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## Research paper

Epidemic and evolutionary characteristics of peste des petits ruminants virus infecting *Procapra przewalskii* in Western China

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## ABSTRACT

Due to the migration or transboundary spread of domestic and wild animals, peste des petits ruminants virus posed a high potential threat to them. In this study, we initially detected that a class of animal named *Procapra przewalskii* was infected with peste des petits ruminants virus (PPRV ChinaGS2018) in Gansu province. According to phylogenetic relationships analysis, we found that ChinaGS2018 comprised of 15,954 nucleotides and was classified into IV genotypes. In addition, indirect immunofluorescence assay (IFA) showed that ChinaGS2018 could infect isolated primary goat tracheal epithelium cells (GTC). Comparing with full-length genome sequences revealed that ChinaGS2018 strain has high identity to the reference complete genomes (87.16–99.55%) at the nucleotide level. Multiple sequence alignment showed that F protein has the highest identity of 99.8%, and H protein has the highest nucleotide substitution ratio. Our study also suggested this strain may be transmitted from Xinjiang, China. Along with the migratory of *Procapra przewalskii*, this wild ruminant infected with PPRV can pose a huge threat to other wild ruminants and domestic ones. This is the first report describing infected with PPRV which will provide insights into the epidemiology and pathogenesis of this important virus.

## 1. Introduction

Peste des petits ruminants (PPR) is an acute, highly contagious disease that primarily infect small ruminants, especially goats and sheep (Abubakar et al., 2009; Gari et al., 2017), characterized by fever, oculo-nasal discharge, diarrhoea, leukopenia, dyspnea and sloughing of the epithelium of oral and nasal mucosa (Kumar et al., 2014). In addition to the susceptibility of goats and sheep, many wildlife are also susceptible to infection, such as buffalo, antelope, camel and hornhorse as well as pig (Hamdy and Dardiri, 1976; Schulz et al., 2018). It has been reported that wild ungulates are also susceptible to it, such as white-tailed deer (Aziz Ul et al., 2018), African grey duiker and Dorcas gazelle (Elzein et al., 2004). PPRV transmission can be divided into contact transmission and non-contact transmission. The PPR disease can cause a serious impact on livestock production in all countries of the world, especially in developing countries. It has been epidemic in > 70 countries in the world, including the Middle East, Africa and most parts of Asia (Baron et al., 2017).

The PPR was first reported in Africa in 1942 in Côte d'Ivoire (Mantip

et al., 2019). During 2007, PPRV infections were first reported in the Ngari region of Tibet in China (Liu et al., 2018). It belonged to lineage IV after laboratory testing through phylogenetic analysis. In 2013, cases of PPRV infection have been reported in Xinjiang, Gansu, Ningxia, Hunan, Jiangxi and Inner Mongolia, which rapidly spread into most of China about > 20 provinces during 2014 (Liu et al., 2018; Wang et al., 2015), resulting in large-scale deaths about > 16,000 sheep and goats (Bao et al., 2017). Those PPRV strains have also been identified as lineage IV. According to incomplete statistics, 53 countries reported PPR outbreaks to the World Organisation for Animal Health (OIE) from 2015 to 2016, including 35 countries in Africa such as Nigeria, Niger, Senegal, Somalia, Sierra Leone, etc., and 18 countries in Asia, for instance, Afghanistan, Bangladesh, Bhutan, China, India, Iran, etc. (Muniraju et al., 2014). Moreover, the number of countries infected with PPRV keeps rising. Now they continue to spread to Central and East Asia, causing major economic losses to livestock farmers (Bao et al., 2017). In December 2016, the disease was diagnosed in several wildlife populations in eastern Mongolia, e.g. saiga antelope (*Saiga tatarica mongolica*), ibex (*Capra sibirica*) and goitred gazelle (*Gazella subgutturosa*), with > 5000

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deaths (Baron et al., 2017).

*Procapra przewalskii* (Przewalski's gazelle), belongs to *Procapra* genus, also named Tan sheep or Mongolian gazelle, has found in Erdos grassland Inner Mongolia, China. The genus *Procapra* is an ancient, monophyletic lineage endemic to Asia. This genus includes three living species: the Mongolian gazelle (MG, *P. gutturosa*), the Przewalski's gazelle (PG, *P. przewalskii*) and the Tibetan gazelle (TG, *P. picticaudata*). *Procapra przewalskii*, animals under first class state protection in China, were distributed in Inner Mongolia, Ningxia, Gansu and Qinghai provinces. Although the population of this species has declined due to human activities and habitat degradation, large sheep tracks have been found in Northwest Gansu in recent years with the improvement of the ecological environment. In October 2018, a small number of sheep were died in northwest Gansu province, caused by infection of PPRV, which was a terrible threaten to the survival of the sheep and incredible challenge to protect rare animals.

In the current study, we first detected *Procapra przewalskii* infected this disease. Therefore, this study aims to detect and analysis the complete genome of PPRV epidemic strain, in order to further investigate evolutionary and epidemiologic dynamics as well as transboundary transmission of PPR virus. The complete genome of ChinaGS2018 from one dead *Procapra przewalskii* was carried out by RT-PCR and splicing. The analysis of genetics and evolution was also performed for tracing infection sources and the disease development. Further, neighbor-joining phylogenetic analysis of full-length PPRV genome and partial genes of PPRV in China between 2007 and 2018 were included in the study.

## 2. Materials and methods

### 2.1. Sample collection

The tissue samples were collected from one dead *Procapra przewalskii* in the western of Gansu province, China. Pathological tissues included spleen, small intestine, intestinal lymph node, lung and kidney as well as heart. Selected samples were shipped on ice bag to the Lanzhou Veterinary Research Institute for confirmation of diagnosis and molecular testing.

### 2.2. Identification for PPRV

Collected tissues were analyzed by RT-PCR to assess the viral load and nucleic acid of PPRV. Total RNA were extracted by Trizol Reagent (Sigma, USA) and quantified with a Nanodrop ND-2000 (Wilmington, DE, USA). Both the PPRV V and N genes were amplified using the One-Step RT-PCR kit (TaKaRa). Primers for amplification N were PPRV-N-F: 5' TCTCGGAAATCGCCTCACAGACTG 3'; PPRV-N-R: 5' CCTCTCCTGTCCTCCAGAATCT 3'(Manjunath et al., 2015).

For indirect immunofluorescence assay (IFA), PPRV ChinaGS2018 was produced by consecutively inoculating homogenized tissue of small intestine tissue onto Vero cells. After 3 consecutive passages, the PPR virus was harvested and stored at  $-80^{\circ}\text{C}$  for later experiments. Primary cells of goat tracheal epithelium cells (GTC) were generously provided by Prof. Chu Yuefeng (Lanzhou Veterinary Research Institute) and cultured in RPMI 1640 Medium containing 10% FBS (Gibco), 100  $\mu\text{g}/\text{mL}$  of streptomycin and 100 IU/mL of penicillin. GTC were infected with 1 MOI ChinaGS2018 for 48 h. Anti-rabbit PPRV-H polyclonal antibody (1: 200) and FITC-conjugated antibody (1: 80) were used in IFA. After washing 5 times of each step, cells were detected under fluorescence microscope.

### 2.3. Reverse transcription-polymerase chain reaction (RT-PCR) and genome sequencing

For full-length genome sequencing, a nested PCR was performed on tissue samples. Total RNA were extracted from these samples using TRI

**Table 1**

Sequences of primers used for PCR amplification.

Gene ID	Primer sequence (5' to 3')	Product Size (bp)
P1	F: ACCAAACAAAGTTGGGTAAGG R: AGTCCACATCGCTGTCTGTCAGATC	2270
P2	F: GATTGAAGGACTCGGAGGTCTGAC R: TGATGATGACATCATCGTAGACACGG	2260
P3	F: ACCTAGAAGATACATAGTCGGCTCATG R: TCTCGTATGGACTTGGCCCTAA	852
P4	F: GGACGCAGAAAGGAAGGAGACAC R: GCTTGGCCTAGTCATCACCTTGT	711
P5	F: GGACCCCTCAAACCAAAG R: GCAACTCAAAGTGCTACCC	1134
P6	F: CCTACCCAACCTTATCTGAGATCAAGG R: GCTAAGTCAAGACTGACCCCTGATC	2250
P7	F: TTCTGGGTGCAAACCTGTCCC R: CTCAGGGTCTTTAAGGTAGGA	2500
P8	F: GCTGCACTGAAGAATGAGTGGGATTC R: AGAGGTCTCAAGGATCCCAAGACC	2610
P9	F: GACATCCCTGTGAGGGTTGCAAGATAC R: ACCAGACAAAGCTGGGAATAGATAC	2760

Reagent® (Sigma, CA, USA). First-strand cDNAs were synthesised using GoScript™ Reverse Transcription System (Promega, CA, USA) according to the manufacturer's instructions. Nine pairs of PCR primers were designed and used to amplify nine overlapping fragments by RT-PCR (Table 1). All primers were synthesised by Shenggong Co, Ltd., China. Then PCR products were purified using the QIA Quick Gel Extraction Kit (QIAGEN) and were sequenced using standard Sanger methods in TsingKe Biological Technology (Xi'an, Shanxi).

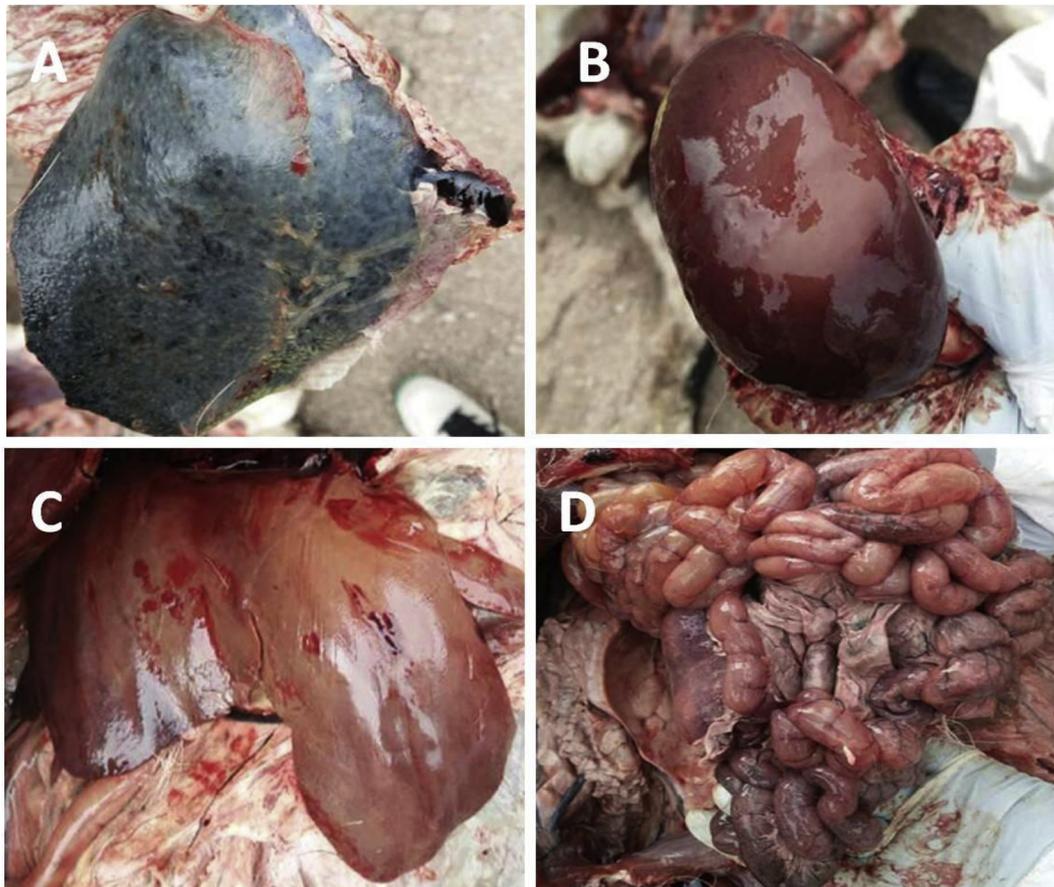
### 2.4. Multiple sequence alignment and phylogenetic analysis

The genome sequences of PPRV obtained in this study have been deposited in GenBank. Both partial N, P, M, F, H, L and full-length sequence were aligned using MegAlign software in Lasergene package. Multiple sequence alignments were carried out using Clustal V within the Megalign program (DNASStar, Madison, USA). The phylogenetic relationships of the whole genome or the individual gene sequences (N, P, M, F, H and L genes) from the 43 PPRV strains were analyzed using MEGA 6.0. The statistical significance of phylogenies constructed was estimated by bootstrap analysis with 1000 repetitions.

## 3. Results

### 3.1. Detection for PPRV prevalence

The liver showed black red with severe necrotic lesions; The kidney appeared oedematous, enlarged, haemorrhagic and red-black; The pulmonary surface mucosa showed varying degrees of congestion and haemorrhagic, and there was mixed yellow-white liquid on the mucosal surface; Pulmonary interstitial pneumonia foci, lesions can be clearly seen on the surface, emphysema and edema, hardening and congestion on the edge of the lung; Intestinal mucosa appeared erosion or haemorrhagic, Zebra-striped bleeding occurred in the large intestine, especially at the junction of the colon and rectum. Congestion in the intestinal veins and necrosis in some segments of the intestine (Fig. 1). The results of IFA indicated that ChinaGS2018 could infect GTC as the green fluorescence can be clearly observed compared with mock-infected GTC (Fig. 2). Via RT-PCR analysis, a total of five samples (spleen, small intestine, intestinal lymph node, lungs and kidney) were collected and analyzed which showed a high viral load for PPRV with an individual cycle threshold (Ct) value < 30. Spleen, small intestine and intestinal lymph node were strongly positive (Ct value < 20) for PPRV. Only heart had a Ct value = 29 (Fig. 3A). RT-PCR results also showed the products of V gene were 176 bp (Fig. 3B). According to the RT-PCR results, the spleen, small intestine, intestinal lymph node, lung, heart



**Fig. 1.** Gross lesions in *Procavia przewalskii* that died as a result of ChinaGS2018 infection. A) The liver showed black red with severe necrotic lesions; B) The kidney appeared oedematous, enlarged, haemorrhagic and red-black appearance; C) The lung appeared interstitial pneumonia foci, lesions can be clearly seen on the surface, emphysema and edema; D) The small intestine showed oedematous, enlarged and haemorrhagic. The scale bar = 0.5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and kidney were all positive for PPRV and N gene amplification size was 351 bp (Fig. 3C). Nine corresponding DNA fragments were detected by 1% agarose gel (Fig. 4) then the PCR products were purified and sequenced.

### 3.2. Multiple sequence alignment and analysis

In order to identify the genome characteristics of ChinaGS2018, we conducted multiple sequence alignments between these 43 PPRV sequences. The genome length of the ChinaGS2018 strain was 15,954 nucleotides (nt). Upon online blast analysis, ChinaGS2018 genome showed nucleotide identities ranging from 87.16 to 99.55% with other PPRV strains deposited in GenBank. The highest identity (99.55%) at a nucleotide level with the PPRV are strain ChinaHN2014 and ChinaSaX2014, the least consensus (87.16%) is KN5/2011.

Compared with the domestic epidemic strains of 2013, 2014 and 2015 in China, the multiple sequence alignment analysis of structural proteins for ChinaGS2018 indicated that N gene had 5 nucleotide substitutions (420 T-C, 432 C-T, 765 C-T, 879 G-A, 981 A-G). The P gene also had 5 nucleotide substitutions (81 C-A, 493 C-T, 773 C-T, 976 C-T, 1111 A-C). M gene had 2 nucleotide substitutions (726 A-G, 873 T-C), F gene had 4 nucleotide substitutions (180 C-T; 838 C-T; 1339 A-G; 1453 A-G), but they all had no amino acid mutations. These synonymous mutations in different viral genes display the roles of synonymous codon usages in the evolutionary processes of ChinaGS2018 strain. In addition, mainly H and L genes had 3 amino acids mutations (162 S-L; 320 V-A; 590 T-I) and 2 amino acid mutations (1031 K-R; 1656 S-T), respectively. A total of 48 nucleotide substitutions were

found in ChinaGS2018 strain, among which 5 sites contributed to amino acid changes. It can be seen from the above data, synonymous mutations predominantly occur in N, M, F and P genes, while meaningful substitutions mainly harbour in H and L genes. The sequence of ChinaGS2018 was submitted to NCBI (accession number MN121838).

### 3.3. Phylogenetic analysis of PPRV

Phylogenetic analysis using Neighbor-Joining tree indicated that ChinaGS2018 strain was clustered into the lineage IV and shared the higher homology with the PPRV strains in China other than foreign countries (Sahu et al., 2017), most of them were circulating during 2013–2014. The analysis of full-length sequence for ChinaGS2018 revealed closest nucleotide identities with China/XJBZ/2015 (Fig. 5A). Also F and H have highest homology with China/XJBZ/2015. Based on the N gene and P gene, with that from PPRV/Mogolia/9/2016 and CH/GDDG/2014 (Fig. 5D, E). The GenBank accession numbers used in this study were listed in Table 2.

## 4. Discussion

Through gene sequence alignment, PPRV epidemic strains can be divided into four gene groups: I, II, III and IV, but only one serotype (Ashraf et al., 2017). Among them I, II and III gene groups are mainly circulating in Africa and IV gene group are mainly distributed in Asia (Banyard et al., 2010). In this study, according to the online blast search, we know that ChinaGS2018 was most similar to the following PPRVs: China/XJYL/2013, China/5/2013, China/2/2013,

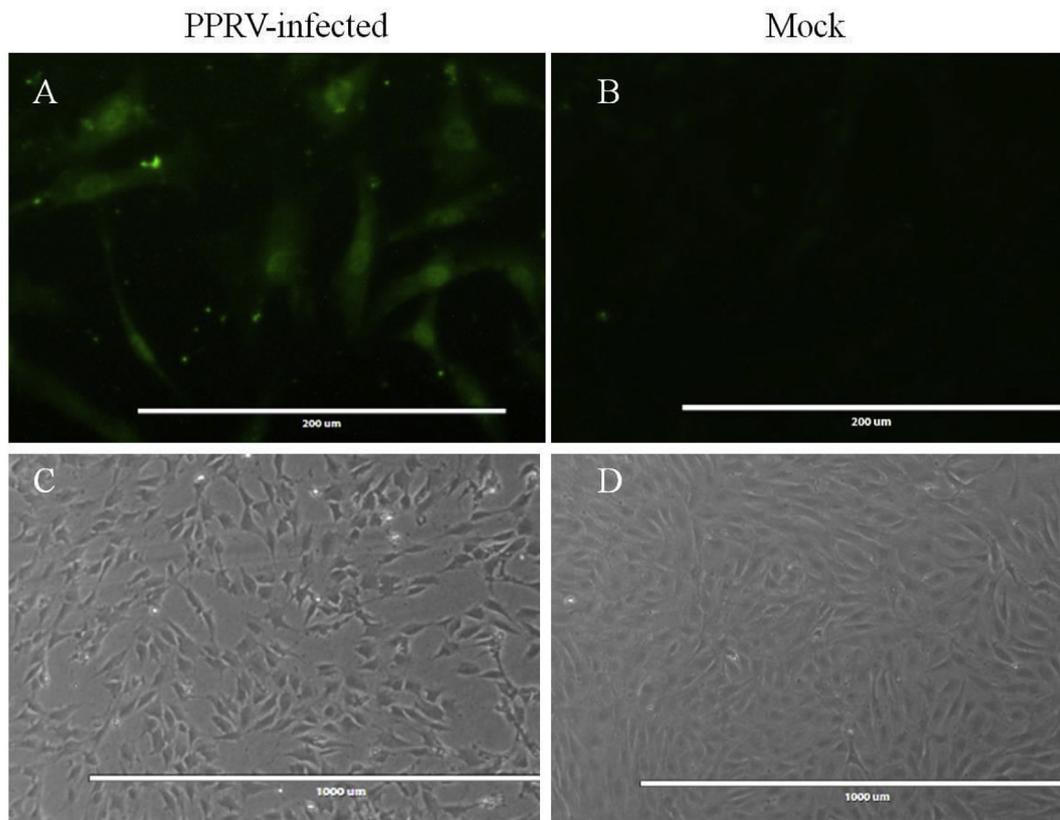


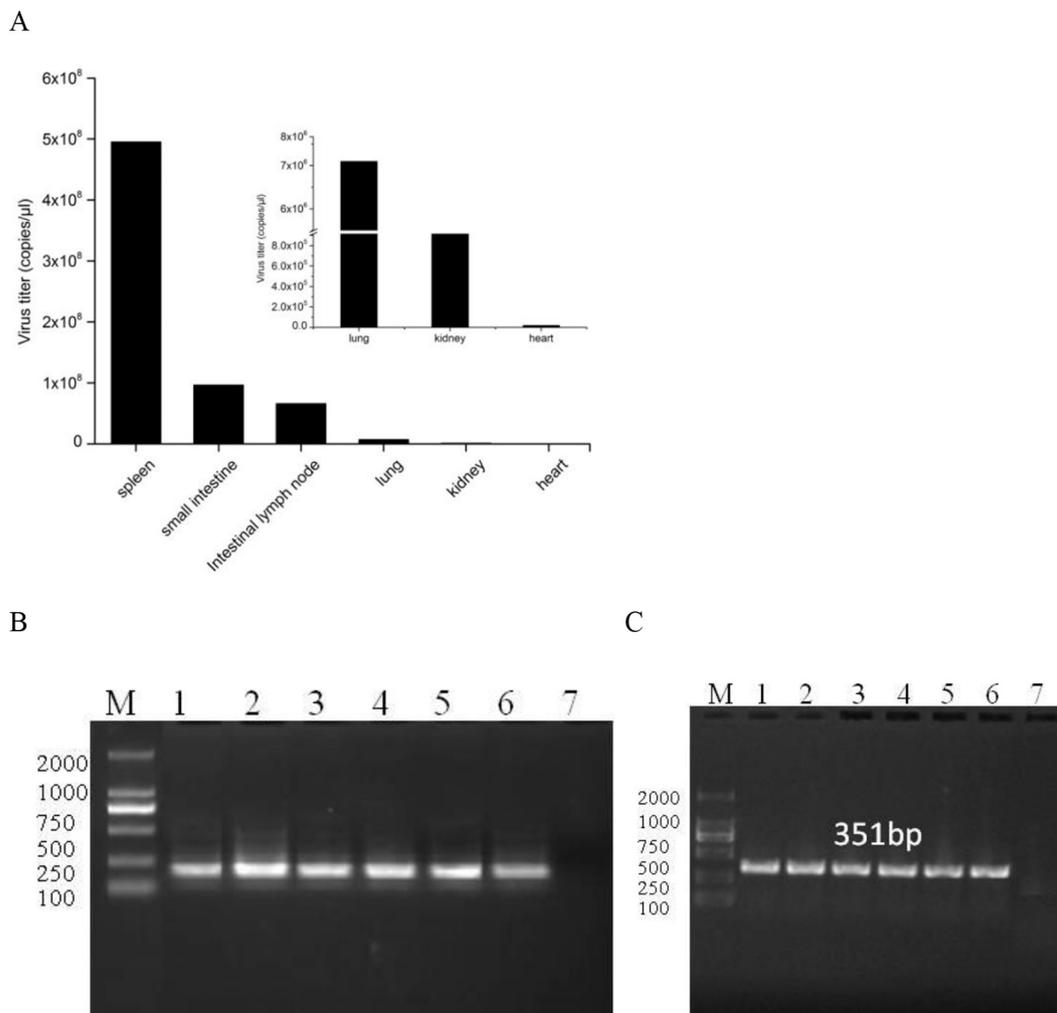
Fig. 2. IFA of viral protein expression in cells infected with PPRV. A: PPRV-infected GTC; B: Mock-infected GTC. C and D were bright field pictures of the cells.

ChinaHeN2014, ChinaGS2014, CH/GDDG/2014 and China/XJBZ/2015. Most of them are in western China. The PPRV ChinaGS2018 was further detected from one *Procapra przewalskii* through clinical signs, RT-PCR and IFA. Our data showed that ChinaGS2018 could infect isolated primary GTC and viral protein expressed in the infected GTC (Fig. 2), which further indicated that *Procapra przewalskii* infected with ChinaGS2018 has potential threat to domestic animals including sheep and goats. It is likely that PPRV was transmitted from domestic or wild goats to *Procapra przewalskii*. Phylogeographic analysis also indicated this strain may be transmitted from Xinjiang, China because of the highest root location state probability (Fig. 5). The phylogeny analysis basically suggested the sequence diversity between ChinaGS2018 and other Chinese PPRV strains. It is possible that the observed diversity is due to ChinaGS2018 is the only virus isolated from *Procapra przewalskii*. Studies have shown that the phylogeny derived from the whole genome sequence is superior than that derived from partial gene sequences (Sahu et al., 2017). So we can draw conclusion in the phylogenetic tree that it might evolved from 2013 to 2014, and it is most closest to China/XJBZ/2015. We further suspected that the virus was transmitted from Xinjiang, China. So the PPR outbreak of 2013–2014 may be responsible for this infection (Mantip et al., 2019).

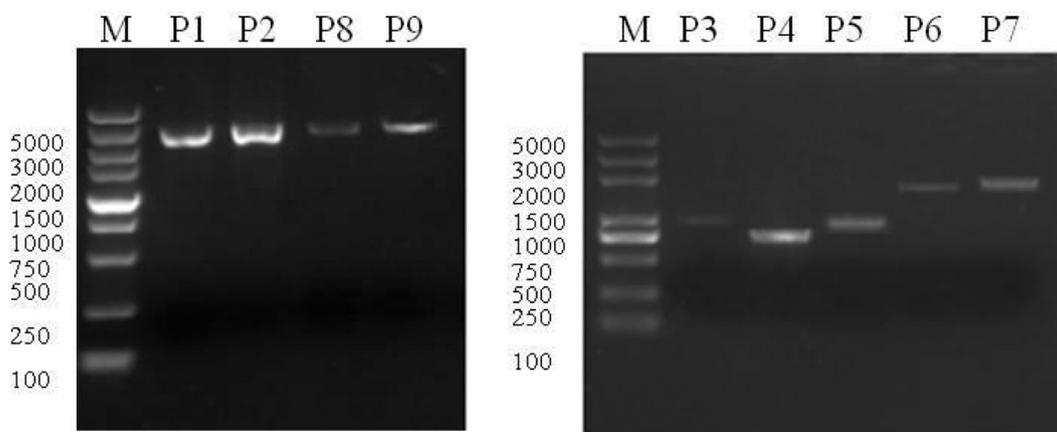
Researchers demonstrated that the length of the M-F UTR of wild-type MeVs is flexible (Bankamp et al., 2014). Wild measles viral strains with seven nucleotide (CCCCCC) insertion and one nucleotide (C or G) deletion between M and F gene had lower TCID<sub>50</sub> than other strains (Bankamp et al., 2014). In this study, ChinaGS2018 also has 6-nt insertion (TCTCCC) at nucleotide 5215 within the 5' UTR of F gene, resulting the reduction of cytopathogenicity and low viral titer, which was consistent with genome characterization of CH/GDDG/2014 (TCCCTC, position 771–776) (Li et al., 2015). In addition, the N, P, M, F, H and L genes had at least one or more nucleotide substitutions. This indicated that PPRV had mutated at the nucleotide level. N protein has a conservative pattern of amino acid utilization (Ma et al., 2018). Some

studies suggested that the H gene to be the most diverse gene among all the genes (Sahu et al., 2017). They also reported that M and F genes had no positive selection sites of Izatnagar/94 isolated in India. Furthermore, M protein was reported to be the most conserved viral protein within the Morbilliviruses (Adombi et al., 2017; Muthuchelvan et al., 2006). Previous studies have shown that H gene plays an important role in the process of virus entry and attachment (Liang et al., 2016). While in this study, the highest ratio of nucleotide substitutes were also found in H gene, and M gene was relatively conserved. The H of ChinaGS2018 has 7 nucleotide substitutions and 3 amino acid mutations (162 S-L; 320 V-A; 590 T-I), the mutations or changes in the H gene suggests that H may have influenced PPRV's pathogenicity and adaptability (Chang et al., 2019; Ma et al., 2017). The point mutation of H may be related to the interspecies transmission of the virus. It also reported that PPRV has very high natural mutation rates, as RNA viruses often represent higher rate than DNA viruses (Jenkins and Holmes, 2003). Of the viral proteins in the Morbilliviruses, the L protein is the largest, which is responsible for the transcription and replication of viral genomic RNA, including initiation, extension, and termination. In this PPRV ChinaGS2018 strain, 2 amino acid mutations (1031 K-R; 1656 S-T) substitute on L protein. Although synonymous substitutions can not change the corresponding amino acid usages in N, P, M and F genes of ChinaGS2018 strain, synonymous codon usage variations might influence gene expression both at the level of translation and regulation transcription (Kumar et al., 2018; Zhou et al., 2019). The PPRV strain ChinaGS2018 might benefit from synonymous substitutions in viral genes to better adapt to its susceptible host.

PPRV was mainly susceptible to goats and sheep, but many wildlife, such as buffalo, antelope, bharals, wild goats, dorcas gazelles and Tibetan gazelle as well as pig and wild boar could also infect to PPRV (Aziz Ul et al., 2018; Munir, 2014). In recently, the researchers also demonstrated that PPRV was able to invade and survive in the tonsil of pig (Schulz et al., 2018). However, interspecies transmission and



**Fig. 3.** Confirmation of viral infection and transcript quantification. A) qRT-PCR for clinical samples. It showed positive for PPRV. B) RT-PCR detection of PPRV-V (176 bp). M: DL2000; 1–6 were spleen, small intestine, intestinal lymph node, lung, heart and kidney, respectively; 7 was negative control. C) N gene amplification (amplicon size 351 bp), M: DL2000; 1–6 were spleen, small intestine, intestinal lymph node, lung, heart and kidney, respectively; 7 was negative control.

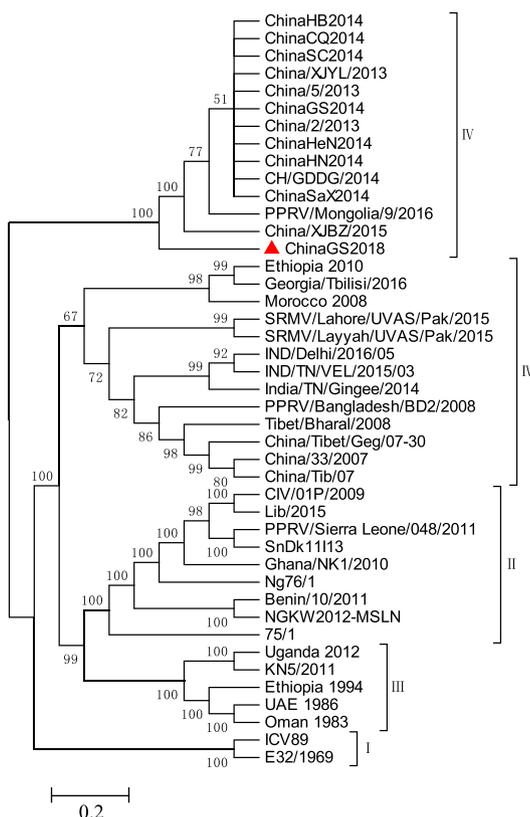


**Fig. 4.** PCR amplification of full-length genome through 9 fragments.

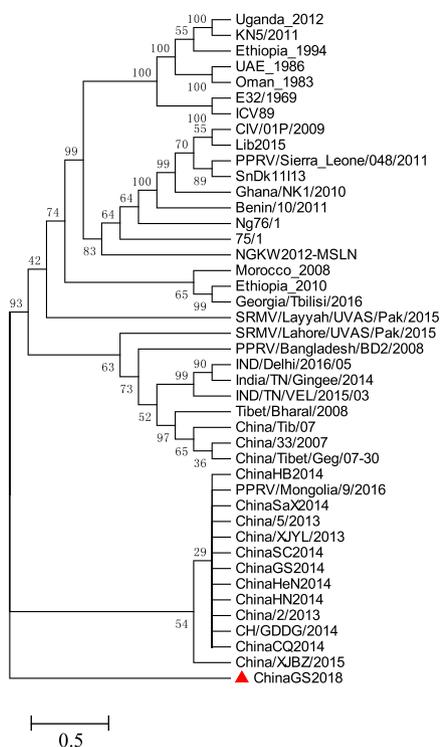
susceptible animals species were also unclear. In the other hand, many wildlife animals were widely distributed in the western China, especially in The Qinghai-Tibet Plateau. Therefore, Along with the migratory of *Procapra przewalskii*, this wild ruminant was a terrible accident which not only seriously influenced the survival of the *Procapra przewalskii* and other rare wild animals but also threatened some domestic animals. Population aggregation occurs during migration

season, which may lead to rapidly spread of PPRV among different wild populations (Li et al., 2017). Due to its seasonal horizontal migration and clustering activity, thus it was also a terrible threaten to the survival of the sheep and was incredible challenge to the rare animals protection. Moreover, some evidences suggest that PPRV is extending its host range (Li et al., 2017; Zhou et al., 2018). This study raises our concerns about the protective efficacy of the PPR vaccine against PPRV

A. Complete genome



B. F gene



C. H gene

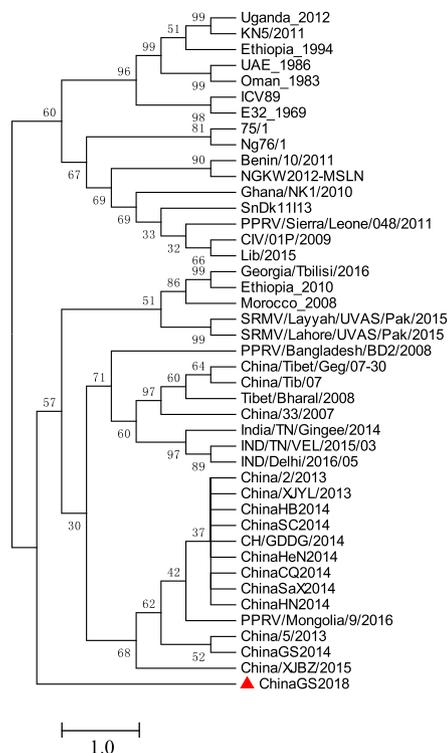
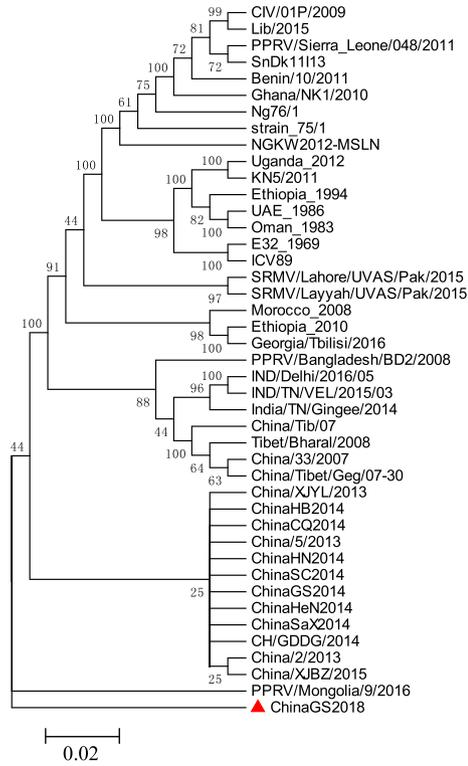
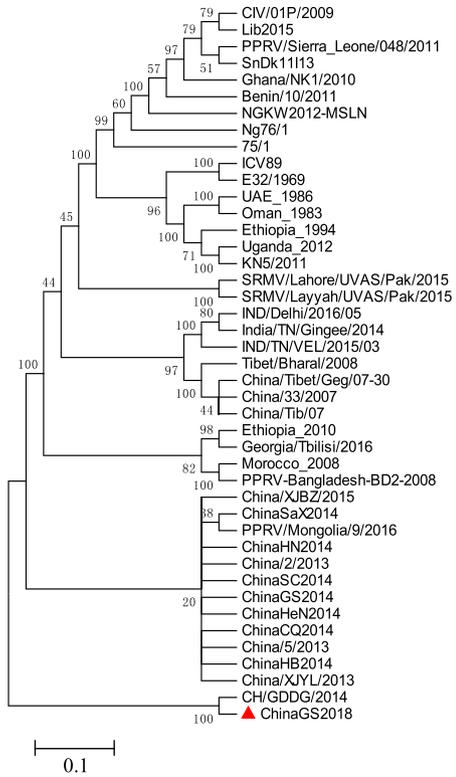


Fig. 5. The phylogenetic tree were constructed using Mega 6.0 using neighbor-joining method. The isolated sequence in this study is marked as a red triangle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

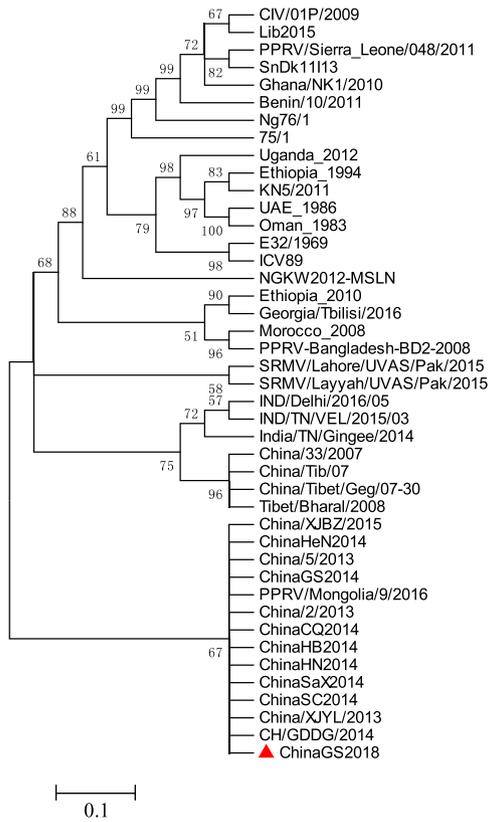
D. N gene



E. P gene



F. M gene



H. L gene

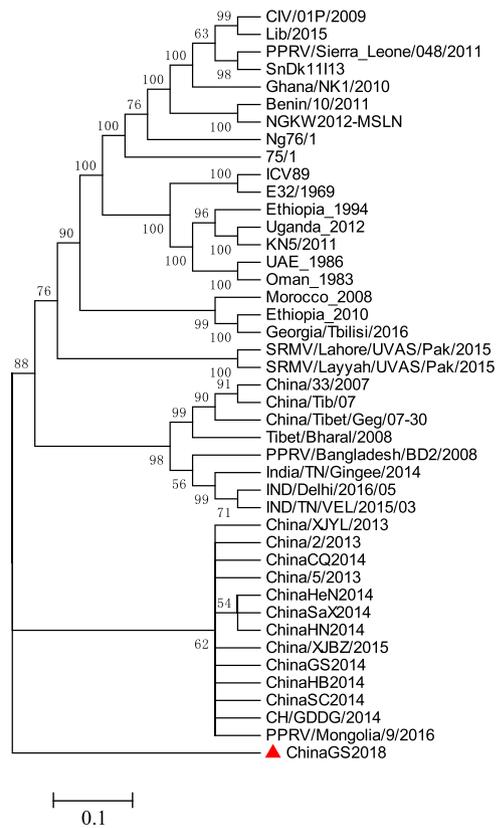


Fig. 5. (continued)

**Table 2**  
Whole genome sequences used in this study.

Strain	Source	Lineage	GenBank nos.
ICV89	Goat/Cote d'Ivoire	I	EU267273
E32/1969	Goat/Senegal	I	KP789375.1
Ng76/1	Goat/Nigeria	II	EU267274
PPRV/Sierra leone/048/2011	Goat/Sierra Leone	II	MF741712.1
SnDk11113	Goat/Senegal	II	KM212177
Lib2015	Goat/Liberia	II	KU236379.1
Benin2011	Goat/Benin	II	KR781449.1
Ghana/Nk1/2010	Sheep/Ghana	II	KJ466104.1
CIV/01P/2009	Goat/Cote d'Ivoire	II	KR781451
75/1	Goat/Nigeria	II	KY628761.1
Uganda 2012	Goat/Uganda	III	KJ867543
KN5/2011	Goat/Kenya	III	KM463083
Oman 1983	Goat/Oman	III	KJ867544
UAE 1986	Goat/UAE	III	KJ867545
Ethiopia 1994	Goat/Ethiopia	III	KJ867540
ChinaHeN2014	Goat/China	IV	MF443347.1
ChinaCQ2014	Goat/China	IV	MF443353.1
ChinaSC2014	Sheep/China	IV	MF443338.1
ChinaGS2014	Sheep/China	IV	MF443351.1
ChinaHN2014	Goat/China	IV	MF443345.1
ChinaHB2014	Goat/China	IV	MF443348.1
ChinaSaX2014	Goat/China	IV	MF443339.1
CH/GDDG2014	Goat/China	IV	KP868655.1
China/2/2013	Goat/China	IV	KX421384.1
China/5/2013	Goat/China	IV	KX421387.1
China/Tibet/Geg/07–30	Goat/China	IV	FJ905304
China/Tib/07	Goat/China	IV	JF939201
Tibet/Bharal/2008	Wild bharal/China	IV	JX217850.1
China/33/2007	Goat/China	IV	KX421388.1
China/XJYL/2013	Goat/China	IV	KM091959
China/XJBZ/2015	Goat/China	IV	KT633939.1
PPRV/Mogolia/9/2016	Goat/Mogolia	IV	KY888168.1
Morocco 2008	Alpine goat/Morocco	IV	KC594074.1
Ethiopia 2010	Goat/Ethiopia	IV	KJ867541
IND/TN/VEL/2015/03	Goat/India	IV	KT860064.1
IND/Delhi/2016/05	Goat/India	IV	KX033350.1
India/TN/Gingee/2014	Goat/India	IV	KR261605.1
Georgia/Tbilisi/2016	Goat/UK	IV	MF737202.1
SRMV/Layyah/UVAS/Pak/2015	Sheep/Pakistan	IV	KY967610.1
SRMV/Lahore/UVAS/Pak/2015	Goat/Pakistan	IV	KY967608.1
PPRV/Bangladesh/BD2/2008	Goat/Bangladesh	IV	MG581412.1

currently circulated in China (Silva et al., 2011). So a vaccine readily available for use in wild animals will be need for preventing possible PPRV epidemic (Mariner et al., 2017; Silva et al., 2011).

PPRV is still prevalent in some local places of the world, so controlling the epidemic disease of PPR is necessary. Nowadays, in order to control and prevent further spread of PPR, China as well as the whole world are implementing the PPR national eradication plan, which aims to eradicate PPR by 2020 (Baron et al., 2017). The importance of new vaccines for wild animals cannot be ignored. However, not all farmers have strong precautions and choose to vaccinate their sheep or goats, so there are still annual outbreaks of PPR in unvaccinated sheep and goats. Vaccine immunization was effectively used to prevent small ruminants disease. Sheep can be vaccinated with attenuated ruminant pest vaccine. Such as Nigeria 75/1 and Sungri 96 were most widely used vaccine strains (Saravanan et al., 2010). Multiple factors can affect transmission of PPRV among goats. Occasional or even frequent contact of wild with domestic animals might facilitate viral transmission. Transboundary transmission may be an additional factor in the high prevalence of PPRV mortality among goats and sheep. So we should prevent the infection of wild animals by delivering vaccine at designated sites in the field. Trying to adopt the centralized captive breeding mode of sheep and goats or any other domestic animals there will be less economic losses of breeding industries.

## 5. Conclusion

In summary, the PPRV ChinaGS2018 isolate was sequenced, assembled and analyzed against all available PPRV genome sequences to estimate nucleotide diversity and the evolutionary relationship. To our knowledge, this is the first report describing *Procapra przewalskii* infected with PPRV, which showed whole-genome sequence of a PPRV strain prevalent in Gansu province, western China. We foresee that the data reported in this study will enrich our knowledge about the evolution and transboundary nature of the PPRV, which will have important implications for effective control of PPRV in the world.

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## Declaration of Competing Interest

The authors declare no competing financial interests or conflicts.

## References

- Abubakar, M., Jamal, S.M., Arshed, M.J., Hussain, M., Ali, Q., 2009. Peste des petits ruminants virus (PPRV) infection; its association with species, seasonal variations and geography. *Trop. Anim. Health Prod.* 41, 1197–1202.
- Adombi, C.M., Waqas, A., Dundon, W.G., Li, S., Daojin, Y., Kakpo, L., Aplogan, G.L., Diop, M., Lo, M.M., Silber, R., Loitsch, A., Diallo, A., 2017. Peste des Petits ruminants in Benin: persistence of a single virus genotype in the country for over 42 years. *Transbound. Emerg. Dis.* 64, 1037–1044.
- Ashraf, W., Unger, H., Haris, S., Mobeen, A., Farooq, M., Asif, M., Khan, Q.M., 2017. Genetic detection of peste des petits ruminants virus under field conditions: a step forward towards disease eradication. *BMC Vet. Res.* 13, 34.
- Aziz Ul, R., Wensman, J.J., Abubakar, M., Shabbir, M.Z., Rossiter, P., 2018. Peste des petits ruminants in wild ungulates. *Trop. Anim. Health Prod.* 50 (8), 1815–1819.
- Bankamp, B., Liu, C., Rivailier, P., Bera, J., Shrivastava, S., Kirkness, E.F., Bellini, W.J., Rota, P.A., 2014. Wild-type measles viruses with non-standard genome lengths. *PLoS One* 9, e95470.
- Banyard, A.C., 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, 2885–2897.
- Bao, J., Wang, Q., Li, L., Liu, C., Zhang, Z., Li, J., Wang, S., Wu, X., Wang, Z., 2017. Evolutionary dynamics of recent peste des petits ruminants virus epidemic in China during 2013–2014. *Virology* 510, 156–164.
- Baron, M.D., Diop, B., Njeumi, F., Willett, B.J., Bailey, D., 2017. Future research to underpin successful peste des petits ruminants virus (PPRV) eradication. *J. Gen. Virol.* 98, 2635–2644.
- Chang, Q., Guo, F., Liu, J., Zhang, D., Feng, Y., Ma, X.X., Shang, Y., 2019. Basal interferon signaling and therapeutic use of interferons in controlling peste des petits ruminants virus infection. *Infect. Genet. Evol.* 103981.
- Elzein, E.M., Housawi, F.M., Bashareek, Y., Gameel, A.A., Al-Afaleq, A.L., Anderson, E., 2004. Severe PPR infection in gazelles kept under semi-free range conditions. *J. Vet. Med. B Infect. Dis Vet. Public Health* 51, 68–71.
- Gari, G., Serda, B., Negesa, D., Lemma, F., Asgedom, H., 2017. Serological investigation of peste des petits ruminants in east Shewa and Arsi Zones, Oromia Region, Ethiopia. *Vet. Med. Int.* 2017, 9769071.
- Hamdy, F.M., Dardiri, A.H., 1976. Response of white-tailed deer to infection with peste des petits ruminants virus. *J. Wildl. Dis.* 12, 516–522.
- Jenkins, G.M., Holmes, E.C., 2003. The extent of codon usage bias in human RNA viruses and its evolutionary origin. *Virus Res.* 92, 1–7.
- Kumar, N., Maherchandani, S., Kashyap, S.K., Singh, S.V., Sharma, S., Chaubey, K.K., Ly, H., 2014. Peste des petits ruminants virus infection of small ruminants: a comprehensive review. *Viruses* 6, 2287–2327.
- Kumar, N., Kulkarni, D.D., Lee, B., Kaushik, R., Bhatia, S., Sood, R., Pateriya, A.K., Bhat, S., Singh, V.P., 2018. Evolution of codon usage bias in Henipaviruses is governed by natural selection and is host-specific. *Viruses* 10.
- Li, X.P., Zhai, S.L., He, D.S., Guo, P.J., Lv, D.H., Wen, X.H., Luo, M.L., Chen, R.A., Wei, W.K., 2015. Genome characterization and phylogenetic analysis of a lineage IV peste des petits ruminants virus in southern China. *Virus Genes* 51, 361–366.
- Li, J., Li, L., Wu, X., Liu, F., Zou, Y., Wang, Q., Liu, C., Bao, J., Wang, W., Ma, W., Lin, H., Huang, J., Zheng, X., Wang, Z., 2017. Diagnosis of peste des petits ruminants in wild and domestic animals in Xinjiang, China, 2013–2016. *Transbound. Emerg. Dis.* 64, e43–e47.
- Liang, Z., Yuan, R., Chen, L., Zhu, X., Dou, Y., 2016. Molecular evolution and characterization of hemagglutinin (H) in peste des petits ruminants virus. *PLoS One* 11, e0152587.

- Liu, F., Li, J., Li, L., Liu, Y., Wu, X., Wang, Z., 2018. Peste des petits ruminants in China since its first outbreak in 2007: a 10-year review. *Transbound. Emerg. Dis.* 65, 638–648.
- Ma, X.X., Chang, Q.Y., Ma, P., Li, L.J., Zhou, X.K., Zhang, D.R., Li, M.S., Cao, X., Ma, Z.R., 2017. Analyses of nucleotide, codon and amino acids usages between peste des petits ruminants virus and rinderpest virus. *Gene* 637, 115–123.
- Ma, X.X., Wang, Y.N., Cao, X.A., Li, X.R., Liu, Y.S., Zhou, J.H., Cai, X.P., 2018. The effects of codon usage on the formation of secondary structures of nucleocapsid protein of peste des petits ruminants virus. *Genes Genomics* 40, 905–912.
- Manjunath, S., Kumar, G., Mishra, B., Mishra, B., Sahoo, A., Joshi, C., Tiwari, A., Rajak, K., Janga, S., 2015. Genomic analysis of host - Peste des petits ruminants vaccine viral transcriptome uncovers transcription factors modulating immune regulatory pathways. *Vet. Res.* 46, 15.
- Mantip, S.E., Shamaki, D., Farougou, S., 2019. Peste des petits ruminants in Africa: meta-analysis of the virus isolation in molecular epidemiology studies. *Onderstepoort J. Vet. Res.* 86, e1–e15.
- Mariner, J.C., Gachanja, J., Tindih, S.H., Toye, P., 2017. A thermostable presentation of the live, attenuated peste des petits ruminants vaccine in use in Africa and Asia. *Vaccine* 35, 3773–3779.
- Munir, M., 2014. Role of wild small ruminants in the epidemiology of peste des petits ruminants. *Transbound. Emerg. Dis.* 61, 411–424.
- Muniraju, M., Munir, M., Parthiban, A.R., Banyard, A.C., Bao, J., Wang, Z., Ayele, C., Ayelet, G., El Harrak, M., Mahapatra, M., Libeau, G., Batten, C., Parida, S., 2014. Molecular evolution of peste des petits ruminants virus. *Emerg. Infect. Dis.* 20, 2023–2033.
- Muthuchelvan, D., Sanyal, A., Sreenivasa, B.P., Saravanan, P., Dhar, P., Singh, R.P., Singh, R.K., Bandyopadhyay, S.K., 2006. Analysis of the matrix protein gene sequence of the Asian lineage of peste-des-petits ruminants vaccine virus. *Vet. Microbiol.* 113, 83–87.
- Sahu, A.R., Wani, S.A., Saminathan, M., Rajak, K.K., Sahoo, A.P., Pandey, A., Saxena, S., Kanchan, S., Tiwari, A.K., Mishra, B., Muthuchelvan, D., Singh, R.P., Singh, Y., Baig, M., Mishra, B.P., Singh, R.K., Gandham, R.K., 2017. Genome sequencing of an Indian peste des petits ruminants virus isolate, Izatnagar/94, and its implications for virus diversity, divergence and phylogeography. *Arch. Virol.* 162, 1677–1693.
- Saravanan, P., Sen, A., Balamurugan, V., Rajak, K.K., Bhanuprakash, V., Palaniswami, K.S., Nachimuthu, K., Thangavelu, A., Dhinakarraj, G., Hegde, R., Singh, R.K., 2010. Comparative efficacy of peste des petits ruminants (PPR) vaccines. *Biologicals* 38, 479–485.
- Schulz, C., Fast, C., Schlottau, K., Hoffmann, B., Beer, M., 2018. Neglected hosts of small ruminant Morbillivirus. *Emerg. Infect. Dis.* 24, 2334–2337.
- Silva, A.C., Carrondo, M.J., Alves, P.M., 2011. Strategies for improved stability of peste des petits ruminants vaccine. *Vaccine* 29, 4983–4991.
- Wang, J., Wang, M., Wang, S., Liu, Z., Shen, N., Si, W., Sun, G., Drewe, J.A., Cai, X., 2015. Peste des petits ruminants virus in Heilongjiang province, China, 2014. *Emerg. Infect. Dis.* 21, 677–680.
- Zhou, X.Y., Wang, Y., Zhu, J., Miao, Q.H., Zhu, L.Q., Zhan, S.H., Wang, G.J., Liu, G.Q., 2018. First report of peste des petits ruminants virus lineage II in Hydropotes inermis, China. *Transbound. Emerg. Dis.* 65, e205–e209.
- Zhou, J.H., Li, X.R., Lan, X., Han, S.Y., Wang, Y.N., Hu, Y., Pan, Q., 2019. The genetic divergences of codon usage shed new lights on transmission of hepatitis E virus from swine to human. *Infect. Genet. Evol.* 68, 23–29.