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LETTER

Identification and Characterization of the First Equine Parainfluenza Virus 5

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Dear Editor,

Parainfluenza virus 5 (PIV5), known as canine parainfluenza virus in the veterinary field, is a negative-sense, nonsegmented, single-stranded RNA virus belonging to the *Paramyxoviridae* family (Chen 2018). The virus was first reported in primary monkey kidney cells in 1954 (Hsiung 1972), then it has been frequently discovered in various hosts, including humans, dogs, pigs, cats, rodents, calves, and lesser pandas (Chatziandreou *et al*. 2004; Lee and Lee 2013; Liu *et al*. 2015; Zhai *et al*. 2017; Jiang *et al*. 2018). So far, PIV5 has not been reported in horse. In this study, using metagenomics analysis we have identified a novel equine PIV5.

Totally 148 fecal samples, 81 sera samples, and 115 nasal swabs were collected in six different cities of Xinjiang and Inner Mongolia, China in 2018 (Supplementary Figure S1). First, Fresh fecal samples were collected from 20 thoroughbred horses (mean 5.6 years) at an equestrian club in Hutubi County, north Xinjiang, China. The cDNA library of fecal specimens was prepared as previously described (He *et al*. 2013). Briefly, viral RNAs were extracted from fecal pooled samples, then reverse transcribed, and randomly amplified using PCR. The tagged and purified PCR products were subjected to Illumina sequencing (HiSeq X-ten, United States, Illumina) in a lane by Shanghai Personal Biotechnology Co., Ltd (Shanghai, China). In total, 76,986,684 reads were generated, of which 197,056 (0.26%) being annotated to mammalian viruses. Sequence analysis showed that 146 reads were closely related to human PIV5, sharing 99.8%–100% identity with the fusion protein (*F*) gene of AGS strain (GenBank no. KX060176).

To confirm the next-generation sequencing results, a pair of primers amplifying the equine PIV5 *F* gene were designed (Supplementary Table S1). RT-PCR results showed that eight fecal samples from 20 thoroughbred horses of the same equestrian club were positive for equine PIV5, indicating that the virus was likely circulating in the thoroughbred horses. Further studies revealed that eight fecal samples from 30 Yili horses in Yining city and five fecal samples from 11 Asian wild horses in Urumqi were positive for *F* gene (Table 1). However, the *F* gene was not detected in Yanqi, Sanhe and Wushi horses (Table 1). Further investigation found that Yili and Asia wild horses were co-bred with thoroughbred horses, but Yanqi, Sanhe and Wushi horses were not co-bred with thoroughbred horses. This suggested that equine PIV5 might be an exotic virus with high prevalence and different geographic distribution in north Xinjiang. In addition, no *F* gene was detected in equine sera and nasal swabs of all samples (Table 1), indicating intestinal cell tropism of equine PIV5. Sequence comparison showed that the *F* genes of all 21 equine PIV5 shared 100% nucleotide identity.

The whole genomes of equine PIV5 were successfully obtained by RT-PCR using primers listed in the Supplementary Table S1. The obtained genome sequence is 15,246 nt and consists of genes encoding nucleocapsid protein (NP), V protein (V), membrane protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN) and L protein (L) (GenBank no. MN604146). Sequence analysis indicated that the novel equine PIV5 shared 98.2%–99.9% homology with six Human PIV5, and 95.6%–98.0% homology with the animal source PIV5 strains (Table 2), indicating that equine PIV5 was closely related to human PIV5.

Phylogenetic analysis based on the whole genome sequences showed that the novel equine PIV5 and six

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Table 1 Information of samples included in this study.

Sampling place	Breed	Samples type	Sample no.	Positive no.
Hutubi County	Thoroughbred horses	Feces/Sera/Nasal swabs	20/40/80	8/0/0
Yining City	Yili horses	Feces/Sera/Nasal swabs	30/30/30	8/0/0
Urumqi City	Asia wild horses	Feces/Sera/Nasal swabs	11/11/5	5/0/0
Hejing County	Yanqi horses	Feces/Sera/Nasal swabs	44/0/0	0
Manzhouli City	Sanhe horses	Feces/Sera/Nasal swabs	11/0/0	0
Wuhai City	Wushi horses	Feces/Sera/Nasal swabs	32/0/0	0

Table 2 Homology analysis of the whole-genome sequences between equine PIV5 and PIV5 reference strains from various hosts.

Reference strains	Nucleotide identity (%)
KX060176/Human PIV5/AGS /USA/1983	99.9
JQ743322/Human PIV5/DEN/UK/1980	98.3
JQ743324/Human PIV5/LN/UK/1980	98.2
JQ743325/Human PIV5/MEL/UK/1980	98.2
JQ743327/Human PIV5/RQ/UK/1976	98.2
JQ743326/Human PIV5/MIL /UK/1980	98.2
Other 11 PIV5 reference strains	95.6–98.0

human PIV5 reference strains formed a single clade and were closely related to the AGS strain, but diverging from the lineages of animal source PIV5 strains (Fig. 1), indicating it may be a human-source virus. Unfortunately, our attempts to isolate the novel equine PIV5 using Vero cells and chicken embryos failed.

In summary, this study provides the first molecular evidence for equine PIV5. While our observations do not permit us to conclude that the virus associates with clinically symptomatic disease, we do indicate that equine PIV5 has a high prevalence and distributed in different geographic areas in Xinjiang, one of the major horse-producing regions in China. Further research is needed to develop

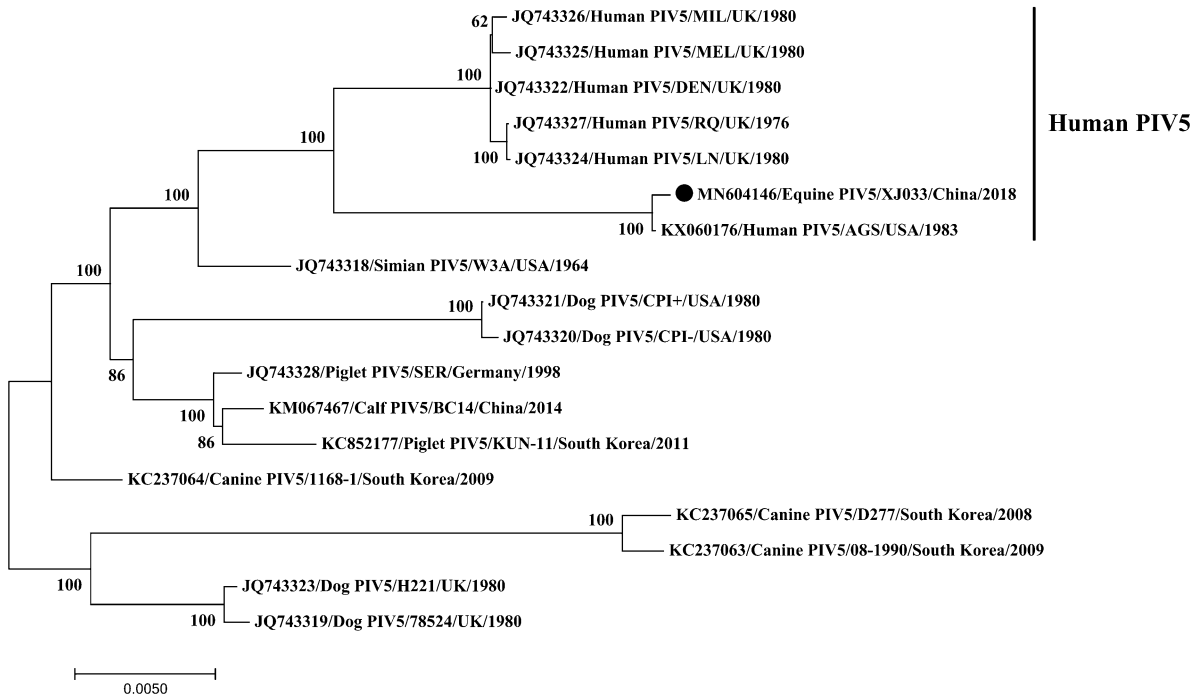

Fig. 1 Phylogenetic analysis of whole genome sequence of equine PIV5. The black circle indicates the virus identified in our study.

new methods for virus isolation, and to investigate the serological epidemiology of PIV5 in horses in China.

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Compliance with Ethical Standards

Conflict of interest The authors have declared no competing interests.

Ethics Statement All experimental procedures involving animals were approved (animal protocol number: 2018005) by the Animal Care and Use Committee of Xinjiang Agricultural University.

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