

Peste Des Petits Ruminants: A First Retrospective Investigation Among Susceptible Animal Species in Qatar

Mohamed Haroun (✉ mismail_99@yahoo.com)

Ministry of Public Health Qatar <https://orcid.org/0000-0002-1234-8034>

Nawal M. Abdulla

Ministry of Municipality and Environment

Mohammed Habeb

Ministry of Municipality and Environment

Elmoubasher A. Farag

Ministry of Public Health Qatar

Research Article

Keywords: Peste des petits ruminants, Retrospective study, Qatar

Posted Date: April 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-371540/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

A retrospective study was conducted to investigate the prevalence of peste des petits ruminants (PPR) in Qatar. Three hundred sixty-eight blood, swabs, and organ tissue samples collected between 2009 and 2016 were screened for PPR viral antibodies, antigens and nucleic acids using ELISA-Ab, ELISA-Ag and rRT-PCR, respectively. Fifteen PPR positive samples were subjected to virus isolation using Vero cell lines. 52% (n=192) of the samples were shown positive for PPR reporting first time infection of 52% (n=71) animal species including sheep, goat, deer, gazelle, addax, Oryx, blackbuck, deer, springbuck and waterbuck. Eight PPR virus (PPRV) field isolates demonstrated classical PPRV cytopathic effect (CPE) and shown positive for the virus antigens proving finally virus isolation. Sheep had the highest infection rate (55%) followed by wild ruminants (54%) and goats (47%). History wise, PPR might exist in Qatar before 2009. A systematic investigation is recommended to identify the risk factors associated with exposure of the susceptible animals to PPR infection, to test the susceptibility of the different species to PPR infection, and to describe the molecular entity and the replicative potentiality of the circulating field strains.

Introduction

Peste des petits ruminants (PPR) is a highly infectious viral disease of domesticated small ruminants (Gibbs et al., 1979) characterized by various clinical manifestations of respiratory and digestive nature (Libeau et al., 2014) which are mostly accompanied by ulcerative inflammations and high morbidity and mortality rates (Hammouchi et al., 2012).

The etiology of the disease PPR virus (PPRV) belongs to the genus *Morbillivirus* under the family *Praramyxoviridae* (Adams et al., 2017). The virus is enveloped, non-segmented, negative sense and single-stranded. The genomes of different isolates are of variable lengths (Chard et al., 2008). However, isolates that have genes organized in the order 3' N-P/C/V-M-F-H-L 5' code for 6 structural and 2 non-structural viral proteins (Bailey et al., 2005). Transmission of the virus is through the respiratory route. Infection is initiated by the surface spike-like H and F structural glycoproteins to induce virus attachment and fusion, respectively (Diallo, 1990). With the aid of the signaling activation lymphocyte molecules (SLAM, CD150) and Nectin-4 (PVRL-4) surface receptors, PPRV exploits the lymphatic and epithelial pathways to establish in vivo or in vitro replication (Pawar et al., 2008; Birch et al., 2013). However, recently, a third endothelial endocytosis pathway was described where the virus replicates potentially to deregulate 146 genes 1-hour post viral attachment and entry (Yang et al., 2018). Distinct from the other members of the group, PPRV is classified with rinderpest virus, dolphin morbillivirus, canine distemper virus, phocid distemper virus, porpoise morbillivirus and human measles morbillivirus (Barrett et al., 1991).

Primarily infecting sheep and goats, several infections and outbreaks were also reported among other host species. Infections among wild ungulates were early observed by Furley et al., 1987; Elzein et al., 2004; Kinne et al., 2010; Abubakar et al., 2011 and Bao et al., 2011. Recent outbreaks in Sagia and naïve

unknown PPR-infecting wild ruminants were also reported (Privot et al., 2020). Apparently healthy ruminants including Oryx (Frölich et al, 2005), cattle (Anderson and McKay, 1994, El Amin and Hassan, 1999; Haroun et al., 2002; Herzog et al., 2019; Rasooli et al., 2019), buffaloes (Govindarajan et al., 1997; Balamurugan et al., 2014), camels (Ismail et al, 1992; Roger et al., 2001; Haroun et al., 2002, Abraham et al., 2005) and yaks (Li et al., 2018) were also shown positive for blood serum PPRV antibodies (Ab) suggesting natural exposure of these animals to circulating PPRV field strains. Several studies have demonstrated retrieval of the virus RNA from tissues of clinically PPR-suspected one-humped camels (*Camelus dromedaries*) assuming natural exposure to the virus (Khalafalla et al., 2010; Kwiatek et al., 2011; Albina et al., 2013; Zakian et al., 2016, Omani et al., 2018). Records also indicated detection of viral nucleic acid among unnatural hosts including dogs (Ratta et al., 2016); Asiatic lion (Balamurugan et al., 2012) and biting midges (Sevik and Oz, 2015) were also evidenced. Successful establishment of clinical and subclinical infections were also reported among experimentally PPRV-infected animals including camels (Fakri et al., 2018), cattle (Couacy-Hymann et al., 2007a; Sen et al., 2014) and pigs (Nawathe and Taylor, 1979; Schultz et al., 2018). In vitro, PPRV isolates have shown to have high affinity to potentially replicate into many cell cultures and cell lines including lamb kidney cell culture (Begum et al, 2020), Vero cell lines (Kumar et al., 2016), Marmoset B95a cell lines (Sreenivasa et al., 2006), Monkey CV1 cell lines (Adombi et al., 2011), and murine 10T1/2 cell lines (Comerlato et al., 2020). However, with a characteristic to only support replication of virulent isolates, 10T1/2 is recommended a tool to differentiate attenuated strains from virulent isolates (Comerlato et al., 2020).

Recognized single serotype in nature, molecular phylogenetic analysis of the small region of the viral N/F gene identified four worldwide distinct PPRV genetic lineages (Banyard and Parida, 2015); lineage I and II in West Africa, lineage III in Arabia and East Africa and lineage IV in Asia and the Middle East (Dhar et al., 2000, Kinne et al, 2010), all of which were diverged from the West African ancestor (Padhi and Ma, 2014). This finding purports to facilitate the geographic speciation of the disease in general and the rapid spread of lineage IV across Asia and Africa. Respected originating in West Africa and endemic in South Asia, the first known breakthrough of PPR to European Turkey was in 2016 (Banyard et al., 2010). Shortly after occurrence of the disease in Turkey in 2018 nearby the European Union borders, PPR was reported in Bulgaria in 2018 recording the first occurrence of the disease in the European Union (Altan et al., 2018). In the Middle East, PPR was reported in most of the Arabian Peninsula countries including Oman (Hedger et al., 1980; Taylor et al., 1990), the United Arab Emirates (Furley et al., 1987) and Saudi Arabia (Abu-Elzein et al., 1990; Elzein et al., 2004; Al-Dubaib, 2009). Despite no conventional or molecular evidences indicating existence of the disease in Qatar, serum anti-PPRV antibodies were repeatedly detected among clinically suspected animals (DAR, 2013).

In Qatar, the total population of PPR susceptible animal species is estimated to be 1,777, 207 (DAR, 2021), 62% (n= 1,115,293) of which are sheep, 26% (n= 464,980) goats, 8% (n=135,976) camels, 3% cattle (n=45958) and 1% (n= 15,000) are wild ruminants. Due to the limited grazing areas in the country, most of the animal species are raised within mixed closures of open farming system, however, each occupies a separate unit. Of the total wild ruminants species, Oryx represents 82% (n=12,400) of the species. These are kept into 3 different separate natural reservation units supervised by the governmental Wild

Ruminants Reservation Authority. The few reminders and the rest of the wild ruminants species are owned by the private sectors and kept in the same manner described for the other animal species.

Recognized animal food importing country, the quarantine veterinary section at of the veterinary authority in Qatar is routinely checking the vaccination certification upon importation from PPR suspected or endemic countries. As a practice, the animal health section used to vaccinate susceptible animals against PPR using the live attenuated PPRV 75-1 Nigerian strain. Usually vaccinations are performed at the discretion of the animal owner, complementation with the animal movement regulations or response to a reported regional PPR outbreak. However, the first incident of PPR in Qatar was reported by Banyard and Parida, 2015; based on viral RNA that had been retrieved from samples collected in 2010 from goats and deer. Nonetheless, to date, there is no single official national or international declaration indicating existence of PPR in Qatar.

With respect to the recent global movement towards preventing, controlling and eradicating PPR, there is a crucial need to raise the global awareness about the disease benefiting from the regulations set forth in the national feedback platforms (FAO, 2015a). Taking this into consideration, it is obvious that there are huge gaps in knowledge concerning the epidemiology of the PPR in Qatar. These gaps include the following questions: 1. Does PPR exist in Qatar? 2. And if so, what is the extent of the disease perpetuation among the susceptible domesticated and captivated wildlife hosts? 3. What are the lineages of the viruses that are circulating among the susceptible animals? 3. And finally, what are the possible factors that are potentially influencing the disease to perpetuate among these animals?

Since PPR is on the World Organization for Animal Health (OIE) list of the notifiable diseases, and it's currently a global threat to animal industry, it needs more attention. The aforementioned setbacks observed in Qatar coupled with the animal species existing in the country, the rearing pattern practiced in the country with the absence of previous vaccination records; have promoted the authors to conduct a first retrospective investigation about the prevalence of the disease. Answers given to the previously raised questions would expect to offer baseline data on the disease and help establishment of an effective sustainable preventive and control programmes.

Materials And Methods

Samples: All samples, which represented the bulk of the material available at the Veterinary Laboratory (VL) facility, Qatar, and examined suitable for testing at the time of the study, were used in this investigation. Three hundred sixty-eight whole blood, blood sera, ocular and nasal swabs, and organ tissues, collected between 2009 and 2016 and stored between -40°C and -80°C were used for this retrospective investigation. The samples were derived from sheep, goats and wild ruminants, which had been examined by the relevant veterinary clinics (VC) upon suspecting PPR and/or foot-and-mouth disease (FMD) and blue tongue (BT). Clinical data of the samples and the putative factors that might account for the risks of exposure of these animals to PPR including duration of the sample acquisition, vaccination record, animal focal point (FP) and VC were obtained from each relevant sample submission

sheet and recorded. Procedures, which had been followed to submit the samples from the VC to the VL, were traced. Briefly, each sample package had been received by the Sample Registration Office (SRO), checked for quality and data relevance before submission to the designated diagnostic unit. Necropsy samples and the relevant data, which had been collected at the VL by the pathologist were submitted to the SRO before redirected to the diagnostic units. Whole blood and blood serum samples, which had been obtained from the life or necropsied animals, were collected each into 5 mL to 8 mL volume by venipuncture of the external jugular vein or by heart puncture, respectively. Blood sera obtained into 2 mL aliquots had been prepared in the relevant VL facility following overnight standing between 18°C and 26°C for coagulation before centrifuged at 800 g for 10min at 10°C.

Enzyme-linked immunosorbent antibody: The PPRC VER1209 GB competitive enzyme-linked immunosorbent assay (cELISA) kit was used to determine the anti-PPRV Ab in each 25- μ L-serum sample following the manufacturer's instructions (IDvet Innovative Diagnostic Kits, Grabels, France). An in-lab pre-prepared and validated internal positive quality control serum was included in each run. Optical densities were obtained using BioTek ELx808 spectrophotometric microplate ELISA reader aided with a 450 nm WL filter. Results were interpreted using Gen5.5 software version and a validated ELISA-Ab spreadsheet results interpretation calculator.

Enzyme-linked immunosorbent antigen: The PPRAG 0313-506 VER0313 GB capture enzyme-linked immunosorbent assay (capELISA) was used to determine the PPR viral Ag in each swab and organ tissue collected for this purpose. About 10% of the viral swab elution and tissue homogenate concentration were used to test each sample following the manufacturer's instructions (IDvet Innovative Diagnostic Kits, Grabels, France). Optical densities were obtained using BioTek ELx808 spectrophotometric microplate ELISA reader aided with a 450 nm WL filter. Results were interpreted using Gen5.5 software version and a validated ELISA-Ab spreadsheet results interpretation calculator.

Determination of PPRV cDNA

Viral RNA extraction: Swab elution and blood samples (140 μ L each) were used for to extract the PPRV RNA using the QIAamp Viral RNA Mini 2010 methodology. About 25-30 mg of each previously collected organ tissue was used to purify the viral RNA following the Qiagen RNeasy® Mini Kit instructions (Qiagen, Hilden, Germany). Finally, each viral RNA extract was obtained in 80- μ L amounts using the kit AVE buffer (Qiagen, Hilden, Germany) as elution buffer. All aliquots were stored at - 20°C till further processing.

Real-time polymerase chain reaction (rRT-PCR): The single-step qualitative real-time reverse transcription polymerase chain reaction (rRT-PCR) described by Qiagen, GmbH, Germany, 2011 was used to detect the PPRV RNA following the manufacturer's instructions. About 100 ng (10 μ L) each RNA template extract was used for amplification to obtain the viral cDNA. Positive and negative controls were treated the same using an approximation of 0.1% PPR positive control preparation and nuclease-free water (NTC), respectively. Using ABI 7300 Real Time Cycler, a program of 48 °C for 10 min (RT step), 95 °C for 10 min

(RT inactivation/denaturation), 40 cycles of 95 °C for 15 s (denaturation) and 60 °C for 45 s (annealing/extension), respectively, was used for amplification of the obtained cDNA fragments.

Virus isolation

Cell culture: The African green monkey (Vero) cell line (American Type Culture Collection, Manassas, VA) cultured at 70-80% semi-confluent monolayers in 25 cm³ cell culture flasks (Corning) was used for cultivation of the PPRV strains. Cell lines maintenance was performed by continuous passage into 25-cm³ cell culture flasks using 4.5 g/L glucose, L-glutamine, sodium pyruvate and NaHCO₃ enriched Dulbecco's modified Eagles medium (DMEM) (Lonza, USA) supplemented with 10% bovine calf serum (BCS), 25 µL/mL 1M HEPES (Lonza, USA), 100 iu/mL penicillin, 10 µg/mL streptomycin and 20 µg/mL fungustatin.

Samples selection and preparation: Based on the chronology of the date of sample acquisition, sample quality, nature of the clinical and necropsy findings and positivity to ELISA-Ag and/or rRT-PCR, 15 tissue samples representing 8 species were selected for virus isolation into Vero cell lines. 10% homogenate concentrations were prepared using sterile phosphate-buffered saline (PBS). All samples underwent filtration using sterile 0.2 µm Millipore filters. Each final 2 mL sample aliquot was supplemented with 100 iu/mL penicillin, 20 µg/mL streptomycin and 20 µg/mL gentamycin, incubated for 1 h at room temperature, and preserved at 4°C before inoculation.

Virus cultivation: Each 500 µL pre-prepared PPRV inoculum was inoculated onto 4-day-old 70-80% semi-confluent 25-cm³ Vero cell line following thrice washing of the cells with 5 mL PBS. All infected cultures were incubated for 1 h at 37°C into CO₂-free cell culture grade incubator. Control flasks received serum-free DMEM were treated the same. Following this, each inoculum was removed from the infected and control flasks. Finally, each flask received 5 mL 2% BCS-supplemented DMEM. All flasks were incubated back at 37°C and guarded daily for change in media colour and development of cytopathic effect (CPE).

Results validation

Samples from animals have had classical clinical signs and/or typical necropsy features and detected positive for PPRV Ag and PPRV RNA or succeeded virus isolation were considered PPR infected. Moreover, samples from animals have had classical clinical signs and/or typical necropsy features and detected positive for PPRV Ab were considered PPR infected. Samples from animals with no clinical features or atypical PPR clinical signs and/or postmortem findings and detected positive or negative for PPRV Ab were considered PPR negative.

Statistical analysis

The proportion of the PPR samples considered positive for the PPR infection and indicated for the positive animals was calculated out of the total PPR-suspected samples. Previous records of the putative factors that might represent risks exacerbating exposure to the PPR-infected cases were evaluated. The

significance of association of each factor to PPR infection was evaluated as the univariate logistic regression. All significances were considered at type I error of $\alpha < 0.10$.

Results

Detection of anti-PPRV Ab: 56% (n=14) serum samples were found positive for PPR Ab (Table 1).

Detection of PPR viral Ag: 100% (n=12) initially rRT-PCR-tested or later cultured samples were demonstrated positive for PPRV Ag (Table 1).

Detection of PPR viral RNA: 51% (n=170) molecularly tested samples retrieved viral RNA fragments (Table 1). Except to samples from blackbuck (PPRV-QBB14), all rRT-PCR results were on cohort with the viral isolation findings.

Virus isolation: All Vero CL-cultured samples demonstrated typical PPRV CPE (Figure 1) and considered PPRV field isolates upon subjection to ELISA viral Ag detection. Eight fields strains were identified from samples belonging to sheep (PPRV-QS09), gazelle (PPRV-QG09, PPRV-QG10-1, PPRV-QG10-2, and PPRV-QG10-3), Addax (PPRV-QA11), Oryx (PPRV-QO14), and blackbuck (PPRV-QBB14).

Study results: The 368-subject samples were collected from 135 PPR-suspected cases, 31 of which were sheep, 36 goats and 86 were wild ruminants (Table 2). Initial screening to detect PPR Ab, Ag, nucleic acid or to isolate the virus has resulted in 57% (n= 211) positive PPR samples; 47% sample from sheep, 61% from goats and 51% from wild ruminants. Validations of the results qualified 52% (n=192) of the screened samples positive for PPR infection and were used to identify the relevant PPR-infected animals (Table 2). 52% (n=71) of the animals were considered infected with the field PPRV strains, 54% (n=17) were sheep, 47% (n=17) goats and 54% (n=37) were wild ruminants. Including sheep and goats, 7 wild ruminant animal species namely; deer, gazelle, addax, Oryx, blackbuck, springbuck and waterbuck (data not shown) were considered positive for PPR infection.

Clinically, 64% (n=45) of the infected animals have had classical infection, 4% (n=3) sudden death and 32% (n=23) non-specific. Of the 59 necropsied PPR-infected animals 13 (22%) were goats, 17 (29%) sheep and 29 (49%) were wild ruminants. 2% (n=1) have had respiratory lesions, 3% (n=2) digestive, 63% (n=37) respiratory-digestive and 32% (n=19) generalized features. Sheep and wild ruminants have had profound respiratory clinical feature compared to goats.

54% (n=38) of the infected cases were reported in summer, 28% (n=20) in winter and 18% (n=13) in spring. All infected cases were distributed over 48 animal focal points, 7 VC and 7 municipalities. 8% (n=4) of the focal points repeatedly reported PPR infection throughout the period of the sample collection (data not shown).

Discussion

In this study, conventional and molecular assays were used to retrospectively investigate the prevalence of PPR in Qatar using samples collected from animals that have been raised between 2009 and 2016. The initial findings confirmed existence of PPR in Qatar with a rate of 52% of the investigated animals being infected with the virus. Further, that 9 different animal species were shown infected with circulating PPRV field isolates reflects the extent of the perpetuation of the disease among the subject animals and assumes endemic status of PPR in Qatar. This observation could be inferred from the facts that: 1. There had been continuous infections throughout the years of collection of the samples. 2. There had been repeated occurrence of PPR infection in 8% of the investigated focal points. 3. And, there had been continuous exposure to the virus verified by seropositivity of some of the unvaccinated animals.

The observational finding that the sample collection date of the sheep isolate PPRV-QS09 was 2009 (data not shown) defies Banyard and Parida, 2015 report that the first Qatari PPR case was in 2010. Considering the earlier regional reports of existence of PPR evidenced by Furley et al., 1987; Hedger et al., 1980; and Taylor et al., 1990, and the infections in neighboring countries documented by Abu-Elzein et al., 1990 and Elzein et al., 2004, it is likely that the disease was introduced prior to 2009 as observed in this investigation. This is probably to happen due to transmission from infected livestock imports, which have been kept in close quarters to susceptible animals. The keeping of camels in some neighboring PPR-endemic countries to reduce overstocking practiced in Qatar might also increase the likelihood to expose to circulating field strains. Failure to detect such infected cases in the meantime might be due to lack of surveillance programmes and/or focus on other officially reported mucosal-like diseases; namely BT (OIE, 2010) and FMD (Nardo et al., 2011; Brito et al., 2015; OIE, 2015; FAO, 2015b; FAO, 2016).

The study observation that 7 wild ungulates were found infected with the PPRV, some of which are reported for the first time, also indicates the wide perpetuation of the disease among these species. One consideration in this study is that gazelle and deer had higher morbidity rate (46%) compared to the other members of the species category (data not shown). This might be due to population biasness, the large number of the tested samples or due to potential moving of these animals for trade purposes subjecting these animal species to stresses. Since 54% of the PPR-infected cases were demonstrated to occur in summer ($P \leq 0.001$), rearing of susceptible unvaccinated animals in an open housing system, PPR endemic conditions, temperatures ranging between 40.7°C and 45.2°C and relative humidity between 41% and 55% might enhance sensitivity to PPR infection. The relatively large number of the involved wild ruminant species in this study correlates with the previous reports indicating expansion of PPR among new wild ruminant members (Pruvot et al., 2020), support the call to investigate the potentiality of PPR infection among wild ruminants (Al-Naeem et al., 2000), and encourages the recommendations to investigate the role of wild ruminants in the epidemiology of PPR and the status of the disease at the wildlife-livestock interface (Fine et al., 2020).

Additionally, camels, which are estimated to comprise 8% of the total PPR susceptible animals in Qatar, should be closely monitored. Since the dromedary camels play an important role in the traditional animal industry, additional investigations are required to determine whether they are reservoir hosts perpetuating PPR (Khalafalla et al., 2010) or atypical dead-end PPR-infected hosts maintaining non-infectious viral

RNA replication (Schulz et al., 2019) and demonstrating serum viral Ab (Ismail et al., 1992; Roger et al., 2001; Haroun et al., 2002, Abraham et al., 2005).

In regards to goats, they are considered to be most sensitive to PPR infection among domesticated species (Lefevre and Diallo, 1990), attributing this to the abundance of the SLAM cell receptors mediating the lymphoid pathway of the virus infection (Prajapati et al., 2019). Sustained by the previous observations of Mahapatra et al (2006) findings, Pope et al (2013) concluded that upon experimentally respiratory infecting goats, PPRV replicates in the local tonsillar and lymphoid tissues before undergoing epithelial replication, a finding concluded by early demonstration of the viral antigens at these sites and the late-mild mucosal demonstrated form of the disease observed among these animals. However, based on the clinical records, our investigation has observed some variations in this sensitivity. Higher infection rates and profound respiratory features were observed among the infected sheep and wild ruminants compared to goats (data not shown). This observation is consistent with the recent reports of an endothelial endocytosis pathway for PPRV infection (Yang et al., 2018), and agrees with the suggestion that mortalities among PPR-infected animals are due to viral pneumonitis. In our view, this logic since the respiratory system is the primary predilection site for PPR infection, rendering the lungs highly susceptible to infection due to its exceptional abundant endothelial cells lining this highly vascularized organ. Diop et al (2005) and Couacy-Hymann et al (2007b), had already reported an impact of breed, and nutritional and health status in virulence among PPRV infections. Further systematic investigation is required to identify the effect of species and breed for sensitivity to PPRV infection. Furthermore, factors including the animal of choice, sample quality (sampling duration, sample of choice, sample number, sample treatment) and reporting system should also be monitored in testing sensitivity to PPR infection among different species during natural infections and outbreaks.

The importance of PPR worldwide has obligated implementation of robust surveillance programmes to continuously guard against the disease. Rapid diagnosis using the most reliable and accurate diagnostic tools is the key factor to reach conclusive results. In this investigation we used ELISA Ag, *r*RT-PCR and virus isolation to differentiate PPR-infected samples from non-infected. Although others advanced techniques are also used, the results obtained are highly compatible. However, the mismatched result for the blackbuck samples isolating the virus (PPRV-QBB14) versus failure to retrieve the virus RNA is an example for seldom-unexpected results. Contamination with RNA inhibitors is common, and it might be the cause for the false negative result we obtained. Despite no specific RNA inhibitor is known to effectively degrade PPRV RNA, exogenous and endogenous RNases of A, B and C types are usually the ascribed degrading agents compromise the quality of the PCR assays. The pancreatic ribonucleases, group of the RNase A (RNase 1), are the most endogenous tissue-sourced RNA inhibitors encountered during molecular assays, and they might be the cause for our negative *r*RT-PCR result. Similar to MMLV and Taq DNA polymerase, heparin, the common used blood anticoagulant, co-purifies with RNA (Jung et al., 1997) during isolation of RNA from blood, plasma, serum, buffy coat and bone marrow samples; and degrade the viral RNA (Beutler et al., 1990; Bai et al., 2000). One additional possible limitation to our result pertains to sample quality. This could be due to the long storage period of the sample prior to conduct testing (Rainen et al., 2005). Notably, the successful virus isolation was achieved from the liver sample

kept as a separate set and experienced optimum storage conditions at the virology unit. Unfortunately, the investigation did not subject this sample to *r*RT-PCR to verify this assumption.

The continuous high rate of spread of PPR around the world is quite evident (Dou et al., 2020). This might be due to the 3-dimensional pathways of replication the virus owns enabling it to potentially infect wide range of hosts (Aziz et al., 2020) and to afflict an estimate of 30 million animal globally every year (OIE, 2018). These observations might also imply either evolution of new genetically modified strains that compromise the efficiency of the current applied vaccines (Mumford, 2007) or indicates gaps in the controlling programmes rendering infected animals introduced inattentively in PPR-free territories (Dormitzer et al., 2011; Tussche et al., 2012). With the recent tendency to establish national food safety and security chain in Qatar, there is a need to undertake policies to implement effective preventive and control programmes against PPR. Endorsement of regular active surveillance programmes coupled with systematic vaccination schedules is a must. The use of effective diagnostic methods and analytic tools including next generation molecular sequencing and virus isolation are advised to ensue infectivity and reveal virus phylogeny. Adoption of proficiency testing with reference laboratories is important to build the diagnostic capacities and to ensue efficiency and certainty.

Abbreviations

PPR: Peste des petits ruminants; PPRV: Peste des petits ruminants virus; ELISA-Ab: Enzyme-Linked Immunosorbent Assay-Antibody detection; ELISA-Ag: Enzyme-Linked Immunosorbent Assay-Antigen detection; *r*RT-PCR: Real-Time Reverse Transcription Polymerase Chain Reaction.

Declarations

Acknowledgements

This investigation is part of the project NPRP8-1854-4-027 sponsored by. The authors are grateful to Qatar National Research Fund (QNRF), QF, for funding and the Department of Animal Resources (DAR), Qatar for conduction of this study at these premises. Our acknowledgement is extended to Professor Hussni Mohammed, Department of Population Medicine, College of Veterinary Medicine, Cornell University Ithaca, NY, USA, for the statistical analysis of the results. We would like to thank Professor Asim Abdel-Mageed, Department of Urology, Tulane University School of Medicine, University School of Medicine, NO, USA and Mr. Shariq Jaffary, CDC and HP, MoPH, Qatar for reading of the manuscript.

Funding: This investigation is part of the project NPRP8-1854-4-027 sponsored by Qatar National Research Fund (QNRF), Qatar.

Conflict of interest: The authors declare no conflict of interest at individual or institutional levels concerning the investigation.

Availability of data and material: Data sharing not applicable to this article, as no datasets were generated during this study.

Code availability: Not applicable

Authors' contributions:

Conceptualization: Mohamed Haroun.

Study design: Mohamed Haroun and Elmoubasher A. Farag.

Methodology: Mohamed Haroun and Nawal M. Abdulla.

Investigation and data collection: Mohammed Habeb and Nawal M. Abdulla.

Original draft writing: Mohamed Haroun.

Manuscript revision: All authors.

Funding acquisition: Mohamed Haroun and Elmoubasher A. Farag.

Ethical declaration

The study was executed according to the IACUC approval number: MoPH-MME-003 issued by The Ministry of Municipality and Environment IACUC Committee for the project NPRP8-1854-4-027. The authors certify that the submitted manuscript was performed in accordance with the ethical approval given above and to the standards laid down in the 1964 Helsinki Declaration and its later amendments.

Consent to participate

All authors approved their consent to participate in this investigation prior to conduction.

Consent for publication

All authors approved their consent for publication of the outcome of the investigation prior to conduction.

References

1. Abraham G, Sintayehu A, Libeau G, Albina E, Roger F, Laekemariam Y, Abayneh D, Awoke KM (2005) Antibody sero-prevalences against peste des petits ruminants (PPR) virus in camels, cattle, goats and sheep in Ethiopia. *Prev Vet Med* 70: 51–57.
2. Abu-Elzein EME, Hassanien MM, Al-Afaleq AI, Abdelhadi MA, Honsawai FMJ (1990) Isolation of peste des petits ruminants from goats in Saudi Arabia. *Vet Rec* 127: 309-310.
3. Abubakar M, Rajput ZI, Arshed MJ, Sarwar G, Ali O (2011) Evidence of peste des petits ruminants virus (PPRV) infection in Sindh Ibex (*Capra aegagrus blythi*) in Pakistan as confirmed by detection of

- antigen and antibody. *Trop Anim Hlth Prod* 43: 745–747.
4. Adams MJ, Lefkowitz EJ, King AMQ, Harrach B, Harrison RL, Knowles NJ et al (2017). Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). *Arch Virol* 162: 2505-2538.
 5. Adombi CM, Lelenta M, Lamien CE, Shamaki D, Koffi YM, Traoré A et al (2011) Monkey CV1 cell line expressing the sheep–goat SLAM protein: A highly sensitive cell line for the isolation of peste des petits ruminants virus from pathological specimens *J Virol Methods* 173: 306-313
 6. Al-Dubaib MA (2009) Peste des petits ruminants morbillivirus infection in lambs and young goats at Qassim region, Saudi Arabia. *Trop Anim Hlth Prod* 41: 217–220. <https://doi.org/10.1007/s11250-008-9178-6>
 7. Al-Naeem A, Abu-Elzein EME, Al-Afaleq AI (2000) Epizootiological aspects of peste des petits ruminants and rinderpest in sheep and goats in Saudi Arabia. *Rev Sci Tech Off Int Epiz* 19:855-858.
 8. Albina E, Kwiatek O, Minet C, Lancelot R, Servan de Almeida R et al (2013) Peste des Petits ruminants, the next eradicated animal disease? *Vet Microbio* 165 (1-2): 38–44.
 9. Altan E, Parida S, Mahapatra M, Turan N, Yilmaz H (2018) Molecular characterization of Peste des petits ruminants viruses in the Marmara Region of Turkey. *Transbound Emerg Dis*. 00:1-8. <https://doi.org/10.1111/tbed.130958>
 10. Anderson J, McKay JA (1994) The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implication to rinderpest control programmes. *Epidemiol Infect* 112: 225-234.
 11. Aziz UR, Kuldeep D, Qasim A, Irshad, H, Muhammad, O, Umar C et al (2020) Peste des petits ruminants in large ruminants, camels and unusual hosts. *Vet Q* 40 (1): 35-42. DOI: [1080/01652176.2020.1714096](https://doi.org/10.1080/01652176.2020.1714096)
 12. Bai X, Fischer S, Keshavjee S, Liu M (2000) Heparin interference with reverse transcriptase polymerase chain reaction of RNA extracted from lungs after ischemia-reperfusion. *Transpl Int* 10:146-150.
 13. Bailey D, Banyard A, Dash P, Ozkul A, Barrett T (2005) Full genome sequence of peste des petits ruminants virus, a member of the *Morbillivirus* *Virus Res* 110:119–124. DOI: [10.1016/j.virusres.2005.01.013](https://doi.org/10.1016/j.virusres.2005.01.013).
 14. Balamurugan V, Krishnamoorthy P, Raju DS, Rajak KK, Bhanuprakash, V., Pandey, AB et al (2014) Prevalence of Peste-des- petits-ruminant virus antibodies in cattle, buffaloes, sheep and goats in India. *Virus Dis* 25 (1): 85-90.
 15. Balamurugan V, Sen A, Venkatesan G, Bhanot V, Yadav V, Bhanuprakash, V et al (2012) Peste des petits ruminants virus detected in tissues from an Asiatic lion (*Panthera leopersica*) belongs to Asian lineage IV. *J Vet Sci* 13 (2): 203-206.
 16. Banyard AC, Parida S (2015) The molecular epidemiology of peste des petits ruminants. Springer-Verlag Berlin Heidelberg 2015 69 M. Munir (ed.), *Peste des Petits Ruminants Virus*. ☒

17. Banyard AC, Parida S, Batten C, Oura C, Kwiatek O, Libeau G (2010) Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. *J Gen Virol* 91 (Pt 12): 2885-2897. DOI: 10.1099/vir.0.025841-0.
18. Bao J, Wang Z, Li L, Wu X, Sang P, Wu G et al (2011) Detection and genetic characterization of peste des petits ruminants virus in free-living bharals (*Pseudois nayaur*) in Tibet, China. *Res Vet Sci* 90: 238-240.
19. Barrett T, Subbarao SM, Belsham GJ, Mahy BW (1991) The molecular biology of the Morbilliviruse. In: *The Paramyxovirus*. Kinsbury DW Ed, pp. 83-102. Plenum, New York.
20. Begum, S., Nooruzzaman, M., Hasnat, A., Parvin M.M., Parvin, R., Islam, M.R., and Chowdhury, E.H., 2020. Isolation of peste des petits ruminants virus using primary goat kidney cell culture from kidneys obtained at slaughter. *Vet Med Sci* 1-8. <https://doi.org/10.1002/vms3.413>
21. Beutler E, Gelbert T, Kuhl W (1990) Interference of heparin with the polymerase chain reaction. *BioTechniques* 9:166.
22. Birch J, Juleff N, Heaton MP, Kalbfleisch T, Kijas J, Bailey D (2013) Characterization of ovine Nectin-4, a novel peste des petits ruminants virus receptor. *J Virol* 87: 4756-4761.
23. Brito BP, Rodriguez LL, Hammond JM, Pinto J, Perez AM (2015) Review of the Global Distribution of Foot-and-Mouth Disease Virus from 2007 to 2014. *Transbound Emerg Dis* 1-17.
24. Chard LS, Bailey DS, Dash P, Banyard AC, Barrett T (2008) Full genome sequences of two virulent strains of peste-des-petits ruminants virus, the Côte d'Ivoire 1989 and Nigeria 1976 strains. *Virus Res* 136(1-2): 192-197.
25. Comerlato J, Albina E, Puech C, Franco AC, Minet C, Eloiflin RJ, et al (2020) Identification of a murine cell line that distinguishes virulent from attenuated isolates of the morbillivirus Peste des Petits Ruminants, a promising tool for virulence studies. *Virus Res* 286:198035.
26. Couacy-Hymann E, Bodjo C, Danho T, Libeau G, Diallo A (2007) Evaluation of the virulence of some strains of peste-des-petits-ruminants virus (PPRV) in experimentally infected West African dwarf goats. *Vet J* 173: 178-183.
27. Couacy-Hymann E, Bodjo SC, Tounkara K, Koffi YM, Ohui AH, Danho T et al (2007a) Comparison of two competitive ELISA for the detection of specific peste des-petits-ruminants antibodies in sheep and cattle population. *Afri J Biotech* 6: 732-733.
28. DAR (2013) Department of Animal Resources Annual Report, State of Qatar, 2013.
29. DAR (2021) Department of Animal Resources Statistical Identification Program, State of Qatar, 2021.
30. Dhar P, Sreenivasa BP, Barrett T, Corteyn M, Singh RP, Bandyopadhyay SK (2000) Recent epidemiology of peste des petits ruminants virus (PPRV). *Vet Microbiol* 88:153-159
31. Diallo A (1990) Morbillivirus group: genome organization and proteins. *Vet Microbiol* 1-4: 155-163. DOI: 10.1016/0378-1135(90)90145-I.
32. Diop M, Sarr J, Libeau G (2005) Evaluation of novel diagnostic tools for peste des petits ruminants virus in naturally infected goat herds. *Epidemiol Infect* 133: 711-717.

33. Dormitzer PR, Galli G, Castellino F, Golding H, Khurana S, Giudice GD et al (2011) Influenza vaccine immunology. *Immunol Rev* 239: 167-177.
34. Dou Y, Liang Z, Prajapati M, Zhang R, Li Y, Zhang Z (2020) Expanding Diversity of Susceptible Hosts in Peste Des Petits Ruminants Virus Infection and Its Potential Mechanism Beyond. *Front Vet Sci* 7:66.
35. El-Amin MAG, Hassan AM (1999) Rinderpest and PPR Surveillance in Sudan (1996-1998). In: The Seromonitoring and Surveillance of Rinderpest Throughout Africa -Phase III. Results for 1998. Proceedings of a ResearchCo-ordination Meeting of the FAO/IAEA/OAU/IBAR/PARC Co-ordinated Research Project organized by the joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Machakos, Kenya, 26-30 April 1999.
36. Elzein EM, Housawi FM, Bashareek Y, Gameel AA, Al-Afaleq AI, Anderson E (2004) Severe PPR infection in gazelles kept under semi-free range conditions. *J Vet Med B* 51 (2): 68-71.
37. Fakri FZ, Bamouh Z, Jazouli M, Tadlaoui KO, Elharrak M (2019) Experimental infection of dromedary camels with virulent virus of Peste des Petits Ruminants. *Vet Microbiol* 235:195–198.
38. FAO (2015a) Global Strategy for the Control and Eradication of PPR. <http://www.fao.org/3/a-i4460e.pdf>
39. FAO (2015b) Foot and mouth disease situation: Monthly report, May 2015.
40. FAO (2016) Foot and mouth disease situation: Monthly report, January 2016.
41. Fine AE, Pruvot M, Benfield CTO, Caron A, Cattoli G, Chardonnet Ph et al (2020) Eradication of Peste des Petits Ruminants Virus and the Wildlife-Livestock Interface. *Front Vet Sci* 7:50. <http://doi.org/10.3389/fvets.2020.00050>
42. Frölich K, Hamblin C, Jung S, Ostrowski S, Mwanzia J, Streich WJ, et al (2005) Serologic surveillance for selected viral agents in captive and free-ranging populations of Arabian oryx (*Oryx leucoryx*) from Saudi Arabia and the United Arab Emirates. *J Wildl Dis* 41(1):67-79. doi: 10.7589/0090-3558-41.1.67.
43. Furley CW, Taylor WP, Obi T (1987) An outbreak of peste des petits ruminants in a zoological collection. *Vet Rec* 121: 443-447.
44. Gibbs EPJ, Taylor WP, Lawman MJP, Bryant J (1979) Classification of peste des petits ruminant's virus as a fourth member of the genus Morbillivirus. *Intervirology* 11: 268-274.
45. Govindarajan R, Koteeswaran A, Venugopalan AT, Shyam G, Sha Una S, Shaila MS et al (1997) Isolation of peste des petits ruminants virus from an outbreak in Indian buffalo (*Bubalus bubalis*). *Vet Rec* 141 (22): 573-574.
46. Hammouchi M, Loutfi C, Sebbar G, Touil N, Chaffai N, Batten C et al (2012) Experimental infection of alpine goats with a Moroccan strain of peste des petits ruminants virus (PPRV). *Vet Microbiol* 160: 240-244.
47. Haroun M, Hajer I, Mukhtar M, Ali BE (2002) Detection of Antibodies Against Peste des Petits Ruminants Virus in Sera of Cattle, Camels, Sheep and Goats in Sudan. *Vet Res Commun* 26: 537-

541.

48. Hedger RS, Barnett ITR, Gray DF (1980) Some virus diseases of domestic animals in the Sultanate of Oman. *TAHP* 12:107–114.
49. Herzog CM, de Glanville WA, Willett BJ, Kibona TJ, Cattadori IM, Kapur V et al (2019) Pastoral production is associated with increased peste des petits ruminants seroprevalence in northern Tanzania across sheep, goats and cattle. *Epidemiol Infec* 147:e2421–9. DOI: <http://doi.org/10.1017/S0950268819001262>
50. Ismail TM, Hassas HB, Nawal MA, Rakha GM, Abd El-Halim MM, Fatebia, MM (1992) Studies on prevalence of rinderpest and peste des petits ruminants antibodies in camel sera in Egypt. *J Vet Med* 10 (2): 49-53.
51. Jung R, Lubcke C, Wagener C, Neumaier M (1997) Reversal of RT-PCR inhibition observed in heparinized clinical specimens. *BioTechniques* 23:24-28.
52. Khalafalla AI, Saeed IK, Ali YH, Abdurrahman MB, Kwiatek O, Libeau G et al (2010) An outbreak of peste des petits ruminants (PPR) in camels in the Sudan. *Acta Trop* 116: 161-165.
53. Kinne J, Kreutzer R, Kreutzer M, Wernery U, Wohlsein P (2010) Peste des petits ruminants in Arabian wildlife. *Epidemiol Infec* 138: 1211-1214.
54. Kumar N, Barua S, Riyesh T, Chaubey KK, Rawat KD, Khandelwal N et al (2016) Complexes in Isolation of Multiple Viruses from Mixed Viral Infections: Viral Interference, Persistence and Exclusion. *PLoS ONE*, 11. DOI:[1371/journal.pone.0156110](https://doi.org/10.1371/journal.pone.0156110)
55. Kwiatek O, Ali YH, Saeed IK, Khalafalla AI, Mohamed OI, Obeida AA et al (2011) Asian lineage of peste des petits ruminants virus, Africa. *Emerg Infec Dis* 17: 1223-1231.
56. Lefevre PC, Diallo A (1990) Peste des petits ruminants. *Rev Sci Tech* 9: 951-965. <https://www.ncbi.nlm.nih.gov/pubmed/2132714>
57. Li XH, Li K, Zhang H, Gan P, Luo HQ, Han ZQ et al 2018. Epidemiological investigation and risk factors of Peste des petits ruminants (PPR) in yaks (*Bos grunniens*) and cattle in five regions of China. *Trop Biomedicine* 35 (3): 736-743.
58. Libeau G, Diallo A, Parida S (2014) Evolutionary genetics underlying the spread of peste des petits ruminants virus. *Anim Frontiers* 4: 14-20.
59. Mahapatra M, Parida S, Baron MD, Barrett T (2006) Matrix protein and glycoproteins F and H of Peste-des-petits-ruminants virus function better as a homologous complex. *J Gen Virol* 87: 2021-2029.
60. Mumford JA (2007) Vaccines and viral antigenic diversity. *Rev Sci Tech* 26 (1): 69-90.
61. Nardo, A.D., Knowles, N.J., and Paton, D.J., 2011. Combining livestock trade patterns with phylogenetics to help understand the spread of foot and mouth disease in sub-Saharan Africa, the Middle East and Southeast Asia. *Rev Sci tech* 30 (1): 63-85. <https://www.ncbi.nlm.nih.gov/pubmed/21809754>

62. Nawathe, D.R., and Taylor W.P., 1979. Experimental infection of domestic pigs with the virus of peste des petits ruminants. *Trop Anim Hlth Prod* 11 (1): 120-122.
63. OIE (2010) OIE World Animal Health Information System
http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Countryreports
64. OIE (2015) Overview of the FMD situation in the Middle East, 2015.
65. OIE (2018) Geographical Distribution of PPR. [(Accessed on 3 February 2021)]; Available online:<http://www.oie.int/en/animal-health-in-the-world/ppr-portal/distribution/>
66. Omani RN, Gitao GC, Gachohi J, Gathumbi PK, Bwihangane BA, Abbey K et al (2019) Peste des petits ruminants (PPR) in dromedary camels and small ruminants in Mandera and Wajir Counties of Kenya. *Adva Virol* 2019, 4028720.<https://doi.org/10.1155/2019/4028720>
67. Padhi A, Ma L (2014) Genetic and epidemiological insights into the emergence of peste des petits ruminants virus (PPRV) across Asia and Afric *Sci Reports* 4: 7040. DOI: 10.1038/srep07040.
<https://pubmed.ncbi.nlm.nih.gov/25391314/>
68. Pawar RM, Raj GD, Kumar TMS, Raja A, Balachandran C (2008) Effect of siRNA mediated suppression of signaling lymphocyte activation molecule on replication of peste des petits ruminants virus in vitro. *Virus Res* 136:118-123. DOI: 10.1016/j.virusres.2008.04.026.
69. Pope RA, Parida S, Bailey D, Brownlie J, Barrett T, et al. (2013) Early Events following Experimental Infection with Peste-Des-Petits Ruminants Virus Suggest Immune Cell Targeting. *PLoS ONE* 8(2): e55830. DOI:10.1371/journal.pone.0055830
70. Prajapati M, Alfred N, Dou Y, Yin X, Prajapati R, Li Y, Zhang Z (2019) Host Cellular Receptors for the Peste des Petits Ruminant Virus. *Viruses*, 11 (8): 729. <https://doi.org/10.3390/v11080729>
71. Pruvot M, Fine AE, Hollinger C, Strindberg S, Damdinjav B, Buuveibaatar B, et al (2020) Outbreak of Peste des Petits Ruminants among Critically Endangered Mongolian Saiga and Other Wild Ungulates, Mongolia, 2016–2017. *Emerg Infect Dis* 26 (1): 51-62. doi: 10.3201/eid2601.181998.
72. Rainen L, Arbique JC, Asthana D, Earley MC, Geiszler RL, Krieg-Schneider F, et al (2005). 1nd ed. Pennsylvania,USA: CLSI; 2005. MM13-A-Collection, transport, preparation, and storage of specimens for molecular methods: approved guideline; pp. 1–51.
73. Rasooli AR, Nouri M, Abad-Shapouri MR, Khalafi E, Daghari M (2019) Seroprevalence of peste des petits ruminants (PPR) virus infection in sheep and cattle in Ahvaz. *J Vet Res* 73 (4): 465-473.
74. Ratta B, Pokhriyal M, Singh SK, Kumar A, Saxena M, Sharma B (2016) Detection of peste des petits ruminants virus (PPRV) genome from nasal swabs of dogs. *Curr Microbiol* 73 (1): 99-103.
75. Roger F, Guebre Yesus M, Libeau G, Diallo YM, Yilma, T (2001) Detection of antibodies of rinderpest and peste des petits ruminants viruses (*Paramyxoviridae*, Morbillivirus), during a new epizootic disease in Ethiopian camels (*Camelus dromedarius*). *Revue de Méd Vét (Toulouse)* 152 (3): 265-268.
76. Schulz C, Fast C, Schlottau K, Hoffmann B, Beer M (2018) Neglected hosts of small ruminant morbillivirus. *Emerg Infec Dis* 24 (12): 2334-2337.

77. Schulz C, Fast C, Wernery U, Kinne J, Joseph S, Schlottau K, et al (2019) Camelids and Cattle Are Dead-End Hosts for Peste-des-Petits-Ruminants Virus. *Viruses* 11(12):1133.
78. Sen A, Saravanan P, Balamurugan V, Bhanuprakash V, Venkatesan G, Sarkar J et al (2014) Detection of subclinical peste des petits ruminants virus infection in experimental cattle. *Virus Dis* 25 (3): 408-411.
79. Sevik M, Oz ME (2015) Peste-des-petits-ruminants virus isolate Culicoides-PPRV 02 nucleocapsid protein (N) gene, partial cds. GenBank: KU325483.1.
<https://www.ncbi.nlm.nih.gov/nuccore/KU325483>
80. Sreenavisa BP, Singh RP, Mondal B, Dhar P, Bandyopadhyay SK (2006) Marmoset B95a Cells: A Sensitive System for Cultivation of Peste des Petits. *Vet Res Commun Jan* 30 (1): 103-108.
DOI:1007/s11259-005-3200-5
81. Taylor WP, Albusaidy S, Barret T (1990) The epidemiology of peste des petits ruminants in the Sultanate of Oman. *Vet Microbiol* (22): 341-352.
82. Tussche C, Steinbruck L, McHardy AC (2012) Detecting patches of protein sites of influenza A viruses under positive selection. *Mol Biol Evol* (29): 2063-2071.
83. Yang B, Qi X, Guo H, Jia P, Chen S, Chen Z et al (2018) Peste des Petits Ruminants Virus Enters Caprine Endometrial Epithelial Cells via the Caveolae-Mediated Endocytosis Pathway. *Front Microbiol* (9): 210. <https://doi.org/10.3389/fmicb.2018.00210>
84. Zakian, A., Nouri, M., Kahroba, H., Mohammadian, B., and Mokhber-Dezfouli M.R., 2016. The first report of peste des petits ruminants (PPR) in camels (*Camelus dromedaries*). *Trop Anim Hlth Prod* 48 (6):1215-1219.

Tables

Table (1) Descriptive summary of the data of the biological samples identified positive for PPR infection

Sample type	PPR positive samples/test				Clinical signs	Necropsy features
	ELISA-Ab	ELISA-Ag	rRT-PCR	Virus isolation		
Blood serum	14	NT	2	NT	Not specific, Classical	Respiratory, Digestive
Peripheral blood EDTA	NAP	NT	10	NT	Classical	Respiratory, Digestive, Generalized
Nasal Swabs	NAP	NAV	12	NAV	Classical	ND
Buccal swabs	NAP	NAV	21	NAV	Classical	ND
Fecal swabs	NAP	NAV	8	NAV	Classical	ND
Pre-scapular lymph node	NAP	NT	3	NT	Classical	Generalized
Mediastinal lymph nodes	NAP	NT	16	2	Classical	Respiratory, Digestive
Mesenteric lymph nodes	NAP	1	20	1	Classical	Respiratory, Digestive
Lung	NAP	6	22	4	Classical	Respiratory, Digestive
Tracheas	NAP	NAV	1	NT	Classical	Respiratory, Digestive
Spleen	NAP	4	19	7	Classical	Respiratory, Digestive, Generalized
Liver	NAP	1	7	1	Classical	Respiratory, Digestive
Kidney	NAP	NAV	3	NAV	Sudden death, Classical	Generalized
Small intestines	NAP	NT	7	NT	Classical	Respiratory, Digestive
Large intestine	NAP	NT	19	NT	Classical	Respiratory, Digestive, Generalized
Total	14	12	170	15		

ELISA-Ab: Enzyme-Linked Immunosorbent Assay -Antibody. ELISA-Ag: Enzyme-Linked Immunosorbent Assay-Antigen. rRT-PCR: *Real-time* reverse transcription polymerase chain reaction. NAP: Not Applicable. NAV: Not Available. ND: Not Done. NT: Not Tested. Not specific: Either rise in temperature, fever, lacrimation, nasal discharge or diarrhea.

Table (2) Subject samples tested positive for PPR infection and used to identify the corresponding infected animal species

Samples			Animals			
Subject	Positive	% Positive	Subject	Positive	% Positive	Species
94	44	47%	31	17	55%	Sheep
75	46	61%	36	17	47%	Goats
199	102	51%	68	37	54%	Wild ruminants
368	192	52%	135	71	52%	Total

Figures

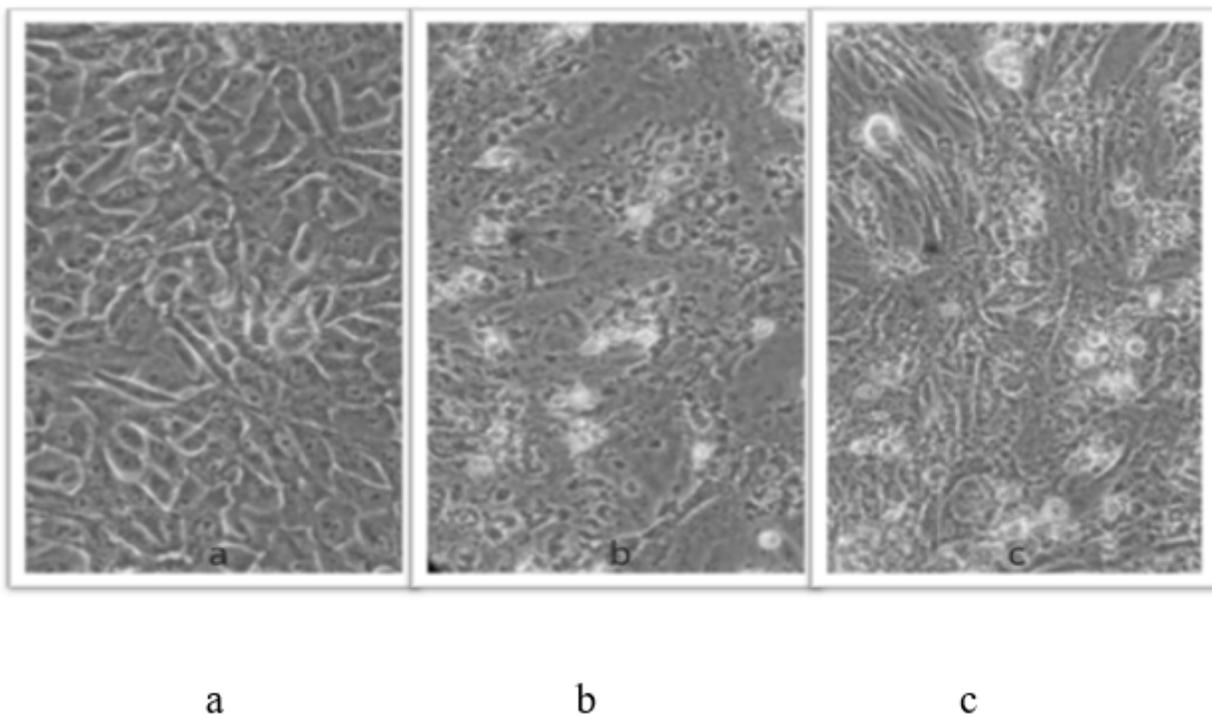


Figure 1

Semi-confluent non-infected control Vero cell lines (a) compared to isolate PPRV-QS09-infected cell lines, 3 days post infection (b) and 6 days post infection (c) exhibiting cellular aggregation and early karyorexis (X20)