

Sequence analysis of peste des petits ruminants virus from ibexes in Xinjiang, China

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ABSTRACT. Peste des petits ruminants (PPR) is an infectious disease caused by peste des petits ruminants virus (PPRV). While PPR mainly affects domestic goats and sheep, it also affects wild ungulates such as ibex, blue sheep, and gazelle, although there are few reports regarding PPRV infection in wild animals. Between January 2015 and February 2015, it was found for the first time that wild ibexes died from PPRV infection in Bazhou, Xinjiang, China, where a total of 38 ibexes (including young and adult ibexes) were found to have died abnormally from PPR-related issues. First, we tested for the presence of the F gene of PPRV by RT-PCR. Then, we compared the sequence of the isolated F gene from the ibex strain, termed PPRV Xinjiang/Ibex/2015, with those previously identified from small domestic ruminants from local areas

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near where the reported isolate was collected as well as those from other regions. The current sequence was phylogenetically classified as a lineage IV virus, and shared a high level of sequence identity (99.7%) with a previously described Xinjiang PPRV isolate.

Key words: Peste des petits ruminants; Ibex; Xinjiang

INTRODUCTION

Peste des petits ruminants (PPR) is an endemic viral disease affecting sheep and goats across many areas of Africa, the Middle East, and Asia, and is caused by peste des petits ruminants virus (PPRV) (Govindarajan et al., 1997). PPR is categorized as a Food and Agriculture Organization of the United Nations and World Organization for Animal Health (FAO/OIE) grade A severe contagious disease, with an incidence and mortality rate as high as 100 and 90%, respectively (Zhu et al., 2009). Additionally, PPR is classified as a firstclass animal contagious disease in China (Zhu et al., 2009). PPRV is a negative-sense, singlestranded RNA virus of the genus Morbillivirus, which is genetically grouped into four lineages (I, II, III, and IV) based on partial sequence analysis of the fusion (F) gene, of which lineages I to III circulate in Africa and lineage IV in Asia (Dhar et al., 2002). There are six structural PPRV proteins that are encoded by 6 genes, namely N-P-M-F-H-I in that order from the 3'to the 5'-terminus of the viral genome (Bailey et al., 2005). The F protein is a fusion protein that is important for successful viral infection. Specifically, the spikes on the viral envelop surface are comprised of the F protein, and are associated with viral adherence and invasion (Muthuchelvan et al., 2006), which are crucial steps for successful infection (Haffar et al., 1999; Jing et al., 2004). PPR was first reported in West Africa in 1940, and is now mainly present in Africa, the Middle East, the Arabian Peninsula, and South Asia (Özkul et al., 2002).

In July 2007, a PPR outbreak was reported in the Ngari region of southwestern Tibet, China (Wang et al., 2009). In December 2013, a new outbreak of PPR was reported in Xinjiang Province, and PPR was also detected in Gansu, Inner Mongolia, Ningxia, Jiangxi, and Hunan Provinces by mid-March 2014 (Wang et al., 2015). The ibex, also known as the Asian antelope, is under First-Grade State Protection in China, and primarily lives in groups distributed among the Xinjiang Tianshan Mountains, northwestern Gansu Province, and the northwestern Inner Mongolia Autonomous Region. Therefore, the aim of the current study was to compare the sequences of the F gene of PPRV infecting ibexes in Xinjiang, China, with other PPRV sequences available in the GenBank database in order to improve our knowledge of the epidemiology of PPR in the country.

MATERIAL AND METHODS

Specimen collection

The Bazhou region is approximately 500 km away from Urumqi, the capital of Xinjiang Province in northwest China. The region is home to a wide range of wild animals including ibex, argali, wild yak, deer, brown bear, and snow leopard. A total of 38 wild ibexes died abnormally in the Bazhou region from January to February 2015. The cause of death seemed to be PPR according to the clinical signs and symptoms. As such, the Xinjiang Academy of

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Animal Science cooperated with the China Animal Health and Epidemiology Center to further investigate the mortalities of ibexes in this region. Lungs, lymph nodes, and swab samples were collected from suspected PPRV-infected ibexes, all of which showed typical symptoms of PPR, including fever, lassitude, anorexia, and diarrhea. Clinical tissues were homogenized for RNA extraction, and the remaining samples were kept at -20°C until further use.

Viral genomic RNA extraction

Homogenized tissue slurry (200 μ L) was mixed with 1 mL TRIzol reagent (Thermo Fisher, Carlsbad, CA, USA) and incubated at room temperature for 6-10 min. Then, 200 μ L chloroform was added and the solution was incubated for 10 min. The mixture was then centrifuged at 12,000 rpm for 20 min and the supernatant was discarded. The pellet was washed with 75% ethanol (v/v), dried at 37°C for 5-10 min, diluted in 20 μ L DEPC water, and stored at -20°C until later use.

Reverse transcription-polymerase chain reaction (RT-PCR) amplification of the target gene

The following primers were designed using the Oligo 6.0 software (Molecular Biology Insights, Cascade, CO, USA) for target F gene amplification based on the genome sequence of PPRV Nigeria 75/1 strain available in the National Institutes of Health (NIH) National Center for Biotechnology Information (NCBI) GenBank: P1, 5'-ATGCTCTGTCGGTGATAAC-3' and P2, 5'-TCGCATGACATTCTATGAAC-3'. The anticipated length of the amplified product was 322 bp. The SuperScript I One-Step RT-PCR Platinum Taq HiFi System (Invitrogen, Carlsbad, CA, USA) reaction mixtures consisted of 3 μ L total RNA, 25 μ L Reaction Mix, 5 μ L dNTPs, 3 μ L primers, 1 μ L RT/Platinum Taq, and 13 μ L RNase Free ddH₂O. The cycling conditions for target F gene amplification were as follows: 1 cycle of RT (50°C for 30 min, and 94°C for 2 min); followed by 30 cycles of PCR including denaturation (94°C for 30 s), annealing (56°C for 30 s), and extension (72°C for 1 m); and then a final extension at 72°C for 10 min.

Target gene cloning and sequencing

The RT-PCR products were purified using a DNA gel extraction kit (Bioteck, Beijing, China) following the manufacturer protocol. The purified products were inserted into the pMD18-T vector and transformed into HD5 α cells. The transformed cells were screened, the plasmid was extracted, and the target gene was detected using 1% agarose gel electrophoresis following PCR. The screened positive clones were then sent to Shanghai Bioengineering Co. Ltd. (Shanghai, China) for sequencing. All data were processed and analyzed with the DNASTAR software (Lasergene, Madison, WI, USA).

RESULTS

RT-PCR F gene amplification

The RT-PCR amplification product of the Xinjiang/Ibex/2015 strain F gene was

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detected by 1% agarose gel electrophoresis. The results showed that the size of the amplified fragment was consistent with that of the anticipated product, and was approximately 322 bp (Figure 1).



Figure 1. RT-PCR products following F gene amplification and 1% agarose gel electrophoresis. *Lane* M = DL2000 bp; *lane* 1 = sample 1; *lane* 2 = sample 2; *lane* 3 = control.

F gene sequencing

F gene homology analysis

Gene homology analysis revealed that the nucleotide sequence homology between the PPRV Xinjiang/Ibex/2015 F gene and domestic and foreign reference strain F genes was 88.5-99.7%, with the lowest sequence homology (88.5%) to that of the F gene of the Yemen_2001b strain, and the highest sequence homology (up to 99.7%) to those of the domestic separated strains China/XJYL/2013, China/Shaanxi/2014, and China/Beijing/2014 (Figure 2).

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Sequence analysis of PPRV from ibexes in China

											Perc	entide	entity												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
1		96.6	98.4	97.8	99.1	99.1	90.7	97.2	98.1	89.1	95.0	98.4	97.5	97,5	97.8	88.5	93.2	99.1	97.8	97.2	97.8	97.8	97.5	1	Nepal 2009
2	3.5		96.9	97.5	97.5	96.3	89.8	97.5	97.8	88.2	94.7	96.9	97.2	97.2	97.5	88.2	91.9	96.9	96.3	95.7	96.3	96.3	96.0	2	Iraq 2002
3	1.6	3.2		98.1	99.4	98.1	91,3	97.5	98.4	89.8	95.3	98.8	97.8	97.8	98.1	89.1	93.8	98.1	99.4	98.8	99.4	99.4	99.1	3	R22 10
4	22	2.5	1.9		98.8	97.5	90.7	98.1	99.1	89.1	96.0	98.1	98.4	98.4	98.8	89.1	93.2	97.5	97.5	96.9	97.5	97.5	97.2	4	Mdn 96
5	0.9	2.5	0.6	1.3		98.8	91.3	98.1	99.1	89.8	96.0	99.4	98.4	98.4	98.8	89.1	93.8	98.8	98.8	98.1	98.8	98.8	98.4	5	Kcch 2000
6	0.9	3.8	1.9	2.5	1.3		90.4	96.9	97.8	88.8	94.7	98.1	97.2	97.2	97.5	88.2	92.9	98.8	97.5	96.9	97.5	97.5	97.2	6	Bsk Guj 05
7	10.1	11.2	9.4	10.1	9.4	10.5		90.1	91.3	97.8	90.1	91.3	91.0	90.4	90.7	97.5	91.9	90.4	91.0	90.7	91.0	91.0	90.7	7	Gedarif KSUD71
8	2.9	2.5	2.5	1.9	1.9	3.2	10.9		98.4	88.5	95.3	97.5	97.8	97.8	98.1	88.5	92.5	96.9	96.9	96.3	96.9	96.9	96.6	8	Egypt 111 2009
9	1.9	22	1.6	0.9	0.9	22	9.4	1.6		89.8	96.6	98.4	98.8	98.8	99.1	89.8	93.8	97.8	97.8	97.2	97.8	97.8	97.5	9	TamilNadu 2011
10	10.7	11.8	9.9	10.7	9.9	11.1	1.0	11.5	9.9		88.2	89.8	89.4	88.8	89.1	96.0	90.1	88.8	89.4	89,4	89.4	89.4	89.1	10	sudan 1972.seq
11	5.2	5.5	4.8	4.2	4.2	5.5	10.9	4.9	3.5	11.8		95.3	95.7	95.7	96.0	88.5	93.2	94.7	94.7	94.1	94.7	94.7	94.4	11	Soba Khartoum KHSUD08
12	1.6	3.2	1.3	1.9	0.6	1.9	9.4	2.5	1.6	9.9	4.9		97.8	97.8	98.1	89.1	93.2	98.1	98.1	97.5	98.1	98.1	97.8	12	Pakistan 2009
13	2.5	2.9	22	1.6	1.6	2.9	9.8	22	1.3	10.3	4.5	22		98.1	98.4	90.1	93.5	97.2	97.2	96.6	97.2	97.2	96.9	13	Morocco 08-9
14	2.5	2.9	22	1.6	1.6	2.9	10.5	22	1.3	11.1	4.5	22	1.9		98.4	88.8	92.9	97.2	97.2	96.6	97.2	97.2	96.9	14	Meh Guj 05
15	22	2.5	1.9	1.3	1.3	2.5	10,1	1.9	0.9	10.6	4.2	1.9	1.6	1.6		89.1	93.2	97.5	97.5	96.9	97.5	97.5	97.2	15	Kuwait 1999
16	12.8	13.1	12.0	12.1	12.1	13.2	2.5	12.8	11.3	2.9	12.8	12.1	10.9	12.5	12.0		90.4	88.2	88.8	88.8	88.8	88.8	88.5	16	Yemen 2001b
17	7.3	8.7	6.6	7.3	6.6	7.7	8.7	8.0	6.6	9.6	7.3	7.3	6.9	7.7	7.3	10.6		92.9	93.2	92.5	93.2	93.2	92.9	17	Nigeria 75-1
18	0.9	3.2	1.9	2.5	1.3	1.3	10.5	3.2	22	11.1	5.5	1.9	2.9	2.9	2.5	13.2	7.7		97.5	96.9	97.5	97.5	97.2	18	CH Tib 07
19	22	3.8	0.6	2.5	1.3	2.5	9.7	3.2	22	10.3	5.5	1.9	2.9	2.9	2.5	12.4	7.3	2.5		99.4	100.0	100.0	99.7	19	CH Shaanxi 2014
20	2.9	4.5	1.3	3.2	1.9	3.2	10.1	3.9	2.9	10.3	6.2	2.5	3.5	3.5	3.2	12.4	8.0	3.2	0.6		99.4	99.4	99.1	20	CH HNZM 2014
21	22	3.8	0.6	2.5	1.3	2.5	9.7	3.2	22	10.3	5.5	1.9	2.9	2.9	2.5	12.4	7.3	2.5	0.0	0.6		100.0	99.7	21	CH BJ 2014
22	22	3.8	0.6	2.5	1.3	2.5	9.7	3.2	22	10.3	5.5	1.9	2.9	2.9	2.5	12.4	7.3	2.5	0.0	0.6	0.0		99.7	22	CH XJYL 2013
23	2.5	4.2	0.9	2.9	1.6	2.9	10.1	3.5	2.5	10.7	5.9	22	3.2	3.2	2.9	12.8	7.6	2.9	0.3	0.9	0.3	0.3		23	CH XJIbex 2015
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		

Figure 2. F gene nucleotide homology analysis.

Evolutionary genetic analysis of the F gene

Evolutionary genetic analysis was conducted on the F gene from 23 PPRV strains. The results showed that the F gene of the PPRV Xinjiang/Ibex/2015 strain was most similar to those of the China/XJYL/2013 and China/Shaanxi/2014 viral strains, suggesting that these genes may share a common origin, and that they belonged to the same branch, lineage IV, along with the Iraq_2002, Nepal_2009, and China/Tibet/07-1 strains (Figure 3).



Figure 3. Phylogenetic analysis based on nucleotide sequences of the F gene.

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DISCUSSION

In the current study, PPRV infection was identified via RT-PCR amplification of the viral F gene from ibex samples collected in Xinjiang, China. For the first time, it was found that 38 ibexes (including young and adult ibexes) died within a 2-month period in 2015 from PPR in Xinjiang. Based on the 322-bp sequence of the F gene characterized in this study, a phylogenetic tree was constructed based on similar sequences identified from GenBank. The phylogenetic analysis indicated that the F gene sequence from the Xinjiang/Ibex/2015 strain was in lineage IV along with PPRV strains from Asia and the Middle East. Moreover, the results showed that the PPRV detected from these ibexes shared a high-level of sequence homology with PPRV strains of the lineage IV type, exhibiting 94.4-99.7% sequence identity at the nucleotide level, especially to strains isolated in China, which exhibited a higher sequence identity of 99.1-99.7% (Figure 1).

In conclusion, for the first time it was found that ibexes in Xinjiang were infected with PPRV, and that the virus was in lineage IV. Meanwhile, these cases were found to be negative for foot and mouth disease, pasteurella, and streptococcus. The results of the current study add to the genetic resources available for PPR, provide a basis for further study of F protein biological functions, and provide meaningful information for future investigations into the molecular epidemiology of PPR in wild animals. Additionally, countries surrounding China, including India, Iran, and Pakistan are all areas severely affected by PPR, and will thus benefit from this information. Since the first discovery of PPR in Tibet in 2007, PPR continues to spread in China, reminding us to stay alert and strengthen PPR immunosurveillance in order to one day eradicate this disease.

Conflicts of interest

The authors declare no conflict of interest.

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