First Detection and Genetic Characterization of Equus Caballus Papillomavirus 1, 2 and 7 in China

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Abstract

Background: Nine species of *Equus caballus papillomavirus* (EcPV) have been reported to infect horses, however, there are so far no reports of such infections in China.

Results: In our pioneer study with Chinese horses, we found EcPV-1 in intranasal papilloma and nasal swabs, EcPV-2 in nasal swabs and semen, and EcPV-7 primarily in semen. This indicates that EcPVs are indeed hosted by horses in China, and that EcPV-2 and 7 may be getting transmitted though breeding. Sequence analyses for complete genomic sequences of EcPV-1 (G2), EcPV-2 (XJ-KS1391) and EcPV-7 (XJ-zs1) were performed which indicated that EcPV-1, 2 and 7, that infect horses in China, share 99.3% nt identity with the already published sequences for EcPV-1, 2 and 7. These observations indicate that three types of EcPVs identified in the current study are highly similar variants of previously known types of EcPV-1, 2 and 7. Phylogenetic analysis based on L1 genes in GenBank showed that EcPV-1, 2 and 7, found in Chinese horses, are closely related to and clustered together with already known EcPV-1, 2 and 7, respectively.

Conclusion: Our study provides a novel evidence for EcPVs infection and circulation in Chinese horses and thus lays the foundation for a systematic and detailed epidemiological study of these infections in Chinese horses.

Background

Papillomaviruses (PVs) are small, non-enveloped viruses harboring 8 kb of circular and double-stranded DNA genomes [1, 2]. They are classified into different species of papillomaviruses according to the diversity of nucleotide sequences in the open reading frame (ORF) of the L1 gene [1, 3, 4]. The viruses infect different animals including humans, horses and bovine [1]. Till now nine species of EcPV, that are capable of infecting horses, have been documented [2, 4–7], however, there is no information on EcPV infection in horses within China.

In June 2018, we found papilloma-like tissue in the nose cavity of an eight years old thoroughbred stallion, of an equestrian club in Changji City, north-Xinjiang, China, that also experienced clinically manifested difficulty in breathing. We designed this present study to perform metagenomic sequencing of the papilloma tissue from that stallion to characterize the virome of the papilloma and to identify the disease-causing pathogen.

Methods

cDNA library preparation

The cDNA library of the intranasal papilloma tissue was prepared according to a previously described method [8]. Briefly, the tissue was grinded and pooled to extract the viral nucleic acids that were reverse

transcribed and then randomly amplified. The tagged and purified PCR products were sequenced at Shanghai Personal Biotechnology Co., Ltd. using one lane Illumina sequencing (HiSeq X-ten).

Pcr Analysis

We developed specific PCR assay for each virus to confirm EcPV-1 in the next-generation sequencing results and to investigate the presence of EcPV2-9 in the horse population of north-Xinjiang. Primers were designed to amplify the various regions of the L1 gene for specific and discriminative amplification of these viruses (Table S1). For amplification of the target genes we used TIANSeq HiFi Amplification Mix (Tiangen Biotechnology Co., Ltd) and used the following PCR program: 94°C for 2 minutes, 35 cycles of denaturation at 98°C for 10 seconds, annealing and extension at 68°C for 15 seconds and final extension at 68°C for 5 minutes. All methods were performed in accordance with the relevant guidelines and regulations.

Results

Different strains of EcPVs are present in a localized population of horses in China

Sequence analysis showed that a total of 61,077,096 sequence reads of 150 bp were generated from the tissue sample, of which 6,054 (0.0099%) could be annotated to known mammalian viruses (EcPV-1, Gemycircularvirus, Picobirnavirus, Murine leukemia virus, Human endogenous retrovirus W, etc). Among the various mammalian virus sequence reads obtained from the tissue sample, 1,690 reads shared more than 99.2% nucleotide identity with E6, E7 and L1 genes of EcPV-1 reference strains (GenBank accession nos. MF288893 and AF498323).

PCR results showed that the intranasal papilloma and seven nasal swabs (3%, 7/230) were positive for EcPV-1, four nasal swabs (1.74%, 4/230) and two semen samples (16.7%, 2/12) were positive for EcPV-2, and three semen samples (25%, 3/12) were found to be positive for EcPV-7 (Table 1).

Table 1
Information of thoroughbred horse nasal swabs and semen included in this study

Sample place	Farm	no[c] N/S	Sex[a] M/F	Average age[b]	EcPV-1	EcPV-2	EcPV-7
Yili	A	37/0	6/31	5.4(3–10)	0	4(10.8%)	0
		0/6	6/0	6.3(5–8)	0	1(16.7%)	1(16.7%)
	B	96/0	30/66	10(0–13)	4(4.2%)	0	0
Changji	C	41/0	7/34	6.3(4–12)	0	0	0
		0/6	6/0	8.1(6–12)	0	1(16.7%)	2(33.3%)
Urumqi	D	56/0	10/46	4.4(0–8)	3(5.4%)	0	0
	Total	242	65/177	7.2(3–13)	7(2.9%)	6(2.5%)	3(1.2%)

[a]M/F: male/female

[b]Age in 2019

[c]N/S: nasal swabs/semen

Complete genomic profiling of the different EcPV strains reveal high sequence identity with the reference strains

The complete genomes for EcPV-1, 2 and 7 were obtained by PCR analysis using primers mentioned in Table S2. The complete genome of EcPV-1 identified in this study is 7611 bp long (GenBank accession no. MN164462) and designated as EcPV-1 G2. It shares 99.3%- 99.4% nucleotide identity with previously submitted genome of EcPV-1 to GenBank. E6, E7, E1, E2, E4, L1 and L2 ORF of EcPV-1 G2 shared high nucleotide (98.8–99.8%) and amino acid identity (98.6–99.8%) with EcPV-1 reference strains. In accordance with a previously published study by Dong et al., 2017 [7], the sequences of the eight L1 genes in the various EcPV-1s found in Chinese horses, share up to 99.8% nucleotide identity (GenBank accession nos. MN164462, MT364343-MT364349).

The complete genome sequences of the EcPV-2, that was identified in the horse semen (7802 nt), named XJ-KS1391, (GenBank accession no. MW410986), shared a 99.87% sequence identity with EcPV-2a (GenBank accession no. EU503122) and a sequence identity of 99.05% with EcPV-2b (GenBank accession no. HM461973) indicating that EcPV-2 XJ-KS1391 and EcPV-2a share a closer genetic relationship. E6, E7, E1, E2, E4, L1 and L2 ORF of EcPV-2 XJ-KS1391 share high nucleotide (98.3–100%) and high amino acid identity (98.4–100%) with EcPV-2 reference strains. The L1 genes in six strains of EcPV-2 from these horses have a nucleotide identity of 99.8–100% (GenBank accession nos. MW410986, MW429199-MW429203).

The complete genomic sequence of the EcPV-7 as identified in the horse semen (7619 nt, named XJ-zs1, MW410987), shares 99.75% sequence identity with the complete genome sequence of EcPV-7 reference strain (GenBank accession no. JX035935). The E6, E7, E1, E2, E4, L1 and L2 ORFs of EcPV-7 XJ-zs1 share high nucleotide (98.9–100%) and amino acid identity (98.2–100%) with EcPV-7 reference strains. Three L1 genes of EcPV-7 found in Chinese horses show nucleotide identity as high as 99.4–99.7% (GenBank accession nos. MW410987, MW429204 and MW429205).

L1 gene sequence comparison show identity with reference L1 genes

Moreover, the sequencing and comparison of the L1 gene in the EcPV-1, 2 and 7 strains in Chinese horses show a 98.8–99.1% nucleotide sequence identity with the reported EcPV-1, 2 and 7 strains.

Phylogenetic Analysis

Phylogenetic tree based on L1 genes analysis of EcPV-1 to 9 reveal that EcPV-1 from Chinese horses were clustered into a dependent branch, together with EcPV-1 reference strains, into *Zatapapillomavirus* lineage. Moreover, Chinese EcPV-2 strain was clustered into EcPV-2a branch of *Dyoiotapapillomavirus* lineage, whereas, EcPV-7 was clearly related to EcPV-7 reference strain of *Dyorhopapillomavirus* lineage (Fig. 1).

Discussion

Previous studies have shown that the EcPV-1 causes cutaneous papillomas in young horses, with age less than three years, that regress spontaneously within one to nine months [2, 7, 9]. However, as a novel finding we report EcPV-1 in the intranasal papilloma of an adult thoroughbred stallion (eight years-old) which unfortunately did not spontaneously return to health after 15 months. Even though it was treated by a veterinarian in November 2019, the horse could not progress with breeding due to breathing difficulties persistent breathing difficulties. In previous studies EcPV-1 has been shown to infect young horses with age as less as one to two years [9, 10], however we were able to detect EcPV-1 in the nasal swabs of seven clinically healthy foals which were not even one year-old (Table 1).

It has been reported that EcPV-2 is present in the tissues of squamous cell carcinomas of sick horses and genitalia and conjunctiva swabs of healthy ones [11–14]. Our study is the first of its kind to report EcPV-2 in nasal swabs and semen of Chinese horses (Table 1). Lange et al. were the first ones to find EcPV-7 in penile mass in horses in 2013 [4] and we also found EcPV-7 in horse semen (Table 1). However, the nasal swabs were devoid of any traces of EcPV-7. Detection of EcPV-2 and − 7 in semen suggests that the primary route for transmission of these two viruses could be through sexual activities in horses. The current study was performed in horses from Changji, Urumqi, and Yili indicating that EcPV-1, 2 and 7 are in circulation within different regions of north-Xinjiang in China.

The complete genomes of Chinese EcPV-1, 2 and 7 shared more than 99.3% nucleotide identity with previously submitted genome of EcPV-1, 2 and 7 to GenBank, respectively, indicating that EcPV-1, 2 and 7 had low genetic diversity. Homology analysis of the L1 genes indicated EcPV-1, 2 and 7 in Chinese horses possess high genetic identity.

According to EcPV taxonomy, that follows the general criteria established by the International Committee on the Taxonomy of Viruses (ICTV), an EcPV strain is recognized as a variant if the nucleotide sequence of the L1 ORF differs by less than 2% homology with the closest known EcPV type [1, 3, 4]. Based on this criterion, it is concluded that the EcPV-1, 2 and 7 strains in Chinese horses are indeed legit variants of referenced EcPV-1, 2 and 7 strains.

Conclusions

In our present study, we discover for the first time three localized strains of EcPVs (EcPV-1, 2 and 7) in China and performed a thorough exploration of their basic genetic characteristics. This study is the first to detect EcPV-1 in intranasal papilloma, EcPV-2 in nasal swabs and semen, and EcPV-7 in semen from localized Chinese population of horses. We demonstrate that the three EcPVs are indeed circulating among thoroughbred horses that have low diversities but different geographic distributions in north Xinjiang. This is also the first report about the presence of EcPV-1, 2 and 7 in China, and thus a founding contributor towards any future molecular and sero-epidemiological and pathological studies for these viruses.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

XJ and WJ made substantial contributions to conception and design, and had the initial idea, was partially guiding the experiments and contributed substantially to the manuscript. TP, SX and RM performed the experiment. JE, ZL and KL analyze sequences. PN, DR and JC collect samples. XJ and TP drafted the manuscript. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All experimental procedures involving animals were approved by the Animal Care and Use Committee of Xinjiang Agricultural University, Urumqi, Xinjiang, China under animal protocol number: 2018005.

Consent for publication

Not applicable.

Competing interests

All authors have declared no competing interests.

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Figures

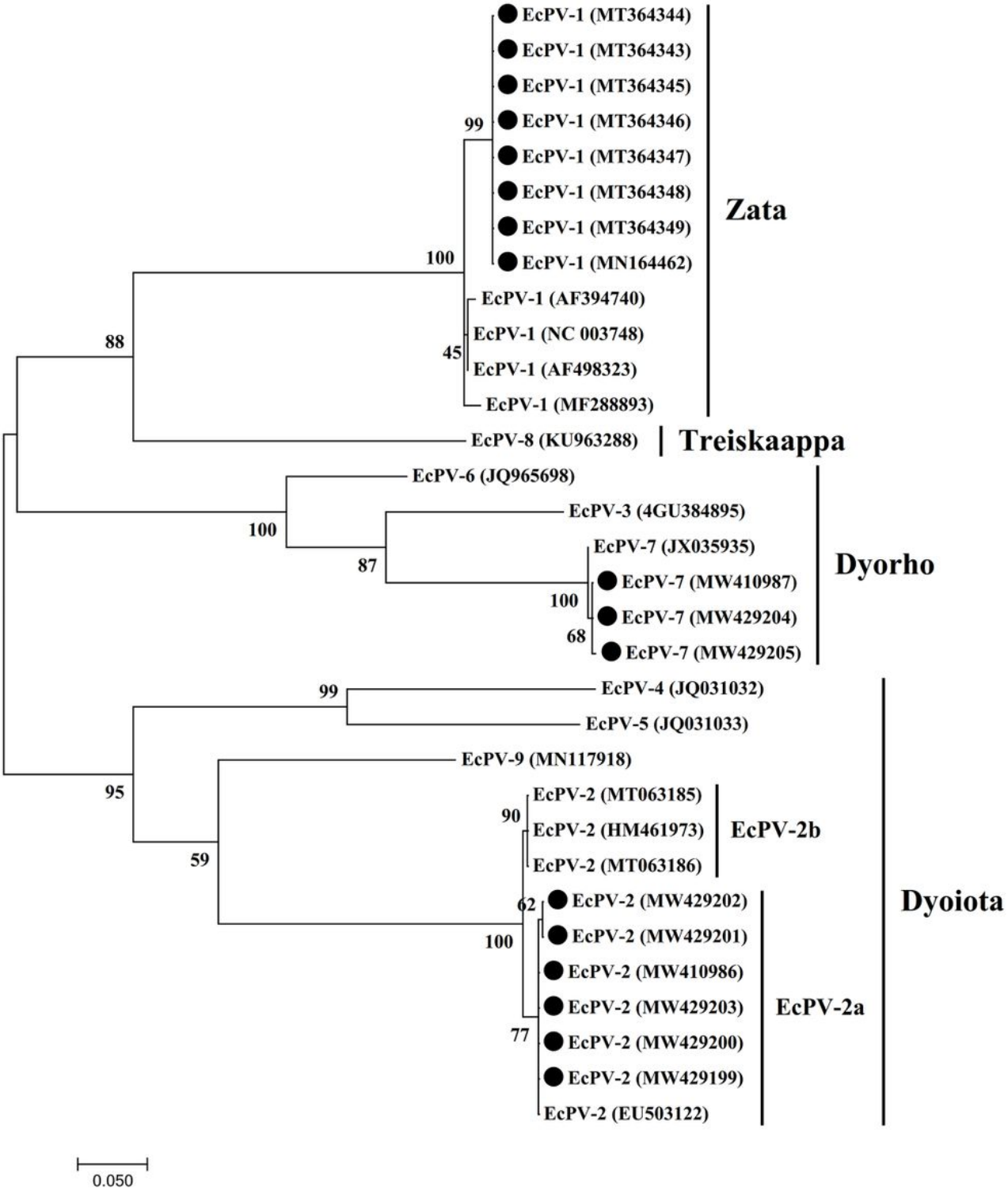

Figure 1

Phylogenetic analysis of EcPVs based on L1 genes. The black circles indicated the EcPV-1, -2 and -7 detected in this study.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx
- TableS2.docx