Molecular evidences show human papillomavirus type 124 can be trans-species infection in horses

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Short Report

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Abstract

Human papillomavirus type 124 (HPV124), a member of betapapillomavirus, is associated with viral warts, actinic keratosis, and squamous cell carcinoma incidence in human. No reports of HPV124 infection in mainland China have been reported to date. In a survey of Chinese horses, we detected HPV124 via next-generation sequencing (NGS) in nasal swabs from 42% (39/93) of Thoroughbred horses, 7% (2/30) of the Akhal-Teke horses, and 17/50 aborted fetal lung tissues of Yili mares that were initially found to be negative for equid herpesvirus 1, which indicated the virus can be trans-species infection in different breeding horses, and suggested that this pathogen may potentially contribute to abortion incidence among horses. Further investigation showed that some workers involved in horse farm were positive for HPV124, which strongly suggested that horse-origin HPV124 strain may be transmissible from human workers to horses. Sequence analyses of *E6* gene sequences from 18 Chinese HPV124 samples were performed, revealing that the 7 of 18 HPV124 strain identified both horses and human in the present study shared 100% sequence identity with previously published HPV124 isolate. Phylogenetic analyses based on *E6* gene sequences in GenBank further revealed that the HPV124 isolate identified herein was closely related to the NJ3900 HPV124 reference strain, clustering together with the established Beta 1 HPV lineage. These results provide first evidence that HPV124 can infect horses, may function as a causative agent for abortions in Yili horses, and come from HPV124-positive humans laboring.

Introduction

Papillomaviruses (PVs) are small, non-enveloped viruses with a circular ~ 8 kb dsDNA genome. PVs exhibit host specificity, and have been reported to infect a wide variety of fish, reptiles, birds, and mammalian species [1, 2]. To date, over 650 animal and human PV (HPV) subtypes have been identified, with ~ 440 HPV subtypes having been detected within the general human population [1]. The L1 gene open reading frame (ORF) nucleotide sequence is used to classify HPV strains into five genera (alpha, beta, gamma, mu, and nu) [3–5]. HPV124, which is a betapapillomavirus, has previously been identified and sequenced by Antonsson et al. [6], and it has further been detected in tissue samples associated with actinic keratosis, squamous cell carcinoma [7], viral warts [8], and breast cancer [9]. In recent reports, HPV124 has also been detected in oral, nasal and nasopharynx samples from healthy volunteers [10–13], and HPV124 has only been reported to be able to infect humans to date [3, 7–13]. To date, no reports have noted the presence of HPV124 in mainland China.

The present study was developed to survey PV infection incidence among horses of North Xinjiang, which is a leading horse-producing region in China, and to explore the potential for viral transmission between detected viruses and disease.

Materials And Methods

From July 2019 – May 2021, 437 total samples, including horse samples (205 nasal swabs, 90 fecal samples, 30 vaginal swabs, and 50 aborted fetal lung tissues), as well as 62 human nasal swabs, were collected from four farms in Yili, Changji and Urumqi in North Xinjiang, China (Table 1). Swabs were transported to the laboratory in tubes containing 10 mL of phosphate buffer and stored at -80°C, while aborted fetus tissue samples were prepared by veterinarians at the individual farms.

Table 1
Information of samples included in this study for HPV124

Sampling place	Farm	N/F/V/L	Breed	HPV124
Yili	A	56/90/0/0	Thoroughbred horse	2(4%)/0/0/0
		8/0/0/0	Human	1(12.5%)/0/0/0
	B	82/0/0/50	Yili horse	0/0/0/17(34%)
		44/0/0/0	Human	5(11.4%)/0/0/0
Changji	C	37/0/0/0	Thoroughbred horse	37(100%)/0/0/0
		10/0/0/0	Human	1(10%)/0/0/0
Urumqi	D	30/0/30/0	Akhal-teke horse	2(7%)/0/0/0
	Total	437		
N/S/V/L: Nasal swab/Feces/Vaginal swab/Lung tissue of aborted fetus				

Nasal swabs from Thoroughbred and Akhal-Teke horses were used to prepare cDNA libraries as in previous report [14]. Briefly, these nasal swabs were pooled together, centrifuged at 100,000 × g, followed by the extraction of viral nucleic acids which were then reverse transcribed and amplified using random primers. Tagged, purified amplification products were then sequenced with an Illumina HiSeq X-ten instrument at Shanghai Personal Biotechnology Co., Ltd.

Prepared samples were initially ground, centrifuged, and vortexed to yield a homogenate from which 200 µL of the obtained supernatant was collected for nucleic acid preparation based on provided directions (Geneaid Biotech Co., LTD). HPV124 partial *E6* gene (237 nt) in these samples was detected via PCR using the following primers: (forward primer) 5'-GGAAGGAGCATTTAGTGTTTG-3'/(reverse primer)5'-CTCCAAGCTCCTCTTACTTTATG-3'. PCR amplification was conducted with the 2× *TransStart*® *FastPfu* Fly PCR SuperMix (TransGen Biotech Co., LTD) using the following thermocycler settings: 95°C for 2 min; 35 cycles of 95°C for 20 s, 72°C for 30 s; 72°C for 5 min (TransGen Biotech Co., LTD). Positive PCR amplicons were ligated into the *pEASY*®-Blunt T vector (TransGen Biotech Co., LTD) for transforming DH5a competent cells (TransGen Biotech Co., LTD) and ten clones of each amplicon were eventually selected for Sanger sequencing (Sangon Biotech Co., LTD).

For further details regarding these sequences, including GenBank nos, see Fig. 1. All HPV124 nucleotide sequences in this study were submitted to GenBank with the accession nos. OK484563-OK484566 and

OL474072-OK474085. Sequences were further assessed using the MegAlign software in Lasergene v7.1, with phylogenetic trees being constructed via the maximum-likelihood approach based on the Tamura–Nei model using 1,000 bootstrap replicates to assess the accuracy of the resultant tree topology [15].

Results And Discussion

A number of previous studies that were conducted in different countries have documented that nine EcPVs and three BPVs can infect the horses [3–5, 16–18]. However, before this study, no one has described about infections caused by these viruses in the Chinese horses. In the current study, we first investigated the existence of PVs in the different samples of Chinese horses. Interestingly, virome results revealed that of the 68,007,096 reads generated from the nasal swabs, 56,054 (0.08%) were annotated to mammalian viruses including papillomavirus, retrovirus, circovirus-like virus, herpesvirus, astrovirus, parvovirus, and paramyxovirus. Of these viral reads, 103 shared > 99.5% nt identity with the HPV124 *E6* gene, suggesting that much like bovine papillomavirus 1 (BPV1), BPV2, and BPV13 [17, 18], HPV124 can also undergo trans-species transmission to infect horses. PCR analyses further confirmed that HPV124 was detectable in nasal swabs from two Thoroughbred horses in farm A in Yili city, 37 Thoroughbred horses in farm C in Changji city, and two Akhal-Teke horses in farm D located in Urumqi city (Table 1), with HPV124 case positivity rates of 4% (2/56), 100% (37/37) and 7% (2/30), respectively (Table 1). In addition, subsequent analysis of 50 samples of Yili horse aborted fetal lung tissue samples from farm B that were negative for EHVs revealed detectable HPV124 viral DNA in 17/50 of these samples via PCR (Table 1). Published reports have convincingly demonstrated HPVs only replication in human to date[1, 2], but our results indicated that HPV124 can readily infect heterologous hosts-horse, and this virus exist in mainland China. In order to avoid human viruses pollute samples of horses, workers wear gloves, masks, and protective clothing in sampling, thus HPV124 infection in horses is believable. These are also the first data to our knowledge demonstrating the presence of PV in aborted fetal tissue from horses, indicating that HPV124 is evidence for vertical transmission in horses as earlier reports of HPVs and BPVs having respectively demonstrated in humans and bovine species [19–27]. Similar to recently one study have demonstrated that EHV-1 cause abortions in Yili mares [28], our findings suggested that HPV124 may contribute to the incidence of Yili horse abortions, which highlight HPV124 as a candidate equine abortion virus worthy of further study.

To determine the potential for this HPV124 strain to transmit from humans to horses, we collected 62 nasal swabs from humans, including eight workers from farm A, 44 workers from farm B, and 10 workers from farm C (Table 1). PCR results confirmed that one worker in farm A, five workers in farm B, and one worker in farm C were positive for HPV124 (Table 1), which suggested that this HPV124 strain may be transmissible from workers to horses at an HPV124-positive farm. These results thus indicate that individuals working on a stud farm should take proper precautions to avoid HPV124 viral transmission. In humans, HPV124 has both been detected in healthy individuals and linked to diseases including viral warts, actinic keratosis, and squamous cell carcinoma [3, 7–11]. All seven HPV124-positive workers identified in this study were free of any clinical signs of disease, suggesting them to be healthy carriers.

Future research should thus focus on the route of HPV124 viral transmission between humans and horses.

HPV124 partial *E6* genes sequences were next amplified with appropriate primers (5'-GGAAGGAGCATTTAGTGTTTG-3'/5'-CTCCAAGCTCCTCTTACTTTATG-3'). Following sequence assembly, the *E6* genes from nine nasal swabs (five workers, two Thoroughbred horses, two Akhal-Teke horses) and nine aborted Yili horse tissue samples were sequenced, with the resultant sequences being deposited in NCBI (GenBank nos. OK484563-OK484566 and OL474072-OK474085).

Multiple sequence alignment of these *E6* gene sequences revealed the 18 sequences HPV124 strains from these human and equine samples to exhibit 96.9–100% nt identity, consistent with genetic diversity. Of these 18 HPV124 strains, sequences of seven (XJ-ZS5-52 from Thoroughbred horse nasal swab, XJ-ZS-A and XJ-ZS-G from human nasal swabs, as well as XJ-ZS2-1, XJ-ZS4-1, XJ-ZS20-1, and XJ-ZSi from aborted tissue samples of Yili mares) were compared with the HPV124 NJ3900 reference strain (GenBank no. GQ845446), revealing 100% DNA sequence similarity, which supported horse-origin HPV124 might be derived from human with HPV124 positive. Moreover, the remaining 11 HPV124 strains exhibited 97.4–99.5% nt similarity with this reference strain. In contrast, the *E6* genes from these Chinese HPV124 strains exhibited just 51.5–62.9% and 29.8–45.6% nt similarity, respectively, with Beta2-Beta5 type HPV and EcPV.

A phylogenetic tree developed based upon *E6* gene sequences from Beta type HPV and EcPV strains indicated that the Chinese HPV124 strain identified herein was closely related to the NJ3900 HPV124 reference strain, with HPV124, HPV8, HPV12, HPV14, and HPV19 all being clustered into the Beta 1 HPV lineage, which diverged from the Beta2-Beta5 HPV and EcPV lineages (Fig. 1).

In summary, this study is the first to have reported the detection of HPV124 among different breeds of horses or in any non-human species. As this virus was detected in aborted fetal lung tissue samples, HPV124 may be a causative agent responsible for abortions in Yili horses. Further studies revealed that this HPV124 isolate could be transmitted from humans working with HPV124 to horses. The results of this study will generate new awareness regarding the potential role of HPV124 in abortion incidence and zoonotic infections in horses and human throughout the world.

Declarations

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Conflict of interest The authors have declared no competing interests.

Ethics approval 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: 2020012, and performed according to the Animal Ethics Procedures and Guidelines of the Ministry of Agriculture of China. Aborted fetal lung tissue samples were collected by farm veterinarians in accordance with approved procedures. The owners provided written consent for the inclusion of these lung tissue samples in this study. All human participants provided written informed consent for the use of nasal swabs in the present study.

Authors' contributions P.T. and J.X. performed the research, analyzed the data, and drafted the manuscript. N.P., X.S., C.J., R.D ., S.T., E.Y., J.P., E.J., L.K. contributed to the collection of samples and detection of PCR. P.T., E.J. 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.

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Figures

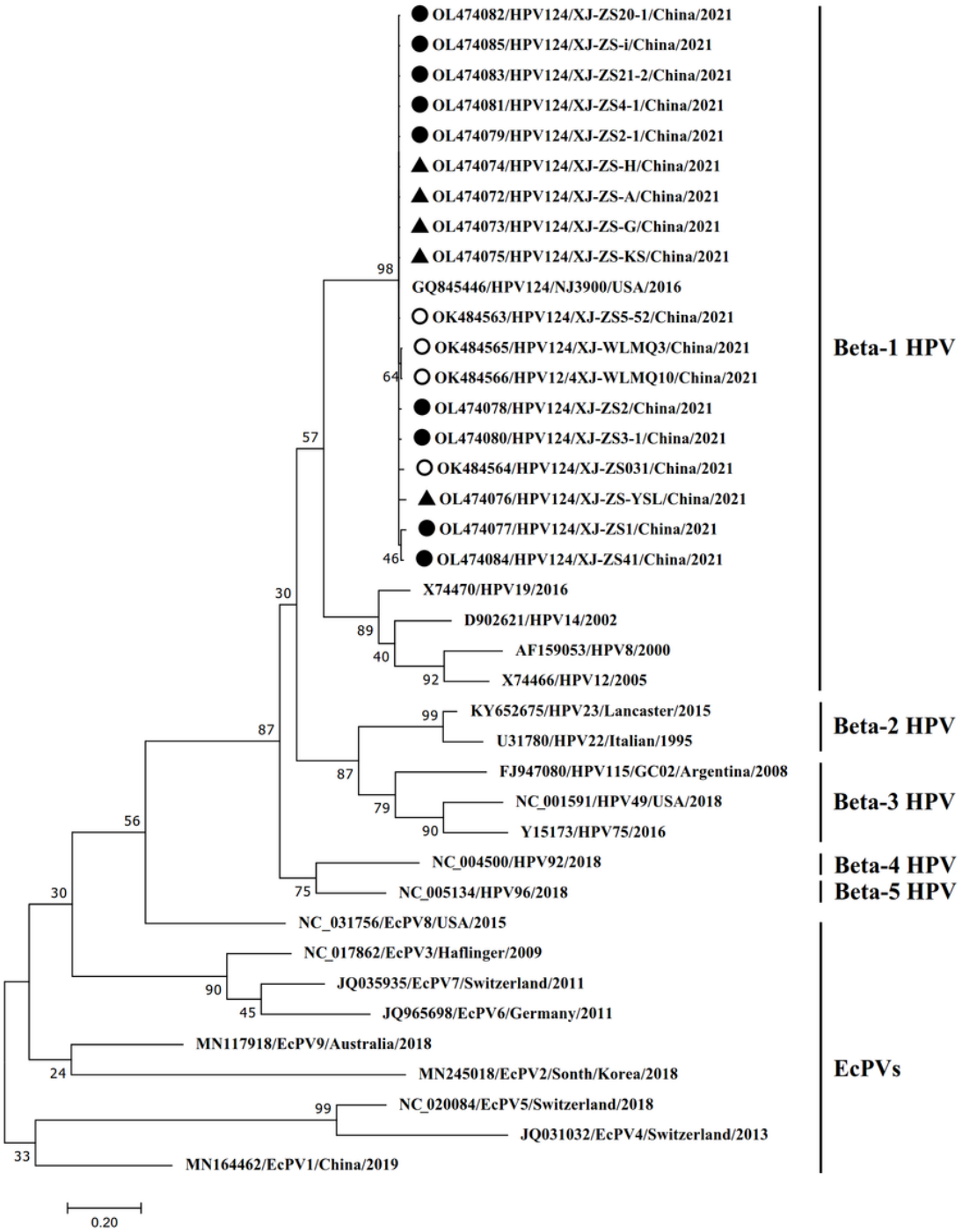

Figure 1

Phylogenetic tree of HPV124 strains identified in China based on partial *E6* gene sequences (237 nt). HPV124 strains identified in aborted fetal lung tissues in Yili mares, worker nasal swabs, and nasal swabs from Thoroughbred or Akhal-Teke horses are respectively denoted by black circles, black triangles, and open circles.