Novel Papillomaviral Sequence Detected within Epidermal Plaques in a Wolf (*Canis lupus*)

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ABSTRACT: We describe numerous, pale plaques affecting the inguinal skin of a grey wolf (*Canis lupus*). Histologically, these were consistent with papillomaviral plaques. Immunohistochemistry confirmed papillomavirus antigens and partial sequencing of the L1 gene suggests this is a novel papillomavirus, most closely related to *Canis familiaris* Papillomavirus 5.

In April 2012, an experienced trapper captured an adult male grey wolf (*Canis lupus*) on a cattle feedlot near Carrot River, Saskatchewan, Canada (53°16'N, 103°35'W). Concerned that the wolf's inguinal skin changes were unusual and would devalue the pelt (D. Sussums, pers. comm.), he submitted the carcass through Saskatchewan Environment to the Western-Northern Region of the Canadian Wildlife Health Cooperative (CWHC) for diagnostic investigation. The wolf was in excellent body condition with abundant fat stores. Small (2–10 mm diameter), pale, flat-topped plaques (Fig. 1) were symmetrically distributed on inguinal skin including the prepuce and inner thigh.

FIGURE 1. Papillomavirus-associated epidermal plaques (arrows) on the inguinal skin adjacent to the prepuce (P) of a wild grey wolf (*Canis lupus*) from Saskatchewan, Canada.

Histologic examination of hematoxylin and eosin-stained sections of the skin lesions revealed plaques that were demarcated from adjacent epidermis by an abrupt, steep-sided, acanthotic, hyperkeratotic epidermis with hypergranulosis (giant keratohyaline granules) and a scalloped deep edge (Fig. 2a). Keratinocyte maturation in the plaques was orderly (Fig. 2b). This histologic appearance was consistent with viral plaques. Most plaques contained some melanin, however pigment content was less than the adjacent skin. A few lymphocytes extended into the plaques, which may indicate that the plaques were regressing. The superficial dermis was infiltrated by few lymphocytes, plasma cells and melanophages.

FIGURE 2A. Histologic features of a papillomavirus-associated epidermal plaque from a wild grey wolf (*Canis lupus*) from Saskatchewan, Canada. The plaque is markedly acanthotic, hypergranular and hyperkeratotic (arrow) compared to adjacent skin (right side) that may be mildly acanthotic with compacted hyperkeratosis but that lacks the somewhat scalloped deep edge present in the plaque. H&E stain. 10X; Bar=100 μ m.

FIGURE 2B. Orderly keratinocyte maturation is present within the plaque. H&E. 60X; Bar=20 μ m.

Immunohistochemical staining for papillomavirus was performed by Prairie Diagnostic Services, Saskatoon, Saskatchewan, Canada using a previously described method with some modifications (Favrot et al. 2009) and an automated staining platform (Code-On Histomatic Stainer, Fisher Scientific, Edmonton, Alberta, Canada) after heat-induced epitope retrieval. The primary antibody (polyclonal goat anti-bovine papillomavirus antigens, Virostat, Portland, Maine, USA) was tested at dilutions of 1:2000 and 1:4000. Binding of the primary antibody was detected using 3,3'-diaminobenzidine tetrahydrochloride chromogen (Electron Microscopy Science, Ft. Washington, Pennsylvania, USA) and an avidin-biotin immunoperoxidase complex reagent (Vector Laboratories, Inc., Burlingame, California, USA) bound to rabbit anti-goat immunoglobulins. Positive immunostaining for papillomavirus antigens was limited to the plaques (Fig. 2c). Stain uptake was heaviest in the stratum corneum, with scattered rare nuclear immunoreactivity in the stratum granulosum.

We extracted DNA from previously frozen skin and used a degenerate primer set (FAP 59/FAP 64) that previously amplified a portion of the papillomavirus L1 gene from a variety of species (Forslund et al. 1999; Antonsson and McMillan 2006) to detect papillomavirus using the protocol of Forslund et al. (1999). The positive and negative controls were DNA extracted from a canine viral papilloma and a no-template, respectively. A 420 DNA base-pair partial segment of the L1 gene was amplified, sequenced (Macrogen, Seoul, Republic of Korea), deposited in GenBank (Accession

FIGURE 2C. Immunoreactivity (golden-brown) is heaviest throughout the stratum corneum (arrow) with focal positive intranuclear staining in the stratum granulosum. Avidin-biotin complex immunoperoxidase technique with hematoxylin counter stain. 60X; Bar=20 μ m.

KT072744) and compared to sequences in GenBank for homology analysis using the Basic Local Alignment Search Tool (BLAST; NCBI 2015). The BLAST analysis revealed that the amplified segment was most similar to *Canis familiaris* Papillomavirus 5 isolate Birkenfeld (CPV5; Accession FJ492743.1; 88%), which is classified as a Chipapillomavirus (Rector and Van Ranst 2013).

We reviewed 488 wolf submissions in the CWHC database for similar cases. In 1987, CWHC pathologists diagnosed oral papillomas in two wolves based on gross and histologic appearance (one each from British Columbia and Alberta). Archival tissues were not available. To our knowledge, this is the first description of papillomavirus-associated lesions affecting haired skin in the wolf and the first published molecular characterization of a papillomavirus in this species.

Papillomaviruses are associated with proliferative lesions in many domestic and wild animals including more than 50 mammalian species, birds, and reptiles (Rector and Van Ranst 2013). These lesions may include hyperplasia, dysplasia, or overt neoplasia. In domestic dogs (*Canis lupus familiaris*) oral papillomas, cutaneous papillomas and pigmented epidermal plaques are lesions attributed to infection with several different canine papillomaviruses (Nicholls and Stanley 1999; Luff et al. 2012). In the grey wolf, the only reported occurrence of oral papilloma is a description of a few small lesions affecting the lips of two wolves from Alberta, Canada (Samuel et al. 1978). The oral papillomas they describe and those found in domestic dogs are exophytic masses with papillary projections of

hyperplastic epidermis, which differs from the plaques seen in this wolf. In domestic dogs, viruses associated with oral papillomas are classified as Lambda-papillomaviruses and are considered genetically distinct from those typically found in lesions affecting haired skin (Rector and Van Ranst 2013). But it is unknown if a similar site specificity exists for papillomavirus infections in wild canids such as the wolf and the coyote (*Canis latrans*), which are also affected by oral papillomas (Samuel et al. 1978).

Partial sequencing of the L1 gene suggests this virus is most closely related to CPV5, which Luff et al. (2012) found in pigmented viral plaques in domestic dogs. The viral plaques in this wolf are histologically similar to those found in domestic dogs, but the key difference is their pigment content is less. The remarkable similarity between the virus we describe and CPV5 is intriguing since most researchers consider papillomaviruses to be host species specific (Rector and Van Ranst 2013). Given the close ancestral relationship between wolves and domestic dogs, it is plausible that the virus we describe may infect both.

Asymptomatic papillomavirus infections are also common in multiple species, with lesion development requiring additional factors such as immunosuppression (Munday and Kiupel 2010), however none was confirmed in this wolf. While the potential for neoplastic transformation in wolves is unknown, squamous cell carcinomas arising from pigmented viral plaques have been reported in domestic dogs (Munday and Kiupel 2010).

This report highlights the importance of experience and training for wildlife disease monitoring. We credit the identification of this unique skin lesion to the astute observations of the experienced trapper. The skin lesions in this wolf are subtle, thus similar cases might have been overlooked in the past, particularly in wolves affected with more substantial skin diseases, such as sarcoptic mange.

The description of papillomavirus-associated plaques in this wolf adds to the growing list of papillomaviruses identified in wildlife. Future research may aim to determine if epidermal plaques occur in wolves elsewhere, further characterize wolf papillomaviruses, and determine whether oral papillomaviruses in wolves are similar or different to that which we describe here.

We thank Donald Sussums for his sharp observational skills and for submitting this case for investigation.

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