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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Detection of Papillomavirus DNA in Cutaneous Squamous Cell Carcinoma and Multiple Papillomas in Captive Reptiles

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Summary

Papillomaviruses (PVs) are small, non-enveloped DNA viruses that cause mucocutaneous tumours including squamous cell carcinoma (SCC) in man. In animals, evidence supports a causal role for PVs in the development of cutaneous and oral SCC in some species. In reptiles, three cases of papilloma or fibropapilloma have been associated with PV infection, but no association has been reported to date with SCC. Two cases of cutaneous epithelial tumours, multiple papillomas in a spiny-tailed lizard (*Uromastyx acanthinura*) and SCC in a Dumeril's boa (*Acrantophis dumerili*), were investigated by polymerase chain reaction. PV DNA was amplified from samples of both lesions. Typical microscopical features suggestive of PV infection (e.g. the presence of koilocytes) were observed in the lesions from the spiny-tailed lizard. This is the first report of an association between PV and SCC in reptiles. Further studies are needed to better clarify the role of PVs in these species and to characterize the PV strains involved.

Keywords: papillomavirus; reptile; snake; squamous cell carcinoma

Papillomaviruses (PVs) are small, non-enveloped DNA viruses with a circular, double-stranded genome belonging to the family Papillomaviridae. PVs infect the epithelium of vertebrates (Bernard *et al.*, 2010) and are generally considered species specific. Most of the PV types identified derive from human isolates, with over 200 types established currently (Ryndock and Meyers, 2014). However, in the last decade there has been an exponential increase in the number of completely characterized animal PV genomes (Rector and VanRanst, 2013). To date, 112 distinct non-human PV types have been characterized genomically from 54 host species, mostly mammals, but also three birds and three reptile species (Rector and VanRanst, 2013). In man, alpha high-risk mucosal PVs cause genital and oral neoplasia; moreover, recent evidence suggests the involvement of beta PVs in the development of cutaneous squamous cell carcinoma (SCC) (Ghittoni *et al.*, 2015).

In animals, evidence supports a causal role for PVs in the development of equine sarcoid as well as mucosal and cutaneous SCC in several species (Munday and Kiupel, 2010). It was recently shown that feline PV type 2 contributes, together with ultraviolet light, to the development of feline SCC, strengthening the hypothesis that PVs act as a causative agent of cutaneous cancer in animals (Altamura *et al.*, 2016). In reptiles, PVs have been associated

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with cutaneous papilloma and fibropapilloma in two sea turtle species (*Chelonia mydas and Caretta caretta*) (Herbst *et al.*, 2009) and with multiple papillomas in a diamond python (*Morelia spilota*) (Lange *et al.*, 2011; Gull *et al.*, 2012). Neoplasia in reptiles appears to be increasing in recent decades, with more reports of sporadic cases and reviews in zoological collections (Catao-Dias and Nichols, 1999; Garner *et al.*, 2004; Sykes and Trupkiewicz, 2006).

This apparent increase could be due to improvements in husbandry and veterinary medicine, resulting in longer lifespans, more accurate post-mortem examinations aimed at definition of the causes of death and an increased attention to the detection of viral infectious agents (Sykes and Trupkiewicz, 2006). Cutaneous epithelial tumours, in particular SCCs, are described in reptiles (Garner *et al.*, 2004; Sykes and Trupkiewicz, 2006; Deetzen von *et al.*, 2012), although they appear less common than cutaneous mesenchymal tumours in these animals. However, the association between SCC and PVs in reptiles has not been reported. Herein we document the detection of PV DNA in cutaneous epithelial tumours of reptiles, including a SCC.

Two cases of cutaneous epithelial tumours, multiple papillomas in a spiny-tailed lizard (*Uromastyx acanthinura*) and a SCC in a Dumeril's boa (*Acrantophis dumerili*), were diagnosed in 2009 and 2010, respectively, in animals maintained at the Zoological Garden of Rome (Bioparco). Information about the animals was retrieved from sample submission forms and post-mortem reports. Archived haematoxylin and eosin stained sections from formalin-fixed paraffin wax-embedded samples were reviewed.

Polymerase chain reaction (PCR) assays were performed with degenerate primers FAP59 (forward; 5′-TAACWGTIGGICAYCCWTATT-3′) and FAP64 (reverse; 5′-CCWATATCWVHCATITCICCATC-3′), which are able to amplify conserved regions of the *L1* gene from a wide range of human and animal PVs (Forslund *et al.*, 1999). Total DNA was recovered from paraffin wax-embedded sections using the DNeasy™ Blood and Tissue Kit (Qiagen, Hilden, Germany) as described by Borzacchiello *et al.* (2008). Purified DNA (100–150 ng) was subjected to PCR using the AmpliTaq™ Gold DNA Polymerase kit (Applied Biosystems, Waltham, Massachusetts, USA) with the following amplification protocol: initial denaturation for 10 min at 94°C, followed by 45 cycles of 1 min and 30 s at 94°C, 1 min and 30 s at 50°C, 1 min and 30 s at 72°C and a final elongation step of 5 min at 72°C. All amplification products were visualized by 1% Tris acetate EDTA agarose gel electrophoresis. The positive control was DNA from a PV-positive bovine cutaneous fibropapilloma (Bocaneti *et al.*, 2013). A fragment of the expected size (480 base pairs) was amplified from the positive control and both reptile specimens.

The Dumeril's boa had an ulcerated and infiltrative mass (3 × 2 cm) on the dorsum and was humanely destroyed after repeated recurrences of the lesion and worsening body condition. Histologically, a well-differentiated SCC was diagnosed (Goldschmidt and Goldschmidt, 2017). Islands and cords of neoplastic epithelial cells, showing a variable degree of squamous differentiation and associated with scirrhous proliferation, were seen. Central degeneration and distinct keratin 'pearls' were noted occasionally (Fig. 1). The mitotic index was 0–1 per ×400 high-power field. The overlying epidermis was multifocally ulcerated; the tumour deeply infiltrated the dermis, but there was no evidence of metastatic spread. In the spiny-tailed lizard, multiple exophytic proliferations (0.4–0.5 cm) on the dorsum were characterized microscopically by islands of epithelial cells, gradually keratinizing towards the periphery and frequently showing central degeneration. Around the islands there was abundant lamellar keratin and many koilocytes were observed between the epithelial cells (Fig. 2).

The tumours reported here have been already reported in reptiles and both have been associated with molecular detection of PV. Papilloma has been reported in lizards (Garner *et al.*, 2004) and SCC has been documented in snakes (Catao-Dias and Nichols, 1999; Garner *et al.*, 2004). Several reports describe the association of skin papillomas

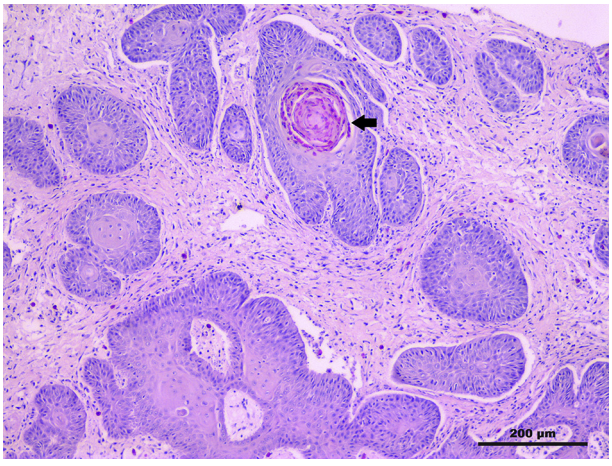

Fig. 1. Cutaneous squamous cell carcinoma in the Dumeril's boa. Islands and cords of neoplastic epithelial cells associated with stromal fibroplasia. Keratin pearls are also shown (arrow). HE.