http://dx.doi.org/10.1590/1678-4162-9277

Arq. Bras. Med. Vet. Zootec., **v.69, n.5, p.1105-1113, 2017**

Confirmation and Sequence analysis of N gene of PPRV in South Xinjiang, China

[*Confirmação e análise de sequência do gene N de PPRV em Xinjiang do Sul, China*]

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ABSTRACT

In China, Peste des petits ruminants (PPR) was officially first reported in 2007. From 2010 until the outbreak of 2013, PPRV infection was not reported. In November 2013, PPRV re-emerged in Xinjiang and rapidly spread to 22 P/A/M (provinces, autonomous regions and municipalities) of China. In the study, suspected PPRV-infected sheep in a breeding farm of South Xinjiang in 2014 were diagnosed and the characteristics of complete sequence of N protein gene of PPRV was analyzed. The sheep showed PPRV-infected signs, such as fever, orinasal secretions increase, dyspnea and diarrhea, with 60% of morbidity and 21.1% of fatality rate. The macroscopic lesions after autopsy and histopathological changes were observed under light microscope including stomatitis, broncho-interstitial pneumonia, catarrhal hemorrhagic enteritis and intracytoplasmic eosinophilic inclusions in multinucleated giantcell in lung. The formalin-fixed mixed tissues samples were positive by nucleic acid extraction and RT-PCR detection. The nucleotide of N protein gene of China/XJNJ/2014 strain was extremely high homology with the China/XJYL/2013 strain, and the highest with PRADESH_95 strain from India in exotic strains. Phylogenetic analysis based on complete sequence of N protein gene of PPRV showed that the China/XJNJ/2014 strain, other strain of 2013-2014 in this study and Tibetan strains all belonged to lineage IV, but the PPRV strains of 2013-2014 in this study and Tibetan strains were in different sub-branches.

Keywords: PPR; diagnosis; N gene; sequence analysis

RESUMO

Na China, Peste des petits ruminants (PPR) foi relatado oficialmente em 2007. De 2010 até o surto de 2013, não houve relato de infecção por PPRV. Em Novembro de 2013, PPRV ressurgiu em Xinjiang e rapidamente se espalhou para 22 P/A/M (províncias, regiões autônomas e municípios) da China. No estudo, ovelhas com suspeita de infecção por PPRV em uma fazenda de reprodução no sul de Xinjiang form diagnosticadas em 2014 e as características da sequência completa da proteína N do gene do PPRV foi analisada. As ovelhas tinham sinais de infecção pelo PPRV, como febre, aumento de secreções oro-nasais, dispneia e diarreia, com 60% de morbidade e 21.1% de fatalidade. As lesões macroscópicas após mudanças histopatológicas foram observadas sob microscópio, incluindo estomatite, pneumonia bronco-intersticial, enterite hemorrágica catarral e inclusões eosinofílicas intracitoplasmáticas em células gigantes multinucleares no pulmão. As amostras de tecido fixadas em formalina testaram positivo para detecção de RT-PCR por extração de ácido nucleico. Os nucleotídeos da proteína N do gene da linhagem China/XJNJ/2014 apresentou extrema homologia com o China/XJYL/2013, e homologia ainda maior com a variedade PRADESH-95 da Índia. Análise filogenética baseada na sequencia completa da proteína N do gene de PPRV mostrou que as variedades China/XJNJ/2014, outra de 2013-2014 mostrada nesse estudo e as Tibetanas todas pertenciam à linhagem IV, mas as PPRV de 2013-2014 nesse estudo e as Tibetanas estavam em diferentes agrupamentos.

Palavras-chave: PPR, diagnóstico, gene N, análise de sequencia

Recebido em 30 de junho de 2016
Aceito em 29 de setembro de 2016
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INTRODUCTION

Peste des petits ruminants (PPR), caused by peste des petits ruminants virus (PPRV), is a kind of acute, highly contagious and infectious disease (Dhinakar *et al*. 2001, Libeau *et al*. 2014). The characteristic of PPR is high fever, oculonasal secretions increase, stomatitis, enteritis, pneumonia, high morbidity mortality (Luka *et al*. 2012). PPRV mainly inf and ected sheep, goats, and wild small ruminants (Libeau *et al*. 2014), but also other ruminants including cattle, buffalo, camel (Kwiatek *et al*. 2011, Balamurugan *et al*. 2012a) and Asiatic lion (Balamurugan *et al*. 2012b). PPRV was classified under *Morbillivirus* of the family *Paramyxoviridae* (Gibbs *et al*. 1979), and has only one serotype (Anees *et al*. 2013). PPRV is an enveloped negative-sense single-stranded RNA virus with the genome of 15948 nucleotides (nt) in length. Recently, novel strains have emerged which contain 15 954 nucleotides and 15 957 nucleotides in China and 15 942 nucleotides in India (Bao *et al*. 2014, Su *et al*. 2015). The viral genome is composed of 6 genes (N, P, M, F, H and L), encoding 6 structural proteins, respectively (Shaila *et al*. 1996, Luka *et al*.2012, Anees *et al*. 2013, Kumar *et al*. 2014, Su *et al*. 2015). According to the literature, PPRVs have been classified into 4 lineages (lineages I, II, III, and IV) based on the N genes (Maganga *et al*. 2013).

PPR was first reported in Ivory Coast of West Africa in 1942 (GARGADENNEC and LALANNE, 1942; Maganga *et al*. 2013). Currently, PPR is mainly prevalent in Africa, Asia and Middle East regions (Munir *et al*. 2012, Kgotlele *et al*. 2014). The China Animal Health and Epidemiology Center, based on descriptive epidemiologic and serologic evidence, first indicated the presence of PPR in Tibet in 2005. Molecular epidemiologic detection confirmed PPR emerged in Tibet from July through October in 2007 (Wang *et al*. 2009). After that, three or more PPR outbreaks were reported between 2008 and 2010 in Tibet. In November 2013, PPR re-emerged in Huocheng County in Xinjiang Uygur Autonomous Region (Bao *et al*. 2014), and was also reported in Beijing (Su *et al*. 2015), Gulang County of Gansu, Wulatehou Banner of Inner Mongolia Autonomous Region, Yanchi County of Ningxia Autonomous Region, and Dongkou County of Hunan Provinces in China, and so on. At least 256 counties or banners in 22 P/A/M in 2014 were infected (Wang *et al*. 2009, Wu *et al*. 2015). Yunnan, Guizhou, Anhui, Hubei and Jiangsu Provinces were PPR-stricken areas. The peak period of outbreaks was in April 2014 in China. More than 70 000 sheep and goats have been culled in September 2014 causing huge loss (Wu *et al*. 2015).

This study reported the diagnosis of sheep suspected with PPR and the characteristics of the complete sequence of N protein gene of PPRV in a breeding farm of South Xinjiang in 2014.

MATERIALS AND METHODS

There were about 10 000 sheep being raised in a breeding farm in the Akesu region of south Xinjiang. Most of these sheep had been bought from the surrounding townships (indigenous breed, Mekit sheep) and Yili region of Xinjiang (exotic breed), a few of them bought from Gansu province (exotic breed). The scattered sheep which were raised by individual farmers (20-50 sheep per household) combined into a large herd, then rotational grazing. In mid-May of 2014, a herd of sheep with 5 goats was infected after grazing near a reservoir. Five of the dead sheep have been examined using autopsy after on-site diagnosis by staff of the South Xinjiang Diagnostic Center for Livestock and Poultry Disease. The viscus tissues samples of the five sheep were fixed in 10% neutral formalin, separately.

The histopathological changes of the formalin-fixed tissues samples which were conventional paraffin-embedded sections and H.E. stained were observed under light microscope. The PPR viral RNA was extracted from the five formalin-fixed mixed tissues samples (lungs, lymph glands, spleens, livers and kidneys) using RNeasy® FFPE kit (QIAGEN, Code No. 217504) in accordance with the instructions provided by the manufacturer. The extracted RNA was used to amplify the DNA by one-step RT-PCR according to the instruction of RNA LA PCRTM Kit (AMV) Ver.1.1 (TaKaRa, Code No. RR012A). The primers of N gene (upstream primer: 5′-AGA GGA GGA GGA GCA AGA-3′ and downstream primer: 5′-GTA TCA GGG TTC GGT GTT-3′) were designed by using Primer PREMIER Version 5.0 software. The optimum annealing temperature was 55℃. The length of amplified products was 1 881 bp. Lyophilized attenuated live PPR vaccine (Nigeria 75/1) bought from Xinjiang Recon Animal Husbandry Bio-Technology was used as the positive control in the study. The PCR amplified products were purified following the instructions of TIANgel Midi Purification Kit (TIANGEN®, Code No. DP209), and sequenced using ABI3730XL sequencer. The SeqMan program and EditSeq program of DNAStar were used to splice and edit

the sequences (Table 1), respectively. Identity of the nucleotide sequence and amino acid sequence were reckoned by the Clustal W method of the MegAlign program in the DNAStar software. The phylogenetic tree was reconstructed by the neighbour-joining method of the Kimura two-parameter model in the MEGA5 version 5. The obtained sequences in the study have been submitted to GenBank under the accession numbers "GenBank accession NO.: KX938427" for N gene.

Table 1. Information of Peste des petits ruminants virus strains and N Genes from GenBank

No.	Strain Name	GenBank accession NO.	Host	District	Time
1	**China/XJYL/2013**	KM091959	goat	Yili of Sinkiang, China	30-Nov-2013
2	China/BJ/2014	KP260624	milk goat	Beijing, China	16-Aug-2014
3	**Ethiopia 1994**	KJ867540	goat	Ethiopia	1994
4	**Ethiopia 2010**	KJ867541	goat	Ethiopia	2010
5	**Ghana/NK1/2010**	KJ466104	sheep	Ghana	2010
6	CH/HNNY/2014	KM089830	goat	Henan, China	08-May-2014
7	CH/HNZK/2014	KM089831	goat	Henan, China	08-May-2014
8	**CH/HNZM/2014**	KM089832	goat	Henan, China	08-May-2014
9	**ICV89**	EU267273	goat	Cote d'Ivoire	1989
10	PRADESH_95	JN647694	not specified	India	1995
11	**Sungri/96**	KF727981	goat	India	1996
12	Sungri 1996 MSD (Netherlands)	KJ867542	goat	India	1996, vaccine strain
13	Jhansi-2003	GU014571	goat	India	2003
14	Jhansi 03	EU344738	goat	India: Jhansi, Uttar Pradesh	2003
15	Revati 2005	FJ750559	sheep	India: Revati, Ballia, Uttrapradesh	2005
16	Revati-2006	GU014574	sheep	India	2006
17	Guj/2007	JN632532	Panthera leo persica (Asiatic lion)	India	2007
18	India/TN/Gingee/2014	KR261605	goat	India	16-Sep-2014
19	Izatnagar/94	KR140086	goat	India	1994, virulent strain
20	KN5/2011	KM463083	goat; sex: female; age: 6 months	Kenya	May-2011
21	Morocco-2008	KC609745	alpine goat	Morocco	2008
22	**Morocco 2008**	KC594074	alpine goat	Morocco	2008
23	**Nigeria/75/1**	HQ197753	caprine goat	Nigeria	1975
24	Nigeria/75/1	X74443	-	Nigeria	vaccine strain
25	Ng76/1	EU267274	goat	Nigeria	1976
26	Oman 1983	KJ867544	goat	Oman	1983
27	DORCAS_87	JN647695	not specified	Oman	1987
28	**E32/1969**	KP789375	goat	Senegal	03-Sep-1969
29	SnDk11I13	KM212177	goat: eye swab from 1 year old female host	Senegal	11-Mar-2013
30	IND/TN/GIN/2014/01	KT270355	goat	India	25-Sep-2014
31	MIELIK_72	JN647693	not specified	Sudan	1972
32	**Tibet/Bharal/2008**	JX217850	wild bharal	Tibet, China	2008
33	**China/Tibet/0701**	EU360596	goat	Tibet, China	2007
34	China/Tib/07	JF939201	goat	Tibet, China	Dec-2007
35	x11	GQ184299	goat	Tibet, China	Aug-2007
36	**China/Tibet/Geg/07-30**	FJ905304	goat	Tibet, China	Aug-2007
37	Turkey 2000	NC_006383	ovis aries	Turkey	2000
38	Turkey 2000	AJ849636	ovis aries	Turkey	2000
39	UAE 1986	KJ867545	dorcas gazelle	United Arab Emirates	1986
40	**Bhopal 2003**	FJ750560	goat	India:Bhopal,Madhyapradesh	2003
41	**Sungri-96**	GQ452013	goat	India	1996
42	Sungri/96	AY560591	goat	India	1996, candidate vaccine virus
43	Turkey/00	AJ563705	not specified	Turkey	2000
44	**Uganda 2012**	KJ867543	goat	Uganda	2012
45	**China/XJNJ/2014**	KX938427	sheep	South Xinjiang, China	May-2014

RESULTS

The morbidity and clinical signs of sheep suspected with PPR. The morbidity was 60 percent (180/300). There were 100 serious cases of 180 (55.6%) PPRV-infected sheep, most of which were 4-6 months and few of them were 1-3 years, and 38 (21.1%) of them were dead. The main manifestations of illness were high fever, dullness, loss of appetite, large amounts of gray smelly orinasal discharge, dyspnea, diarrhea and emaciation. It is important to note that PPRV-infected sheep were all indigenous breed. Abnormal sheep were also found by investigation in other herds of the region.

The main clinical signs included fever, dullness, diarrhea, lacrimation, matting of eye lids, purulent oculonasal discharges, cutaneous nodules, erosions on the soft palate and gums, and laboured breathing were observed (Fig. 1a). Color inconsistency in different areas, multiple dark red and firm areas in the lungs were detected (Fig. 1b). Red foamed liquid flowed from section of lungs. The other results of pathological autopsy included haemorrhages and congestion of the intestines, watery intestinal contents (Fig. 1c), uneven section of grey-red lymph nodes, patches of grey-yellow on kidney surfaces (Fig. 1d). There were no apparent macroscopic lesions in other organs.

The histopathological changes of dead sheep suspected with PPR: Lung lesions of broncho-interstitial pneumonia were seen obviously in the sheep. The epithelial cells of the bronchi, bronchioles and alveoli showed hydropic degeneration. The alveoli lumina were filled with edematous fluid, desquamated epithelial cells, erythrocyte and inflammatory cells. The infiltration with larger macrophages and lymphocytes were observed in alveoli and interstitial tissue of the lungs. Hyperplasia of typical syncytial cells both focal and scattered were observed in the alveoli lumina. Intracytoplasmic eosinophilic inclusions in macrophages, syncytial cells and alveolar type II epithelial cells of lung and epithelial cells of the bronchi and bronchioles (Fig. 1e) were observed. The lesions of intestines consisted of widely degeneration and necrosis of epithelial cells of the intestinal mucosa and enteraden, edema, congestion and hemorrhage of the lamina propria mucosae and submucosa which were infiltrated with inflammatory cells mainly consisting of macrophages and lymphocytes. Extensive cellular degenerations and necrosis existed in the parenchyma cells of organs such as liver, spleen, lymph nodes and kidney. In addition, macrophages, lymphocytes and plasmocytes infiltrated in interstitial tissue of the liver, spleen, lymph nodes and kidney were observed.

Result of RT-PCR. Three (60%) of the five formalin-fixed mixed tissues samples were positive by agarose gel electrophoretic identification.

Result of sequencing and sequence analysis of N protein gene. The sequences of the N protein gene of the three positive samples were absolutely accordant. One of strains, named China/XJNJ/2014 was composed of 1 578 nucleotides encoding a protein of 525 amino acids and submitted at GenBank. GenBank accession number was “GenBank accession NO.: KX938427”. The nucleotide of N protein gene of China/XJNJ/2014 strain had extremely high homology with China/XJYL/2013 strain (99.9%) from the Yili region of Xinjiang reported as the first strain in the PPR outbreak, with 99.0-99.8% nucleotide identity with the PPRV strains of China in 2014, with the lowest homology of 88.8% with Uganda 2012 strain of PPRV from Uganda, with homology of 97.7-98.0% and 93.6% with Tibet strains in 2007-2008 and Nigeria/75/1 which was used as Chinese vaccine strain, respectively, and with the highest homology of 98.0% with PRADESH_95 strain from Indian in exotic strains. The amino acid of N protein of China/XJNJ/2014 strain exhibited 100% identity with three strains (China/XJYL/2013 strain, CH/HNNY/2014 strain and CH/HNZM/2014 strain), followed by other strains from China in 2014 including CH/HNZK/2014 strain (99.8%) and China/BJ/2014 strain (99.2%), and the lowest (92.6%) was Oman 1983 strain and DORCAS_87 strain of PPRV from Oman, with Tibet strains in 2007-2008 and Nigeria/75/1 strain were 98.7-98.9% and 95.0% respectively, with PRADESH_95 strain from India was the highest (98.5%) in exotic strains.

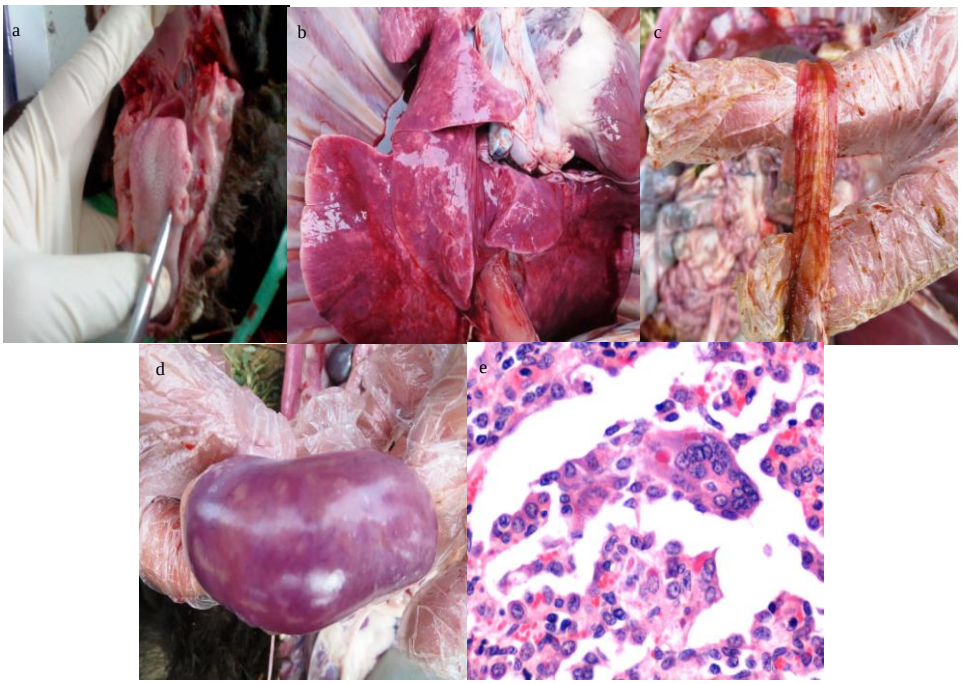

Figure 1. Post mortem and histopathological changes in sheep with PPR. (a) Gray-white ulceration on tongue. (b) Multiple dark red and firm areas on lung. (c) Red stripe on intestinal mucosa. (d) patches of grey-yellow on kidney surface. (e) Intracytoplasmic eosinophilic inclusions in syncytial cell and alveolar type II epithelial cells of lung (H.E.×400).

The 45 PPRV strains analyzed in the study were divided into 4 lineages (I-IV) based on the complete sequence of N protein genes and all involved Chinese strains clustered into lineage IV along with 14 strains from India, 2 strains from Morocco, 1 strain from Ethiopia, and 3 strains from Turkey (Fig. 2). The China/XJNJ/2014 strain and other involved Chinese PPRV strains of 2013-2014 in the study were in same sub-branches, but Tibetan strains from China in 2007-2008 were in other sub-branches. Moreover, Phylogenetic analysis revealed that the China/XJNJ/2014 strain was the most closely related to the two Indian strains in 2014.

DISCUSSION

The herd of sheep in the study had PPR symptoms such as fever, orinasal secretions increase, dyspnea and diarrhea. Morbidity and fatality rate of the disease was 60% and 21.1%, respectively. The fatality rate may be above this figure because culled PPRV-infected sheep have not been included. The macroscopic lesions after autopsy and histopathological changes under light microscope included stomatitis, broncho-interstitial pneumonia, catarrhal hemorrhagic enteritis and intracytoplasmic eosinophilic inclusions in multinucleated giantcell of lung. The positive rate of the formalin-fixed mixed tissues samples was 60% by RT-PCR detection. The results of sequencing and sequence analysis of N protein gene were consistent with PPRV gene. It was confirmed that the herd was infected with PPRV when the outbreak occurred. In the study, it should be noted that PPRV were detected from the formalin-fixed tissues samples which have several advantages: to prevent the spread of the virus, do not need cold chain transport and archived pathological tissues samples can be used for retrospective study, without affecting detection of PPRV (Kihu *et al*. 2015).

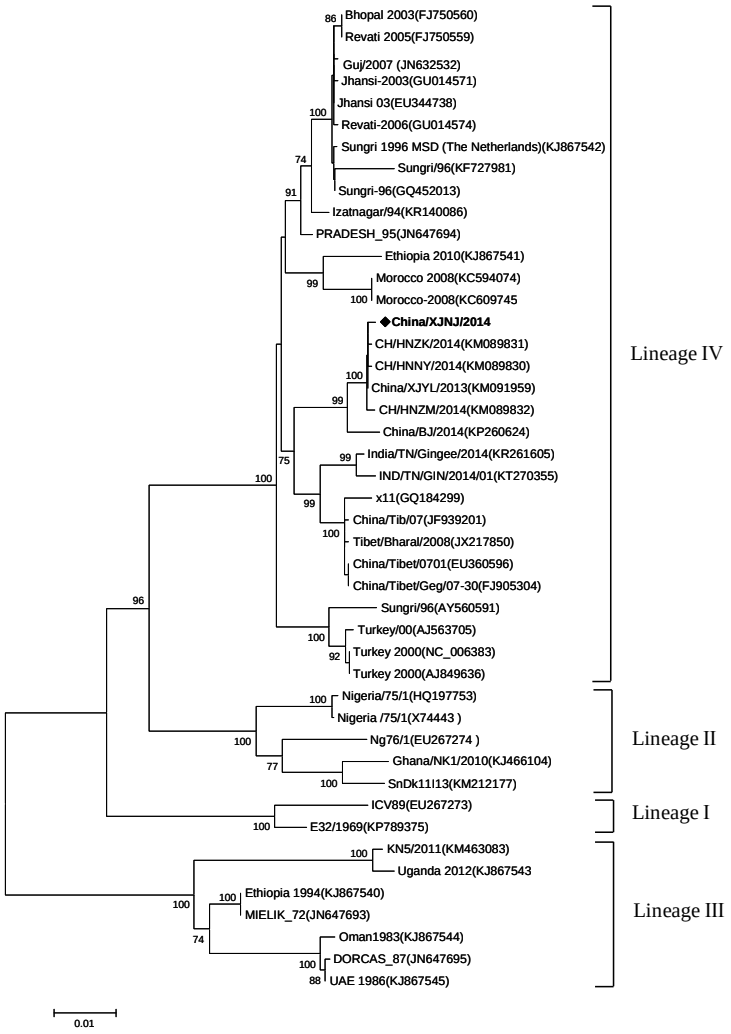

Figure 2. Phylogenetic tree based on the complete sequence of N protein gene constructed, it can show actual time and region sources of PPRV strain. The neighbor-joining method and the Kimura two parameter model were used for phylogenetic analysis in MEGA5 version 5 at bootstrap value 1000 replicates, and values <70% were not shown. Horizontal ines were proportional to sequence distances. Figure indicates the clear division of the four lineages of PPRV. Sequences of south Xinjiang strains were marked with black rhombus (◆).

In 2013-2014, China had suffered the unprecedented outbreak of PPR, including at least 256 counties or banners in 22 P/A/M and over 70 000 sheep and goats have been culled caused huge loss for sheep farming (Wu *et al*. 2015). Although the presence of PPR was indicated in Tibet as early as 2005 using the descriptive epidemiologic and serologic method and confirmed by molecular epidemiologic detection in Tibet from July through November 2007. Afterwards, three or more outbreaks of PPR were reported between 2008 and 2010 in Tibet. However, from 2010 until the outbreak of 2013, PPRV infection was not officially reported in China (Wang *et al*., 2009).

Phylogenetic analysis showed that all involved Chinese strains in the study belonged to lineage IV, but in different sub-branches with Tibetan strains of 2007-2008 (Bao, *et al*., 2014, Wu *et al*., 2015). Outbreaks of PPR have been reported in 7 countries (Mongolia, Russia, Kazakhstan,

Tajikistan, Afghanistan, Pakistan and India) adjacent to Xinjiang of China, which means that PPRV was present in these countries. The New Silk Road strategy further drove the development of sheep raised in Xinjiang and led to sheep production increase. Uighurs are overwhelmingly Muslims who have greater need for sheep products in the Xinjiang Uygur Autonomous Region. There were abundant and various wild small ruminant animals in the border of Xinjiang, and PPRV can spread between domestic animals and wild small ruminants, so wild small ruminants in the vicinity of the boundaries of Xinjiang probably played a role in cross-border dissemination of PPRV. In addition, before and after the Chinese New Year, the consumption of mutton was considerable causing frequent sheep transportation to different places. After comprehensive analysis of these reasons, we draw a conclusion that the re-emergence of PPRV in the end of 2013 was probably introduced from neighbouring countries into Xinjiang rather than from Tibet of China. It was reported in the literature that the outbreak of PPR in 2013-2014 in China was probably introduced from Tajikistan and Pakistan (Bao, *et al*. 2014, Wu *et al*. 2015). The herd of PPRV-infected sheep in the study belonged to a new sheep breeding farm. Most of sheep in the farm were bought from the surrounding towns (indigenous breed, Mekit sheep) and Yili region of Xinjiang (exotic breed), a few of them bought from Gansu province (exotic breed). Because of the significantly higher price of mutton in South Xinjiang compared to North Xinjiang, sheep and products were transported from North Xinjiang to South Xinjiang. In mid-May 2014, the herd of sheep was infected with PPR after the outbreak of PPR in Yili of North Xinjiang. Considering the above analysis, the outbreak of PPR in South Xinjiang was possibly introduced from North Xinjiang.

Our investigation showed that the sheep in most counties and regions of South Xinjiang suffered a severe pneumoenteritis before outbreaks of PPR in the Yili region of North Xinjiang in 2013. Meanwhile, according to local large veterinary drugs sales departments in South Xinjiang, the records showed that many sheep farmers bought various types of drugs to treat pneumoenteritis in 2013. PPR possibly existed at that time without being recognized, because the disease was yet in the public eye and was misdiagnosed as other pneumoenteritis in small ruminants by local veterinarians. Besides, the transportation vehicles, which may be contaminated by excretions and secretions from infected sheep, were a major risk factor for spread of PPR. Although compulsory immunization with PPR vaccine (Nigeria 75/1) was required by the Ministry of Agriculture of China in all Xinjiang in 2013, some sheep have not developed effective antibodies against PPRV and were susceptible to PPRV for the following possible reasons: (1) The sheep might be missing vaccination because sheep were relatively scattered in large geographical area of South Xinjiang. (2) Some local farmers with backward ideology did not realize the severity of PPR and didn't cooperate in compulsory immunization. (3) Since some local veterinary departments had poorer facilities and sanitation, some vaccines without proper preservation led to low efficacy. In addition, Tajikistan and Pakistan from which the outbreak of PPR in 2013-2014 in China was probably introduced based on literature are nearer to South Xinjiang than North Xinjiang. According to the above analysis, this outbreak of PPR may be have been earlier in South Xinjiang than that in North Xinjiang. Namely, PPR was likely spread from South Xinjiang to North Xinjiang. The sheep which had received the PPR vaccine in North Xinjiang were less susceptible to PPR. The sheep which were introduced from inland provinces were un-vaccinated and susceptible to PPR, since they were outside the scope of compulsory immunization; however, there were few of these in the herd. All this explains why sheep infected with PPR were mainly indigenous breed.

The spread of PPR from South Xinjiang to North Xinjiang has not clearly been established yet and requires more extensive data to determine. PPRV was introduced from Xinjiang to central-eastern provinces and then spread between inland provinces of China (Wang *et al*. 2015). Yunnan, Hubei, Anhui, Guizhou and Jiangsu provinces were more severely struck by PPR than Xinjiang although that is where PPR emerged first (Wu *et al*. 2015). The sheep were relatively less susceptible to PPRV because the compulsory immunization with PPR vaccine was required in all Xinjiang, as early as 2013. However, the un-vaccinated sheep were susceptible to PPR in other provinces except Xinjiang. Phylogenetic

analysis revealed that the highest homology of the China/XJNJ/2014 strain shared with the strain from India in September 2014, speculated possible transmission of PPR from China to India.

The number of sheep was about 10 000 around the herd of PPRV-infected sheep in the study, but fortunately, no PPRV-infected sheep were reported or found in the area. It was highly correlated with that the epidemic was under fine control. Because after the outbreak of PPR at the end of 2013, Ministry of Agriculture and relevant authorities of the People's Republic of China highly valued it and taken the following measures (1) forbidding the transportation of sheep and goat in affected area; (2) emergency vaccination of all susceptible animals in threatened area; (3) timely reporting of suspected PPRV-infected animals; and (4) culling of all animals in PPRV-infected herds and on all farms within a radius of 5km.

It should be noted that, according to reports, large ruminates (cattle, buffalo and camel) and Asiatic lion (Panthera leo persica) can also be infected with PPRV besides small ruminates, and morbilliviruses have the inclination to adapt to new host species. Therefore, if PPRV were frequently spread between different host species, this might increase the likelihood of genetic variation of PPRV. Spread of PPR by wild small ruminates should not be ignored and should be closely monitored. Relevant authorities should detect PPRV on host species, region and time by molecular epidemiology to provide a scientific basis for prevention and control of PPR. In addition, the emergence of novel PPRV strains should be monitored in order to avoid it. The vaccine cannot provide protection for animals which were infected with PPRV variants. Currently, 2-3 lineages of PPRV in a region or country have emerged (Maganga *et al*. 2013) and it was reported that there were lineage II and lineage IV in China (Wang *et al*. 2015).

ACKNOWLEDGEMENTS

This study was funded by the National Natural Science Foundation of China (No. 31460655), Tarim University and Huangzhou Agricultural University Joint fund project of scientific research (HNTDLH1404).

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