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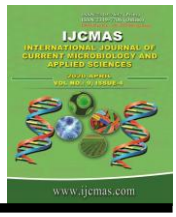

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Detection of Bovine Papillomavirus in Cutaneous Lesions by Polymerase Chain Reaction (PCR) in Cattle

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A B S T R A C T

Bovine papillomavirus (BPV) causes benign tumours in the mucosal and cutaneous epithelium and is characterized by the presence of warts. The present study includes the molecular identification of BPV strains in samples of warts using degenerate polymerase chain reaction (PCR) primers FAP59/64. Wart samples were collected from the cattle having typical lesions on various parts of the body. The present study showed that PCR amplification with the primers FAP59/64, which partially amplify the L1 gene and showed the 470bp amplicon size, indicating BPV. The results in this study are important for the development of prophylactic and therapeutic measures that contribute to reducing the economic losses associated with BPV.

Introduction

Bovine papillomaviruses (BPV) are causative agents of benign and malignant tumors in cattle, such as cutaneous papillomas, fibropapillomas, and urinary bladder and esophageal cancers, causing significant economic losses (Stocco dos Santos *et al.*, 1998; Vázquez *et al.*, 2012; Carvalho *et al.*, 2013). There are 14 types of BPV, which have been classified into 3 separate genera: *Delta*, *Epsilon*, and *Xi*. Each can cause type-specific lesions (Borzacchiello *et al.*, 2008). BPV-1, BPV-2, and BPV-13, for example, are classified in the *Deltapapilloma virus* genus and induce fibropapilloma (Lunardi *et al.*, 2013). They are also capable of infecting diverse host species, causing equine sarcoid (Nasir and Reid, 1999).

Lesions of the teats and udders in cattle are commonly related to BPV-1 (Jarrett *et al.*, 1984). Fibropapilloma in the penis is also associated with BPV-1 and leads to necrosis and loss of reproductive function (Gardiner *et al.*, 2008).

In cutaneous fibropapilloma, BPV-2 is a causative agent of malignant bladder tumors (Jarrett *et al.,* 1984). Most of these types of viruses have also been detected in the peripheral blood and reproductive tissue samples of cattle, resulting in vertical transmission (Diniz *et al.,* 2009). BPV *Xipapillomavirus* types (Carvalho *et al.,* 2013) are considered selective epitheliotropic viruses, inducing the formation of true papillomas (Hatama *et al.,* 2011). In contrast, the BPV *Epsilonpapillomavirus* types can induce fibropapillomas and true papillomas (Tomita *et al.,* 2007). BPV-7 is grouped separately (Ogawa *et al.*, 2007).

While hundreds of human papillomavirus (HPV) types have been identified, only six BPV types had been characterized until the early 1980s (Jarret *et al.,* 1984, Bernard 2005). However, recent studies employing PCR with generic primers FAP59/FAP64 in combination with cloning and sequencing, have described 15 putative new BPV types (Forslund *et al.,* 1999, Antonsson and Hansson 2002, Ogawa *et al.,* 2004).

After characterization of their complete genome sequences, four of these Japanese isolates were recently recognized as new viral types (BPV- 7, -8, -9, and -10) (Ogawa *et al.,* 2007, Tomita *et al.,* 2007, Hatama *et al.,* 2008). In addition, four putative new BPV types have been identified in cutaneous lesions from cattle herds in Parana state, Brazil (Claus *et al.,* 2008).

In cattle, bovine papillomavirus (BPV) induces exophytic lesions (papillomas, warts) and flat lesions (flat warts, cervical intraepithelial neoplasia) in cutaneous and mucosal epithelia (de Villiers *et al.,* 2004; Dyne *et al.,* 2018).

BPV-induced benign lesions regress spontaneously; however, they may develop into cancer, especially in the presence of cofactors such as environmental carcinogens (Corteggio *et al.,* 2013). BPV diagnosis usually includes a clinical examination, histopathology, and immunohistochemistry (Betiol *et al.,* 2012).

Polymerase chain reaction (PCR) has been used as a sensitive method for the identification and genotyping of BPV (Leto *et al.,* 2011). Specific primers have also been successfully employed mainly for BPV identification in blood (Araldi *et al.,* 2014). BPV contains a double-stranded, circular, 8-kb DNA genome divided into the following 3 regions: an early region, a long control region, and a late region, which encodes several important proteins (Zheng and Baker, 2006).

Although infections caused by BPV in cattle do not cause much damage, they produce great economic losses due to their impact on aesthetics and the quality of cattle in livestock shows and hinder the commercialization of products derived from animals infected with BPV, such as leather for the production of footwear and other clothing (Catroxo *et al.,* 2013; Araldi *et al.,* 2014). However, superinfections in lesions and milking difficulties when papillomas appear on the udders can cause considerable health and management complications, and finally, some genotypes are associated with the development of carcinogenic lesions (Campo *et al.,* 1992; Borzacchiello *et al.,* 2003).

The molecular characterization described in this report will establish a guide for subsequent studies with a greater number of samples. The results of this research are important because they contribute to the development of prophylactic and therapeutic measures that minimize economic losses associated with the presence of papillomavirus in cattle.

Materials and Methods

Animal and sample collection

Samples are collected from animals showing cutaneous papillomatous lesion that were brought to clinics by the owners. The collected tissue samples had varying diameters (5 - 10 cm) and came from different parts of the body (e.g., udder, teat, abdomen, and back). All the samples were immediately stored at -21°C until processing in the laboratory. The clinical specimens were taken by hand (wearing gloves, changed for each sample), packed individually, and maintained at 4°C until the DNA extraction procedure was completed.

Fragments from each skin wart were triturated in phosphate-buffered saline solution (PBS pH 7.2), and the suspensions (10-20%, w/v) were centrifuged for 15 min at 3000 x *g* at 4°C. Aliquots (250 µL) of the supernatant were treated with lysis buffer [10 mM Tris; 1 mM EDTA; 0.5%Nonidet P40; 1% SDS; and 0.2 mg/mL proteinase K (Invitrogen, Life Technologies, USA)]. After homogenization, the samples were incubated at 56°C for 30 min. Total DNA was extracted from bacterial isolates by using commercially genomic DNA mini kit (Qiagen - Germany) following the mini spin protocol according to the manufacturer's instructions.

PCR amplification and electrophoresis

PCR was performed in the region of the FAP gene that encodes the viral protein L1 (Carvalho *et al.*, 2013). The reaction was performed using a final volume of 20 µl, which included 1 to 5 ng of DNA, 0.5 µM each primer, FAP59 (5´-TAAC WGTIGGICAYCCWTATT-3´) and FAP 64 (5´- CCWATATCWVHC ATITC ICCATC-3´), 0.2 mM each DNTP, 1X PCR buffer, 1.5 mM $MgCl_2$ and 1 U of Taq DNA polymerase. Amplification consisted of an initial denaturation of 5 min at 95°C, followed by 35 cycles of 60econds at 95°C, 60 seconds at 52°C and 60seconds at 72°C, with a final extension of 5 min at 72°C. The amplification products were analysed by electrophoresis in a 1.2% agarose gel in TBE buffer pH 8.4 (89mM Tris; 89mM boric acid; 2mM EDTA) at constant voltage (50V) for approximately 45min, stained with ethidium bromide (0.5µg/ml), and visualized under UV light. The presence of a band of 470 base pairs (bp) indicated that the virus was present.

Results and Discussion

The presence of the 470 bp fragment established as indicative of infection caused by the virus is shown in Figure 1. This fragment size is similar to that reported by Carvalho *et al.,* (2013), who studied the virus in a herd of Holstein cattle affected by chronic cutaneous papillomatosis. However, Araldi *et al.,* (2014), using the same set of primers, reported a 478 bp fragment in cutaneous papillomas in samples of Angus Red cattle in Sao Paulo, Brazil.

Similarly, Claus *et al.*, (2009), in a study conducted in beef cattle in Paraná, Brazil, reported a 480 bp amplicon size using the same sets of primers as in the present study (FAP59/FAP64). However, all amplicons, after subsequent sequencing, were confirmed as positive for BPV. The variability in the size of base pairs was previously described by Carvalho *et al.,* (2013), who reported sizes ranging between 469 and 484 bp in different viral strains. This fact highlights the importance of using sequencing, in addition to using specific segments of the viral fragment that we wish to amplify because it allows comparative studies of the different genotypes found in an outbreak where the presumptive diagnosis is BPV.

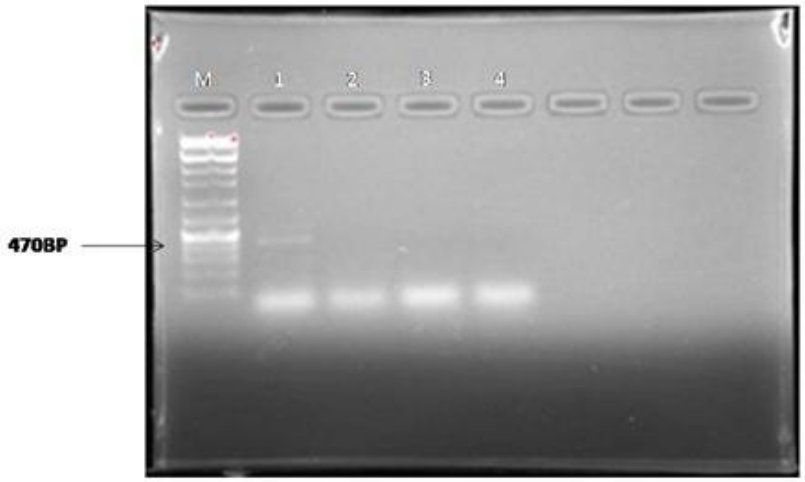

Figure.1 PCR products (470 bp) for the L1 gene of bovine papillomavirus in wart samples using primers FAP59/64. Lane 1 represent positive samples; Lane 2, 3 and 4 represents negative sample and Lane M represents molecular weight markers from 100 to 1000 base pairs

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