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Detection of bovine papillomavirus type 14 DNA sequences in urinary bladder tumors in cattle

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ABSTRACT

Bovine papillomavirus type 14 (BPV-14) is a novel *Deltapapillomavirus* (δPV) which is most closely related to BPV-1, -2, and -13, well-known members of the δPV genus. So far BPV-14 has been detected in cutaneous neoplastic lesions in cattle and in feline sarcoids. As BPV-14 may share biological and pathological properties with BPV-1, -2 and -13, it has been hypothesized that, like other δPVs, BPV-14 could be associated with bovine bladder neoplasia.

In this study, 50 tumors of the urinary bladder of cattle were diagnosed. DNA was extracted from all tumor samples as well as from 25 normal bladder samples and submitted to BPV-14 L1 PCR and subsequent amplicon sequencing analysis. BPV-14 L1 DNA sequences of specific 195 bp amplicons were obtained from 17 of 50 (34%) tumor DNA isolates; no BPV-14 DNA was detected from 25 normal samples. Amplicons revealed a 99% homology with the corresponding BPV-14 L1 DNA region (GenBank accession number KP276343.1). Co-infections by two or three δPV types were also seen. This study reveals the presence of BPV-14 DNA alone or in combination with other δPV DNA in bovine bladder tumors alone and suggests that BPV-14 could also be involved in bladder neoplasia as its E5 oncoprotein has the potential to induce cell proliferation. Furthermore, this is the first study to show the presence of BPV-14 in Europe, suggesting that BPV-14, like other δPVs, has a worldwide distribution.

1. Introduction

Papillomaviruses (PVs) are double-stranded DNA viruses that infect mucosal and cutaneous epithelia of vertebrates in a species-specific manner. PV infection can persist asymptomatically or can result in cell transformation (Bernard et al., 2010).

Bovine papillomaviruses (BPVs) comprise fourteen types, which have been well characterized and classified into the genera *Deltapapillomavirus* (δPV; BPV-1, -2, -13, 14) (Lunardi et al., 2013; Munday et al., 2015), *Xipapillomavirus* (χPV; BPV-3, -4, -6, -9, 11, -12) (Zhu et al., 2012), *Epsilonpapillomavirus* (εPV; BPV-5, -8) (Bernard et al., 2010; Tomita et al., 2007). BPV-7 has not been assigned but it has been proposed to belong to the genus *Dyoxipapillomavirus* (Rector and Van Ranst, 2013).

Only bovine δPVs are known to infect both epithelial and mesenchymal tissues and show cross-species infection. Bovine δPV infection is thought to cause tumors in horses (Bergvall, 2013), Cape mountain zebras, giraffes and sable antelopes (van Dyk et al., 2012; Williams et al., 2011). In addition, bovine δPVs have a role in bladder tumor in buffaloes (Pangty et al., 2010; Roperto et al., 2013; Somvanshi, 2011).

Occasionally BPV-1 and very commonly BPV-2 and BPV-13 are also associated with bladder carcinogenesis in adult cattle grazing on lands rich in bracken fern (Roperto et al., 2015). It is well known that this plant contains toxic compounds like ptaquiloside (PT) and quercetin, which have immunosuppressant, mutagenic and carcinogenic effects (Borzacchiello et al., 2003; Campo et al., 1992; Peretti et al., 2007). BPV-14 is a novel virus, the genome of which has been recently determined (Munday et al., 2015). BPV-14 DNA has been detected in five captive African lions (*Panthera leo*) in

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Australia (Orbell et al., 2011), in feline sarcoids in North America and New Zealand (Munday et al., 2010) and in some fibropapillomas as well as inflammatory skin lesions in cattle (Munday and Knight, 2010).

As some evidence suggests that BPV-14 is an oncogenic virus (Munday et al., 2015), it was hypothesized that BPV-14, such as other bovine δPVs, could contribute to the development of bladder tumors in cattle.

Therefore, the aim of this study was to evaluate a large series of bovine bladder tumors for the presence of BPV-14 DNA. To the authors' knowledge, this is the first time that bovine bladder tumors have been screened for BPV-14 infection.

2. Materials and methods

2.1. Ethics statement

All the samples were collected *post-mortem* at private and public slaughterhouses in southern Italy; the animals were slaughtered following a mandatory clinical *ante-mortem* examination as required by European Union (EU) legislation.

2.2. Tumor samples

Fifty bladder tumor and twenty-five normal bladder samples were collected, the latter serving as negative controls, with the permission of the medical authorities in the slaughterhouses named "Barbara Rocco sas" of Simbario (Calabria Region), "Macello Comunale" of Muro Lucano (Basilicata Region), "Cestari Carni srl" of Montesano sulla Marcellana (Campania Region), "Frigo Sud srl" of Nocera Superiore (Campania Region), "Real Beef srl" of Flumeri (Campania Region). The animals were 3–14 years old and had grazed on pasture containing bracken fern. Both tumor and normal bladder samples were immediately bi-dissected and either frozen in liquid nitrogen and stored at −80 °C for subsequent molecular biological analysis or fixed in 10% buffered formalin for histopathology.

2.3. Histopathology

The formalin-fixed tissues were routinely embedded in paraffin wax and hematoxylin-eosin (HE) sections were prepared. A histologic diagnosis for each sample was made according to an established classification scheme (Roperto et al., 2010).

2.4. Detection of BPV-14 DNA by Polymerase Chain Reaction (PCR)

DNA was extracted from fifty urothelial tumors and five normal bladder samples using a DNeasy Tissue kit (Qiagen, Germany) according to instructions of manufacturer. The presence of BPV-2 and BPV-13 DNA had been previously evaluated in these samples (Roperto et al., 2015).

Specific PCR primers (jmpSA-F 5′-GGAACAAACCTCACAATCAC-3′; jmpSA-R 5′- CCAGTTCTCTAATACTGAGG-3′) as described by Munday et al. (2015) were used to amplify a 195 bp region within the BPV-14 L1 gene.

PCR reaction mixtures used containing AmpliTaq Gold DNA Polymerase (Applied Biosystems, USA) were prepared, according to the manufacturer's protocol. Amplification conditions and sequencing analysis have been published previously (Roperto et al., 2015). Briefly, PCR conditions were as follows: denaturation for 5 min at 94 °C, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 60 °C for 45 s and extension at 72 °C for 5 min. DNA extracted from a sarcoid of a lion was used as positive control whilst a no template control (sterile water) was added to the five negative controls.

To confirm the specificity of the primers, PCR products were purified by Qiaquick PCR purification Kit (Qiagen GmbH) and bidirectionally sequenced using a BigDye_Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) following manufacturer's recommendations. Sequences were dye-terminator removed by DyeEx_ 2.0 spin kit (Qiagen GmbH) and run on a 3500 Genetic Analyzer (Applied Biosystems). Electropherograms were analyzed using Sequencing analysis v5.2 and sequence scanner v1.0

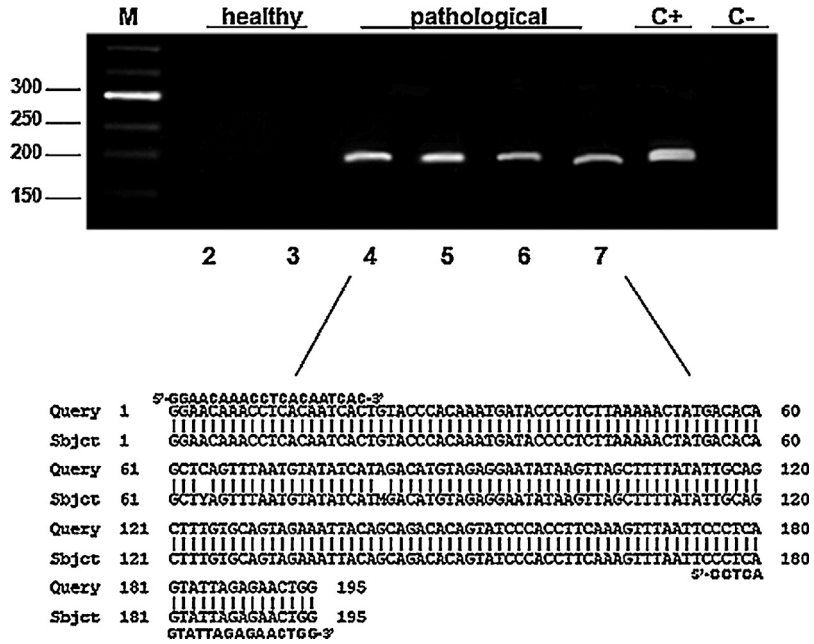

Fig. 1. PCR analysis for detection of BPV-14 L1 DNA. Lane M, DNA marker ladder; Lane 2,3 two normal (control) samples from healthy cattle; Lane 4–7 four representative tumor samples; Lane C+, positive control; Lane C−, negative control in which the template was replaced with distilled water. The lower part of the figure shows 99% homology between the sequence of the amplicons and the sequence of BPV-14 L1 deposited in GenBank (KP276343.1).