Transmission of Human Papillomavirus Type 124 from Horses to Humans Working on a Stud Farm

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Research Article

Keywords: Human papillomavirus type 124, Horse, Abortion storm, Zoonotic virus

Posted Date: January 25th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1272524/v1>

Abstract

Human papillomavirus type 124 (HPV124) is a betapapillomavirus that is associated with viral warts, actinic keratosis, and squamous cell carcinoma incidence. 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 4% (2/56) of thoroughbred horses, and 7% (2/30) of the Akhal-Teke horses, suggesting that horses can serve as a reservoir species for this virus. In addition, HPV124 was detected in 17/50 lung tissue samples from aborted Yili horse fetuses that were initially found to be negative for equid herpesviruses and equine arteritis virus, suggesting that this pathogen may potentially contribute to abortion incidence among horses. Further investigation showed that all workers involved in Yili mare production of farm B were positive for HPV124, while all other assessed individuals in farm B or without any history of contact with horses were negative for this virus. indicating that this HPV124 strain may be transmissible from horses to human workers at an HPV124-positive farm. Sequence analyses of E6 gene sequences from 18 Chinese HPV124 samples were performed, revealing that the 7 of 18 HPV124 strain identified among horses 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 new evidence that HPV124 can infect Chinese horses, may function as a causative agent for abortions in Yili horses, and may also infect humans laboring in Yili mare stables.

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]. 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) [2-4]. HPV124, which is a betapapillomavirus, has previously been identified and sequenced by Antonsson et al. [5], and it has further been detected in tissue samples associated with actinic keratosis, squamous cell carcinoma [6], viral warts [7], and breast cancer [8]. In recent reports, HPV124 has also been detected in oral samples from healthy volunteers [9, 10], and HPV124 has only been reported to be able to infect humans to date [2, 6-10]. To date, no reports have noted the presence of HPV124 in mainland China.

The present study was developed to survey HPV 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, 419 total samples, including 205 nasal swabs, 90 fecal samples, 30 vaginal swabs, and 50 aborted fetal lung tissue samples from horses negative for equid herpesvirus (EHV) and equine arteritis virus (EAV), as well as 44 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 (Nong Yi Fa [2017] No. 25).

Nasal swabs from thoroughbred and Akhal-Teke horses were used to prepare cDNA libraries as in previous reports [11]. Briefly, these nasal swabs were pooled together, centrifuged at 100,000 xg, 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 viral DNA in these samples was detected via PCR using the following primers: (forward primer) 5'-GGAAGGAGCATTTAGTGTTTG-3'/(reverse primer) 5'-CTCCAAGCTCCTTACTTTATG-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) and used to transform *E. coli* DH5a competent cells (TransGen Biotech Co., LTD). Sanger sequencing was then performed for 10 individual clones per amplicon (Sangon Biotech Co., LTD).

For further details regarding these sequences, including GenBank accession numbers, see Figure 1. All HPV124 nucleotide sequences in this study were submitted to GenBank with the accession numbers 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 [12].

Results And Discussion

In our previous study surveying different regions in north Xinjiang, we found that high-risk HPV18 was able to infect a range of different breeding horses [13], suggesting the potential for horses to carry many different HPV subtypes. To test this possibility, we herein employed an NGS-based approach to survey the HPV infection status 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, BPV13, and HPV18 [13-15], 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 and two Akhal-Teke horses in farm D located in Urumqi city (Table 1), with HPV124 case positivity rates of 4% (2/56) and 7% (2/30), respectively (Table 1). This virus was not detectable in any analyzed fecal samples or vaginal swabs, suggesting that much like HPV18 [13], HPV124 can readily circulate in different horse breeds across a range of geographical regions in Xinjiang province.

Subsequent analysis of 50 samples of Yili horse aborted fetal lung tissue samples from farm B that were negative for EHV and EAV revealed detectable HPV124 viral DNA in 17/50 of these samples via PCR (Table 1). These are the first data to our knowledge demonstrating the presence of PV in aborted fetal tissue from horses, suggesting that this virus may contribute to the incidence of Yili horse abortions. These results are consistent with earlier reports of HPV11 and BPV2 having respectively caused abortions in humans and bovine species [16, 17]. Several prior studies have also demonstrated that EHV and EAV can cause equine abortions [18-21]. As such, our results highlight HPV124 as a candidate equine abortion virus worthy of further study.

To determine the potential for this HPV124 strain to transmit from horses to humans, we collected 44 nasal swabs from humans, including 20 workers from farm B, 2 individuals that participated in a short internship at farm B, and 22 individuals without any history of contact with horses (Table 1). PCR results confirmed that only five workers from farm B were positive for HPV124, while all other assessed individuals were negative for this virus (Table 1). Further analyses revealed that all five of these HPV124-positive workers were directly involved in Yili mare production. This suggests that HPV124 infection status is closely tied to workplace setting and/or work-related duties, indicating that this HPV124 strain may be transmissible from horses to human workers at an HPV124-positive farm. The 15 workers in the same farm but not directly involved in mare production were negative for this virus, suggesting an absence of sustained human-to-human transmission. These results thus indicate that individuals working on a stud farm should take proper precautions to avoid HPV124 infection or 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 [2, 6-10]. All five 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 horses and humans.

HPV124 E6 genes sequences were next amplified with appropriate primers (5'-GGAAGGAGCATTTAGTGTTTG-3'/5'-CTCCAAGCTCCTCTTACTTTATG-3'). Following sequence assembly, the E6 genes from 9 nasal swabs (5 workers, 2 thoroughbred horses, 2 Akhal-Teke horses) and 9 aborted Yili horse tissue samples were sequenced, with the resultant sequences being deposited in the GenBank database under accession numbers: 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% nucleotide identity, consistent with genetic

diversity. Of these 18 HPV124 strains, sequences of 7 (1 from thoroughbred horse nasal swabs, 2 from human nasal swabs, and 4 from aborted Yili horse tissue samples) were compared with the HPV124 NJ3900 reference strain (accession no. GQ845446), revealing 100% DNA sequence similarity. Moreover, the remaining 11 HPV124 strains exhibited 97.4-99.5% nucleotide 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% nucleotide 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 Beta 2-Beta 5 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 horses to humans working in the context of Yili mare production. The results of this study will generate new awareness regarding the potential role of HPV124 in abortion incidence and zoonotic infections in horses throughout the world.

Declarations

Acknowledgments This study was supported by a National Natural Science Foundation of China (32060808), China Postdoctoral Science Foundation (2020T130555, 2019M653901XB), The Natural Science Foundation of Xinjiang Uyghur Autonomous Region (2019D01A47) and Post-doctoral Science Foundation of Xinjiang Agricultural University (XJAU20180723).

Compliance with ethical standards

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.

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Tables

Table 1 Information of samples included in this study for HPV124

Sampling place	Farm	N/F/V/A	Breed	HPV124
Yili	A	56/90/0/0	Thoroughbred horse	2(4%)/0/0/0
	B	82/0/0/50	Yili horse	0/0/0/17(34%)
		44/0/0/0	Human	5(11%)/0/0/0
Changji	C	37/0/0/0	Thoroughbred horse	0
Urumqi	D	30/0/30/0	Akhal-teke horse	2(7%)/0/0/0
Total		419		

N/S/V/A: Nasal swab/Feces/Vaginal swab/Lung tissue of aborted fetus

Figures

Figure 1

Phylogenetic tree of HPV124 strains identified in China based on partial E6 gene sequences. HPV124 strains identified in aborted fetal lung tissue, worker nasal swabs, and nasal swabs from thoroughbred or Akhal-Teke horses are respectively denoted by black circles, black triangles, and open circles.

Supplementary Files

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- [Sequencesfor18HPV124E6.docx](#)