Research Article

Sequence Analysis of N Gene of Peste Des Petits Ruminants Virus from Hail, Saudi Arabia

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Abstract | Peste des petits ruminants (PPR) is an endemic disease in Hail, Saudi Arabia where outbreaks are regularly reported. This study was carried out to analyze nucleoprotein (N) gene of PPRV from fatal infections in small ruminants. For this purpose, RNA was extracted from suspected animal samples (n=18) and were screened by real time-PCR. Additionally, to assess the phylogenomics, the N gene was amplified and sequenced from sheep and goats (n=12). Nucleotide identity among Hail strains was identified to be 96.2-100%, while identity with previously sequenced Saudi Arabian strains and with reference PPRV retrieved from GenBank was found to be 94.1-98% and 81.2-100%, respectively. Phylogenetic analysis revealed that Hail PPRV strains clustered in four separate groups within lineage IV indicating genetic divergence based on N gene. These findings further support the continuous characterization of PPRV strains in small ruminants to support the global eradication plan in 2030.

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Introduction

Peste des petits ruminants (PPR) is a highly infectious disease of small ruminants and is characterized by high morbidity and mortality rate in both domestic and wild small ruminants (Elzein et al., 2004; Munir et al., 2013). PPRV belongs to the genus Morbillivirus within the family Paramyxoviridae (Gibbs et al., 1979), and its genome encodes six structural proteins including nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin protein (HA), and RNA-dependent RNA polymerase (L), and two nonstructural proteins (V and C). Based on the nucleotide sequence of either the N or F gene, PPRV strains are grouped into four genetic lineages (Bailey et al., 2005; FAO, 2016). Lineages I, II, and III have been found primarily in Africa, while, lineage IV is prevalent in Asian countries (Baron et al., 2016). N gene is the most divergent and appropriate for molecular characterization (Kwiatek et al., 2007). In Saudi Arabia, PPR was first reported in 1990 (Abu Elzein et al., 1990), the disease has since spread to different parts of the country (Housawi et al., 2004; Al-Afaleq et al., 2004; Boshra et al., 2015). PPR outbreaks are regularly occurring in Hail district (Mahmoud et al., 2016, 2017), which demands the characterization of these viruses to assess the genetic nature of circulating field strains of PPRV.

The study was designed to analyze PPRV-N gene

Links Researchers

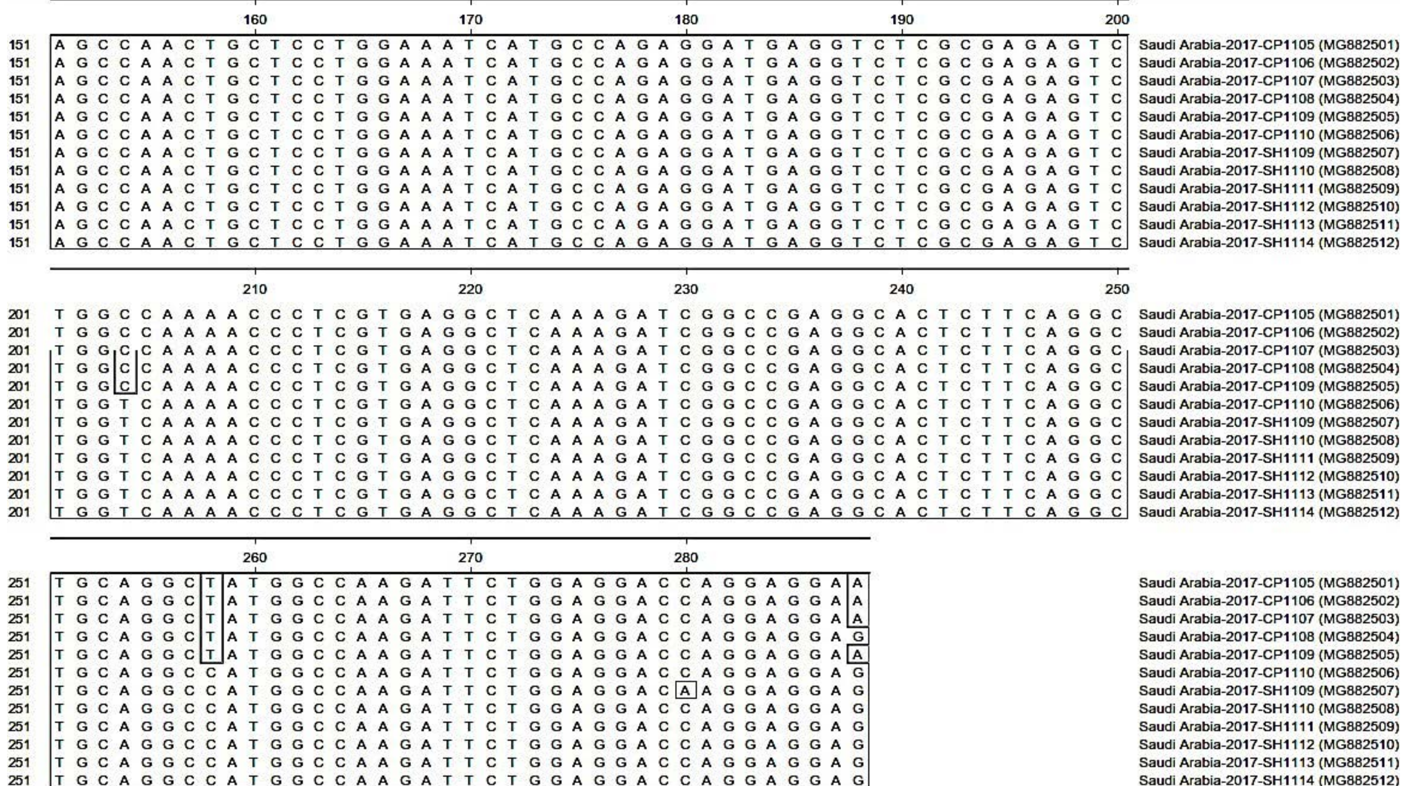
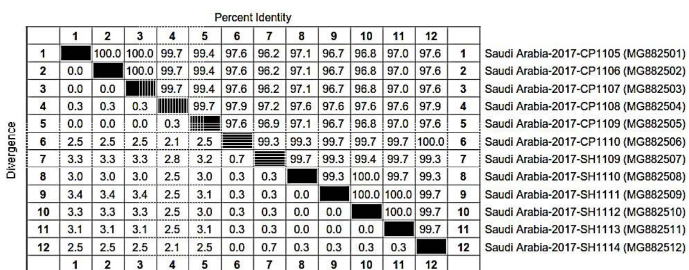


Figure 1: *Sequence alignment produced by Clustal W among PPRV strains identified from outbreaks occurred in Hail, Saudi Arabia.*

sequence from strains, which were causing regular outbreaks in Hail. Saudi Arabia.



Percent Identity (upper triangle); Divergence (lower triangle)

	1	2	3	4	5	6	7	8	9	10	11	12		
1		100.0	100.0	99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	1	Saudi Arabia-2017-CP1105 (MG882501)
2	0.0		100.0	99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	2	Saudi Arabia-2017-CP1106 (MG882502)
3	0.0	0.0		99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	3	Saudi Arabia-2017-CP1107 (MG882503)
4	0.3	0.3	0.3		99.7	97.9	97.2	97.6	97.6	97.6	97.6	97.9	4	Saudi Arabia-2017-CP1108 (MG882504)
5	0.0	0.0	0.0	0.3		97.6	96.9	97.1	96.7	96.8	97.0	97.6	5	Saudi Arabia-2017-CP1109 (MG882505)
6	2.5	2.5	2.5	2.1	2.5		99.3	99.3	99.7	99.7	99.7	100.0	6	Saudi Arabia-2017-CP1110 (MG882506)
7	3.3	3.3	3.3	2.8	3.2	0.7		99.7	99.3	99.4	99.7	99.3	7	Saudi Arabia-2017-SH1109 (MG882507)
8	3.0	3.0	3.0	2.5	3.0	0.3	0.3		99.3	100.0	99.7	99.3	8	Saudi Arabia-2017-SH1110 (MG882508)
9	3.4	3.4	3.4	2.5	3.1	0.3	0.3	0.0		100.0	100.0	99.7	9	Saudi Arabia-2017-SH1111 (MG882509)
10	3.3	3.3	3.3	2.5	3.0	0.3	0.3	0.0	0.0		100.0	99.7	10	Saudi Arabia-2017-SH1112 (MG882510)
11	3.1	3.1	3.1	2.5	3.1	0.3	0.3	0.0	0.0	0.0		99.7	11	Saudi Arabia-2017-SH1113 (MG882511)
12	2.5	2.5	2.5	2.1	2.5	0.0	0.7	0.3	0.3	0.3	0.3		12	Saudi Arabia-2017-SH1114 (MG882512)
	1	2	3	4	5	6	7	8	9	10	11	12		

Figure 2: *Percentage identity and divergence among PPRV viruses identified from outbreaks occurred in Hail, Saudi Arabia.*

Materials and Methods

Detection of PPRV RNA

Nasopharyngeal swabs (n=18) were collected from sick sheep (n=6), goats (n=6) and clinically healthy camels (n=6) as described by Mahmoud et al. (2017). Swabs were suspended in 500µl of 0.9% saline solution and the viral RNA was extracted from 140µl of supernatant. The total RNA was eluted from Mini-spin column using QIAamp ® Viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions.

Real-time reverse transcriptase PCR (rtRT-PCR), targeting the large protein gene, was used to screen the presence of viral RNA in samples using genesig® Kit (Genesig®, Primerdesign™ Ltd).

RT-PCR and Sequencing.

To provide genetic material for N gene sequence analysis, samples were amplified by RT-PCR (RT-PCR) using NP3 (5´-TCTCGGAAATCGCCT-CACAGACTG-3´) and NP4 (5´CCTCCTCCTG-GTCCTCCAGAATCT-3´) primers as described by Couacy-Hymann et al. (2002).

The cDNA was prepared using Transcriptor First Strand cDNA Synthesis Kit (Roche, Inc.). Briefly, 5µl of RNA was mixed with 1 µl random hexanucleotide primers (600 pmole/µl), 4 µl 5x reaction buffer (8 mM Mgcl2), 0.5 µl RNase inhibitor (40 U/µl), 0.5 µl reverse transcriptase (20 U/µl), 2 µl 10 Mm dNTP mix, 7 µl nuclease free water and the reaction was carried out as per the manufacturer's instructions.

PCR reaction using RT-PCR kit (QIAGEN) were made as follows: including 2 µl of the cRNA as template: 5 µl of dNTP mixture (200 mM for each dNTP), 5 µl of 10× Taq buffer, 5 µl of primers mixture (5 pmoles/µl for each primer), 32 µl of water and 1 µl of Taq polymerase (1.25 U/µl), Thermal cycling conditions was then carried out as described by Couacy-Hymann et al. (2002).

Purified products were then sequenced using Big Dye-Terminator® v.3.1, AB (Applied Biosystems, USA) following the manufacturer's instructions.

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
1		100.0	100.0	99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	95.5	99.7	96.5	97.6	97.3	94.2	96.8	93.4	94.1	95.7	95.7	99.0	98.4	1	Saudi Arabia-2017-CP1105 (MG882501)
2	0.0		100.0	99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	95.5	99.7	96.5	97.6	97.3	94.2	96.8	93.4	94.1	95.7	95.7	99.0	98.4	2	Saudi Arabia-2017-CP1106 (MG882502)
3	0.0	0.0		99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	95.5	99.7	96.5	97.6	97.3	94.2	96.8	93.4	94.1	95.7	95.7	99.0	98.4	3	Saudi Arabia-2017-CP1107 (MG882503)
4	0.3	0.3	0.3		99.7	97.9	97.2	97.6	97.6	97.6	97.6	97.9	95.8	99.3	96.9	94.8	98.3	94.4	97.9	93.4	94.1	95.7	95.7	99.0	98.3	4	Saudi Arabia-2017-CP1108 (MG882504)
5	0.0	0.0	0.0	0.3		97.6	96.9	97.1	96.7	96.8	97.0	97.6	95.6	99.7	96.6	97.6	97.3	94.1	97.5	93.4	94.1	95.7	95.7	99.1	98.4	5	Saudi Arabia-2017-CP1109 (MG882505)
6	2.5	2.5	2.5	2.1	2.5		99.3	99.3	99.7	99.7	99.7	100.0	97.9	97.9	99.0	96.9	99.7	95.8	100.0	95.7	96.5	98.0	98.0	97.6	99.0	6	Saudi Arabia-2017-CP1110 (MG882506)
7	3.3	3.3	3.3	2.8	3.2	0.7		99.7	99.3	99.4	99.7	99.3	97.5	97.2	98.4	99.7	98.7	95.3	99.4	95.3	96.1	97.6	97.6	96.6	98.4	7	Saudi Arabia-2017-SH1109 (MG882507)
8	3.0	3.0	3.0	2.5	3.0	0.3	0.3		99.3	100.0	99.7	99.3	97.7	97.4	98.7	99.7	98.7	95.8	99.7	95.3	96.1	97.6	97.6	96.7	98.7	8	Saudi Arabia-2017-SH1110 (MG882508)
9	3.4	3.4	3.4	2.5	3.1	0.3	0.3	0.0		100.0	100.0	99.7	97.4	97.0	98.4	99.7	99.3	95.4	99.3	95.3	96.1	97.6	97.6	96.4	98.4	9	Saudi Arabia-2017-SH1111 (MG882509)
10	3.3	3.3	3.3	2.5	3.0	0.3	0.3	0.0	0.0		100.0	99.7	97.4	97.1	98.4	99.7	99.3	95.5	99.4	95.3	96.1	97.6	97.6	96.5	98.4	10	Saudi Arabia-2017-SH1112 (MG882510)
11	3.1	3.1	3.1	2.5	3.1	0.3	0.3	0.0	0.0	0.0		99.7	97.7	97.3	98.7	99.7	99.0	95.7	99.7	95.3	96.1	97.6	97.6	97.0	98.7	11	Saudi Arabia-2017-SH1113 (MG882511)
12	2.5	2.5	2.5	2.1	2.5	0.0	0.7	0.3	0.3	0.3	0.3		97.9	97.9	99.0	96.9	99.7	95.8	100.0	95.7	96.5	98.0	98.0	97.6	99.0	12	Saudi Arabia-2017-SH1114 (MG882512)
13	4.7	4.7	4.7	4.3	4.5	2.1	2.5	2.3	2.7	2.6	2.4	2.1		96.8	97.0	97.9	97.3	98.0	97.4	96.5	95.7	97.3	97.3	95.9	97.3	13	China-Tibet-0701 (Eu360596)
14	0.3	0.3	0.3	0.7	0.3	2.1	2.9	2.7	3.0	3.0	2.7	2.1	3.3		97.0	97.9	97.6	95.7	97.4	93.8	94.5	96.1	96.1	99.3	99.0	14	Culicoides-PPRV 02 (KU325483)
15	3.7	3.7	3.7	3.2	3.5	1.0	1.6	1.3	1.7	1.6	1.3	1.0	3.0	3.1		99.0	98.3	95.6	98.6	94.5	95.3	96.9	96.9	96.3	94.0	15	IR-ZHN336-11-2012 (JX898862)
16	2.5	2.5	2.5	2.2	2.5	0.0	0.3	0.3	0.3	0.3	0.3	0.0	2.1	2.1	1.1		99.0	95.8	100.0	95.7	96.5	98.0	98.0	97.6	99.3	16	Iraq-2017- L81 (LT882721)
17	2.8	2.8	2.8	1.8	2.4	0.3	1.0	0.7	0.7	0.7	0.7	0.3	2.8	2.4	1.7	0.4		95.3	99.3	95.3	96.1	97.6	97.6	97.3	99.0	17	Iraq-2017- N90 (LT882728)
18	6.1	6.1	6.1	5.9	6.3	4.3	4.9	4.4	4.8	4.7	4.5	4.3	2.1	4.5	4.7	4.4	5.0		95.7	93.4	94.1	95.7	95.7	94.7	96.0	18	Jhansi-2003 (GU014571)
19	2.6	2.6	2.6	2.1	2.6	0.0	0.6	0.3	0.3	0.3	0.3	0.0	2.6	2.6	1.4	0.0	0.3	4.5		95.7	96.5	98.0	98.0	96.9	98.6	19	Kurdistan-2011 (JF969755)
20	7.1	7.1	7.1	7.1	7.1	4.5	4.9	4.9	4.9	4.9	4.9	4.5	3.6	6.6	5.7	4.5	4.9	7.1	4.5		94.9	96.5	96.5	93.4	94.9	20	Pak_Faisalabad-2010 (JN009673)
21	6.2	6.2	6.2	6.2	6.2	3.6	4.1	4.1	4.1	4.1	4.1	3.6	4.5	5.8	4.9	3.6	4.1	6.2	3.6	5.3		96.9	96.9	94.1	95.7	21	Saudi Arabia-99-7 (DQ840195)
22	4.5	4.5	4.5	4.5	4.5	2.0	2.4	2.4	2.4	2.4	2.4	2.0	2.8	4.1	3.2	2.0	2.4	4.5	2.0	3.6	3.2		100.0	95.7	97.3	22	Saudi Arabia-99-8 (DQ840197)
23	4.5	4.5	4.5	4.5	4.5	2.0	2.4	2.4	2.4	2.4	2.4	2.0	2.8	4.1	3.2	2.0	2.4	4.5	2.0	3.6	3.2	0.0		95.7	97.3	23	Saudi Arabia-99-9 (DQ840-96)
24	1.0	1.0	1.0	1.1	0.9	2.5	3.5	3.4	3.7	3.7	3.1	2.5	4.3	0.7	3.8	2.5	2.8	5.6	3.2	7.1	6.2	4.5	4.5		98.5	24	TR-ANTALYA-2015-1506 (KU325492)
25	1.6	1.6	1.6	1.8	1.6	1.0	1.6	1.3	1.7	1.6	1.3	1.0	2.8	1.0	2.1	0.7	1.0	4.1	1.4	5.3	4.5	2.8	2.8	1.5		25	Turkey-2012- ADANA (JQ388616)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		

Divergence

Figure 3: *Percentage identity and divergence of HAIL PPRV with representative lineage IV strains retrieved from GenBank.*

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
1		100.0	100.0	99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	83.1	83.9	81.2	82.4	81.2	84.3	84.3	81.2	1	Saudi Arabia-2017-CP1105 (MG882501)
2	0.0		100.0	99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	83.1	83.9	81.2	82.4	81.2	84.3	84.3	81.2	2	Saudi Arabia-2017-CP1106 (MG882502)
3	0.0	0.0		99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	83.1	83.9	81.2	82.4	81.2	84.3	84.3	81.2	3	Saudi Arabia-2017-CP1107 (MG882503)
4	0.3	0.3	0.3		99.7	97.9	97.2	97.6	97.6	97.6	97.6	97.9	83.1	83.9	81.2	82.3	81.2	84.3	84.3	81.2	4	Saudi Arabia-2017-CP1108 (MG882504)
5	0.0	0.0	0.0	0.3		97.6	96.9	97.1	96.7	96.8	97.0	97.6	83.1	83.9	81.2	81.9	81.2	84.3	84.3	81.2	5	Saudi Arabia-2017-CP1109 (MG882505)
6	2.5	2.5	2.5	2.1	2.5		99.3	99.3	99.7	99.7	99.7	100.0	84.3	85.1	82.4	83.4	82.4	85.5	85.5	82.4	6	Saudi Arabia-2017-CP1110 (MG882506)
7	3.3	3.3	3.3	2.8	3.2	0.7		99.7	99.3	99.4	99.7	99.3	83.9	84.7	82.0	82.9	82.0	85.1	85.1	82.0	7	Saudi Arabia-2017-SH1109 (MG882507)
8	3.0	3.0	3.0	2.5	3.0	0.3	0.3		99.3	100.0	99.7	99.3	83.9	84.7	82.0	83.3	82.0	85.1	85.1	82.0	8	Saudi Arabia-2017-SH1110 (MG882508)
9	3.4	3.4	3.4	2.5	3.1	0.3	0.3	0.0		100.0	100.0	99.7	83.9	84.7	82.0	83.0	82.0	85.1	85.1	82.0	9	Saudi Arabia-2017-SH1111 (MG882509)
10	3.3	3.3	3.3	2.5	3.0	0.3	0.3	0.0	0.0		100.0	99.7	83.9	84.7	82.0	83.2	82.0	85.1	85.1	82.0	10	Saudi Arabia-2017-SH1112 (MG882510)
11	3.1	3.1	3.1	2.5	3.1	0.3	0.3	0.0	0.0	0.0		99.7	83.9	84.7	82.0	83.1	82.0	85.1	85.1	82.0	11	Saudi Arabia-2017-SH1113 (MG882511)
12	2.5	2.5	2.5	2.1	2.5	0.0	0.7	0.3	0.3	0.3	0.3		84.3	85.1	82.4	83.4	82.4	85.5	85.5	82.4	12	Saudi Arabia-2017-SH1114 (MG882512)
13	19.9	19.9	19.9	19.9	19.9	18.2	18.8	18.8	18.8	18.8	18.8	18.2		94.5	92.2	92.2	92.2	96.9	96.9	92.2	13	Ethiopia-94 (DQ840183)
14	18.9	18.9	18.9	18.9	18.9	17.3	17.8	17.8	17.8	17.8	17.8	17.3	5.8		91.8	91.8	91.8	96.5	96.5	91.8	14	Ethiopia-96 (DQ840175)
15	22.6	22.6	22.6	22.6	22.6	20.9	21.4	21.4	21.4	21.4	21.4	20.9	8.3	8.8		99.6	100.0	95.3	95.3	99.6	15	Oman 1983 (DQ840168)
16	20.9	20.9	20.9	21.0	21.6	19.5	20.2	19.5	20.1	19.7	19.9	19.5	8.3	8.8	0.4		99.6	95.3	95.3	100.0	16	Oman 1987 (JN647695)
17	22.6	22.6	22.6	22.6	22.6	20.9	21.4	21.4	21.4	21.4	21.4	20.9	8.3	8.8	0.0	0.4		95.3	95.3	99.6	17	Oman-Ibri-83 (DQ840168)
18	18.3	18.3	18.3	18.3	18.3	16.7	17.3	17.3	17.3	17.3	17.3	16.7	3.2	3.6	4.9	4.9	4.9		100.0	95.3	18	Sudan-Mielik-72 (DQ840159)
19	18.3	18.3	18.3	18.3	18.3	16.7	17.3	17.3	17.3	17.3	17.3	16.7	3.2	3.6	4.9	4.9	4.9	0.0		95.3	19	Sudan-Sinar-72 (DQ840158)
20	22.6	22.6	22.6	22.6	22.6	20.9	21.4	21.4	21.4	21.4	21.4	20.9	8.3	8.8	0.4	0.0	0.4	4.9	4.9		20	UAE-86-Dorcas (DQ840169)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		

Divergence

Figure 4: *Percentage identity and divergence of HAIL PPRV with representative lineage III strains retrieved from GenBank.*

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
1		100.0	100.0	99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	87.8	87.8	88.6	88.6	87.8	88.2	88.6	87.8	87.5	1	Saudi Arabia-2017-CP1105 (MG882501)
2	0.0		100.0	99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	87.8	87.8	88.6	88.6	87.8	88.2	88.6	87.8	87.5	2	Saudi Arabia-2017-CP1106 (MG882502)
3	0.0	0.0		99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	87.8	87.8	88.6	88.6	87.8	88.2	88.6	87.8	87.5	3	Saudi Arabia-2017-CP1107 (MG882503)
4	0.3	0.3	0.3		99.7	97.9	97.2	97.6	97.6	97.6	97.6	97.9	87.8	87.8	88.6	88.6	87.8	88.2	88.6	87.8	87.5	4	Saudi Arabia-2017-CP1108 (MG882504)
5	0.0	0.0	0.0	0.3		97.6	96.9	97.1	96.7	96.8	97.0	97.6	87.8	87.8	88.6	88.6	87.8	88.2	88.6	87.8	87.5	5	Saudi Arabia-2017-CP1109 (MG882505)
6	2.5	2.5	2.5	2.1	2.5		99.3	99.3	99.7	99.7	99.7	100.0	88.6	88.6	90.2	90.2	88.6	89.8	90.2	90.2	89.8	6	Saudi Arabia-2017-CP1110 (MG882506)
7	3.3	3.3	3.3	2.8	3.2	0.7		99.7	99.3	99.4	99.7	99.3	88.2	88.2	89.8	89.8	88.2	89.4	89.8	89.8	89.4	7	Saudi Arabia-2017-SH1109 (MG882507)
8	3.0	3.0	3.0	2.5	3.0	0.3	0.3		99.3	100.0	99.7	99.3	88.2	88.2	89.8	89.8	88.2	89.4	89.8	89.8	89.4	8	Saudi Arabia-2017-SH1110 (MG882508)
9	3.4	3.4	3.4	2.5	3.1	0.3	0.3	0.0		100.0	100.0	99.7	88.2	88.2	89.8	89.8	88.2	89.4	89.8	89.8	89.4	9	Saudi Arabia-2017-SH1111 (MG882509)
10	3.3	3.3	3.3	2.5	3.0	0.3	0.3	0.0	0.0		100.0	99.7	88.2	88.2	89.8	89.8	88.2	89.4	89.8	89.8	89.4	10	Saudi Arabia-2017-SH1112 (MG882510)
11	3.1	3.1	3.1	2.5	3.1	0.3	0.3	0.0	0.0	0.0		99.7	88.2	88.2	89.8	89.8	88.2	89.4	89.8	89.8	89.4	11	Saudi Arabia-2017-SH1113 (MG882511)
12	2.5	2.5	2.5	2.1	2.5	0.0	0.7	0.3	0.3	0.3	0.3		88.6	88.6	90.2	90.2	88.6	89.8	90.2	90.2	89.8	12	Saudi Arabia-2017-SH1114 (MG882512)
13	13.8	13.8	13.8	13.8	13.8	12.7	13.2	13.2	13.2	13.2	13.2	12.7		100.0	94.9	94.9	100.0	97.6	97.3	94.1	93.7	13	Ghana-Accra-76 (840163)
14	13.8	13.8	13.8	13.8	13.8	12.7	13.2	13.2	13.2	13.2	13.2	12.7	0.0		94.9	94.9	100.0	97.6	97.3	94.1	93.7	14	Ghana-Accra-78 (840167)
15	12.8	12.8	12.8	12.8	12.8	10.8	11.3	11.3	11.3	11.3	11.3	10.8	5.4	5.4		100.0	94.9	96.5	96.1	97.6	97.3	15	Mali-99-1 (DQ840192)
16	12.8	12.8	12.8	12.8	12.8	10.8	11.3	11.3	11.3	11.3	11.3	10.8	5.4	5.4	0.0		94.9	96.5	96.1	97.6	97.3	16	Mali-99-366 (DQ840193)
17	13.8	13.8	13.8	13.8	13.8	12.7	13.2	13.2	13.2	13.2	13.2	12.7	0.0	0.0	5.4	5.4		97.6	97.3	94.1	93.7	17	Nigeria 76-1 (DQ840164)
18	13.3	13.3	13.3	13.3	13.3	11.3	11.8	11.8	11.8	11.8	11.8	11.3	2.4	2.4	3.7	3.7	2.4		99.6	95.7	95.3	18	Nigeria-75-2 (DQ840161)
19	12.8	12.8	12.8	12.8	12.8	10.8	11.3	11.3	11.3	11.3	11.3	10.8	2.8	2.8	4.1	4.1	2.8	0.4		95.3	94.9	19	Nigeria-75-3 (DQ840162)
20	13.8	13.8	13.8	13.8	13.8	10.8	11.3	11.3	11.3	11.3	11.3	10.8	6.2	6.2	2.4	2.4	6.2	4.5	4.9		99.6	20	Senegal10 (HQ131963)
21	14.3	14.3	14.3	14.3	14.3	11.3	11.8	11.8	11.8	11.8	11.8	11.3	6.7	6.7	2.8	2.8	6.7	4.9	5.4	0.4		21	SierraLeone 2009 (JN602080)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		

Divergence

Figure 5: *Percentage identity and divergence of HAIL PPRV with representative lineage II strains retrieved from GenBank.*

Links Researchers

Percent Identity

Divergence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
1		100.0	100.0	99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	87.5	88.2	86.3	87.8	88.2	1	Saudi Arabia-2017-CP1105 (MG882501)
2	0.0		100.0	99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	87.5	88.2	86.3	87.8	88.2	2	Saudi Arabia-2017-CP1106 (MG882502)
3	0.0	0.0		99.7	99.4	97.6	96.2	97.1	96.7	96.8	97.0	97.6	87.5	88.2	86.3	87.8	88.2	3	Saudi Arabia-2017-CP1107 (MG882503)
4	0.3	0.3	0.3		99.7	97.9	97.2	97.6	97.6	97.6	97.6	97.9	87.5	88.2	86.3	87.8	88.2	4	Saudi Arabia-2017-CP1108 (MG882504)
5	0.0	0.0	0.0	0.3		97.6	96.9	97.1	96.7	96.8	97.0	97.6	87.5	88.2	86.3	87.8	88.2	5	Saudi Arabia-2017-CP1109 (MG882505)
6	2.5	2.5	2.5	2.1	2.5		99.3	99.3	99.7	99.7	99.7	100.0	87.1	87.8	86.7	87.5	88.6	6	Saudi Arabia-2017-CP1110 (MG882506)
7	3.3	3.3	3.3	2.8	3.2	0.7		99.7	99.3	99.4	99.7	99.3	87.5	88.2	87.1	87.8	89.0	7	Saudi Arabia-2017-SH1109 (MG882507)
8	3.0	3.0	3.0	2.5	3.0	0.3	0.3		99.3	100.0	99.7	99.3	87.5	88.2	87.1	87.8	89.0	8	Saudi Arabia-2017-SH1110 (MG882508)
9	3.4	3.4	3.4	2.5	3.1	0.3	0.3	0.0		100.0	100.0	99.7	87.5	88.2	87.1	87.8	89.0	9	Saudi Arabia-2017-SH1111 (MG882509)
10	3.3	3.3	3.3	2.5	3.0	0.3	0.3	0.0	0.0		100.0	99.7	87.5	88.2	87.1	87.8	89.0	10	Saudi Arabia-2017-SH1112 (MG882510)
11	3.1	3.1	3.1	2.5	3.1	0.3	0.3	0.0	0.0	0.0		99.7	87.5	88.2	87.1	87.8	89.0	11	Saudi Arabia-2017-SH1113 (MG882511)
12	2.5	2.5	2.5	2.1	2.5	0.0	0.7	0.3	0.3	0.3	0.3		87.1	87.8	86.7	87.5	88.6	12	Saudi Arabia-2017-SH1114 (MG882512)
13	14.2	14.2	14.2	14.2	14.2	14.7	14.2	14.2	14.2	14.2	14.2	14.7		96.9	94.9	96.5	93.7	13	Cote devoir-CI-89 (DQ840199)
14	13.1	13.1	13.1	13.1	13.1	13.6	13.1	13.1	13.1	13.1	13.1	13.6	3.2		96.1	99.6	94.5	14	Guinea 89 (DQ840171)
15	15.7	15.7	15.7	15.7	15.7	15.2	14.7	14.7	14.7	14.7	14.7	15.2	5.3	4.1		95.7	93.3	15	Guinea-88 (DQ840170)
16	13.6	13.6	13.6	13.6	13.6	14.2	13.6	13.6	13.6	13.6	13.6	14.2	3.6	0.4	4.5		94.1	16	Senegal 94 (DQ840174)
17	13.2	13.2	13.2	13.2	13.2	12.7	12.2	12.2	12.2	12.2	12.2	12.7	6.6	5.8	7.1	6.2		17	Senegal-68 (DQ840165)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		

Figure 6: *Percentage identity and divergence of HAIL PPRV with representative lineage I strains retrieved from GenBank.*

Sequence analysis

Obtained sequences were edited and assembled using EditSeq and SeqMan within Lasergene (version 7.1.0, DNASTAR,Inc,Madison,WI,USA). Sequence alignment was performed using ClustalW, neighbor-joining method to determine the phylogenetic relationship with sequences retrieved from GenBank using BLASTn tool (https://blast.ncbi.nlm.nih.gov4/Blast).

Results

Detection of PPRV RNA

PPRV RNA was identified in all samples by rtRT-PCR. In contrast, N gene was amplified and sequenced from sheep and goat's swabs (n=12). Sequences were submitted to the GenBank and are available under accession numbers MG882501-MG882512.

Sequence analysis

HAIL strains sequences displayed multiple nucleotide substitutions (Figure 1), nucleotide identity among sequences is 96.2-100% (Figure 2), while identity with previously sequenced Saudi Arabia strains and reference PPRV retrieved from GenBank was 94.1-98% and 81.2-100%, respectively (Figure 3,4,5). Phylogenetic tree showed that local strains clustered in four different groups within lineage IV, sequenced viruses clustered in separate groups when compared with previously identified Saudi Arabia strains (Figure 7).

Discussion

Several outbreaks of PPR in Hail district were reported based on clinical picture, antigen or antibody detection (Mahmoud et al., 2016, 2017). However, literature shown that analysis of N gene improves the image of PPRV epidemiology and is considered better than F gene-based distribution of PPRV strains (Shaila et al., 1996; Banyard et al., 2010). PPRV was identified in all samples, but when amplified by RT-PCR only sheep and goats were positive, camel samples were negative, which may be attributed to variable sensitivity and/or specificity. Therefore, routine diagnostic tests must be validated.

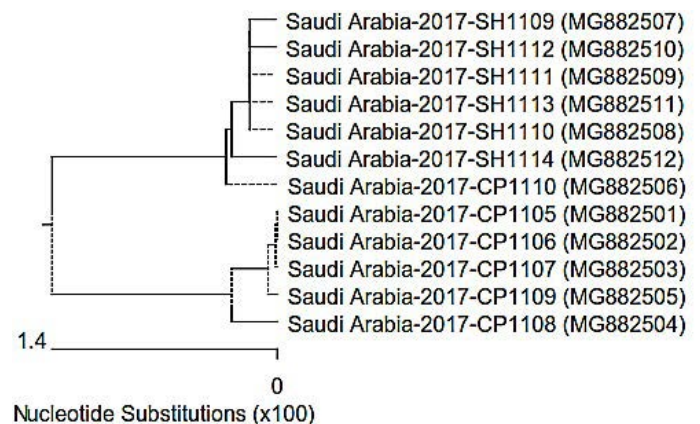


Figure 7: *Phylogenetic analysis of PPRV identified from outbreaks occurred in Hail district. Saudi Arabia.*

Sequence analysis identified four PPRV genotypes circulating in the region, Hail strains differ from previously reported viruses in the country, phylogenetic tree showed that Hail PPRV clustered in four different places within lineage IV which may be due to introduction of new strains by importing live infected animals or the evolution of the wild type viruses. Large-scale studies involving analysis of viral genomes collected from different outbreaks will help to understand the nature of the circulating lineage.

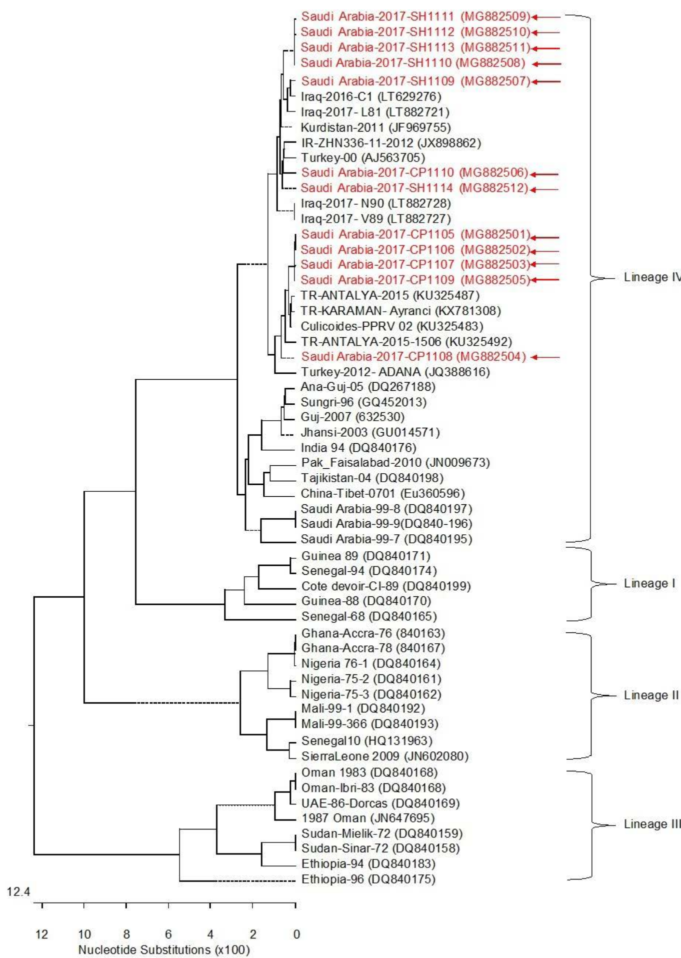


Figure 8: *Phylogenetic homology tree based on multiple sequence alignments of variable region of the N gene sequences from HAIL PPRV compared to previously sequenced strains representing all lineages, local strains are written in red and marked with arrows.*

Conclusion

Conventional and real time PCR were used for identification of PPRV, which varied in sensitivity and/or specificity. Analysis of partial sequence of PPRV-N gene revealed multiple nucleotide substitutions among Hail strains. Phylogenetic tree clustered local PPRV strains in four separate groups within lineage IV. Molecular characterization of PPRV strains in emerging outbreaks will help to improve prevention and control strategies to ultimately eradicate the disease in 2030 from the globe.

Author Contributions

AZ collected samples, performed RNA extraction and amplification, participated in the design of the study. MM participated in the design of the study, lab work, sequencing and analyses and draft the manuscript. MA participated in the design of the study and revised the manuscript.

References

- Abu Elzein, E.M.E., Hassanien, M.M., Alafaleq, A.I., Abdelhadi, M.A. and Housawi, F.M.T. 1990. Isolation of PPR virus from goats in Saudi Arabia. Vet. Rec. 127: 309–310.
- AL-Afaleq, A., Abu-Elzein, E., Al-Naeem, A. and Mahmoud. 2004. Sero surveillance for peste des petits ruminants (PPR) and rinderpest antibodies in naturally exposed Saudi sheep and goats. Veterinarski Arhiv. 74 (6): 459–465.
- Bailey, D., Banyard, A., Dash, P., Ozkul, A. and Barrett, T. 2005. Full genome sequence of peste de petite ruminants virus, a member of morbillivirus genus. Virus Res. 110 (1-2): 119–124. https://doi.org/10.1016/j.virusres.2005.01.013
- Banyard, A.C., Parida, S., Batten, C., Oura, C., Kwiatek, O. and Libeau, G. 2010. Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. J. Gen. Virol. 91: 2885–2897. https://doi.org/10.1099/vir.0.025841-0
- Baron, M.D., Diallo, A., Lancelot, R. and Libeau, G. 2016. Peste des petits ruminants virus. Adv. Virus Res. 95: 1–42. https://doi.org/10.1016/bs.aivir.2016.02.001
- Boshra, H., Truong, T., Babiuk, S. and Hemida, M.G. 2015. Seroprevalence of Sheep and Goat Pox, Peste Des Petits Ruminants and Rift Valley Fever in Saudi Arabia. PLOS ONE. 13: 1–12. https://doi.org/10.1371/journal.pone.0140328
- Couacy-Hymann, E., Roger, F., Hurard, C., Guillou, J.P., Libeau, G. and Diallo, A. 2002. Rapid and sensitive detection of peste des petits ruminants virus by a polymerase chain reaction assay. J. Virol. Meth. 100: 17–25. https://doi.org/10.1016/S0166-0934(01)00386-X
- Elzein, E.M., Housawi, F.M., Bashareek, Y., Gameel, A.A., Al-Afaleg, A.I. and Anderson, E. 2004. Severe PPR infection in gazelles kept under semi-free range conditions. J. Vet. Med. B, Infect. Dis. Vet. Public Health. 51(2): 68–71. https://doi.org/10.1111/j.1439-0450.2004.00731.x
- FAO, 2016. World Organisation for Animal Health. Peste des petits ruminants global eradication programme: contributing to food security, poverty alleviation and resilience. Five years (2017–2021). Food and Agriculture Organization of the United Nations. Rome, Italy: http://www.fao.org/3/a-i6316e.pdf.

- Gibbs, E.P., Taylor, W.P., Lawman, M.J. and Bryant, J. 1979. Classification of peste des petits ruminants virus as the fourth member of the genus Morbillivirus. Intervirol. 11(5): 268–274. https://doi.org/10.1159/000149044
- Housawi, F., Abu Elzein, E., Mohamed, G., Gameel, A., Al-Afaleq, A., Hegazi, A. and Al-Bishr, B. 2004. Emergence of Peste des Petits Ruminants in Sheep and Goats in Eastern Saudi Arabia. Revue d'élevage et de médecine vétérinaire des pays tropicaux. 57(1-2): 31–34. https://doi.org/10.19182/remvt.9901
- Kwiatek, O., Mint, C., Grillet, C., Hurard, C., Carlsson, E., Karimov, B., Albina, E., Diallo, A. and Libeau, G. 2007. Peste des petits ruminants (PPR) outbreak in Tajikistan. J. Comp. Pathol. 136: 111–119. https://doi.org/10.1016/j.jcpa.2006.12.002
- Mahmoud, A.Z., Abdellatif, M. and Abdalla, A. 2017. High seroprevalence of PPRV-antibodies among sheep and goats in Hail, Saudi Arabia. Vet. Sci. Res. Rev. 3(1): 1–5. https://doi.org/10.17582/journal.vsrr/2017.3.1.1.5
- Mahmoud, A.Z.E., Abdellatif, M. and Abdalla. M.A. 2017. Outbreaks of PPR-FMD among sheep and goats in Hail, Saudi Arabia. Veterinary Sciences: Res. Rev. 3(2): 38–44. https://doi.org/10.17582/journal.vsrr/2017/3.2.38.44
- Mahmoud, A.Z.E., Abdellatif, M.M. and Abdalla, M.A. 2016. Prevalence of PPR-virus antibodies in sheep, goats and camels in Hail, Saudi Arabia. Br. J. Virol. 3(3s): 86–89. https://doi.org/10.17582/journal.bjv/2016.3.3s.86.89
- Munir, M., Zohari, S. and Berg, M. 2013. Molecular biology and pathogenesis of Peste des Petits ruminants virus. Springer Briefs Anim. Sci. 151.
- Shaila, M.S., Shamaki, D., Forsyth, M.A., Diallo, A., Goatley, L., Kitching, R.P. and Barrett, T. 1996. Geographic distribution and epidemiology of peste des petits ruminants virus. Virus Res. 43(2): 149–153. https://doi.org/10.1016/0168-1702(96)01312-3