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ORIGINAL PAPER

Genetic characterization of a putative new type of bovine papillomavirus in the *Xipapillomavirus* 1 species in a Brazilian dairy herd

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Abstract

Currently, bovine papillomavirus types are divided into five genera, namely, *Deltapapillomavirus*, *Epsilonpapillomavirus*, *Xipapillomavirus*, *Dyoxipapillomavirus*, and *Dyokappapapillomavirus*. In the recent decades, the characterization of numerous putative and novel bovine papillomavirus types from cattle in several geographic regions, has revealed the occurrence of a high viral diversity. In this study, we describe the identification and characterization of a putative new bovine papillomavirus type within species *Xipapillomavirus* 1 of *Xipapillomavirus* genus. The detection of the viral types identified in the skin warts was obtained by polymerase chain reaction assays targeting the L1 gene, followed by direct sequencing of the generated amplicons. The partial L1 sequences revealed that bovine papillomavirus types 6, 10, and 11, the putative new bovine papillomavirus type designated BPV/CHI-SW2, and an unreported putative new bovine papillomavirus type (named BPV/BR-UEL08) were associated with cutaneous papillomatosis in the cows from the dairy herd investigated. Phylogenetic reconstruction based on the L1 gene revealed that the BPV/BR-UEL08 isolate clustered with other bovine papillomaviruses classified in the *Xipapillomavirus* genus, being closely related to representatives of the species *Xipapillomavirus* 1. Investigations focusing on the molecular epidemiology of bovine papillomaviruses related to clinical outcomes in cattle are of fundamental importance to determine the actual genetic diversity and prevalent viral types to be included in vaccines for cattle.

Keywords Cattle · BPV · PCR · L1 gene · Phylogenetic analysis

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Introduction

Papillomaviruses (PVs) are small, nonenveloped oncoviruses containing a genome represented by a circular double-stranded DNA molecule that varies from 5748 to 8607 bp in length [1]. In cattle, bovine papillomaviruses (BPVs) may result in subclinical infections, benign cutaneous lesions commonly known as warts, and benign or malignant proliferations of the mucosal lining of the upper digestive tract or urinary bladder of cattle fed with braken fern [2–4].

PVs are classified in *Papillomaviridae* family that comprises subfamilies *Firstpapillomavirinae* and *Secondpapillomavirinae* which include more than 50 genera and 130 viral species [1]. BPV types are divided into at least five well-established known genera, where BPVs 1, 2, 13, and 14 are grouped in *Deltapapillomavirus* genus; BPVs 5 and 8 are classified in *Epsilonpapillomavirus* genus; BPVs 3, 4, 6, 9, 10, 11, 12, 15, 17, 20, and 23 belong to *Xipapillomavirus* genus; BPV 7 is the only member that represents

Dyoxipapillomavirus genus, while BPVs 16 and 22 are representatives of *Dyokappapapillomavirus* genus [5–9]. In addition, fully characterized BPV types 18, 19, and 21 remain in yet undefined genera [7, 9].

Several molecular strategies have allowed the genotyping of BPVs associated with diverse clinical outcomes in cattle. The PCR identification using type-specific primers is normally performed [2, 10]. Alternatively, PCR assays with degenerate primers which target highly conserved regions of L1 gene of PVs in general are also frequently used to study viral diversity related to cattle with cutaneous papillomatosis [11–13]. With this method, viral types are predominantly defined by direct sequencing of the obtained amplicons [4, 14–16]. However, genotyping is also done in association with the observation of distinct digestion profiles by RFLP technique [17, 18].

In the recent decades, the identification and characterization of numerous putative and novel BPV types from beef and dairy cattle in several geographic regions worldwide, including cattle herds from Brazil, have observed the occurrence of a high viral diversity, primarily associated with animals presenting skin warts [4, 7, 13, 15, 16, 19].

In this study, we describe the identification and characterization of a putative new BPV type within species *Xipapillomavirus* 1 of *Xipapillomavirus* genus.

Materials and methods

Lesion samples and histopathologic evaluation

Five skin warts were individually and surgically removed from distinct anatomic locations of three (A–C) adult dairy cows with cutaneous papillomatosis. These animals were from a cattle herd in the state of Paraná, Southern Brazil (Table 1). All efforts were made to minimize animal suffering including local anesthesia. The procedures involving animals used during this investigation were approved by the Ethics Committee for Animal Experimentation of the Universidade Estadual de Londrina, Brazil, under protocol no. 5730.2016.83.

Sections of the collected skin warts were fixed by immersion in 10% buffered formalin solution and routinely processed for histopathologic evaluation while part of the specimens were maintained at − 80 °C until used in molecular analysis.

Papilloma fragments were ground in phosphate-buffered solution (PBS) pH 7.2, and suspensions (10–20% w/v) were centrifuged at 3000×*g* for 5 min. Aliquots of 250 µL from the supernatants were treated with lysis buffer (10 mM Tris, 1 mM EDTA, 0.5% Nonidet P40, 1% SDS, and 0.2 mg/mL of proteinase K) (Invitrogen, Carlsbad, USA). After vigorous homogenization, samples were incubated at 56 °C for 30 min.

Nucleic acid purification

For nucleic acid extraction, a combination of the phenol/chloroform/isoamyl alcohol and silica/guanidine isothiocyanate methods was performed [20]. Purified DNA was eluted in 50 µL of ultrapure sterile water and maintained at − 20 °C until further use in the PCR assays. Aliquots of ultrapure sterile water were included as negative controls in all DNA extraction procedures.

PCR amplification and sequencing of partial L1 gene fragment

To detect the BPV types present in all skin warts evaluated during this study, the modified PCR assay [21], using the primer pair FAP59 (forward: 5′-TAACWGTIGGICAY CCWTATT-3′) and FAP64 (reverse: 5′-CCWATATCWVH-CATITCICCATC-3′), which amplifies a partial fragment of approximately 480 bp of L1 gene of PV in general [12], was used. Aliquots from the PCR-amplified products were analyzed by electrophoresis in a 2% agarose gel stained with ethidium bromide (0.5 mg/mL) and examined under UV light.

Amplicons representing the partial fragments of the L1 gene (FAP products) were excised from agarose gels and purified by means of PureLink Quick Gel Extraction kit (Invitrogen, Carlsbad, USA). Direct sequencing was then done using the BigDye Terminator v.3.1 Cycle Sequencing

Table 1 Characteristics of the cows and lesions evaluated for the presence of bovine papillomavirus

Cow#	Breed	Age (years)	Sample	Anatomic location	Viral type	GenBank Accession No.
A	Jersey	5	SC01	Back	BPV10	MH729199
			SC02	Neck	BPV11	MH729200
B	Jersey	6	SC03	Udder	BPV6	MH729201
			SC04	Teat	BPV/CHI-SW2	MH729202
C	Holstein	4	SC05	Thigh	BPV/BR-UEL08	MH729203

Kit (Applied Biosystems, Carlsbad, USA), with the primers FAP59 and FAP64, in a 3500 Genetic Analyzer (Applied Biosystems, Carlsbad, USA), according to the manufacturer's instructions. When the nucleotide (nt) sequence generated for a DNA sample presented identities lower than 90% with other PV sequences deposited on GenBank database, the DNA sample was subjected to PCR amplification of the entire length of L1 gene.

PCR amplification of the complete L1 gene

To amplify the whole extension of the L1 gene of PV DNA detected in the skin wart of cow C, a PCR assay using primers that anneal to the initial (forward: 5′-GGGCCA TGGATGTCATACTGGCTACCAAGTTC-3′) and final (reverse: 5′-GGGGGTCGACTTATGCATTTTTCCGCC TAC-3′) regions of BPV6 L1 gene was used. These primers were originally selected from the BPV6 genome to amplify the full-length of the L1 gene of BPV6 initially intended to express and purify this protein but are being used for the first time in molecular identification.

The PCR assays were prepared by adding 5 µL of purified DNA and a mix containing 1 µM of each primer, 100 µM of each dNTP (Invitrogen, Carlsbad, USA), 2.5 U of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, USA), 5 µL of 10× PCR buffer (20 mM Tris HCl pH 8.4 and 50 mM KCl), 1.5 mM of $MgCl_2$, and ultrapure sterile water to a final volume of 50 µL. Amplification was performed under the following conditions: 94 °C for 10 min followed by 40 cycles of 1 min at 94 °C, 1 min at 56 °C, 1 min at 72 °C, and a final step of 7 min at 72 °C.

The amplicon obtained, representing the entire L1 gene and with the expected length of approximately 1500 bp, was purified by using a PureLink Quick Gel Extraction Kit (Invitrogen, Carlsbad, USA). Molecular cloning using the TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, USA) was performed according to the manufacturer's instructions. Inserts from three selected clones were then sequenced in both directions using the T7 and T3 primers.

Phylogenetic analysis

The quality analysis of chromatogram readings generated was examined with the Phred application. The sequences were accepted if the base quality was ≥ 20. The consensus sequences were determined using CAP3 software, and the derived sequences were then compared with all sequences in the GenBank database using the BLASTn program.

Pairwise and multiple sequence alignments at the nt and amino acid (aa) levels and sequence similarities were calculated using ClustalW in MEGA v5 software [22]. The L1 nt sequences derived from 24 fully sequenced BPV types were included in the phylogenetic analysis. Phylogenetic trees were reconstructed from the alignment of complete L1 nt sequences using the Maximum Likelihood method with the General Time Reversible model using MEGA v5 software. There were a total of 1327 positions in the final dataset. Bootstrap support values were determined for 1000 replications.

The partial nt sequences of L1 gene, as well as the complete nt sequence of L1 gene of the putative new BPV type identified and characterized in this study, referred to as BPV/BR-UEL08, are deposited in GenBank under the accession numbers MH729199, MH729200, MH729201, MH729202, and MH729203 (Table 1).

Results

The skin warts evaluated were true papillomas, while histopathology revealed a benign neoplastic cutaneous growth formed by the severe proliferation of epithelial cells (Fig. 1). Within the papilloma, there were severe orthokeratotic hyperkeratosis of the stratum cornea, moderate ballooning degeneration, and hyperplasia of epithelial cells of the stratum spinosum. In addition, there were reduced mitotic activity and apoptosis, and several epithelial cells of the stratum spinosum had enlarged, condensed nuclei.

Partial fragments of the L1 gene of BPV were successfully amplified by the PCR assay using the FAP59/64 primer pair from all DNA samples purified from the skin warts evaluated, generating PCR products with the expected length of approximately 480 bp. All negative controls included in the PCR assays were not amplified.

Direct sequencing of the FAP amplicons obtained from the skin warts revealed that the cows from this dairy herd were infected by several types of BPV, including fully characterized BPV types, a previously identified putative new BPV type, and an unreported putative new BPV type. BPV genomic sequences amplified from two skin warts collected from cow A had similarities in the partial L1 fragment of 100 and 99% with BPV types 10 and 11, respectively. Cow B was infected by BPV strains sharing 98% identity with a previously described putative new BPV type (designated BPV/CHI-SW2) and 99% identity with BPV6. However, an unreported putative new BPV type (BPV/BR-UEL08) was identified in the skin wart from cow C (Table 1).

Comparison of the partial L1 fragment of BPV/BR-UEL08, obtained with the FAP PCR assay, revealed elevated nt sequence similarity (72%) with the nt sequence of BPV6. When the complete nt sequence of the L1 gene of BPV/BR-UEL08 (1521 nt) was used in the comparative analysis, 75.8% of similarity was shared with L1 gene of BPV23, having identities that varied between 60.4 and 75% with other representatives of the *Xipapillomavirus* genus.

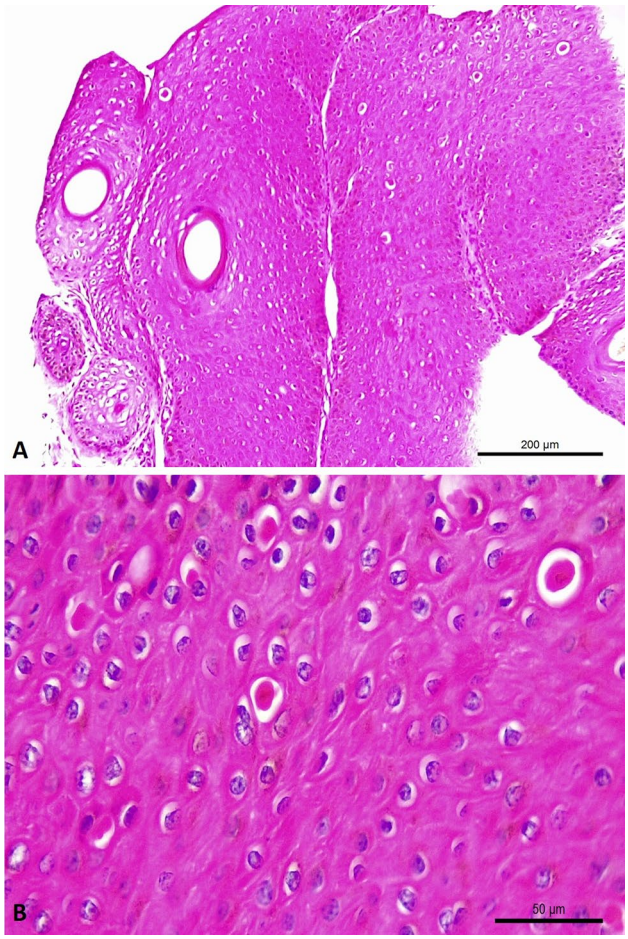

Fig. 1 Histopathologic features of cutaneous bovine papilloma infected with putative new BPV type BPV/BR-UEL08; there is proliferation of epithelial cells at the stratum spinosum with varying degrees of ballooning degeneration (**a**). Several epithelial cells have enlarged, condensed nuclei, and some are apoptotic (**b**). Hematoxylin and eosin stain; Bar, **a** 200 µm, **b** 50 µm

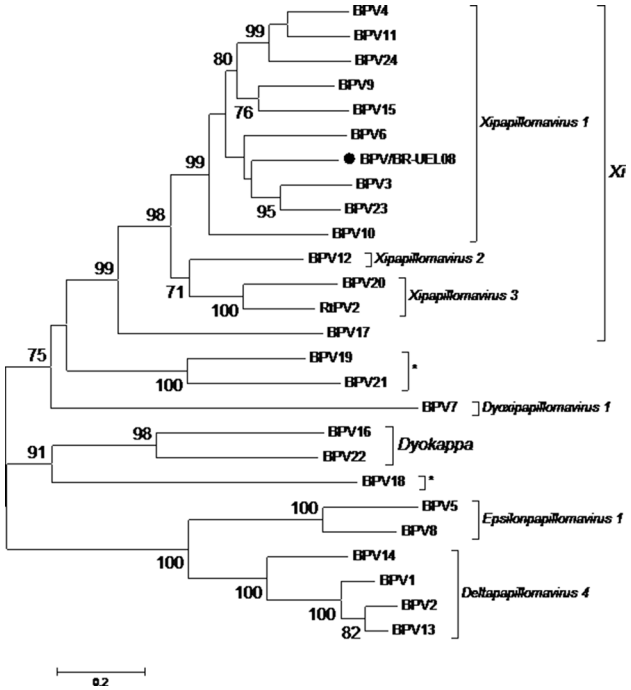

Fig. 2 Molecular phylogenetic analysis between bovine papillomavirus types based on complete L1 gene nucleotide sequences. The evolutionary history was inferred by means of the Maximum Likelihood method based on the General Time Reversible model. Numbers at internal nodes represent the bootstrap support values (percentages) determined for 1000 replications (values less than 60 are not shown). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 26 nucleotide sequences. All positions containing gaps and missing data were eliminated. The putative new BPV type identified in this study is represented by a black circle symbol. The five well-established genera where BPV types are classified as well as the six corresponding viral species are shown: *Xipapillomavirus 1*, *2*, *3* (*Xipapillomavirus* genus), *Dyoxipapillomavirus 1* (*Dyoxipapillomavirus* genus), *Epsilonpapillomavirus 1* (*Epsilonpapillomavirus* genus), *Deltapapillomavirus 4* (*Deltapapillomavirus* genus). BPV types that remain with undefined classification are demonstrated with asterisks

Phylogenetic reconstruction, based on the nt sequences of L1 gene of the previously fully sequenced BPV types, revealed that the BPV/BR-UEL08 isolate clustered with BPVs classified in the *Xipapillomavirus* genus, being closely related to representatives of the species *Xipapillomavirus* 1 (Fig. 2).

Discussion

In this study, the evaluation of papillomaviral DNA identified in five skin warts by PCR assays followed by sequence analysis revealed five distinct BPV types associated with warts from three dairy cows belonging to the same cattle herd. A putative new BPV type, previously unreported and referred herein as BPV/BR-UEL08, was identified from a skin wart excised from the thigh of one of these cows. In addition, three previously characterized viral types, BPVs 6, 10, and 11, and the putative new BPV type BPV/CHI-SW2, initially described in a cattle herd from China and most closely related with BPV3 [23], were also identified.

In our study, two of the three cows evaluated had two different skin warts as confirmed by BPV DNA detection. Direct sequencing of the FAP amplicons showed the occurrence of multiple infections in skin warts from diverse anatomic sites in these animals. The occurrence of multiple infections in cattle with papillomatosis was also identified in other studies [17, 19, 24].

In addition, since five BPV types were identified from the five skin warts evaluated, this finding indicates the occurrence of a high diversity of viral types associated with cutaneous papillomatosis in this cattle herd. Similar results of an elevated diversity in viral genotypes infecting cattle with

skin warts from individual cattle herds were described in Japan [13], Italy [25], and in cattle from the Northern [15], Northeastern [10], and Southern [16] regions of Brazil.

Since the L1 gene is considered the most conserved among PV genes, the nt sequence has been used for identification and classification of new PVs. The criteria for PV systematics determine that a strain is considered as a new PV type if the L1 gene nt sequence differs by more than 10% when compared to the closest known PV type. Furthermore, PVs that share between 60 and 70% nt similarity in the L1 ORF are considered as distinct species, while PVs of different genera share less than 60% nt sequence identity in the L1 gene. Consequently, when the nt sequence used for the comparison represents a partial fragment of L1 ORF, the strain is designated as a putative new viral type [26].

In our investigation, the phylogenetic reconstruction demonstrated that the newly described putative BPV type BPV/BR-UEL08 clustered with other viral types previously classified in the *Xipapillomavirus* genus that is known to group most of BPV types identified in cutaneous papillomatosis from cattle herds [9]. Our findings suggest that the phylogenetic position for this new isolate is in species *Xipapillomavirus* 1 of *Xipapillomavirus* genus. Since BPV types 6 and 23 were the closest known PV types for putative novel BPV (BPV/BR-UEL08) and the identities shared in the complete L1 gene nt sequence were 75 and 75.8%, BPV/BR-UEL08 should be considered as a new BPV type classified in this viral species, where BPVs 3, 4, 6, 9, 10, 11, and 15 are other representatives classified [9].

When compared with all well-defined genera where BPV types are classified, the *Xipapillomavirus* genus has the higher number of known fully characterized BPV types (BPVs 3, 4, 6, 9, 10, 11, 12, 15, 17, 20, and 23) [6, 9]. Curiously, all BPV types identified from the skin warts in cows evaluated during this study were classified within the *Xipapillomavirus* genus. It is worthy to mention that most putative new BPV types characterized by sequencing of the partial fragments of the L1 gene from skin warts of cattle with papillomatosis were classified within the *Xipapillomavirus* genus [4, 14, 15, 17, 23].

The use of degenerate primers targeting the L1 gene followed by direct sequencing of the obtained amplicons has been considered an useful tool to identify new BPV types classified in diverse PV genera as well as viral diversity associated with cutaneous papillomatosis in cattle [4, 12, 14–17, 27]. Herein, the strategy using the FAP primers, which was originally designed to amplify HPV types with tropism to skin [12] and subsequently used to amplify PV DNA from many animal species [27], enabled the identification of three previously characterized BPV types, a putative previously described new BPV type, and an unreported putative new BPV type. In other surveys that used the same primer pair to amplify the partial L1 gene sequences of BPV types from skin warts of cattle, a broad range of viral types classified in all known BPV genera were successfully identified [4, 14, 15, 17, 18].

In Brazil, throughout the utilization of PCR assays with degenerate or specific primers targeting the L1 gene, a high genetic diversity in viral types was associated with cases of cutaneous papillomatosis in both beef and dairy cattle herds from various geographic regions. In the Northeastern region, BPVs 1–10, a BPV11 subtype, and putative new BPV types were detected [10, 19]. BPVs 1, 2, 6, 7, 8, 9, and 10, as well as six putative new BPV types were identified with use of FAP PCR assay in the South [4, 16, 28], culminating in the characterization of BPV13 complete genome [5]. In addition, four putative new BPV types infecting dairy cattle maintained at the Amazon biome within the Northern region of Brazil, and classified in three distinct genera, were identified [15]. Moreover, by randomly primed rolling circle amplification (RCA) followed by next-generation sequencing (NGS), six new BPV types, named BPVs 16, 17, 18, 19, 20, 21, that were coinfecting a bovine from the Northern region of Brazil, were fully characterized from a single skin wart [7]. In addition, the complete genome sequence of BPV23 was identified by the same strategy in cattle with papillomatosis from the state of Acre, Northern Brazil [6].

Currently, 23 distinct BPV types are recognized via characterization of their entire genomes [9]. Differently, the recognition of more than 200 HPV types, detected in association with benign and malignant lesions from the skin and mucosa membranes, suggests that studies performed in humans have been more extensive compared to those done in this animal species (https://pave.niaid.nih.gov/#home). Therefore, additional investigations focusing on the molecular epidemiology of BPVs related to clinical outcomes in cattle are of great importance to reveal the actual genetic diversity and prevalent viral types, so that studies relating to the development of adequate vaccines based on viral proteins of most commonly detected BPV types are considered for use in cattle.

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Author contributions AAA and AFA conceived the study; SEIC and RAAO performed the experiments; SAH performed the pathologic evaluation of the cutaneous lesions; SEIC and ML analyzed the data; and SEIC and ML wrote the manuscript. All authors revised and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted (Ethics Committee for Animal Experimentation of the Universidade Estadual de Londrina, Brazil, under protocol no. 5730.2016.83).

References

1. Van Doorslaer K, Chen Z, Bernard H, Chan PKS, DeSalle R, Dillner J, Forslund O, Haga T, McBride AA, Villa LL, Burk RD, ICTV Report Consortium (2018) ICTV virus taxonomy profile: *Papillomaviridae*. J Gen Virol 99:989–990
2. Wosiacki SR, Claus MP, Alfieri AF, Alfieri AA (2006) Bovine papillomavirus type 2 detection in the urinary bladder of cattle with chronic enzootic haematuria. Mem Inst Oswaldo Cruz 101:635–638
3. Borzacchiello G, Roperto F (2008) Bovine papillomaviruses, papillomas and cancer in cattle. Vet Res 39:45
4. Claus MP, Lunardi M, Alfieri AF, Ferracin LM, Fungaro MH, Alfieri AA (2008) Identification of unreported putative new bovine papillomavirus types in Brazilian cattle herds. Vet Microbiol 132:396–401
5. Lunardi M, Alfieri AA, Otonel RA, de Alcântara BK, Rodrigues WB, de Miranda AB, Alfieri AF (2013) Genetic characterization of a novel bovine papillomavirus member of the Deltapapillomavirus genus. Vet Microbiol 162:207–213
6. Da Silva FR, Cibulski SP, Daudt C, Weber MN, Guimarães LL, Streck AF, Mayer FQ, Roehe PM, Canal CW (2016) Novel bovine papillomavirus type discovered by rolling-circle amplification coupled with next-generation sequencing. PLoS ONE 11:e0162345
7. Daudt C, Da Silva FR, Streck AF, Weber MN, Mayer FQ, Cibulski SP, Canal CW (2016) How many papillomavirus species can go undetected in papilloma lesions? Sci Rep 6:36480
8. Bauermann FV, Joshi LR, Mohr KA, Kutish GF, Meier P, Chase C, Christopher-Hennings J, Diel DG (2017) A novel bovine papillomavirus type in the genus Dyokappapapillomavirus. Arch Virol 162:3225–3228
9. Daudt C, Da Silva FRC, Lunardi M, Alves CBDT, Weber MN, Cibulski SP, Alfieri AF, Alfieri AA, Canal CW (2018) Papillomaviruses in ruminants: an update. Transbound Emerg Dis 65:1381–1395
10. Carvalho CC, Batista MV, Silva MA, Balbino VQ, Freitas AC (2012) Detection of bovine papillomavirus types, co-infection and a putative new BPV11 subtype in cattle. Transbound Emerg Dis 59:441–447
11. Manos MM, Ting Y, Wright DK, Lewis J, Broker TR, Wolinsky SM (1989) The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. Cancer Cells 7:209–214
12. Forslund O, Antonsson A, Nordin P, Stenquist B, Hansson BG (1999) A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. J Gen Virol 80:2437–2443
13. Maeda Y, Shibahara T, Wada Y, Kadota K, Kanno T, Uchida I, Hatama S (2007) An outbreak of teat papillomatosis in cattle caused by bovine papilloma virus (BPV) type 6 and unclassified BPVs. Vet Microbiol 121:242–248
14. Ogawa T, Tomita Y, Okada M, Shinozaki K, Kubonoya H, Kaiho I, Shirasawa H (2004) Broad-spectrum detection of papillomaviruses in bovine teat papillomas and healthy teat skin. J Gen Virol 85:2191–2197
15. Da Silva FR, Daudt C, Streck AF, Weber MN, Filho RV, Driemeier D, Canal CW (2015) Genetic characterization of Amazonian bovine papillomavirus reveals the existence of four new putative types. Virus Genes 51:77–84
16. Lunardi M, Tozato CC, Alfieri AF, de Alcântara BK, Vilas-Boas LA, Otonel RAA, Headley SA, Alfieri AA (2016) Genetic diversity of bovine papillomavirus types, including two putative new types, in teat warts from dairy cattle herds. Arch Virol 161:1569–1577
17. Carvalho RF, Sakata ST, Giovanni DN, Mori E, Brandão PE, Richtzenhain LJ, Pozzi CR, Arcaro JR, Miranda MS, Mazzuchelli-de-Souza J, Melo TC, Comenale G, Assaf SL, Beçak W, Stocco RC (2013) Bovine papillomavirus in Brazil: detection of coinfection of unusual types by a PCR-RFLP method. Biomed Res Int 2013:270898
18. Melo TC, Carvalho RF, Mazzucchelli-de-Souza J, Diniz N, Vasconcelos S, Assaf SL, Araldi RP, Ruiz RM, Kerkis I, Beçak W, Stocco RC (2014) Phylogenetic classification and clinical aspects of a new putative Deltapapillomavirus associated with skin lesions in cattle. Genet Mol Res 13:2458–2469
19. Batista MV, Silva MA, Pontes NE, Reis MC, Corteggio A, Castro RS, Borzacchiello G, Balbino VQ, Freitas AC (2013) Molecular epidemiology of bovine papillomatosis and the identification of a putative new virus type in Brazilian cattle. Vet J 197:368–373
20. Alfieri AA, Parazzi ME, Takiuchi E, Medici KC, Alfieri AF (2006) Frequency of group A rotavirus in diarrhoeic calves in Brazilian cattle herds, 1998-2002. Trop Anim Health Prod 38:521–526
21. Claus MP, Vivian D, Lunardi M, Alfieri AF, Alfieri AA (2007) Phylogenetic analysis of bovine papillomavirus associated with skin warts in cattle herds from the state of Parana. Pesq Vet Bras 27:314–318
22. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739
23. He Z, Meng Q, Qiao J, Peng Y, Xie K, Liu Y, Cai X, Zhang J, Chen C (2014) Mixed nipple infections caused by variant of BPV3 and a putative new subtype of BPV in cattle. Transbound Emerg Dis 63:e140–e143
24. Claus MP, Lunardi M, Alfieri AA, Otonel RAA, Sartori D, Fungaro MHP, Alfieri AF (2009) Multiple bovine papillomavirus infections associated with cutaneous papillomatosis in Brazilian cattle herds. Braz Arch Biol Technol 52:93–98
25. Grindatto A, Ferraro G, Varello K, Crescio MI, Miceli I, Bozzetta E, Goria M, Nappi R (2015) Molecular and histological characterization of bovine papillomavirus in North West Italy. Vet Microbiol 180:113–117
26. De Villiers EM, Fauquet C, Broker TR, Bernard HU, Zur Hausen H (2004) Classification of papillomaviruses. Virology 324:17–27
27. Antonsson A, Hansson BG (2002) Healthy skin of many animal species harbors papillomaviruses which are closely related to their human counterparts. J Virol 76:12537–12542
28. Tozato CC, Lunardi M, Alfieri AF, Otonel RA, Di Santis GW, de Alcântara BK, Headley SA, Alfieri AA (2013) Teat papillomatosis associated with bovine papillomavirus types 6, 7, 9, and 10 in dairy cattle from Brazil. Braz J Microbiol 44:905–909