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Lambdapapillomavirus 2 in a Gray Wolf (*Canis lupus*) from Minnesota with Oral Papillomatosis and Sarcoptic Mange

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ABSTRACT: Oral papillomatosis was diagnosed in a gray wolf (*Canis lupus*) with sarcoptic mange from Minnesota, USA found dead in February 2015. Intranuclear inclusion bodies were evident histologically, and papillomaviral antigens were confirmed using immunohistochemistry. Sequencing of the L1 papillomavirus gene showed closest similarity to *Lambdapapillomavirus 2*.

A 33-kg adult male gray wolf (*Canis lupus*) was found dead in February 2015 in a black spruce bog (48°17'N, 93°11'W) near Voyageurs National Park in Koochiching County, Minnesota, USA. Age was estimated at 2–3 yr old by tooth wear. The wolf had been captured by National Park Service staff in May 2014, ear tagged and fitted with a very high frequency radio collar. In mid-February 2015, Minnesota Department of Natural Resources' pilots detected a mortality signal from the wolf's collar and a ground search a week later allowed for carcass recovery and submission to the US Geological Survey's National Wildlife Health Center (Madison, Wisconsin, USA) for necropsy.

Fur was absent from approximately 40% of the skin of the left and right flanks, abdomen, fore- and hind limbs, head, and tail, and the underlying skin was crusty with multiple scabs (Fig. 1A). Multiple cauliflower-like raised tan masses measuring up to 0.9 × 0.4 × 0.4 cm were present on upper and lower left labial mucosa and at the commissure of the oral cavity (Fig. 1B). A single mass was present on the upper right labial mucosa. Internally, there was minimal subcutaneous, visceral, perirenal and epicardial fat, indicating poor body condition. Multiple small, cauliflower-like tan masses as observed on the labial mucosa were present on the soft palate near

the tonsils and epiglottis (Fig. 1C), and a single mass was present on the mid-esophageal mucosa. The esophagus was empty, but the stomach was full of fur, meat, and bone fragments. Intestines contained a small amount of hair and yellow creamy digesta. Numerous cestodes, identified as *Taenia* sp., were present in the jejunum.

Examination of H&E-stained, alopecic skin sections showed moderate to severe parakeratotic hyperkeratosis, multifocal intracorneal pustules, acanthosis with multifocal intracellular edema, and dermal inflammation with a mite consistent with *Sarcoptes scabiei* observed within epidermal adnexa in the deep dermis (Fig. 2A–C, Fig. 2C inset). Histologically, the oral masses extended from the labial mucosa and were composed of hyperplastic epithelial cells that formed papillary projections supported by a fibrovascular stroma (Fig. 2D). Cells in the stratum basale were aligned perpendicularly to the stroma and there were rare mitoses. Keratinocytes of the stratum spinosum had prominent intracellular bridges, a moderate amount of eosinophilic cytoplasm with round to oval nuclei with finely stippled chromatin, and one or two magenta nucleoli (Fig. 2E, Fig. 2E top inset). Occasional cells in this layer were enlarged with abundant pale eosinophilic cytoplasm with hyperchromatic eccentric nuclei surrounded by a clear space (koilocytes). Within the stratum granulosum, cells had numerous large, irregular intracytoplasmic keratohyalin granules and occasionally contained basophilic intranuclear inclusion bodies that marginated nuclear chromatin (Fig. 2E bottom inset). There was moderate orthokeratotic and parakeratotic hyperkeratosis with multifocal surface bacterial colonies.

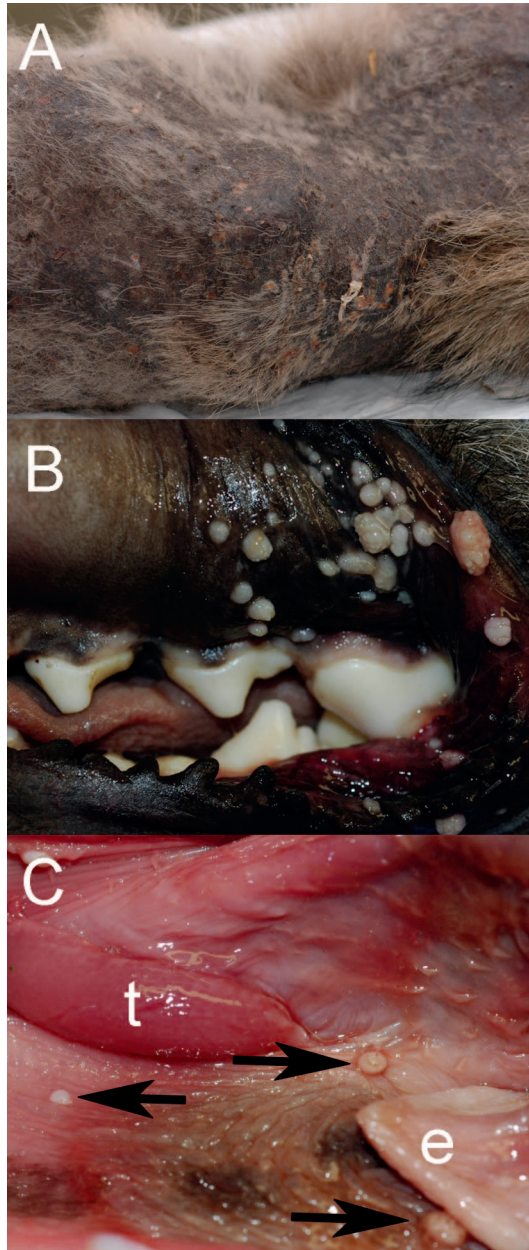


FIGURE 1. Gross images of a gray wolf (*Canis lupus*) found dead near Voyageurs National Park in Koochiching County, Minnesota, USA. (A) Alopecia and crusting on the skin of the left flank and dorsum due to *Sarcophaga scabiei*. (B) Multiple variably-sized papillomas on the left labial mucosa and commissure of the mouth. (C) Several small papillomas (arrows) on the soft palate adjacent to the tonsil (t) and epiglottis (e).

Immunohistochemical staining was performed at the University of Georgia, Athens, Georgia, USA using an automated stainer (Nemesis 3600, Biocare, Concord, California, USA). Reveal Decloaker, 10X (Biocare) was

used for antigen retrieval. Primary antibody (monoclonal mouse antiovine papillomavirus antigens, Biocare), directed against the L1 open reading frame, at a 1:80 dilution was bound to 4plus biotinylated Universal Goat

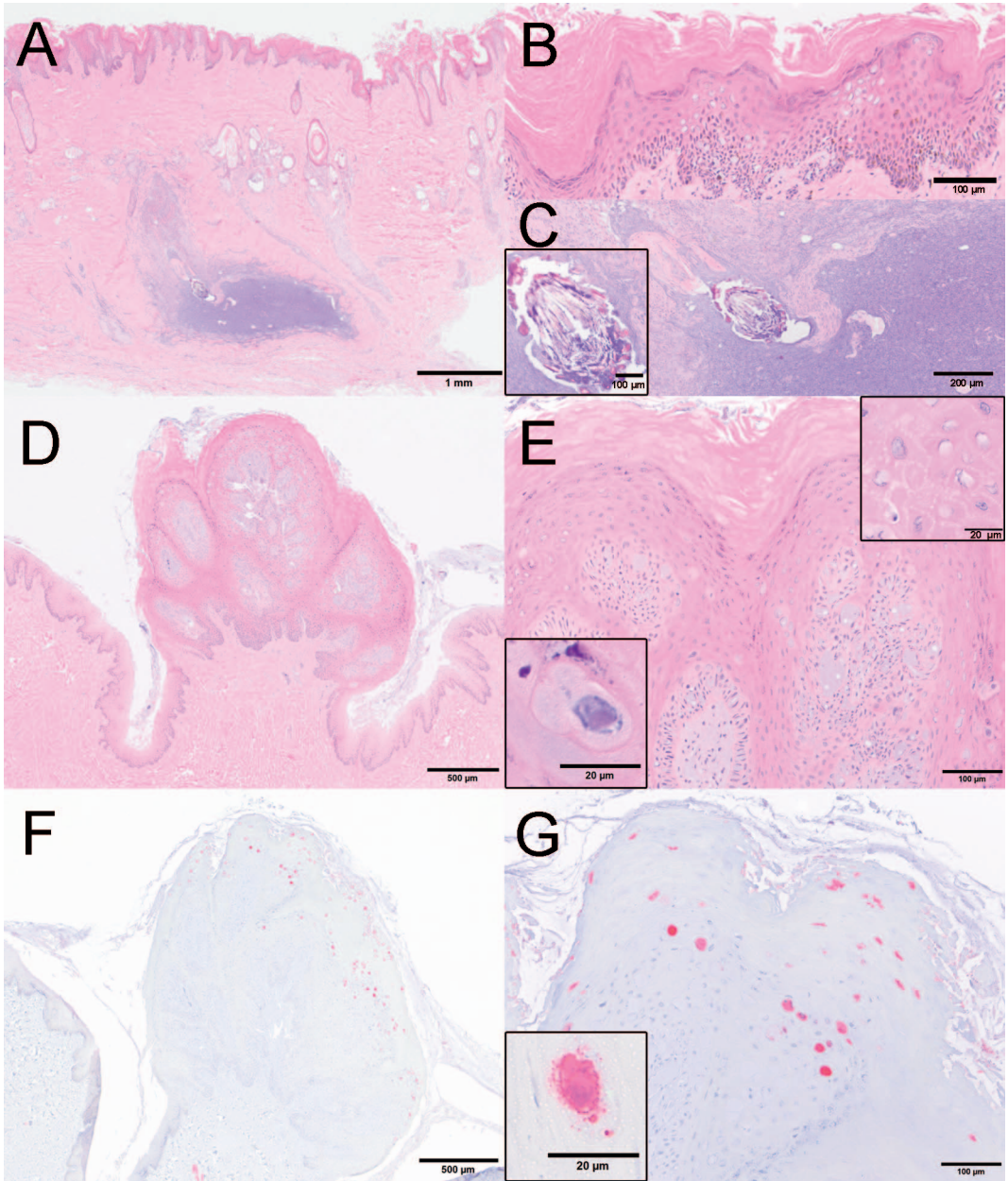


FIGURE 2. Photomicrographs of a gray wolf (*Canis lupus*) found dead near Voyageurs National Park in Koochiching County, Minnesota (USA). (A) Skin showing moderate hyperkeratosis, acanthosis and deep dermal inflammation with a single mite in the epidermal adnexa. H&E stain. (B) Higher magnification of skin showing hyperkeratosis and acanthosis. H&E stain. (C) Higher magnification of skin showing deep dermal inflammation and mite. Inset: *Sarcoptes scabiei* mite. H&E stain. (D) The oral papilloma extends from the labial mucosa and is composed of hyperplastic epithelial cells that form papillary projections. H&E stain. (E) Higher magnification of oral papilloma showing orthokeratotic hyperkeratosis with surface bacteria, a prominent stratum granulosum, a thickened stratum spinosum, and cells in the stratum basale aligned perpendicularly to the stroma. Top inset: keratinocytes of the stratum spinosum with prominent intracellular bridges. Bottom inset: Keratinocyte in the stratum granulosum that contains a basophilic intranuclear inclusion body that marginates nuclear chromatin. H&E stain. (F) Oral labial papilloma showing nuclear immunoreactivity for papillomavirus antigen at the

Link (goat anti-mouse IgG, Biocare), and detected using 4plus Streptavidin Alkaline Phosphatase (Biocare). Nuclear immunoreactivity was the strongest at the junction of the stratum corneum and stratum granulosum (Fig. 2F–G, Fig. 2G inset).

We extracted DNA from the frozen labial mass and performed PCR to amplify the L1 open reading frame of papillomavirus using the methods of Forslund et al. (1999). The 478 base-pair PCR fragment was sequenced at the University of Wisconsin at Madison Biotechnology Center (Madison, Wisconsin, USA) on an ABI 3730xl sequencer (ThermoFisher, Waltham, Massachusetts, USA), primer sequences trimmed, deposited in GenBank (accession number KY092172), and used in the Blast Local Alignment Search Tool (BLAST) aligner to interrogate GenBank at the National Center for Biotechnology Information (NCBI 2016) sequence database and Papillomavirus Episteme (2016). The amplified sequence was most closely related to *Lambdapapillomavirus 2* strain BR-UEL1 (GenBank KF199909.1; 99%) and *Lambdapapillomavirus 2* complete genome (PaVE CPV1REF; 99.8%), respectively, which are classified in the genus *Lambdapapillomavirus* and species *Lambdapapillomavirus 2* (Bernard et al. 2010).

Papillomas typically occur in young or immunocompromised animals and usually spontaneously regress (Head et al. 2002). They are generally host specific, but some, such as bovine papillomavirus and *Lambdapapillomavirus 2*, are known to infect multiple species (Sundberg et al. 2001). *Lambdapapillomavirus 2* is known to infect both dogs and coyotes (Sundberg et al. 1991). While long thought to infect wolves, the virus had not yet been sequenced in this species (Fitzgerald et al. 2000).

The only report of oral papillomatosis in wolves is from two pups in Alberta, Canada in good body condition with mild infections (Samuel et al. 1978). In those cases, the gross and histologic findings were similar to the present case, including the presence of intranuclear viral inclusions within the papilloma. Oral papillomatosis has been found previously in coyotes and wolves with sarcoptic mange (Samuel et al. 1978). Mange can result in emaciation and secondary infections and this debilitation can allow for development of papillomatosis (Fuchs et al. 2016).

The viral sequence in this case was most similar to *Lambdapapillomavirus 2* and distinctly different from a papillomavirus found in epidermal plaques in wolves (Rothenberger et al. 2016). Due to the species similarities, it is likely that *Lambdapapillomavirus 2* can be transmitted among wolves, dogs, and coyotes. Although usually self-limiting and of little clinical significance, large papillomas could interfere with feeding. Oral papillomatosis caused by *Lambdapapillomavirus 2* can progress to squamous cell carcinoma in dogs, but this has not been reported in wolves (Sundberg et al. 2001). Due to virus host specificity, this virus is likely of little concern to humans.

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junction of the stratum corneum and stratum granulosum using the 4plus Streptavidin Alkaline Phosphatase technique. (G) Higher magnification of oral labial papilloma showing nuclear immunoreactivity for papillomavirus antigen at the junction of the stratum corneum and stratum granulosum. Inset: A keratinocyte with strong nuclear immunoreactivity for papillomavirus demonstrates with the 4plus Streptavidin Alkaline Phosphatase technique.

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