BRIEF COMMUNICATIONS and CASE REPORTS

The Electron Microscopic and Immunohistochemical Demonstration of a Papillomavirus in Equine Aural Plaques

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Key words: Aural plaque; horses; papillomavirus.

Aural plaques in horses are raised, flat-surfaced, nonpigmented lesions that often have a scaly or encrusted surface. They occur on the inner aspect of the pinna either as solitary lesions or as multiple, often confluent lesions affecting one or both ears." In a previous study, the cause of aural plaques was not determined but an association between blackflies (Simulium vittatum and S. argus) and aural plaques was suggested. These insects were commonly observed feeding within the ears of many affected horses. Aural plaques are presumed to be caused by a papillomavirus; however, there is no published evidence confirming the presence of virus in the plaques. Based on the gross and microscopic similarity of aural plaques to flat warts (verucca plana), a study was conducted to look for papillomavirus within aural plaques.

The plaques were incidental lesions obtained from four horses submitted for necropsy to the Western College of Veterinary Medicine, Saskatoon, Canada. Case No. 1 was a 10-year-old Clydesdale gelding with a solitary 3-mm diameter, 1-mm elevated plaque without any surface scale. Case No. 2 was a 15-year-old Arabian mare; case No. 3 was a 13-year-old Thoroughbred; and case No. 4 was an adult Quarter horse of unknown age. These three horses had multiple, confluent plaques in both ears. The plaques varied from 4 to 20 mm in diameter and from 2 to 4 mm in height and were encrusted with surface scale.

Microscopically, the plaques had all the features described previously. In addition, enlarged cells (koilocytes) with clear cytoplasm and keratohyalinlike granules of various sizes were present in the outer stratum spinosum and stratum granulosum. Koilocytes were numerous in the solitary plaque from case No. 1 (Fig. 1), whereas in case Nos. 2-4 koilocytes occurred singly or as small clusters of 2-4 cells.

Immunohistochemical staining was performed on serial sections of the blocks from which hematoxylin and eosin-stained sections were cut. Papillomavirus antigens were detected with an avidin biotin complex immunoperoxidase method using I: 1,000 and I: 2,000 dilutions of rabbit antihematoxylin and eosin-stained sections of aural plaques in which the antiserum to papillomavirus virus antigens was omitted or was substituted with nonimmune rabbit serum or antiserum to influenza A virus antigens. Miscellaneous skin lesions with epidermal hyperplasia and hyperkeratosis from 15 horses were also used as controls. A canine cutaneous papilloma acted as a positive control.

Papillomavirus was demonstrated immunohistochemically within the lesions of all four horses. Dark-brown positive staining was confined to nuclei and occurred in koilocytes in the stratum granulosum and outer stratum spinosum (Fig. 2) and in shrunken nuclear remnants of the stratum corneum (Fig. 3).

Positive staining was absent in the adjacent histologically normal epidermis, in the negative controls, and in the epidermis of the other skin conditions in which there was epidermal hyperplasia and hyperkeratosis.

The material for electron microscopic examination was obtained from the sections that were immunohistochemically positive for papillomavirus. A published technique was used to retrieve and prepare the material.

Electron microscopic examination performed on plaques from case Nos. 1 and 2 demonstrated intranuclear crystalline arrays of hexagonal viral particles approximately 38-42 nm in diameter (Fig. 4). The morphologic appearance of the viral particles was typical for papillomavirus, and the size of the particles was similar to that reported in tissue sections in equine cutaneous papillomas.

This study provides strong evidence for a papillomavirus as the cause of aural plaques. The histologic and cytologic features (koilocytes) are typical of lesions induced by papillomaviruses, and electron microscopic examination demonstrated a virus consistent with a papillomavirus in the lesion. The antiserum used in this study detects group-specific antigens of papillomaviruses. Using this antiserum, viral antigen was demonstrated in the stratum corneum and stratum granulosum and in the outer stratum spinosum, i.e., in the expected location for a papillomavirus-induced lesion. The exact identity of this papillomavirus and its relationship to other equine papillomaviruses were not determined by this study.

Papillomaviruses infect skin by being inoculated or by entering abraded, scarified, or otherwise damaged skin. The inner aspect of the pinna, where aural plaques occur, is not obviously prone to trauma. How the virus enters the skin and how it might be transmitted between horses are unknown. Direct transmission between horses is physically unlikely or impossible. In cottontail rabbits, the Shope papilloma virus can be mechanically transmitted by mosquitoes.
Fig. 1. Aural skin; horse No. 1. The granular layer has numerous enlarged cells with clear cytoplasm and kerato-hyalinlike granules (koilocytes) of various sizes. HE.

Fig. 2. Aural skin; horse No. 1. Nuclei of cells in the granular cell layer stain intensely with the antibody to papillomavirus. The focus of positive staining in the stratum corneum is a disintegrating nucleus (arrow). Avidin biotin peroxidase complex method, hematoxylin counterstain.

Fig. 3. Aural plaque; horse. The hyperkeratotic stratum corneum has rows of nuclei that stain positively for papillomavirus. Avidin biotin peroxidase complex method, hematoxylin counterstain.

Fig. 4. Aural plaque; horse. Arrays of intranuclear viral particles are present in a cell of the stratum granulosum. Bar = 0.25 μm.

and assassin bugs, and papillomas develop in the recipient rabbits.' Other biting arthropods may also be capable of transmitting papillomaviruses. As simuliid blackflies feed in the ears of horses, these flies may transmit the papillomavirus in aural plaques.

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References

Reports of Schwannomas in cattle indicate that these tumors are usually multiple and are found most commonly in the thoracic cavity. In this study, we present a case of a solitary Schwannoma in a bovine cecum that had unusual morphologic features.

A solitary spherical tumor, approximately 2 cm in diameter, was detected in the submucosa of the cecum of a 5-year-old Holstein-Friesian cow (Fig. 1). The tumor was elastic and firm, well-demarcated, but not clearly encapsulated, and had a white-gray cut-surface with foci of mucinous degeneration and hemorrhage. No other gross lesions were observed at necropsy, and the central nervous system was normal. The tumor was fixed in 10% neutral buffered formalin. Dissected tumorous tissues were dehydrated, embedded in paraffin, sectioned at 5 μm, and then stained with hematoxylin and eosin for routine evaluation.

Microscopically, the tumor appeared to have originated from the inner circular smooth muscle layer of the cecum and it extended to the submucosa. The tumor had well-defined borders but was not encapsulated. It was composed in some areas of loosely packed spindle cells and in other areas of densely packed spindle cells. The characteristic fasciculated arrangement of cells usually observed in Schwannomas was replaced by distinctive, whorled, neuroid structures of varied sizes and cellularity. These structures were reminiscent of nerve bundles or tactile corpuscles and revealed the concentric arrangement of the spindle cells (Fig. 2). The spindle cells had oval to ellipsoid hyperchromatic nuclei (Fig. 2, inset). The number of spindle cells in mitosis was low. A mucoid material that stained with alcian blue often was found in the intercellular slitlike spaces of the spindle cell whorls. Foci of cells that contained hemosiderin also were present. Some whorls contained nuclear palisading or Wagner-Meissner bodies (Fig. 3). In general, reticulin fibers haphazardly encircled spindle cells that formed the whorls. Continuity of the tumor with normal nerve fibers was not observed, and the tumor did not contain melanin pigments or argyrophilic granules.

Immunostaining patterns of the tumor with anti-S-100 protein antibody demonstrated that spindle cells in the whorls were stained much stronger and more intensely than those of tumors in the other areas (Fig. 4). Immunoreactivity with anti-S-100 protein antibody was visualized with diaminobenzidine-hydrogen peroxide solution. Normal nerve fibers and muscle cells were used as positive controls.

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For ultrastructural evaluation, small pieces of the forma-

A Solitary Schwannoma in the Cecum of a Cow

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Key words: Cattle; cecum; immunohistochemistry; Schwannoma.