Virus Research 244 (2018) 6–12

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Original Article

Genomic comparison of bovine papillomavirus 1 isolates from bovine, equine and asinine lesional tissue samples

C. Koch[a,*], A.S. Ramsauer[b], M. Drögemüller[c], M. Ackermann[b], V. Gerber[a], K. Tobler[b]

[a] Swiss Institute of Equine Medicine, Vetsuisse Faculty Berne, University of Berne, and ALP-Haras, Länggasstrasse 124, Postfach 8466, CH-3001 Berne, Switzerland
[b] Institute of Virology, Vetsuisse Faculty Zurich, University of Zurich, Winterthurerstrasse 266a, CH-8057 Zürich, Switzerland
[c] Institute of Genetics, Vetsuisse Faculty Berne, University of Berne, Bremgartenstrasse 109a, Postfach 8466, CH-3001 Berne, Switzerland

ARTICLE INFO

Keywords:
Bovine papillomavirus
Horse
Donkey
Sarcoid
Species-specific variants

ABSTRACT

Several attempts have been made to categorize equid- and bovid-specific bovine papillomavirus 1 (BPV1) isolates based on sequence tags. This study includes newly determined sequence information from 33 BPV1 isolates of equine, asinine and bovine origin and investigates sequence bias due to host species. Twenty of the viral genomes were sequenced over their entire length and a further thirteen were sequenced, including flanking sequences, at two specific sites, the LCR and the E5 ORF. Alignment and analyses of the sequences did not reveal statistically significant site differences between the sequences of bovine and equid origin. None of the proposed sites of divergence noted by other authors demonstrated significant species-specific characteristics. Our results suggest that BPV1 is shared between equine, asinine and bovine host species, and that viral transfer between bovines and equids is a repeated and ongoing phenomenon.

1. Introduction

Papillomaviruses (PVs) are non-enveloped DNA viruses and among the most widespread animal viruses. Host-specific PVs have been identified in mammals, reptiles and birds (Bernard et al., 2010). Approximately 180 different human and 160 animal-specific PVs have been characterized so far; 21 of these are bovine in origin.[1] Phylogenetic studies indicate that PVs and their hosts co-evolve. They are therefore highly host-specific viruses that usually do not cross species barriers. New variants, however, emerge if mutations accumulate within the virus genome. Closely related PVs are found in different host species indicating possible cross-species infection, which may lead to the emergence of new PV types (Gottschling et al., 2011). To date the only PVs clearly documented to infect more than one species are bovine PV types 1, 2, and 13 (BPV1, BPV2, and BPV13) of the genus Deltapapillomaviruses. These types cause fibropapillomas in bovines and are associated with skin tumours, termed sarcoids, in equids (Chambers et al., 2003; Mayr and Kaaden, 2007; Nasir and Campo, 2008; Lunardi et al., 2013).

Papillomavirus infection may be asymptomatic or cause benign skin tumours, known as papillomas or warts. However, some PVs such as human PV types 16 or 18 (HPV16, HPV18) have a causal role in the development of cancers in conjunction with other co-factors (Muñoza et al., 2006).

In the bovine host, BPV1 causes benign fibropapillomas involving the epithelium and underlying dermis. These fibropapillomas predominantly develop in the paragenital region, including prepuce, udder and teats and usually regress spontaneously. Regression is driven by a local cell-mediated immune response, as the antibody response to BPV1 in bovines is very poor and often absent (Bocaneti et al., 2016; Chambers et al., 2003; Ragland and Spencer, 1968; Barthold and Olson, 1974). Typically, BPV1-induced fibropapillomas contain large numbers of virus particles (Tajima et al., 1968) and the natural infectious cycle is maintained by transmission of virions to other susceptible bovine hosts. This has also been reproduced experimentally (Lee and Olson, 1968).

Equine sarcoids (ES) account for up to 90% of all skin tumours diagnosed in equids (Scott and Miller, 2003; Marti et al., 1993). Unlike fibropapillomas in bovids, ES are usually persistent tumours that tend to recur in a more severe form following accidental or iatrogenic trauma (Bergvall, 2013). A cross-species infection with BPV1 and/or BPV2 is widely accepted as the main extrinsic factor responsible for the development of ES lesions. More recently, BPV13 has also been identified in ES tissues collected from horses in southern Brazil (Lunardi et al., 2013), and this group of investigators speculated that further papillomavirus types might be associated with ES development in other geographic areas.

* Corresponding author.
E-mail address: christoph.koch@vetsuisse.unibe.ch (C. Koch).
[1] See: https://pave.niaid.nih.gov/#search/search_database (Accessed 12 April 2017)

https://doi.org/10.1016/j.virusres.2017.11.005
Received 28 April 2017; Received in revised form 1 November 2017; Accepted 3 November 2017
Available online 04 November 2017
0168-1702/

BPV1 and/or BPV2 DNA are consistently detected in ES, where present as multiple episomes (Amtmann et al., 1980). Moreover, viral mRNA and transforming proteins are intralesionally expressed, supporting the role of BPV1/2 as major aetiological agents of ES (Wilson et al., 2013; Borzacchiello et al., 2008; Carr et al., 2001; Nasir and Reid, 1999). The mode of transmission for ES, however, remains unclear and equids, bovids and environmental sources for infectious BPV1/2 virions need to be considered (Bogaert et al., 2008; Nasir and Campo, 2008). Although BPV1/2 do not undergo productive infection in ES at levels comparable to those reported for cow warts (Wilson et al., 2013), recent publications suggest that ES tumours may contain infectious virions at low concentrations (Brandt et al., 2008a,b; Wilson et al., 2013) and therefore serve as a potential source of infection. This is substantiated by observations of a gradual spread of ES within a small population of donkeys in the absence of a bovine virus source (Reid et al., 1994; Nasir and Campo, 2008). Moreover, Wilson et al. (2013) have visualized intralesional BPV1 virions by transmission electron microscopy (Wilson et al., 2013) suggesting that BPV1/2 infection may be productive at least at some stages of ES disease. To further strengthen the concept of direct BPV1 and/or BPV2 transmission between equids, the observed presence of apparently equine-specific genetic BPV1 variants has been brought into play (Chambers et al., 2003; Nasir et al., 2007; Szczerba-Turek et al., 2011; Wilson et al., 2013; Trewby et al., 2014; Savini et al., 2015).

The double-stranded circular DNA genome of BPV1 and BPV2 is about 8000 base pairs long (Chen et al., 1982; Groff and Lancaster, 1986; unpublished, GenBank ID M20219). Functionally, the BPV1 and BPV2 genomes can be divided into three regions: (1) the long control region (LCR) for replication and transcription, (2) an early region (E) coding for early regulatory and transforming proteins, and (3) a late region (L) containing open reading frames for the major L1 and the minor L2 capsid proteins (Nasir and Campo, 2008). The LCR, because of its crucial function in viral replication and transcription, and E5 as the major oncoprotein of BPV1 and BPV2, have been extensively studied and assessed for possible genetic aberrations that may explain the unique epidemiological and pathological features of ES.

The aim of the present study was comprehensively investigate the genomic sequence variations of BPV1 isolated from bovine, equine and asinine lesional tissue samples. Based on previously published observations, we expected to find equid-specific BPV1 variants, common to horses and donkeys but consistently differing from variants derived from bovines, and to observe possible associations with geographic origin.

2. Materials and methods

2.1. Tissue sampling and DNA extraction

Equine sarcoids and bovine fibropapillomas were collected from 42 subjects between March 2012 and April 2014. All 12 equine samples and 7 asinine samples were collected after surgical excision at the ISME equine clinic Bern, Switzerland. An additional 9 asinine samples were collected at the Donkey Sanctuary in Sidmouth, Devon, U.K. Fourteen bovine fibropapillomas were collected from young dairy cattle (*Bos taurus*) by two Swiss large animal practitioners. Ethical approval for this work was granted by the committee for animal experiments of the Canton Bern, Switzerland (BE 68/12).

The following data were recorded for each subject: age, sex, coat colour, ES type, and localization of the lesion for equids; and age, sex and localization of the lesion with a morphological description provided by the collaborating practitioners for bovids. In the event that several tumours were excised from one animal, a piece of the largest tumour was subjected to DNA isolation using a QIAamp DNA Mini Kit according to the manufacturer's instructions (Qiagen). Aliquots of tumours excised at the ISME equine clinic Bern, Switzerland, were subjected to histopathological examination confirming ES diagnosis.

Table 1
Primer sequences used for sequencing.

Primer name	Sequence (5′-3′)
BPV1_F01	ACTGCTGCAGACATGGTTCA
BPV1_F02	GCCAGGATGAGGATTTTGTT
BPV1_F03	GGCGAGTTTCGAACTCCTAA
BPV1_F04	GGGAAGGAAGCTGGAAGTCT
BPV1_F05	CATAGTCGGGTGCAAACCTT
BPV1_F06	TTTGTACATGCGCACAGAGG
BPV1_F07	TCAAGGCAGGAAGAAGAGGA
BPV1_F08	CAGGTCTGCCCTTTTAATGC
BPV1_F09	CAGTATAGGTGCGGGCATTC
BPV1_F10	CCACGGAAGATCCTGAAGTG
BPV1_F11	TGGGCACACAGTTGATTTGT
BPV1_F12	CACCACCCAAACAACAGATG
BPV1_F13	TTAATCGGCCCTACTGGCTA
BPV1_F14	GGCAGGATGTTCAACTGTGA
BPV1_F15_2	ACATAGCGGGACCGAACAC
BPV1_F16	TCACCGAAACCGGTAAGTAAA

2.2. Amplification and sequencing of viral DNA

Whole viral genomes were amplified by rolling circle amplification (RCA) using 1 µl total DNA as template and an Illustra Templi Phi Kit (GE Healthcare) according to the manufacturer's instructions. Of note, the Phi 29 polymerase used for RCA has proofreading activity. The BPV type was determined by restriction enzyme analysis of purified amplicons using *Bam*HI (Roche). Subsequently RCA products were subjected to direct sequencing performed on an ABI 3730 capillary sequencer (Life Technologies) using BigDye Terminator Sequencing Kit 3.1 (Life Technologies). Sixteen primers were used for sequencing. The primer sequences are listed in Table 1. Sequences were assembled with Sequencher 5.1 (GeneCodes).

In case RCA did not result in amplicons of sufficient quantity or quality, corresponding DNA isolates were subjected to E5 and LCR PCR. The LCR region was amplified using a previously published primer set (Nasir et al., 2007). The E5 region was amplified using a newly designed primer pair (BPV1_E5_for 5′-GAC AAG CAC AAA TAC TGA TCA CC-3′ and BPV1_E5_rev 5′-AGG GGT TGC AGC TCA AGA AC-3′). PCR was performed in a total volume of 25 µl containing 8 µl sterile water, 2 µl of forward and reverse primer (10 µM each), 1 µl DNA as template and 12 µl REDTaq ReadyMIX (Sigma-Aldrich). The cycling program for both PCR assays started with a denaturation step of 3 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C. The nucleotide sequences of all amplimers were determined (Microsynth) using the PCR primers by an ABI 377 sequencer (Applied Biosystems/Invitrogen). Three sequencing runs including both directions and at least two PCR reactions were assembled to final contigs using CLC Main Workbench version 6.5 (Qiagen Bioinformatics). Sequences determined in this study were deposited in GenBank. The accession numbers of the complete genomes are MF384275-MF384294, those of the LCR-regions are MF384295-MF384307, and those of the E5 regions are MF384308-MF384320.

2.3. Bioinformatic analyses of sequences

Sequences were aligned pairwise by Needleman-Wunsch-algorithm (EMBOSS) to the reference sequence (GenBank accession number X02346) and subsequently combined to a multiple sequence alignment (MSA). From the manually edited MSA, positions showing sequence variations between samples from bovine vs. equine or asinine origin were extracted and used for further analyses.

Two parts of the alignment covering sixty viral sequences, 33 newly sequenced in the present study and 82 BPV1 sequence segments of variable size from GenBank were extracted from the MSA and used to infer phylogeny. These parts of the genome corresponded to the