Neuroaxonal dystrophy in raccoons (Procyon lotor) from Iowa

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Abstract. During a 12-month period (1998–1999), microscopic evidence of neuroaxonal dystrophy (NAD) in medullae oblongata of raccoons (Procyon lotor) was observed in 17/39 (47% prevalence in adults) from Iowa, USA. Three of the animals were kits (<3 months), 26 were between 1 and 2 years, and 10 were over 7 years. Lesions were not seen in the medullae of the 3 kits. In young adults, the lesions were mild and were seen in 7 animals. More severe lesions were present in the 10 older raccoons. Grossly, the brains were unremarkable. Microscopically, NAD was confined to the dorsal caudal medulla, where certain nuclei (predominantly gracilis and cuneate) were bilaterally affected. Severely affected animals had vacuolar degeneration of neurons or neuronal loss and extensive areas of spongiosis. Tests for the presence of PrPres in the brain were negative. Spongiotic areas often contained axonal spheroids. Degenerate neurons and axons occasionally contained amorphous periodic acid–Schiff-positive granular material. There was a paucity of inflammatory cells in the affected areas. Since lesions were not present in kits, were either absent or mild in young adults, and were severe in older raccoons, the findings may be related to advancing age. Neuroaxonal dystrophy has not been previously reported in raccoons. Retrospective examination of raccoon brains from the eastern and northwestern areas of the country revealed very low prevalence of NAD. Because of the apparently high prevalence of this condition at this geographic location, factors other than age (genetic, nutritional, and/or environmental) may influence this degenerative process in the brains of raccoons in Iowa.

Raccoons (Procyon lotor) are found throughout North America and in some parts of Europe and Asia where they were introduced in the early 1900s from the United States. They are highly adaptable omnivores and have managed to survive and increase their numbers in many urban and suburban areas. Since raccoons share environments with humans, they have been used as monitors of zoonotic diseases and environmental contamination.

Neuroaxonal dystrophy (NAD) is a degenerative disorder of the central nervous system (CNS). The characteristic findings of NAD are disseminated axonal swellings (spheroids) in terminal and preterminal areas of long axons. Although affected animals generally show gait abnormalities, many cases of NAD with severely affected gracilis and cuneate nuclei did not have any significantly abnormal clinical signs. Among domestic animals, naturally occurring NAD has been described in cats, dogs, sheep, and horses. Such degenerative lesions have not been documented in raccoons. This communication describes the lesions of NAD and its prevalence in a population of raccoons in Iowa, USA.

During a 12-month period (September 1998–August 1999), a total of 39 raccoons (19 females and 20 males) from central Iowa were obtained from wildlife authorities (n = 29) and from a local breeder (n = 10). The latter raccoons were older breeding animals (>7 years) that originated either from the wild from Iowa or were the progeny of parents obtained from the same geographical location. In this older group of raccoons, some had unilateral or bilateral cataracts. The animals obtained from the wildlife authorities were nuisance animals and, except for 3 kits (<3 months), all were young adults (estimated by dental examination to be 1–2 years old). These raccoons were live-trapped and submitted to the National Animal Disease Center (NADC), Ames, Iowa, for necropsy. Complete postmortem examination was performed and representative tissue samples of all major organs, including the entire brain, were immersion fixed in 10% neutral buffered formalin.

The fixed brains were cut into 3–4-mm-wide coronal sections, and 5 sections (1 each of cerebrum, cerebellum, and caudal medulla and 2 sections of brain stem) were selected. For the purpose of this report, lesions confined to the caudal...
medullae are documented. The selected tissues were processed for routine histology, sectioned at 5 μm, and stained with hematoxylin and eosin (HE) for microscopic examination. Selected sections were stained with periodic acid–Schiff (PAS) stain. Sections of brain stem and medulla of 5 raccoons with NAD (2 young adults and 3 older) were examined by immunohistochemistry (IHC) for detection of abnormal prions (PrP\textsuperscript{res})\textsuperscript{1,6} using 2 monoclonal antibodies, F89/160.1.5 and F99/97.6.1.\textsuperscript{1,8} These antibodies would recognize PrP sequences conserved in most mammalian species in which naturally occurring transmissible spongiform encephalopathies (TSEs) have been reported.\textsuperscript{1,8} However, since these monoclonal antibodies have not been tested in raccoon tissues, formalin-fixed brain (caudal medulla) of selected cases (n = 5) were examined by negative-stain electron microscopy for scrapie-associated fibrils (SAFs) by a modified SAF extraction technique.\textsuperscript{6}

Gross lesions were not identified in the brains of any raccoons. Microscopic evidence of neuronal and/or axonal degeneration was seen in 17/39 raccoons. Seven of the affected animals were females and 10 were males. None of the younger animals (3 kits) had lesions of NAD. Excluding these 3 kits, the prevalence of the condition was 47%.

In all positive cases, the lesions of NAD were confined to the dorsal caudal medulla. The lesions were bilateral and consistently involved the gracilis and medial cuneate nuclei. Occasionally, the accessory cuneate nuclei were also involved and, in 1 raccoon, the lesion also extended to the dorsal gray matter of the proximal cervical spinal cord. Morphologic lesions consisted of variable numbers of swollen and hypereosinophilic degenerate axons (spheroids) and neurons that lacked nuclei. Many of the affected neurons had single or multiple clear vacuoles in their perikarya (Fig. 1). In some brains, neurons were focally absent and the neuropil contained clear areas (Figs. 2–4). Microscopic lesions were classified as mild, moderate, or severe as shown in Figs. 2–4, respectively. Mild lesions consisted of the presence of a few degenerate neurons and axonal spheroids (Fig. 2). Periodic acid–Schiff-positive granular material (lipofuscin) was present within some of the affected neurons and in neuropil surrounding degenerate axons. In severe cases, there was extensive bilateral vacuolar degeneration of neurons, often resulting in complete disappearance of the cell and status spongiosus of the neuropil (Fig. 4). Moderate lesions were intermediate in severity (Fig. 3). Older animals (over 7 years) had moderate to severe lesions (Figs. 3, 4), whereas in the young adults, the lesions were mild (Figs. 1, 2).

In all cases with lesions of NAD, there was no significant cellular inflammatory infiltration or increased presence of glial cells in the affected areas. None of the examined raccoons revealed vacuolated neurons in pontine nuclei, as described in raccoons from Oregon.\textsuperscript{9} PrP\textsuperscript{res} was not detected by immunohistochemistry nor were SAFs demonstrated by negative-stain electron microscopy in all 5 cases that were examined.

The microscopic lesions observed in the medulla of the affected raccoons support the diagnosis of NAD. It is presumed that, in such conditions, the terminal parts of the axons are initially affected and the lesion progresses to degeneration of the neurons.\textsuperscript{1,9} However, in the presently described raccoons, the lesions were more severe and extensive in the
neuronal cell bodies (Figs. 2, 3), which suggests that the degenerative process may have been initiated in neuronal perikarya. Neuroaxonal dystrophy has not previously been described in raccoons, and a retrospective examination of a large number of raccoon brains (>500) from the eastern and northwestern United States (A. N. Hamir, unpublished data) revealed evidence of mild NAD lesions in only 3 of these animals (2 at Pennsylvania and 1 at Oregon). Therefore, the observation of a high prevalence of NAD (>40%) in the present study indicates that the condition appears to be widespread in raccoons of central Iowa.

Neuroaxonal dystrophy affecting various nuclei of the caudal brainstem and medulla has been reported in animals and in man.3,5,7,11,12,17,21 Such lesions have been associated with the normal aging process as well as with a number of inherited or acquired neurodegenerative disorders and in experimental conditions.11,20 In the present study, not only did all the older (geriatric) raccoons have severe NAD, but milder, similar lesions were present in the medulla of some of the younger adults. This suggests that neuroaxonal degeneration in medullae of raccoons is most likely a progressive condition and can be expected to be present in the brains of a large proportion of raccoons in central Iowa. Although most raccoons in the wild die during their first 2 years of life,14 it would be of interest to examine brains of older raccoons from geographical locations other than Iowa for lesions of NAD. Such specimens could be obtained from raccoons that are kept in zoos and other wildlife facilities where they may live to an old age.

The pathological changes observed in raccoons in this study are most likely age related. However, factors other than age (genetic, nutritional, or environmental) may influence and accelerate degeneration of neurons and axons in the CNSs of raccoons in Iowa. The observation of lesions in both groups of raccoons (wild-caught and captive) suggests that environmental and/or genetic factors rather than nutrition may be associated with the pathological changes.

In this investigation, the lesions observed in the affected raccoons were either mild in young adults or moderate to severe in geriatrics. Since none of these wild-caught or confined raccoons were subjected to clinical neurological examinations, it is not possible to indicate whether any of the animals had subtle or overt neurological signs present prior to euthanasia. However, in some of the older raccoons, the lesions were so severe and advanced that it is likely most would have shown some clinical evidence of neurological disease. Neuronal vacuolation and other morphological changes observed at this location in the medulla may suggest other pathological conditions, such as those associated with transmissible spongiform encephalopathies (TSEs). In this study, 5/5 raccoons with either mild or severe lesions of NAD were negative for the presence of abnormal PrPsc and SAFs.

Since in the present study only the brain and the medulla were examined, future investigations should include detailed examinations of the spinal cord and peripheral nerves. Also, further studies are required to document the extent, prevalence, and etiology of NAD and to understand the basis for the apparent geographic restriction of this condition in raccoons.

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References