The presence of EcPV-2 DNA in aborted samples is evidence for vertical transmission of EcPV

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Short Report

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Abstract

Nine different species of *Equus caballus papillomavirus* (EcPV) and three bovine papillomaviruses (BPV) have been reported to infect horses, however, there are so far no describing such infections in China. In our pioneer study with Chinese horses, we first found EcPV-2 in the nasal swabs (4/230, 1.7%) of Yili horses, and semen (3/18, 16.7%) of the Thoroughbred horses. This indicated that EcPV can be indeed hosted by horses in China, and that EcPV-2 might be transmitted though breeding. Further detection of EcPVs and BPVs in the aborted fetal lung tissues of Yili mares, which were originally negative for equid herpesviruses, demonstrated that EcPV-2 was positive in 19 out of 50 samples, thereby indicating this finding is first evidence for vertical transmission of EcPV, simultaneous suggesting that EcPV-2 might be a new pathogen causing of responsible for abortions. Thereafter, the sequence analyses for *L1* genes sequences of 26 China's EcPV-2 were performed which indicated that EcPV-2, that primarily infected the horses in China, shared 98.3%-99.9% nt identity with the already published sequences for EcPV-2. These observations indicated that EcPV-2 identified in the current study were highly similar variants of the previously identified strains of EcPV-2. Phylogenetic analysis based on *L1* genes in GenBank showed that EcPV-2, found in the Chinese horses, was closely related to and clustered together with an already known EcPV-2a lineage. Our study provides the early evidence related to EcPV-2 infection in the Chinese horses, and describes the vertical transmission of EcPV for the first time, which can act as a causative agent for Yili horse abortions. Overall, the findings might lay the foundation for a systematic and detailed epidemiological study of this infection in the Chinese horses.

Introduction

Papillomaviruses (PVs) are small, non-enveloped viruses which harbor 8 kb of circular and double-stranded DNA genomes, that are prevalent amongst a wide range of the host species, including mammals, birds, reptiles, and fish [1]. They can be classified into the different species of PVs according to the diversity of nucleotide sequences in the open reading frame (ORF) of the L1 gene [2–4]. Moreover, now, more than 650 distinct animal and human papillomavirus (HPV) types have been identified, and the 440 HPV types have been discovered within the human population. Among other 210 PV types, nine different species of *equus caballus papillomavirus* (EcPV-1 to EcPV-9), and three species of bovine papillomavirus (BPV-1, BPV-2 and BPV-13) have been reported to be capable of infecting the horses worldwide [1, 3–8], however, there is no information available about the potential EcPVs and BPVs infection in the horses within China.

We have designed this present study to detect EcPVs and BPVs infections in the horses of North Xinjiang, which is one of the major horse-producing regions in China, and found a possible association between the viruses detected and diseases in the horses.

Materials And Methods

During July 2020 to May 2021, a total of 298 horse samples, including 230 nasal swabs, 18 semen, and 50 aborted fetus lung tissues of Yili horses negative for equid herpesviruses (EHV), were collected from the four farms located in Yili, Changji and Urumqi of North Xinjiang (Table 1). All the samples were, therefore, transferred to a tube containing 10 mL phosphate buffer for storage at -80°C until required for analysis. The bodies of the various aborted fetuses were subjected to the harmless treatment by the farm's veterinarian.

For nucleic acid extraction, each sample was first ground, then vortexed and after centrifugation, viral nucleic acids were extracted from 200 µL of sample supernatant based on the manufacturer's instructions (Geneaid Biotech Co., LTD). The presence of EcPVs and BPVs DNA in these nucleic acids was subsequently detected by PCR using the primers listed in table S1. PCR was carried out using the 2× *TransStart® FastPfu* Fly PCR SuperMix (TransGen Biotech Co., LTD) with the following PCR conditions: An initial denaturation at 95°C for 2 minutes, followed by 35 cycles, and with a denaturation step at 95°C for 20 seconds, as well as both an annealing step and an extension step at 72°C for 30 seconds each respectively. Eventually a final extension at 72°C for 5 minutes was performed (TransGen Biotech Co., LTD). The positive PCR amplicons were thereafter ligated into a *pEASY*®-Blunt T vector (TransGen Biotech Co., LTD) for transforming DH5a competent cells (TransGen Biotech Co., LTD) and ten distinct clones of each amplicon were eventually selected for the Sanger sequencing (Sangon Biotech Co., LTD).

All the nucleotide sequences of EcPV-2 which were generated in this study were submitted to NCBI (GenBank nos. MW410986, MW429199-MW429203 and OK362333-OK362352). The sequences were thereafter analyzed by MegAlign software in Lasergene v7.1 and phylogenetic trees of all the different target sequences were generated using the maximum-likelihood method based on the Tamura–Nei model. The accuracy of the tree topologies was also evaluated using 1,000 bootstrap replicates [9].

Results And Discussion

A number of previous studies that were conducted in the different countries have documented that nine EcPVs and three BPVs can infect the horses [1, 3-8]. However, before this study, no one has described about infections caused by EcPVs and BPVs in the Chinese horses. In the current study, we first investigated the existence of these viruses in the different samples of Chinese horses. EcPV-2 has been earlier detected in the penile, vulvar, clitoral and oro-pharyngeal papillomas, and the tissues of squamous cell carcinomas [10-13]. Our study is the first of its kind to report the presence of EcPV-2 in the nasal swabs of Yili horses of farm A located in Yili city which did not display any significant clinical signs (Table 1). However, whether Chinese EcPV-2 can potentially cause papillomas and squamous cell carcinomas (SCC) of horses needs further epidemiological investigation. Moreover, based on the previous studies which have reported the existence of EcPV-9 in the semen from a Thoroughbred stallion with a penile lesion [6], and identification of BPV nucleic acids in the semen of healthy cattle [14], we first detected the presence EcPV-2 in the semen from three clinically normal Thoroughbred stallions in farm A and farm D belonged to Yili city and Changji city respectively (Table 1). These results suggested that the primary route for transmission of EcPV-2 could be possibly through the sexual activities in horses, and

that the occurrence of EcPV-2 in the semen of Thoroughbred stallions should be cause of potential concern. In the present study, the semen, collected from Yili and Changji, was found to be positive for EcPV-2, thereby indicating that the virus could have been in circulation in the different regions of North Xinjiang (Table 1).

Table 1

Information of equine samples included in this study

Sampling place	Location	N/S/L	M/F	Breed	Average age	EcPV-2
Yili	A	37/0/0	6/31	Yili horse	5.4(3-10)	4(10.8%)
		0/6/0	6/0	Thoroughbred stallion	6.3(5-8)	1(16.7%)
		0/0/50	21/29	Yili horse	<0	19(38%)
	B	96/0/0	30/66	Thoroughbred horse	10(0-13)	0
Changji	C	41/0/0	7/34	Thoroughbred horse	6.3(4-12)	0
		0/12/0	12/0	Thoroughbred stallion	8.1(6-12)	2(16.7%)
Urumqi	D	56/0/0	10/46	Akhal-teke horse	4.4(0-8)	0
	Total	298	92/206		7.2(3-13)	26(2.5%)
M/F: Female/Male, N/S/L: Nasal swab/Semen/Lung tissue of aborted fetus						

In addition, to the nasal swabs of Yili horses and the semen of Thoroughbred stallions in farm A positive for EcPV-2, and aborted samples of Yili horses without EHVs in the same farm, a single prior study has found the existence of EcPV-2 DNA in the genital, oral mucosa, eyelid and skin from muzzle of dead equine fetuses due to dystocia [13]. Based on these initial observations, we decided to examine these aborted samples for the presence of EcPV-2 DNA. PCR results clearly showed that the EcPV-2 DNA was significantly amplified in 19 out of 50 lung tissues. Our data first detected the presence of EcPV-2 in aborted samples, which supported our hypothesis that EcPV-2 could possibly be spread by the vertical transmission route, and thereby clearly indicating that the virus might contribute to Yili horse abortions. This finding is not surprising given that vertical transmission of HPV-11, BPV-1, -2, and -4 has been documented earlier [15, 16]. A number of the previously published studies have shown that EHV-1, EHV-2, EHV-4, EHV-5, EHV-8 and EAV can be detected in horse aborted samples [17-20]. The results of our current study also suggested that the EcPV-2 could possibly be considered to be a new equine abortion-related virus.

The fragments of EcPV-2 complete genome sequences were thereafter amplified using the primers listed in Table S1 and Table S2. After the sequence assembling, the complete genome of the EcPV-2 obtained from the Thoroughbred stallion named as XJ-YLKS1391 (GenBank no. MW410986) was found to be

7802 nt in length which was rather similar to other EcPV-2 strains. In addition, the *L1* genes of other 25 Chinese EcPV-2 were sequenced, and then deposited into the GenBank database under accession nos: MW429199-MW429203 and OK362333-OK362352.

Based on *L1* gene-phylogenetic characteristics, EcPV-2 reported so far have been assigned to EcPV-2a and EcPV-2b [4]. The complete genome sequences of the EcPV-2, which was identified in the horse semen (7802 nt), named XJ-KS1391, (GenBank no. MW410986), shared a 99.87% sequence identity with EcPV-2a (GenBank no. EU503122) and a sequence identity of 99.05% with EcPV-2b (GenBank no. HM461973) thereby indicating that EcPV-2 XJ-KS1391 and EcPV-2a share a relatively closer genetic relationship. E6, E7, E1, E2, E4, L1 and L2 ORF of EcPV-2 XJ-KS1391 share high nt (98.3%-100%) and high aa identity (98.4%-100%) with EcPV-2 reference strains. The *L1* genes in 26 different strains of EcPV-2 from these horses possess a nt identity of 99.8%-100% (GenBank nos. MW410986, MW429199-MW429203), thereby indicating that these strains found in China were highly similar in their genetic makeup. In addition, these identified sequences were also compared with those of EcPV-2 reference strains Zurich_2009 (GenBank no. HM461973), IZS PLVA_tomach (GenBank no. MT063185), IZS PLVA_vulva (GenBank no. MT063186), and UK isolate (GenBank no. EU503122), with the results indicating a percentage similarity level of 98.3%-99.9% for the DNA sequences. According to PV taxonomy, that follows the general criteria established by the International Committee on the Taxonomy of Viruses (ICTV), an PV strain is potentially recognized as a variant if the nucleotide sequence of the L1 ORF differs by less than 2% homology with the closest known PV type [2-4]. Based on this criteria, it can be concluded that the EcPV-2 strains in the Chinese horses could be indeed considered as legit variants of referenced EcPV-2 strains.

Based on *L1* gene-phylogenetic characteristics, Lange et al proposed that EcPV-2 in the GenBank could be clustered into the lineages of EcPV-2a and EcPV-2b [4]. The results of the current study indicated that the Chinese EcPV-2 derived from the nasal swabs, semen and aborted fetuses were clearly related to EcPV-2 reference strain of EcPV-2a lineage (Fig. 1). In fact, *L1* genes of EcPV-2 found in the Chinese horses share higher nucleotide (99.6%-99.9%) with EcPV-2a reference strain (GenBank no. EU503122) than the known EcPV-2b ones (98.3%-98.8%) (GenBank nos. HM461973, MT063185 and MT063186), these results support that Chinese EcPV-2 belonged to new EcPV-2a variants.

In summary, EcPV-2 was first detected from the samples of the semen and aborted fetuses, and as such, it was shown that the virus could be the probable cause behind the Yili horse abortions. Further characterization of the *L1* genes provided substantial evidence that Chinese EcPV-2 belonged to a known EcPV-2a variant. It is expected that the findings of this study will create awareness about the possible role of EcPV-2 in the horse abortions and its detection could be used for the differential diagnosis of abortions in the horses worldwide.

Declarations

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Conflict of interest

The authors have declared no competing interests.

Ethics approval

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

Authors' contributions

P.T. and J.X. performed the research, analyzed the data, and drafted the manuscript. S.T., X.S., E.Y., W.M., J.W., L.K. contributed to the collection of samples and detection of PCR. P.T., J.W. and J.X. revised the manuscript. J.X. conceived the study, carried out additional analyses and finalized the manuscript. All authors have contributed to the editing of the manuscript. The authors have also read and approved the final manuscript.

References

1. Della Fera AN, Warburton A, Coursey TL, Khurana S, McBride AA (2021) Persistent Human Papillomavirus Infection. Viruses 13(2):321.
2. Bernard HU, Burk RD, Chen ZG, van Doorslaer K, zur Hausen H, de Villiers EM (2010) Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 401(1):70–79.
3. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H (2004) Classification of papillomaviruses. Virology 324(1):17–27.
4. Lange CE, Vetsch E, Ackermann M, Favrot C, Tobler K (2013) Four novel papillomavirus sequences support a broad diversity among equine papillomaviruses. J Gen Virol 94(Pt 6):1365–1372.
5. Peters-Kennedy J, Lange CE, Rine SL, Hackett R P (2019) Equus caballus papillomavirus 8 (EcPV8) associated with multiple viral plaques, viral papillomas, and squamous cell carcinoma in a horse. Equine Vet J 51(4):470–474.
6. Li CX, Chang WS, Mitsakos K, Rodger J, Holmes EC, Hudson BJ (2019) Identification of a novel equine papillomavirus in semen from a thoroughbred stallion with a penile lesion. Viruses 11(8):713.
7. Otten N, von Tscharner C, Lazary S, Antczak DF, Gerber H (1993) DNA of bovine papillomavirus type 1 and 2 in equine sarcoids: PCR detection and direct sequencing. Arch Virol 132(1-2):121–131.

8. Lunardi M, de Alcantara BK, Otonel RA, Rodrigues WB, Alfieri AF, Alfieri AA (2013) Bovine papillomavirus type 13 DNA in equine sarcoids. J Clin Microbiol 51(7):2167–2171.
9. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870–1874.
10. Sykora S, Samek L, Schönthaler K, Palm F, Borzacchiello G, Aurich C, Brandt S (2012) EcPV-2 is transcriptionally active in equine SCC but only rarely detectable in swabs and semen from healthy horses. Vet Microbiol 158(1-2):194–198.
11. Bogaert L, Willemsen A, Vanderstraeten E, Bracho MA, De Baere C, Bravo IG, Martens A (2012) EcPV2 DNA in equine genital squamous cell carcinomas and normal genital mucosa. Vet Microbiol 158(1-2):33–41.
12. Kainzbauer C, Rushton J, Tober R, Scase T, Nell B, Sykora S, Brandt S (2012) Bovine papillomavirus type 1 and Equus caballus papillomavirus 2 in equine squamous cell carcinoma of the head and neck in a Connemara mare. Equine Vet J 44(1):112–115.
13. Greenwood S, Chow-Lockerbie B, Epp T, Knight C, Wachoski-Dark G, MacDonald-Dickinson V, Wobeser B (2020) Prevalence and prognostic impact of equus caballus papillomavirus type 2 infection in equine squamous cell carcinomas in Western Canadian Horses. Vet Pathol 57(5):623–631.
14. Lindsey CJ, Almeida ME, Vicari CF, Carvalho C, Yaguiu A, Freitas AC, Beçak W, Stocco RC (2009) Bovine papillomavirus DNA in milk, blood, urine, semen, and spermatozoa of bovine papillomavirus-infected animals. Genet Mol Res 8(1):310–318.
15. Skoczyn´ski M, Goz´dzicka-J´ozefiak A, Kwas´niewska A (2014) Risk factors of the vertical transmission of human papilloma virus in newborns from singleton pregnancy—preliminary report. J Matern Fetal Neonatal Med 27(3):239–242.
16. Yaguiu A, Dagli MLZ, Birgel Jr EH, Alves Reis BCA, Ferraz OP, Pituco EM, Freitas AC, Beçak W, Stocco RC (2008) Simultaneous presence of bovine papillomavirus and bovine leukemia virus in different bovine tissues: in situ hybridization and cytogenetic analysis. Genet Mol Res 7(2):487–497.
17. Marenzoni ML, Lepri E, Casagrande Proietti P, Bietta A, Coletti M, Timoney PJ, Passamonti F (2012) Causes of equine abortion, stillbirth and neonatal death in central Italy. Vet Rec 170(10):262.
18. Leon A, Laugier C, Foucher N, Tapprest J, Sevin C (2011) A 24-Year retrospective study of equine abortion in normandy (France). J Equine Vet Sci 31(3):116–123.
19. Garvey M, Suárez NM, Kerr K, Hector R, Moloney-Quinn L, Arkins S, Davison AJ, Cullinane A (2018) Equid herpesvirus 8: Complete genome sequence and association with abortion in mares. PLoS One 13(2):e0192301.
20. Tong PP, Duan RL, Palidan NEL, Deng HF, Duan LY, Ren ML, Song XZ, Jia CY, Tian SY, Yang EH, Kuang L, Xie JX (2022) Outbreak of neuropathogenic equid herpesvirus 1 causing abortions in Yili horses of Zhaosu, North Xinjiang, China. BMC Vet Res 18:83.

Figures

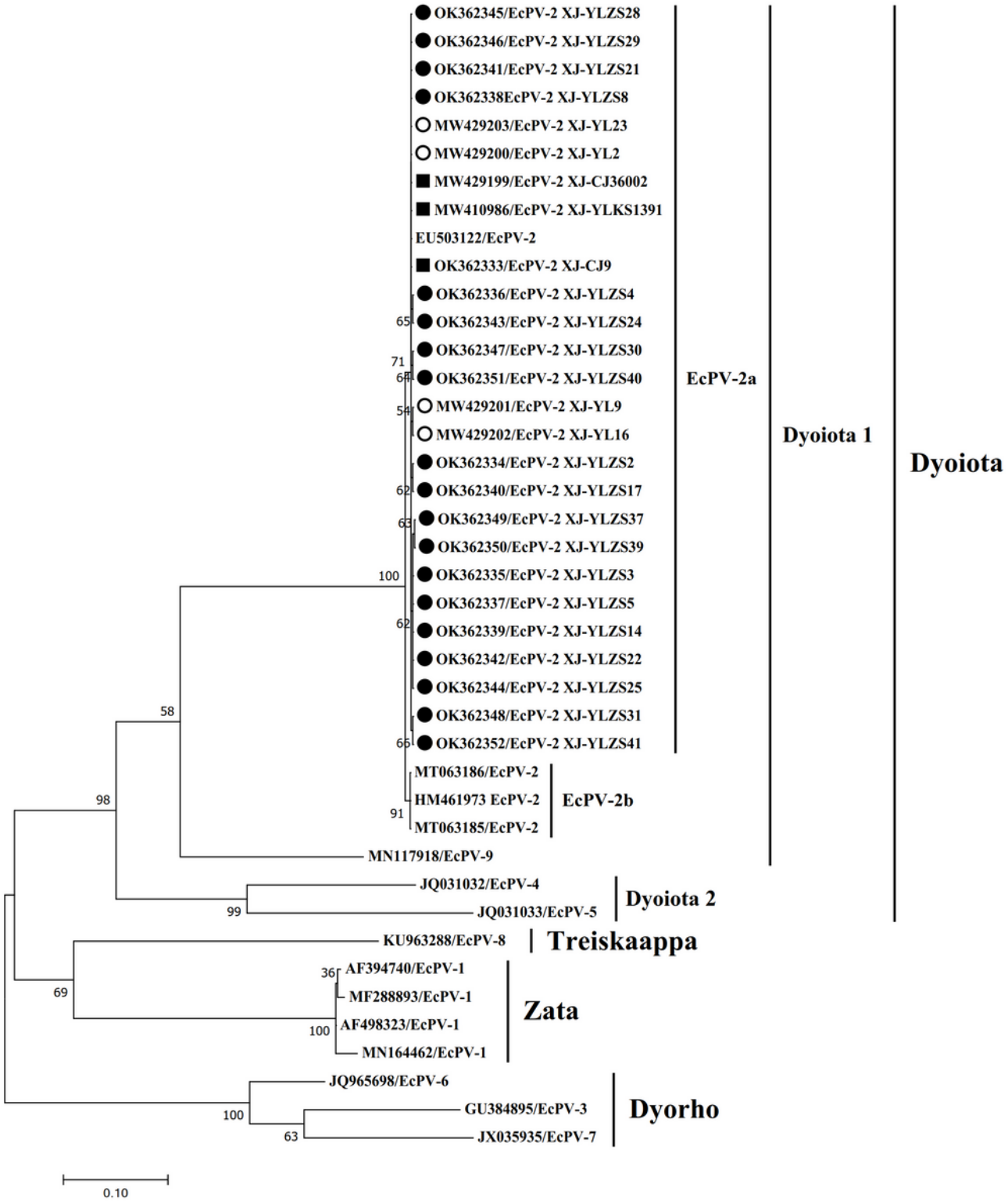

Figure 1

Phylogenetic tree of EcPV-2 strains found in China based on partial L1 gene sequences. Filled circles indicate the EcPV-2 strains identified in aborted fetus lung tissues of Yili mares; Filled boxes indicate the EcPV-2 strains identified in semen of Thoroughbred stallions; The open circles indicate the EcPV-2 strains identified in the nasal swabs of Yili horses.

Supplementary Files

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

- TableS1.docx
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