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Short Communication

Detection and Phylogenetic Study of *Peste des Petits Ruminants* in Iran, 2019: Updated Data

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Abstract

Peste des Petits Ruminants (PPR) is caused by a morbillivirus from the Paramyxoviridae family and the infected animals, especially goats, that show clinical signs of necrotic stomatitis, enteritis, and pneumonia. The PPR virus has four lineages closely related to the geographical regions. Sufficient awareness of the lineage of the virus helps monitor the disease in different regions of a country. Phylogenetic studies have led to implementing strategies against new lineages that may enter a given country from the neighboring countries. The present research aimed to study the PPR virus (PPRV) detected phylogenetically by PCR in a small ruminant flock with PPR clinical signs. The goats in a flock in Alborz province showed clinical signs of PPR, and 10% died. Oral swabs and blood samples were taken from two affected goat flocks. The RT-PCR was conducted to detect PPRV RNA, and the sequence of the obtained RNA was analyzed phylogenetically. Moreover, all the samples were positive for the presence of PPRV and belonged to lineage IV. The isolates had high homology with each other and with the isolates from different countries. To inhibit the entrance of new isolates to Iran and reduce the incidence of outbreaks in Iran, it is essential to control the animals' movement across the borders and increase the vaccination coverage throughout the country. To eradicate PPR, an extensive vaccination program should cover small ruminant populations throughout the country.

Keywords: Goat, Iran, Lineage, Peste des Petits Ruminants, Phylogenetic, RT-PCR

Détection et Étude Phylogénétique de La Peste des Petits Ruminants en Iran, 2019: Données Mises à Jour

Résumé: La Peste des Petits Ruminants (PPR) est causée par un morbillivirus de la famille des *Paramyxoviridae* et les animaux infectés, en particulier les chèvres, qui présentent des signes cliniques, tels que stomatite nécrotique, entérite et pneumonie. Le virus PPR a quatre lignées étroitement liées aux régions géographiques. Une connaissance suffisante de la lignée du virus permet de surveiller la maladie dans différentes régions d'un pays. Les études phylogénétiques ont conduit à la mise en œuvre de stratégies contre de nouvelles lignées qui peuvent entrer dans un pays donné en provenance des pays voisins. La présente recherche visait à étudier le virus PPR (PPRV) détecté phylogénétiquement par PCR dans un petit troupeau de ruminants présentant des signes cliniques de PPR. Les chèvres d'un troupeau de la province d'Alborz ont montré des signes cliniques de PPR et 10% sont mortes. Des écouvillons oraux et des échantillons de sang ont été prélevés sur deux chèvres touchées. La RT-PCR a été réalisée pour détecter l'ARN du PPRV, et la séquence de l'ARN obtenu a été analysée phylogénétiquement. De plus, tous les échantillons étaient positifs pour la présence de PPRV et

appartenaient à la lignée IV. Les isolats avaient une forte homologie entre eux et avec les isolats de différents pays. Pour empêcher l'entrée de nouveaux isolats en Iran et réduire l'incidence des foyers en Iran, il est essentiel de contrôler le mouvement des animaux à travers les frontières et d'augmenter la couverture vaccinale dans tout le pays. Pour éradiquer la PPR, un vaste programme de vaccination devrait couvrir les populations de petits ruminants dans tout le pays.
Mots-clés: Chèvre, Iran, Lignée, Peste des Petits Ruminants, Phylogénétique, RT-PCR

Introduction

Peste des Petits Ruminants (PPR) is caused by a morbillivirus from the Paramyxoviridae family and results in serious economic losses in the sheep and goat industry. The disease is highly contagious, with a death rate approaching 100%. It should be noted that goats are more infected by PPR than sheep. The affected animals' predominant clinical signs include necrotic stomatitis, enteritis, and pneumonia (Smith and Sherman, 2009). The PPR has been reported as an endemic disease in many countries in some parts of Asia and Africa (Kumar et al., 2014). Furthermore, THE PPR is also endemic in Iran, and several outbreaks have been reported in different provinces of this country (Esmaelizad et al., 2011). As a negative-sense RNA virus, PPR comprises six genes that encode 6-8 proteins (Kumar et al., 2014), including the nucleocapsid protein (N), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), and the haemagglutinin protein (H) (WHO, 2018b). Based on the partial sequencing of F and N genes, the PPRV has four lineages meticulously pertinent to the geographical regions (Libeau et al., 2014). The lineage IV has been found in the Middle East countries, including Turkey, Iraq, Bangladesh, Iran, and countries surrounding Iran. The cluster analysis of the virus circulating in Pakistan, Saudi Arabia, Kuwait, and Iran showed that the virus in these countries (Dhar et al., 2002) was the same.

1. The first report of PPR in Iran dates back to 1995 in Ilam province, Iran. In 2004, the disease had spread throughout the country (Rasooli et al., 2019) and has been reported mostly in Alborz, Zanjan, Markazi, Khuzestan, and Tehran provinces (IVO, 2016). The chief control program against PPR is based on vaccination. Therefore, the lack of vaccination results in the persistence of the disease in Iran (Esmaelizad et al., 2011; Rasooli et al., 2019)

Moreover, knowledge about the virus's lineage is advantageous for monitoring the disease in different regions of a country (Esmaelizad et al., 2011). Even though PPR is endemic in many of Iran's neighboring countries (Azizi and Farid, 2010; Munir et al., 2012; Güler et al., 2014), most of these countries do not apply appropriate control strategies against infectious diseases. Therefore, it is necessary to have epidemiological studies to understand the virus's lineage in Iran due to livestock smuggling in these countries. Phylogenetic studies may lead to implementing strategies against new lineages that enter the country (Esmaelizad et al., 2011). The current study aimed to evaluate the PPRV detected phylogenetically using PCR in a small ruminant flock with PPR clinical signs.

Material and Methods

Sample History. During winter 2019, two unvaccinated goat flocks in Alborz province were considered a PPR case. The affected goats had clinical signs, including pneumonia, ocular discharge, diarrhea, and necrotic stomatitis. Oral swabs were taken from two affected adult goat flocks. Then, the samples were examined for the presence of PPRV RNA using the RT-PCR method.

RNA Extraction and PCR Method. According to the manufacturer's protocol, RNA was extracted from the samples using the Maxwell RNA extraction kit. Subsequently, cDNA was synthesized using the

Fermentas kit. RT-PCR was conducted according to Forsyth and Barrett's method(1995). Amplification was achieved using PPRV primers F1 and F2 designed against the PPRV Fusion gene. Forward and reverse primers included 5754-5778 and 6125-6101, respectively, and The sequences of primers used in this protocol for F1 and F2 were 5'-ATCACAGTGTTAAAGCCTGTAGAGG-3' and 5'-GAGACTGAGTTTGTGACCTACAAGC-3', respectively. The RT-PCR was performed using 12.5 µl of the 2x reaction mix, 5 µl of RNase-free distilled water, and 1 µl of 10pmol each primer. The reaction mixture's final volume amounted to 25 µl, including 22.5 µl master mix and 2.5µl cDNA. Amplification was performed in the automated RNA thermal cycle using the following cycling parameters: Denaturation was performed at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min. Subsequently, annealing was conducted at 55°C for 1 min, followed by primer extension at 72°C for 1 min. The final extension was performed at 72°C for 10 min. Eventually, PPRV RNA was amplified to give a DNA fragment of 371 bp.

Sequence Analysis. The PCR products were sequenced using the Sanger sequencing method (Bioneer Company) to determine the detected virus's lineage. Afterward, the results were aligned with the reference sequences of the PPRV available in NCBI GenBank using the Clustal W method. Subsequently, the neighbor-joining method (MEGA 7) was used with 1000 bootstrap replicates to determine phylogenetic relationships using the p-distance model.

Results

The PPRV RNA was detected in all the samples taken from the two affected goat flocks. Phylogenetic analysis of the sequences revealed that the isolates belonged to lineage IV, known as Asian lineage (Figure 1). The isolates' names were UT-Mard Abad 1 and UT-Mard Abad 2, deposited under the accession number MN036450 and MN036451. The isolates shared high homology at 96-98%. Comparing the results of the sequence with available PPRVs in GenBank revealed that the obtained isolates shared the same cluster with the Iranian ones in the previous studies. Moreover, our results were similar to the isolates from China and Pakistan. UT-Mard Abad 1 and isolates from Pakistan shared the same cluster (Table 1).

Table 1. Nucleotide sequence variation for PPRV variants isolated from the studied flock.

		1	2	3	4	5	6	7	8	9	10	11
1	UT-Mard_Abad_1	####										
2	UT-Mard_Abad_2	96.31										
3	Pakistan/PPR/MM49/2011(KC191628)	98.02	97.01									
4	Peste-des-petits-ruminants_virus_isolate_4_(JX443708.1_)	95.90	94.52	97.30								
5	Peste-des-petits-ruminants_virus_isolate_2_(JX443706.1_)	97.96	95.16	98.30	96.83							
6	PAK-Bpr-07-NARC10(GU980867.1)	96.37	94.68	97.71	98.99	97.28						
7	PAK-Veh-05-NARC1_(GU980865)	96.37	94.68	97.71	98.99	97.28	100.00					
8	PAK-Ict-06-NARC2_(GU980862)	95.71	94.02	97.06	98.31	96.60	98.69	98.69				
9	Xinjiang_2015-61_(KY434264.1)	97.75	98.52	98.52	96.59	97.29	97.04	97.04	96.30			
10	Xinjiang_2015-22(KY434230.1)	97.36	96.01	98.04	96.62	96.94	97.06	97.06	96.41	100.00		
11	ChinaGX2014_(MF443350.1)	97.75	98.52	98.52	96.59	97.29	97.04	97.04	96.30	100.00	100.00	

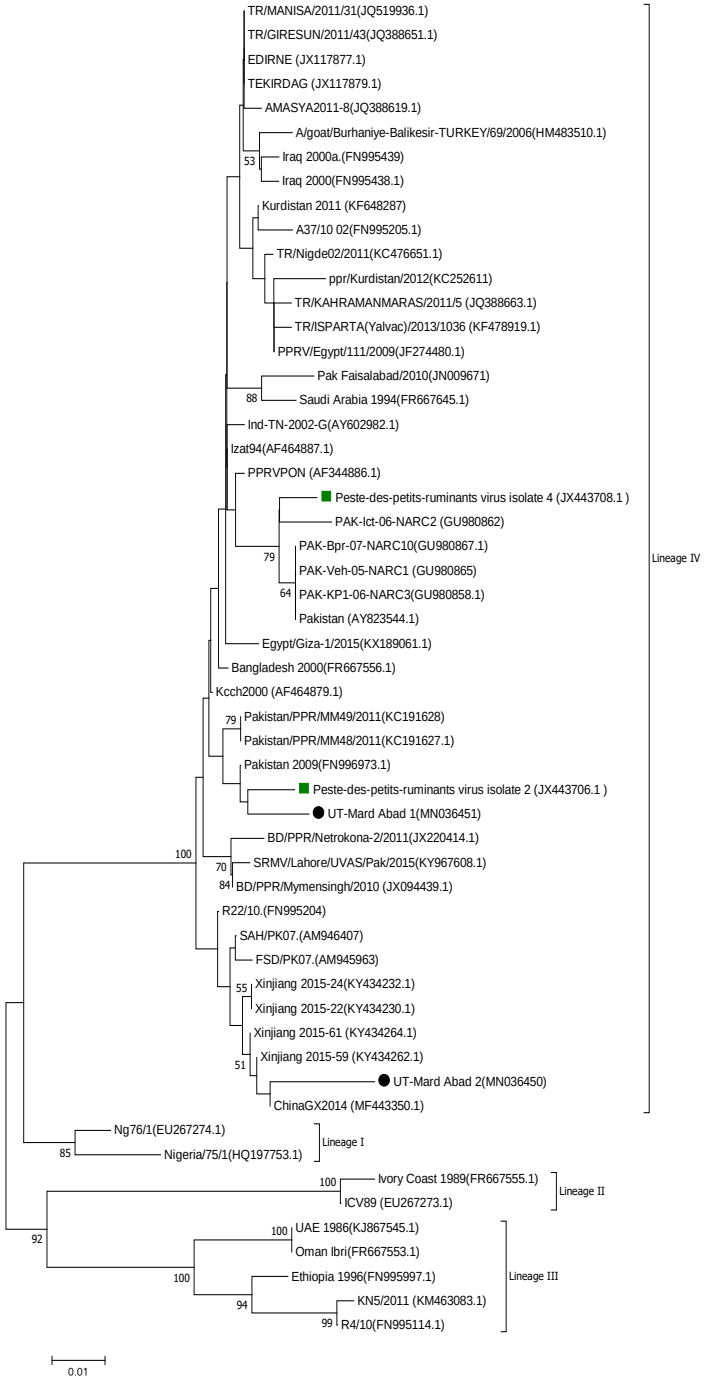

Figure 1. Phylogenetic tree of PPR virus isolated based on the F gene. The alignments had been done by the Clustal W method. Comparison of the isolates with the reference strains from GenBank reveals that our isolates belonged to strains from Pakistan, China, and the strains that had been isolated previously in Iran. All four divergent clusters are shown on the tree.

■: Isolates of PPR virus in the present study.

●: Isolates of the PPR virus in the previous study had been done in Iran

Discussion

In the present study, PPR was detected in a flock with typical clinical signs of the disease. Sheep were not affected by the PPR; however, goats were affected to the extent that even cases of abortion were observed among them. The PPR is endemic in Iran, and its mild form is expected to occur (Zakian et al., 2016). However, catastrophic deaths and severe clinical signs were observed among the studied flock. Such conditions can be attributed to the poor hygiene and lack of vaccination in the studied flock that has a prominent role in the prevalence of PPR in different provinces of Iran (Azizi and Farid, 2010). The increase of mass vaccination coverage against PPR in Iran during 2016 waned the number of outbreaks compared to the previous years (Barani et al., 2017).

The control of small ruminant movement in nomadic flocks is difficult (Libeau et al., 2014), and these animals have an important role in the transmission of PPR throughout Iran. Moreover, Iran is bordered by Afghanistan and Pakistan in the east, Iraq in the west, and Armenia, Azerbaijan, and Turkey in the north. Animals with poor health conditions also move in these regions. Therefore, some of these animals' illegal entrance from other countries increases the number of new infectious agents in Iran.

It has been demonstrated that the PPRV in Iran's neighboring countries shares the same lineage as the Iranian virus (Barani et al., 2017), and molecular characterization of the two infected goat flocks in the present study showed that lineage IV is circulating in Asia. The previous study in Iran showed that the PPRV in this lineage shared the same cluster with Pakistan's strains (Figure 1) (Kumar et al., 2014). In a study performed in 2014 in Turkey, all the detected viruses belonged to the lineage IV (WHO, 2018b). In the same line, another phylogenetic study conducted in Pakistan in 2011 showed the same lineage, and researchers concluded that the Pakistani isolates shared clusters with isolates from Tajikistan, Iran, and China (Güler et al., 2014). The present study results demonstrated that UT-Mard Abad 2 isolate shared the same cluster with the MF443350.1 isolate from China.

There was a genetic relationship between the strains in Iran and the isolated lineage from Pakistan and China in the current study. The UT-Mard Abad 1 isolate had a 98% homology with the isolate from Pakistan. Moreover, this isolate had high homology with JX443706.1 strain, which was isolated previously in Iran and shared the same cluster (Figure. 1.). It is worth mentioning that previous studies showed that although UT-Mard Abad 2 isolate did not share the same cluster with the Iranian isolates, the homology between these isolates was high nonetheless. The homology table shows that UT-Mard Abad 2 isolate has 97% homology with the KC191628 isolate from Pakistan (Table 1), indicating that the same strain is the cause of outbreaks in these countries. In a similar study conducted in Iran during 2011, the results revealed more than 97% homology between Iranian isolates and 16 other isolates from different geographical regions (Smith and Sherman, 2009).

In a study conducted by Güler et al. (2014), no subdivision was found from different provinces in Turkey (WHO, 2018a), indicating that the lineage of the virus in Turkey had remained stable and homologous. In another study conducted by Munir et al. (2012), a clear division was detected in two groups in terms of the sequences of PPRVs isolated from an outbreak, indicating that the genetic divergence among PPRVs was slightly higher in the N gene (6.2%), compared to the F gene (5.2%) (Güler et al., 2014).

The present study results indicated the presence of lineage IV in Iran, which is circulating in all Iran's neighboring countries. Moreover, this study showed high homology between Iranian PPRVs and those from different countries (e.g., Nigeria) in different continents. It should be noted that monitoring PPRV during the outbreaks and controlling animals' movement across borders, such as the Pakistan border, can reduce the prevalence of PPR in Iran. According to the eradication program of OIE, Iran is attempting to

control and eradicate the disease until 2030 (WHO, 2018b, a). Accordingly, the expansion of the PPR vaccination program is one strategy to eradicate the disease in Iran.

Conclusion

To eradicate PPR, an extensive vaccination program should cover small ruminant populations throughout the country.

Authors' Contribution

Study concept and design: N. A. and A. Gh.
Acquisition of data: Z. Z.
Analysis and interpretation of data: A. Gh.
Drafting of the manuscript: M. H. and L. A.
Critical revision of the manuscript for important intellectual content: Z. Z.
Statistical analysis: M. H. F. M.
Administrative, technical, and material support: L. A.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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