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Herpesvirus-like Infection in a Raccoon (*Procyon lotor*)

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ABSTRACT: During March 1990, a subadult raccoon found dead in northeastern Pennsylvania (USA) had gross lesions of multifocal hepatitis. Microscopically, multifocal randomly distributed areas of acute necrosis with intranuclear viral inclusions were seen in liver, spleen, adrenal glands, and tongue. Ultrastructural and immunoperoxidase results of formalin fixed liver were compatible with herpesvirus infection. This virus could be unique to the raccoon or may have been acquired from another species.

Key words: Herpesvirus, Pennsylvania, raccoons, *Procyon lotor*.

During March 1990 while field testing a vaccinia-rabies glycoprotein (V-RG) recombinant virus vaccine for raccoons (*Procyon lotor*) at a state gamelands in northeastern Pennsylvania, USA (76°30′N, 41°20′W) (Rupprecht et al., 1992), a dead subadult female raccoon was encountered with lesions that were compatible with an infection by a herpesvirus. This raccoon had been live-trapped by a Tomahawk live trap (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) at least two times previously at approximately 5 and 6 mo prior to being found dead in the field. At the time of first capture, it was sedated with a mixture of 10 mg/kg ketamine (Veterinary Products, Bristol Laboratories, Division of Bristol-Meyers Co., Syracuse, New York, USA) and 0.4 mg/kg xylazine (Haver, Bayvet Division, Miles Laboratory, Inc., Shawnee, Kansas, USA) administered intramuscularly. It was examined, ear-tagged, and bled for serologic testing of rabies virus neutralizing antibodies. On second capture, it was examined and released without obtaining any samples.

At necropsy, the carcass was in a good nutritional state and had gross lesions confined to the liver. The edges of the liver were slightly rounded, and numerous white, multifocal, randomly distributed areas (approximately 1 mm in diameter) were seen throughout the parenchyma (Fig. 1). Representative sections of heart, lung, liver, kidney, spleen, tongue, skin, adrenal glands, and whole brain were placed in 10% buffered formalin for histopathologic examination. A portion of proximal spinal cord was collected fresh and was examined for rabies virus antigen by a fluorescent antibody (FA) test (Dean and Abelseth, 1973). No evidence of rabies was found in this test.

The fixed brain was cut in approximately 2-mm transverse sections; brain sections and all other tissue sections were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin prior to light microscopic examination. Paraffin-embedded sections of liver also were stained by the streptavidin-biotin complex (ABC) immunoperoxidase method (Hsu et al., 1981) to detect pseudorabies viral antigens. Primary antibody for the ABC test was obtained from National Veterinary Services, Ames, Iowa (USA) and consisted of polyclonal antiserum against pseudorabies. The antiserum was prepared by injecting a virulent field strain of pseudorabies virus into a pig (*Sus serofa*). Serum was collected 84 days after inoculation and diluted 1:4 in Earl's basic salt medium. As described in Hamir et al. (1992), formalin-fixed samples of liver were post-fixed in 1% osmium tetroxide, stained in blocks with 0.5% uranyl acetate, dehydrated in alcohol, and embedded in epon-araldite plastic (Electron Microscopy Sciences, Fort Washington, Pennsylvania 19034, USA). Sections were cut at 60 to 90 nm, stained with uranyl acetate and lead

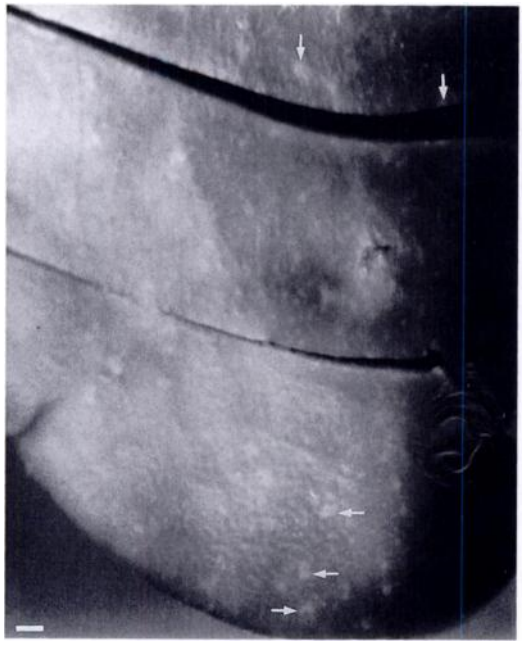

FIGURE 1. Formalin-fixed liver from a raccoon, Pennsylvania, 1990. Note multifocal randomly distributed areas (arrows) of necrosis. Bar = 1 mm.

citrate, and were examined by Philips 301 electron microscope (Philips Electronics Instrument Co., 85 McKee Drive, Mahwah, New Jersey, USA).

Microscopically, liver lesions consisted of many randomly distributed foci of acute hepatocellular necrosis (Fig. 2). Some of the viable hepatocytes at the periphery of these lesions had marginated chromatin and contained central eosinophilic intranuclear inclusion bodies (Fig. 3). Within and around the necrotic foci there were minimal inflammatory cellular infiltrates consisting of mononuclear cells (Fig. 2). Similar necrotic foci and intranuclear inclusions also were present in cortical areas of the adrenal glands and in the spleen. In the tongue, there were small, deep ulcerations; intranuclear inclusions were observed in epithelial cells present at the margins of these ulcers.

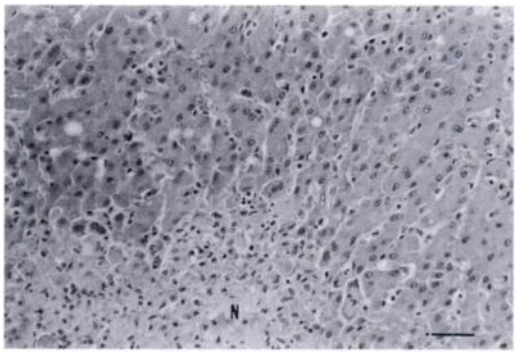

FIGURE 2. Liver from a raccoon, Pennsylvania, 1990. Note focus of necrosis (N) and the presence of minimal inflammatory cellular infiltrate. H&E. Bar = 100 μm.

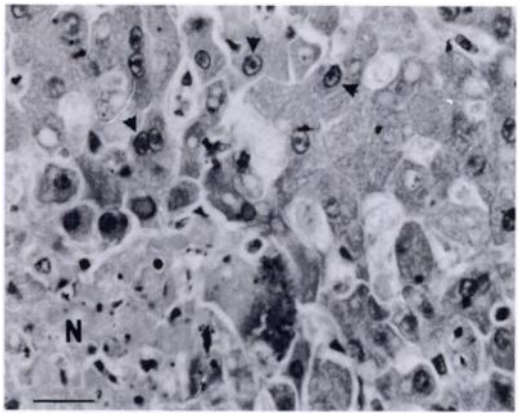

FIGURE 3. Liver from a raccoon, Pennsylvania, 1990. Higher magnification of Figure 2 with intranuclear inclusions in hepatocytes (arrow heads) at the periphery of necrotic focus (N). H&E. Bar = 50 μm.

Incidental lesions in other tissues included moderate numbers of protozoal parasites resembling *Sarcocystis* sp. in striated muscles (heart, diaphragm, tongue, and masseter muscle) and nematode parasites resembling *Capillaria* sp. in epithelial mucosa of the tongue. The latter parasites did not incite observable inflammatory cellular responses and were not associated with the ulcerations.

Immunohistochemical tests with polyclonal antisera to pseudorabies antigen were negative. On ultrastructural examination of the inclusion-containing hepatocytes, we observed many intranuclear viral particles which had dense central cores (Fig. 4). The virus particles measured approximately 100 nm and were icoso-

hedral in shape, thus they were morphologically compatible with herpesviruses (Fenner et al., 1987).

We are aware of only two brief reports which documented multifocal necrotic lesions and characteristic herpetic inclusions in raccoons (Sanger et al., 1978; Maurer and Nielsen, 1981). In both reports, although the affected raccoon tissues were examined by electron microscopy, the authors did not test the tissues for the presence of pseudorabies viral antigen.

Pseudorabies virus can infect many mammalian species including cattle, sheep, goats, dogs, cats, and many wild animals (Fenner et al., 1987). On the basis of experimental studies, the raccoon is considered a major reservoir of pseudorabies virus (Wright and Thawley, 1980). Microscopically, pseudorabies cases had typical herpes-like lesions in neuronal as well as non-neuronal tissues (Goyal et al., 1986). Considering the economic consequences of pseudorabies to swine production and present control programs, it is essential to differentiate histopathologic inclusions of pseudorabies from other herpesvirus infections. In our case the brain was normal and pseudorabies antigen was not demonstrated in affected tissues by the ABC immunoperoxidase method.

Since herpesvirus infection in raccoons may involve diffuse nonsuppurative encephalitis (Maurer and Nielsen, 1981), it is important to differentiate this condition from rabies encephalitis. Rabies has continued to spread unabated in the northeastern United States (Krebs et al., 1992). To counteract the rabies epizootic, several oral recombinant viral vaccines for rabies have been developed (Hamir et al., 1992; Rupprecht et al., 1986). However, in addition to conferring protection against rabies, such proposed biologicals must be rigorously tested for inadvertent vaccine-induced effects in target and non-target animals (Hamir et al., 1992).

Although typical herpetic lesions were seen by light microscopy in this raccoon, and ultrastructurally viral particles compatible with herpesvirus were observed, antigen against the pseudorabies virus was not detected by the ABC immunoperoxidase technique. We were unable to isolate the etiologic agent because neither fresh tissues nor acute serum was available from this raccoon. Since the geographic location of the gamelands is quite remote, it is unlikely that domestic animals were the source of infection for the raccoons. Nevertheless, the specific etiology and epizootiology of this herpes viral infection in raccoons requires further clarification.

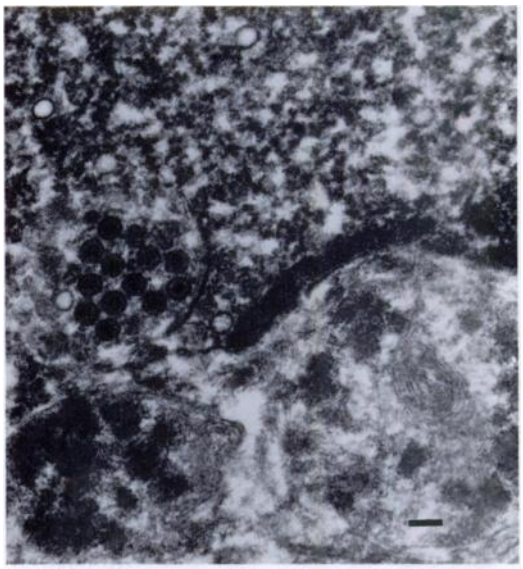

FIGURE 4. Electron micrograph. Liver from a raccoon, Pennsylvania, 1990. Aggregates of herpesviral particles are present within an hepatocyte. Bar = 100 nm.

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