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Cottontail Rabbit Papillomavirus Infection in a Desert Cottontail (*Sylvilagus audubonii*) from Colorado, USA

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ABSTRACT: A wild-caught desert cottontail rabbit (*Sylvilagus audubonii*) from Colorado was observed to have large, pedunculated, dark cutaneous lesions on its abdomen and cylindrical masses on its mouth. Morphologically, the masses were consistent with previous reports of virally induced papillomas. Subsequent DNA analysis indicated widespread infection with cottontail rabbit papillomavirus.

A male cottontail rabbit (*Sylvilagus* sp.) was captured in Larimer County, Colorado, during August 2011 for experimental purposes. It weighed 0.93 kg at capture and was subsequently assessed as not young of the year. Initial examination revealed several large, pigmented, pedunculated, round masses on the animal's ventral side, primarily on the abdomen (Fig. 1) and several pigmented cylindrical masses on the animal's head near the mouth (Fig. 2). Several other cottontails were captured or observed (ca. 20) at the same study locations, and none were noted to have similar masses. The rabbit was held in captivity for 43 days for an experimental study, and we observed that the masses appeared not to negatively affect its health and welfare, matching previous reports of cottontails exhibiting similar lesions (Shope and Hurst, 1933). During this observation period, the abdominal masses remained largely intact, while most of the facial masses were resolved.

In Larimer County, three species of cottontail are potentially sympatric: The desert cottontail (*Sylvilagus audubonii*), the eastern cottontail (*Sylvilagus floridanus*), and the mountain cottontail (*Sylvilagus nuttallii*; Armstrong et al., 2011). A morphologic examination was conducted to assess tentative species identification. We used PCR and DNA sequencing to confirm species identification. The lesions were excised during necropsy, and pathogen identification was determined by PCR and DNA sequencing. Portions of each lesion from the abdomen and head were placed into cryovials containing viral transport media (BA-1). The vials were stored at −80 C until PCR was conducted.

Genomic DNA was isolated from the rabbit and the frozen lesions using a DNeasy Blood and Tissue Kit (Qiagen, Germantown, Maryland, USA). Cottontail species identification was accomplished through amplification of a 459-base pair (bp) segment of the mitochondrial cytochrome *b* gene using primers MVZ 04 and 05 and a published protocol (Smith and Patton, 1991). Based on the morphology of these lesions (Weiner et al., 2010), we selected primers developed for amplifying 439 bp of cottontail rabbit papillomavirus (CRPV) DNA (Xiao and Brandsma, 1996). The PCR reactions were performed using Illustra® Puretaq® Ready To Go PCR Beads (GE Healthcare, Pittsburgh, Pennsylvania, USA), 1.5 µl of template DNA, and 0.4 µM of primers. The PCR profile included an initial denaturation at 95 C for 3 min, then 38 cycles of 94 C for 30 sec, 52 C for 30 sec, 72 C for 30 sec, and a final extension of 72 C for 10 min. The DNA sequencing of all PCR products was accomplished through cycle sequencing using ABI BigDye 3.1® (Life Technologies, Grand Island, New York, USA) following the manufacturer's protocols, and sequences were visualized on a genetic analyzer (ABI 3130xl; Life Technologies). Resulting sequences were identified through a standard nucleotide-nucleotide Basic Local Alignment Search Tool search (National Center for Biotechnology Information, 2013). DNA sequences for the cottontail

FIGURE 1. Photograph of the inguinal abdomen of a desert cottontail rabbit (*Sylvilagus audubonii*) from Colorado, infected with cottontail rabbit papillomavirus, August 2011. Many large, dark lesions projected from the ventrum of this animal, primarily on the abdomen.

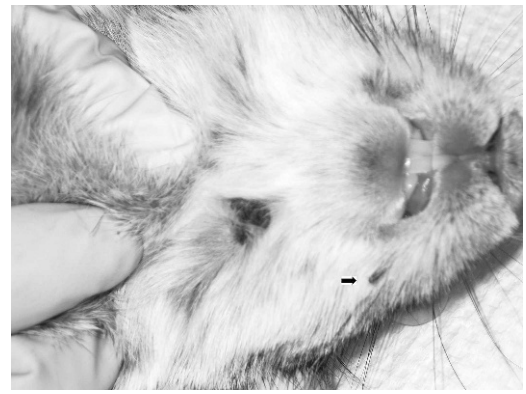

FIGURE 2. Photograph of the head of a desert cottontail rabbit (*Sylvilagus audubonii*) from Colorado, infected with cottontail rabbit papillomavirus, August 2011. At capture, there were many small cylindrical masses near the mouth, many of which were absent 43 days later, when this photograph was taken.

were a 99% match to 323 bp of *S. audubonii* cytochrome *b* (accession No. HQ901072), which confirmed the morphological assessment. The viral DNA sequences (accession No. KC797688) matched 100% to CRPV subtype B (accession No. AJ243287). As a result of genetic sequences confirming a CRPV infection, additional potential etiologies were not investigated further.

Although CRPV has been used for cancer research for decades (Rous and Beard, 1935), it has not been extensively studied in wildlife host populations. Accounts of this virus in natural populations are important to assess the natural dynamics of this pathogen. Cottontail rabbit papillomavirus was first described in Iowa from cottontails hunted there during the 1930s, and soon thereafter specimens positive for this virus were detected in Kansas (Shope and Hurst, 1933). Although the species of cottontail rabbit was not listed in this initial report (Shope and Hurst, 1933), the likely species was *S. floridanus*, which is presently the only *Sylvilagus* sp. known to occur in Iowa (Kaufman, 1998). Species identifications were also not listed for the cottontail rabbits received from Kansas (Shope and Hurst, 1933). Many workers have generically described CRPV as a natural virus of "cottontail rabbits" (Shope and Hurst, 1933; Rous and Beard, 1935), but few have described the species of cottontail rabbit that it infects. However, an early account described this virus as causing papillomas in "western" cottontail rabbits (*S. floridanus*; Beard and Rous, 1935). In addition, others have described tumors associated with CRPV infection as a disease of *S. floridanus,* principally occurring in the midwestern United States (Lancaster and Olson, 1982). Additional accounts of CRPV have been identified in eastern cottontails (*S. floridanus*) from Whidbey Island, Washington State (Evans and Rashad, 1967), a population originally imported from Kansas during 1931 (Dalquest, 1941). Thus, according to our knowledge, previously confirmed CRPV infections in *S. audubonii* appear to be lacking.

An occurrence of CRPV was recently reported from a wildlife sanctuary by the Colorado State University Pathology Service (Weiner et al., 2010). However, the species and collection location were not reported. The presentation of CRPV we report was quite different when compared with a recent report (Weiner et al., 2010). We found the majority of papillomas on the abdomen, whereas the majority of the

papillomas were on the head in the previous study (Weiner et al., 2010). *Sylvilagus floridanus* and *S. audubonii* are thought to be sympatric in a narrow band in northeastern Colorado (Armstrong et al., 2011), an area that includes the current study site. If CRPV is indeed primarily a pathogen of *S. floridanus* (Lancaster and Olson, 1982), sympatric populations of these two species may have facilitated transmission to *S. audubonii*. This report documents CRPV in *S. audubonii* in Colorado and highlights the importance of taxonomy in understanding pathogen-host systems, as presentation of the CRPV described in the current study differed from a recently reported case.

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