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Evolutionary dynamics of recent peste des petits ruminants virus epidemic in China during 2013–2014

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ABSTRACT

Peste des petits ruminants virus (PPRV) causes a highly contagious disease, peste des petits ruminants (PPR), in sheep and goats which has been considered as a serious threat to the local economy in Africa and Asia. However, the in-depth evolutionary dynamics of PPRV during an epidemic is not well understood. We conducted phylogenetic analysis on genomic sequences of 25 PPRV strains from China 2013–2014 outbreaks. All these strains clustered into a novel clade in lineage 4. An evolutionary rate of 2.61×10^{-6} nucleotide substitutions per site per day was estimated, dating the most recent common ancestor of PPRV China 2013–2014 strains to early August 2013. Transmission network analysis revealed that all the virus sequences could be grouped into five clusters of infection, suggesting long-distance animal transmission play an important role in the spread of PPRV in China. These results expanded our knowledge for PPRV evolution to achieve effective control measures.

Peste des petits ruminants (PPR) is one of the most important diseases of sheep and goats, which is caused by a morbillivirus, peste des petits ruminants virus (PPRV). Alongside sheep and goats, PPRV also affects wildlife species including captive, wild small ruminants and sub-clinically cattle and buffalo (Parida et al., 2015). PPR can cause a very high mortality reaching up to 100% in immunologically naïve populations. Thus, PPR is regarded as a significant hurdle to the development of sustainable agriculture. In March 2015, Office Internationale des Epizooties (OIE) and Food and Agriculture Organization (FAO) officially launched a global program to eradicate PPR by 2030. The genome of PPRV is 15,948 or 15,954 nucleotides (nt) in length and organized into six transcriptional units encoding six structural proteins, the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the hemagglutinin protein (H) and the polymerase or large protein (L) (Bailey et al., 2005; Bao et al., 2014). The P transcription unit also encodes two non-structural proteins, C and V.

After the first report of PPR in 1942 in Côte d'Ivoire, the disease has been reported in most parts of sub-Saharan Africa, North Africa, the Middle East, South Asia, Central Asia, and East Asia (Banyard et al., 2014; Dhar et al., 2002; Kwiatek et al., 2011). The first outbreak of PPR in sheep and goats in China was reported in Tibet, China in 2007 (Wang et al., 2009). Another outbreak of PPR in wild small ruminants in Tibet, China was reported in 2008 (Bao et al., 2011). No further spread of the disease has been reported in China until late 2013. On November 30th, 2013, an outbreak of PPR was reported by the Ministry of Agriculture (MOA) of China in a farm in Huocheng county of Yili region in Xinjiang province. Four more infected farms were identified in December 2013 in the same province. The disease has rapidly and widely spread to 21 provinces along with the movements of goats and sheep (Banyard et al., 2014; Wang et al., 2015; Wu et al., 2015). This epidemic of PPR in China continued to the end of June 2013, causing death of more than 16,000 sheep and goats. The spread of PPR in China has posed serious threat of this highly contagious disease on the large population of domestic small ruminants (more than 300 millions sheep and goats) and different species of wild small ruminants in China and neighboring countries.

Phylogenetic studies using partial N or partial F gene sequences have grouped PPRV strains into four lineages (Kwiatek et al., 2007; Shaila et al., 1996). The first three lineages were distributed in Africa. And lineage IV included strains from Asia and recently northern Africa (Kwiatek et al., 2011). Recent phylogenetic analysis revealed that the Chinese Tibetan strains and the Chinese 2013–2014 strains were separately grouped into two clusters in lineage IV (Wu et al., 2015). Historically, PPRV molecular epidemiology has focused on the partial N (255 nt region; nucleotide site 1360–1614 of PPRV genome) or partial F gene (322 nt region; nucleotide site 5779–6100 of PPRV genome) sequences, preventing the in-depth evolutionary analysis

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among strains (Cosseddu et al., 2013; Munir et al., 2011; Ozkul et al., 2002; Padhi and Ma, 2014; Soltan and Abd-Eldaim, 2014). Genome sequences for PPRV have recently been used for an evolutionary study on a global scale (Muniraju et al., 2014c). However, the evolutionary dynamics of PPRV in an epidemic outbreak has not been studied.

Analysis on the evolution of PPRV genome during the course of an epidemic may help the interpretation of field epidemiology data and the implementation of efficient control measures. Such in-depth analysis has been conducted for several other RNA viruses, such as human respiratory syncytial virus, hepatitis C virus, highly pathogenenic avian influenza virus and food-and-mouth disease virus (Agoti et al., 2015; Bataille et al., 2011; Lu et al., 2015; Otieno et al., 2016; Valdazo-Gonzalez et al., 2012). The outbreak of PPR in China during 2013–2014 provided a unique opportunity to study the in-depth epidemiological and evolutionary dynamics of PPRV during an epidemic. The diagnosis and the genetic characterization of the PPR viruses being involved in this epidemic have been reported (Wu et al., 2015). However, the molecular evolution of PPRV between infected farms during the outbreak remains unclear.

In this study, we present full-genome sequence data for the PPR epidemic in China from November 2013 to June 2014. Phylogenetic analysis was used to study the evolutionary dynamics of PPRV during an epidemic.

1. Material and methods

1.1. Field samples

Full-length PPRV genome sequences were achieved directly from clinical samples of infected animals taken from 25 infected farms in 21 provinces. The infected farms included in this study were identified from MOA's Animal Disease Surveillance PPR Data Archive (http://www.syj.moa.gov.cn/dwyqdt/). Clinical samples were screened with real-time RT-PCR with amplification targeting N gene for confirmation. Although 3–5 animals were sampled for diagnosis in each infected farms, samples from one PPR-confirmed animal from each infected farm were used for genome amplification. We used clinical samples of nasal swabs, mesenteric lymph node and spleen. All clinical samples used for genome amplification were detailed in Table 1.

1.2. Genome sequencing

Viral RNA was extracted and used directly for viral genome determination. Fourteen pairs of oligonucleotide primers were used to amplify 14 overlapping fragments by reverse transcription-PCR as previously described (Bao et al., 2014). The PCR products were purified and sequenced with an ABI 3730XL genome sequencer (Applied Biosystems, USA). An addition of 15 PPRV genome sequences representing viral strains from different endemic country was obtained from GenBank. The GenBank accession number, the year and country of isolation were listed in Table 2. Vaccine strains were not included in this study.

1.3. Phylogenetic analysis

All the genome sequences were aligned using MegAlign software in Lasergene package. A phylogenetic tree was inferred by maximum-likelihood method from the nucleotide alignment of genome sequences using MEGA version 4 software, assuming a TN93 model of base substitution (equal substitution rates among sites and between transitional and transversional substitutions) (Tamura et al., 2007). The statistical significance of phylogenies constructed was estimated by bootstrap analysis with 1000 repetitions.

1.4. The analysis of evolutionary rates and times to common ancestry

Temporal dynamics of PPRV were analyzed with time-resolved phylogenies using the Bayesian Markov Chain Monte Carlo (MCMC) method in the BEAST package 1.7.1 (Drummond et al., 2002). The JModelTest program was used to select the best-fitting nucleotide substitution model (Posada, 2009). The datasets were analyzed using the GTR+G substitution model under an uncorrelated exponential relaxed clock with an exponential growth model. The MCMC chains were run for 2×10^8 generations and sampled every 20,000 generation. Convergence was assessed from the effective sample size (ESS) with a 10% burn-in using Tracer v1.6. ESS value above 200 was accepted.

Table 1
Details of the 25 PPRV strains analyzed in this study.

Strain	Collection date	Collection location	Species	Sex[a]	Sample	Accession no.	Clade
China/XJYL/2013	11/30/2013	Xinjiang	goat	M	mesenteric lymph node	KM091959	A
China/XJ2/2013	12/20/2013	Xinjiang	goat	M	spleen	KX421384	D
China/XJ3/2013	12/21/2013	Xinjiang	sheep	F	mesenteric lymph node	KX421385	C
China/XJ4/2013	12/22/2013	Xinjiang	goat	F	spleen	KX421386	D
China/XJ5/2013	12/29/2013	Xinjiang	goat	M	mesenteric lymph node	KX421387	E
ChinaGS2014	1/22/2014	Gansu	sheep	M	mesenteric lymph node	MF443351	E
ChinaNX2014	2/17/2014	Ningxia	sheep	M	mesenteric lymph nod	MF443340	A
ChinaLN2014	3/17/2014	Liaoning	goat	M	mesenteric lymph node	MF443341	E
ChinaCQ2014	3/30/2014	Chongqing	goat	M	mesenteric lymph node	MF443353	B
ChinaHLJ2014	3/31/2014	Heilongjiang	goat	F	nasal swab	MF443346	D
ChinaYN2014	4/1/2014	Yunnan	goat	M	spleen	MF443336	B
ChinaSaX2014	4/1/2014	Shaanxi	goat	F	mesenteric lymph node	MF443339	D
ChinaJX2014	4/1/2014	Jiangxi	goat	F	mesenteric lymph node	MF443342	E
ChinaJL2014	4/1/2014	Jilin	sheep	F	nasal swab	MF443344	D
ChinaJS2014	4/2/2014	Jiangsu	goat	F	mesenteric lymph node	MF443343	B
ChinaHeN2014	4/3/2014	Henan	goat	M	nasal swab	MF443347	D
ChinaHB2014	4/3/2014	Hubei	goat	M	mesenteric lymph node	MF443348	B
ChinaAH2014	4/3/2014	Anhui	goat	F	spleen	MF443354	E
ChinaSX2014	4/5/2014	Shanxi	goat	F	nasal swab	MF443337	E
ChinaGX2014	4/16/2014	Guangxi	goat	F	mesenteric lymph node	MF443350	E
ChinaGZ2014	4/21/2014	Guizhou	goat	M	nasal swab	MF443349	B
ChinaZJ2014	4/25/2014	Zhejiang	goat	F	nasal swab	MF443335	B
ChinaHN2014	4/25/2014	Hunan	goat	M	mesenteric lymph node	MF443345	D
ChinaGD2014	5/15/2014	Guangdong	goat	F	mesenteric lymph node	MF443352	E
ChinaSC2014	6/10/2014	Sichuan	goat	F	nasal swab	MF443338	B

[a] F = Female; M = Male.