the virus's evolution and transmission mechanisms. The integration of serological, epidemiological, and comprehensive molecular data in future work would significantly enhance surveillance of national PPRV.

CONCLUSION

The present study provides initial molecular and phylogenetic evidence of the active circulation of PPRV in goats in Wasit, Thi Qar, and Maysan. The detection of viral RNA in over a quarter of sampled animals, including some that were healthy, also indicates silent circulation. The local isolates were all lineage IV and showed substantial genetic similarity to the Turkish isolates, suggesting they were transmitted locally and that the virus may have moved across borders. These results underscore the need to increase national-level molecular surveillance, enhance training on specific vaccination routes, and control animal

movement, especially in high-incidence regions like Wasit. The fact that clinical symptoms and molecular findings appear incongruent also underscores the need for routine laboratory diagnostics in an effective PPR control program.

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NOVELTY STATEMENT

The present research describes the primary molecular observations and sequencing of PPRV in central Iraq. It presents the earliest evidence of the provincial specificity of clustering and shows disparities in the virus's circulation across Wasit, Thi Qar, and Maysan. It is the first report of silent PPRV circulation in Iraqi goats, as indicated by qPCR Ct values and a high percentage of asymptomatic and infected animals.

AUTHOR'S CONTRIBUTION

ASSA: Clinical examination, collection of blood samples, and serological examination. NMHA: Designation of study and statistical analysis of the obtained data.

ETHICAL APPROVAL

The entire study of animals was carried out in accordance with the institution's norms and was approved by the Scientific Committee of the Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad (Approval ID: 3219/2024).

FUNDING

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GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors confirm that no generative AI or AI-assisted technologies were used in this work.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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Supplementary Table S1: NCBI-BLAST homology sequence identity percentage between local PPRV goat isolates and NCBI-BLAST closely related Turkey isolate.

Local isolate	Access No.	Homology sequence identity (%)		
		Country	Variation (%)	Identity %
PPR IQG No.1 Wasit	PQ287405.1	Turkey	0.33%	99.67%
PPR IQG No.2 Wasit	PQ287406.1	Turkey	0.33%	99.67%
PPR IQG No.3 Wasit	PQ287407.1	Turkey	0.33%	99.67%
PPR IQG No.4 Wasit	PQ287408.1	Turkey	0.33%	99.67%
PPR IQG No.5 Wasit	PQ287409.1	Turkey	0.33%	99.67%
PPR IQG No.1 Maysan	PQ287410.1	Turkey	0.10%	99.90%
PPR IQG No.2 Maysan	PQ287411.1	Turkey	0.33%	99.67%
PPR IQG No.3 Maysan	PQ287412.1	Turkey	0.67%	99.33%
PPR IQG No.4 Maysan	PQ287413.1	Turkey	0.10%	99.90%
PPR IQG No.5 Maysan	PQ287414.1	Turkey	0.33%	99.67%
PPR IQG No.1 Thi-qar	PQ287415.1	Turkey	0.10%	99.90%
PPR IQG No.2 Thi-qar	PQ287416.1	Turkey	0.33%	99.67%
PPR IQG No.3 Thi-qar	PQ287417.1	Turkey	0.33%	99.67%
PPR IQG No.4 Thi-qar	PQ287418.1	Turkey	0.33%	99.67%
PPR IQG No.5 Thi-qar	PQ287419.1	Turkey	0.33%	99.67%

Supplementary Table S2: NCBI-BLAST homology sequence identity percentage between local PPRV goat isolates and the NCBI-BLAST closely related Ethiopian isolate.

Local isolate	Access No.	Homology sequence identity (%)		
		Country	Variation (%)	Identity %
PPR IQG No.1 Wasit	PQ287405.1	Ethiopia	2.02%	97.98%
PPR IQG No.2 Wasit	PQ287406.1	Ethiopia	2.02%	97.98%
PPR IQG No.3 Wasit	PQ287407.1	Ethiopia	2.02%	97.98%
PPR IQG No.4 Wasit	PQ287408.1	Ethiopia	2.02%	97.98%
PPR IQG No.5 Wasit	PQ287409.1	Ethiopia	2.02%	97.98%
PPR IQG No.1 Maysan	PQ287410.1	Ethiopia	1.68%	98.32%
PPR IQG No.2 Maysan	PQ287411.1	Ethiopia	2.02%	97.98%
PPR IQG No.3 Maysan	PQ287412.1	Ethiopia	2.36%	97.64%
PPR IQG No.4 Maysan	PQ287413.1	Ethiopia	1.68%	98.32%
PPR IQG No.5 Maysan	PQ287414.1	Ethiopia	2.02%	97.98%
PPR IQG No.1 Thi-qar	PQ287415.1	Ethiopia	1.68%	98.32%
PPR IQG No.2 Thi-qar	PQ287416.1	Ethiopia	2.02%	97.98%
PPR IQG No.3 Thi-qar	PQ287417.1	Ethiopia	2.02%	97.98%
PPR IQG No.4 Thi-qar	PQ287418.1	Ethiopia	1.68%	98.32%
PPR IQG No.5 Thi-qar	PQ287419.1	Ethiopia	2.02%	97.98%

Supplementary Table S3: NCBI-BLAST homology sequence identity percentage between local PPRV goat isolates and the NCBI-BLAST closely related Indian isolate.

Local isolate	Access No.	Homology sequence identity (%)		
		Country	Variation (%)	Identity %
PPR IQG No.1 Wasit	PQ287405.1	India	2.02%	97.98%
PPR IQG No.2 Wasit	PQ287406.1	India	2.02%	97.98%
PPR IQG No.3 Wasit	PQ287407.1	India	2.02%	97.98%
PPR IQG No.4 Wasit	PQ287408.1	India	2.02%	97.98%
PPR IQG No.5 Wasit	PQ287409.1	India	2.02%	97.98%
PPR IQG No.1 Maysan	PQ287410.1	India	1.68%	98.32%

Table continues on next page.....

Local isolate	Access No.	Homology sequence identity (%)		
		Country	Variation (%)	Identity %
PPR IQG No.2 Maysan	PQ287411.1	India	2.02%	97.98%
PPR IQG No.3 Maysan	PQ287412.1	India	2.36%	97.64%
PPR IQG No.4 Maysan	PQ287413.1	India	1.68%	98.32%
PPR IQG No.5 Maysan	PQ287414.1	India	2.02%	97.98%
PPR IQG No.1 Thi-qar	PQ287415.1	India	1.68%	98.32%
PPR IQG No.2 Thi-qar	PQ287416.1	India	2.02%	97.98%
PPR IQG No.3 Thi-qar	PQ287417.1	India	2.02%	97.98%
PPR IQG No.4 Thi-qar	PQ287418.1	India	1.68%	98.32%
PPR IQG No.5 Thi-qar	PQ287419.1	India	2.02%	97.98%

Supplementary Table S4: NCBI-BLAST homology sequence identity percentage between local PPRV goat isolates and the NCBI-BLAST closely related China isolate.

Local isolate	Access No.	Homology sequence identity (%)		
		Country	Variation (%)	Identity %
PPR IQG No.1 Wasit	PQ287405.1	China	2.36%	97.64%
PPR IQG No.2 Wasit	PQ287406.1	China	2.36%	97.64%
PPR IQG No.3 Wasit	PQ287407.1	China	2.36%	97.64%
PPR IQG No.4 Wasit	PQ287408.1	China	2.36%	97.64%
PPR IQG No.5 Wasit	PQ287409.1	China	2.36%	97.64%
PPR IQG No.1 Maysan	PQ287410.1	China	2.02%	97.98%
PPR IQG No.2 Maysan	PQ287411.1	China	2.36%	97.64%
PPR IQG No.3 Maysan	PQ287412.1	China	2.69%	97.31%
PPR IQG No.4 Maysan	PQ287413.1	China	2.02%	97.98%
PPR IQG No.5 Maysan	PQ287414.1	China	2.36%	97.64%
PPR IQG No.1 Thi-qar	PQ287415.1	China	2.02%	97.98%
PPR IQG No.2 Thi-qar	PQ287416.1	China	2.36%	97.64%
PPR IQG No.3 Thi-qar	PQ287417.1	China	2.36%	97.64%
PPR IQG No.4 Thi-qar	PQ287418.1	China	2.02%	97.98%
PPR IQG No.5 Thi-qar	PQ287419.1	China	2.36%	97.64%

Supplementary Table S5: NCBI-BLAST homology sequence identity percentage between local PPRV goat isolates and the NCBI-BLAST closely related Egypt isolate.

Local isolate	Access No.	Homology sequence identity (%)		
		Country	Variation (%)	Identity %
PPR IQG No.1 Wasit	PQ287405.1	Egypt	2.69%	97.31%
PPR IQG No.2 Wasit	PQ287406.1	Egypt	2.69%	97.31%
PPR IQG No.3 Wasit	PQ287407.1	Egypt	2.69%	97.31%
PPR IQG No.4 Wasit	PQ287408.1	Egypt	2.69%	97.31%
PPR IQG No.5 Wasit	PQ287409.1	Egypt	2.69%	97.31%
PPR IQG No.1 Maysan	PQ287410.1	Egypt	2.36%	97.64%
PPR IQG No.2 Maysan	PQ287411.1	Egypt	2.69%	97.31%
PPR IQG No.3 Maysan	PQ287412.1	Egypt	3.03%	96.97%
PPR IQG No.4 Maysan	PQ287413.1	Egypt	2.36%	97.64%
PPR IQG No.5 Maysan	PQ287414.1	Egypt	2.69%	97.31%
PPR IQG No.1 Thi-qar	PQ287415.1	Egypt	2.26%	97.64%
PPR IQG No.2 Thi-qar	PQ287416.1	Egypt	2.69%	97.31%
PPR IQG No.3 Thi-qar	PQ287417.1	Egypt	2.69%	97.31%
PPR IQG No.4 Thi-qar	PQ287418.1	Egypt	2.36%	97.64%
PPR IQG No.5 Thi-qar	PQ287419.1	Egypt	2.69%	97.31%