Spontaneous Lesions in Aged Captive Raccoons (*Procyon lotor*)

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In nature, free-ranging raccoons typically do not live longer than 2 y; most raccoons in the wild die young due to accidents and diseases. Therefore, few data are available regarding lesions associated with advancing age in raccoons. This communication documents the lesions present in raccoons (7 male; 3 female) that were older than 7 y and had been used as breeders at a commercial facility in central Iowa. The most frequent microscopic lesions in these raccoons included accumulation of iron pigment in livers and spleens (10 of 10 animals evaluated), neuroaxonal degeneration in caudal medulla (10 of 10), vascular mineralization (psammoma body) in choroid plexus (9 of 10), myocardial inclusions (7 of 8), and cystic endometrial hyperplasia (2 of 3). Other conditions were seen with less prevalence. Except for the detection of gastritis with bacteria in the gastric mucosa of 1 raccoon, the presence of inflammatory cells in 3 choroid plexuses, and the presence of Lafora bodies in the brain of 1 animal, all conditions observed had previously been reported in raccoons. Surprisingly, islet-cell amyloidosis, previously observed as common incidental finding in older captive raccoons, was not seen in any of the raccoons we examined. Because free-ranging raccoons are distributed over wide geographic areas, their local environment may have considerable influence on the range of spontaneous lesions that would occur in raccoons obtained from a specific location. Therefore, the lesions found in these raccoons from central Iowa may differ from those of other raccoon populations.

Raccoons are native to North America, where they range from southern Canada through most of the continental United States and into Central America.⁶ Raccoons currently also are found in the former Soviet countries,² Central Europe,⁵ and Japan;¹³ raccoons were introduced after 1930 and have now become largely feral in these countries.

The age-related lesions in several 6-y-old laboratory-confined raccoons (intact females and castrated males) that were inoculated with the agent of chronic wasting disease have previously been documented.⁹ Compared with the previously described animals,⁹ the current raccoons were older, male raccoons were intact, and the animals had not been inoculated with any infectious agent and were used as breeders by a commercial raccoon supplier.

Materials and Methods

Adult raccoons (n = 10; 7 male and 3 female; nos. 850 and 500 through 508; Table 1) were obtained from a commercial raccoon breeder in central Iowa. All raccoons had been live-trapped in the surrounding area and used as breeders for several years. The owner did not know the exact ages of the raccoons but was certain that all were older than 7 y. The raccoons were maintained on waste from meat-packing plants in the surrounding area.

None of the raccoons showed any clinical signs at euthanasia. Except for one raccoon (no. 850), whose brain was sent in formalin by the owner, all animals were submitted live to the National Animal Disease Center (Ames, IA), where they were euthanized with pentobarbital overdose. A complete necropsy was conducted on each carcass. Representative samples of liver,

kidney, spleen, skin, striated muscles (heart, tongue, diaphragm, masseter), thoracic aorta, thyroid gland, trachea, lung, tonsils, esophagus, stomach, intestine (duodenum, jejunum, ileum, rectoanal area), anal sacs, salivary gland, adrenal gland, urinary bladder, mesenteric lymph node, and nerves (optic, trigeminal) and the whole brain were immersion-fixed in 10% neutral buffered formalin. The formalin-fixed brain was cut into coronal sections (width, 2 to 4 mm). Sections of various anatomic sites (a minimum of 5 hemisections of brain per animal), rostral cerebrum, hippocampus, superior colliculus, cerebellum, and brainstem were processed for routine histopathology, embedded in paraffin wax, sectioned at 5 µm, and stained with hematoxylin and eosin. Sections of stomach were stained by the Stiner method for bacteria such as *Helicobacter* spp.; heart and medulla were stained by using periodic acid-Schiff; liver and spleen sections were stained with Pearl Prussian blue for iron; selected sections of the pancreas were stained with Congo red for the detection of amyloid; and the choroid plexus was stained with Masson trichrome for collagen. All processing and staining of tissues was done according to standard methods.

Results

All the carcasses were in good to fat body condition. All had lightened coat color (almost brown to yellowish instead of black). None of the carcasses had any gross lesions; histopathologic lesions are summarized in Table 1 and Figure 1.

Microscopically, all 6 of the male raccoons evaluated showed testicular and epididymal aspermia, which is considered a normal finding in raccoons⁶ for the time of the year (mid-August). Livers of all 10 raccoons revealed extensive multifocal random accumulations of granular dark-brown to black pigment in Kupffer cells; lesser amounts of this material also were seen within hepatocytes. Similar pigment was seen in spleen. Pearl Prussian blue staining revealed that this material was positive for iron.

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	_			Myocardial		Cystic endometrial
Animal no.	Sex	Neuroaxonal degeneration	Psammoma bodies	inclusions	Choroid plexitis	hyperplasia
850	male	+	+	not examined	_	not applicable
500	male	+	—	+	—	not applicable
501	male	+	+	+	—	not applicable
502	male	+	+	+	+	not applicable
503	female	+	+	+	—	+
504	female	+	+	+	—	+
505	male	+	+	+	—	not applicable
506	female	+	+	—	+	—
507	male	+	+	+	—	not applicable
508	male	+	+	not examined	+	not applicable
Total (no. posi	itive/					
no. examin	ed)	10/10	9/10	7/8	3/10	2/3

Table 1. Common microscopic findings in 10 aged raccoons (older than 7 y) used as breeders in a commercial breeding establishment in central Iowa

	no. positive/
	no. examined
Myocardial fibrosis	2/8
Adrenal cortical adenoma	1/9
Extratesticular vasculitis	1/6
Lymphocytic bacterial gastritis	1/9
Cerebro-vascular lymphoid cuffs	1/9
Cerebrovascular mineralization of	1/10
globus pallidus	
Lafora bodies in brain	1/10

Figure 1. Infrequent microscopic findings in 10 aged raccoons (older than 7 y) used as breeders in a commercial breeding establishment in central Iowa.

Lungs of all raccoons had mild multifocal anthrocosis, generally in the vicinity of the air passages. In addition, one raccoon (no. 502) had foci of foamy macrophages in the alveoli at the periphery of the lungs, subjacent to the parietal pleura. This cellular reaction extended into the adjacent alveolar walls, distending these septa. This lesion was diagnosed as foci of lipid pneumonia. In addition, this raccoon showed 2 small foci of concentric bone formation within the alveolar space thereby distending the alveolar space. Within these boney tissues were viable cells in the lacunae. The lesion was considered to be likely an early lesion of a pulmonary idiopathic alveolar ossification, which has previously been reported in a single raccoon.¹¹

The most frequent lesions were present in the brain, heart, and uterus (Table 1). Three different lesions-neuroaxonal degeneration (10 of 10 animals examined), presence of psammoma bodies (9 of 10), and choroid plexitis (3 of 10)-were seen in the brains of these raccoons. Lesions of neuroaxonal degeneration were confined to the dorsal medulla of all raccoons. Bilateral discrete foci with swollen axons (spheroids) and isolated foci of vacuolation in the neuropil were present (Table 1). The severity of the lesion varied from mild (n = 3), to moderate (n = 3) or severe (n = 4). Psommoma bodies consisted of the presence of foci of mineralization in blood vessels of the choroid plexus (n = 6) or meningeal vessels (n = 3); one raccoon showed foci at both locations (Table 1). Affected vessels were small capillaries, and lesions were distributed multifocally. Some mineralized foci had the characteristic laminated appearance of psammoma bodies. The choroid plexuses of 3 raccoons revealed multifocal areas infiltrated with mononuclear inflammatory cells; in all 3 of these animals, the choroid showed segmental thickening with amorphous eosinophilic material (Figure 2; Table 1), which

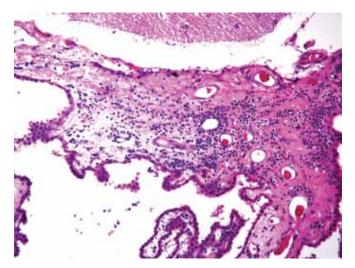


Figure 2. Raccoon brain. Choroid plexus with segmental thickening with amorphous eosinophilic material and multifocal mononuclear cell infiltrate. Hematoxylin and eosin stain; magnification, 160×.

was negative for presence of amyloid on Congo red staining but positive for collagen with Masson trichrome stain.

Lesions were seen in the myocardium of 7 of 8 raccoons. All 7 animals with myocardial lesions had multifocal, randomly distributed inclusions (either eosinophilic or basophilic) in the cytoplasm of myocardiocytes (Table 1). Occasional inclusions appeared to be located extracellularly. On cross-section, inclusions appeared generally round and varied from 20 to 70 μ m in diameter. However, on longitudinal section, they were cigar-shaped (maximum length, 100 μ m), and their ends appeared to blend with the longitudinal striations of the myofibers. The basophilic inclusions were positive on periodic acid–Schiff staining but diastase-resistant. All 3 female raccoons showed mild uterine cystic dilatation and hyperplasia of the endometrial glands.

Infrequent microscopic lesions are summarized in Figure 1. In 2 of the hearts, multifocal areas of fibrosis were present and more prominent in the left ventricular myocardium. These areas of fibrosis were not necessarily associated with the myocardial inclusions mentioned previously. In addition, single cases of extratesticular vasculitis (no. 507), adrenal gland with a cortical adenoma (no. 507), cerebrovascular lymphoid cuffs of unknown Vol 50, No 3 Journal of the American Association for Laboratory Animal Science May 2011

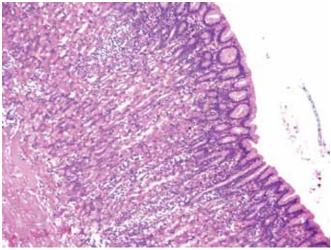


Figure 3. Raccoon stomach, with lymphocytic inflammatory infiltrate in the mucosa. Hematoxylin and eosin stain; magnification, 80×.

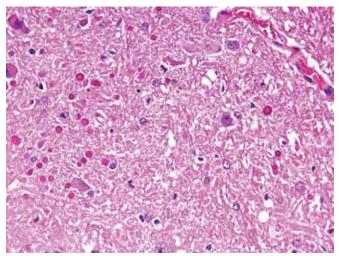


Figure 5. Raccoon brainstem showing aggregates of magenta-colored polyglucosan inclusions (Lafora bodies) in the neuropil. Periodic acid-Schiff stain; magnification, 320×.

Islet-cell amyloidosis, previously observed as common incidental finding in free-ranging³ and aged captive raccoons,⁹ was not seen in any of the raccoons examined in the present study.

Discussion

Although the life expectancy of the raccoon can exceed 10 y in captivity, free-ranging raccoons usually do not survive beyond 2 y because most succumb to predation and diseases in the wild.¹² Therefore, lesions associated with advancing age in raccoons have not been reported frequently.

The reason for the lightened coat color of all 10 raccoons in the study was not determined. Because advancing age can be associated with loss of hair pigment, the age factor may be responsible for this change. Alternatively, these raccoons had been in captivity for extended periods, and the lightened coat color may be due to the artificial indoor environment. In addition, a large variety of foods would have been available to free-ranging raccoons; the restricted nutrition in captivity may have influenced the animals' hair color.

The deposition of iron in liver and spleen most likely was related to the diet of these raccoons. Because they were fed a meat-rich diet, they would have accumulated excess iron from this source in their organs.

There has been one previous report of neuroaxonal degeneration in raccoons,¹⁰ a report that similarly included animals from central Iowa and documented a prevalence rate of 47% in adult raccoons. In young adults, the lesions were mild, whereas they were more severe in older animals.¹⁰ Similar to the current findings, the lesions were bilateral and were confined to the gracilis and cuneate nuclei. The apparently high prevalence of neuroaxonal degeneration in raccoons from central Iowa suggests that factors other than age (genetic, nutritional, and environmental) may contribute to the development of this lesion in these animals.¹⁰

A case of cerebrovascular mineralization in an aged (older than 10 y) female raccoon with uremia associated with polycystic kidney disease has been reported.⁸ The vascular lesions were bilateral and consisted of multifocal mineralized foci in the walls of blood vessels in globus pallidus. The affected vessels were patent and showed no inflammatory cellular response to the mineralized foci. This lesion appears to be similar to a nonsymptomatic condition that has been documented in horses.¹⁴

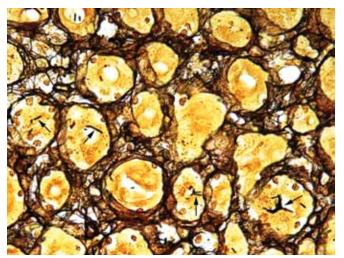


Figure 4. Raccoon stomach. Note the presence of rod-shaped bacterial rods (arrows) in the mucosal cells. Stiner silver stain; magnification, 500×.

etiology (no. 508), and cerebrovascular mineralization of globus pallidus (no. 850) were seen.

In addition, 3 raccoons demonstrated lesions that have not previously been documented in this species. One raccoon (no. 2503; Figure 3) had lymphocytic gastritis with many silver-positive bacteria that sometimes were located within the mucosal cells (Figure 4). Morphologically, these bacteria were rod-shaped and did not resemble spiral Helicobacter-like organisms. The second previously unrecorded lesion in raccoons was the presence of a thickened choroid plexus with mononuclear inflammatory cell infiltration in 3 animals (Table 1; Figure 2). No etiologic agent was demonstrated within the affected choroid plexuses. The third unusual lesion was in the brain of raccoon no. 850. The lateral area of the pons had bilateral, nearly symmetrical areas with small, round, magenta-colored bodies (maximal diameter, 25 µm; polyglucosan inclusions or Lafora bodies; Figure 5). These Lafora bodies were positive on periodic acid-Schiff staining and were present in the neuropil of a few (predominantly trigeminal and facial) nuclei; the inclusions did not incite any cellular inflammatory infiltrate.

Incidental findings of multifocal mineralization (psammoma bodies) in the blood vessels of the choroid plexus and meninges have frequently been reported to occur in free-ranging and laboratory-confined raccoons.7 Previously reported psammoma bodies were seen in brains of 62% of raccoons from Parramore Island, VA, that were necropsied.⁷ These mineralized foci revealed concentric laminations and were present in small capillaries of meninges of the brain, choroid plexus, or both of these sites. In 2 raccoons from the cited study,⁷ the lesions were confined to the meninges of the proximal cervical spinal cord. In most cases, the affected vessels appeared to have been occluded completely. However, no evidence of ischemic changes in the brain parenchyma was seen, and none of the raccoons showed abnormal neurologic signs prior to euthanasia. Mineralization and subsequent occlusion of larger blood vessels likely result in cerebral ischemia. Although the exact cause of this condition is unknown, a primary vascular insult with resultant dystrophic mineralization of the affected vessels was suspected.⁷ None of the present cases had evidence of vascular blockage.

As indicated earlier, Lafora bodies (polyglucosan inclusions) have not previously been documented in raccoons. Lafora bodies are inclusions of complex glycoprotein polymers in tissues.¹⁴ Although not reported often in wild animals, Lafora bodies have frequently been reported in dogs and in a few other domestic animals. These inclusions may or may not be associated with abnormal neurologic signs.¹⁴ The raccoon in the current study had numerous Lafora bodies, which were confined to the trigeminal and facial nuclei.

Although rare in wild free-ranging animals, cystic endometrial hyperplasia and hydrometra have been documented in captive wild animals,¹ including raccoons.^{8,9} Cystic endometrial hyperplasia and hydrometra are thought to result from prolonged, noncyclic estrogen stimulation or excessive levels of circulating estrogens.¹ In some species, nulliparity and aging are associated with progressive proliferative changes in the endometrium,¹ leading to development of cystic endometrial hyperplasia. However, no such predisposing conditions were present in the current study.

In the current study, 7 of 8 raccoons had myocardial inclusions. The inclusions resembled those observed in inclusion-body myositis, as seen in humans.⁴ The human condition is age-related (seen after midlife) and may be associated with certain metabolic disorders.⁴ These inclusions are reported to consist of polysaccharide complexes, and similar inclusions have been observed in skeletal muscles.¹⁵ Two cases of such inclusions in the skeletal muscles of dogs with sarcomas have been documented recently.¹⁵ None of the raccoons in this or the previously reported study⁹ had inclusions in skeletal muscles. The condition most likely is associated with advancing age and appears to be an incidental finding.

Raccoons in captivity can live for well over 10 y. However, the present study and a previous publication⁹ appear to be the only available reports of spontaneous lesions in older raccoons. Although the raccoons of both studies were similar in age and from the same geographic area (central Iowa), the lesions observed in these 2 studies varied somewhat. Except for the detection of silver-positive bacteria in gastric mucosal inflammation of a single raccoon, inflammatory infiltrate in 3 choroid plexuses, and Lafora bodies in the brain of one animal

in the current study, the lesions observed have been reported in other raccoons previously.

Surprisingly, islet-cell amyloidosis, previously observed as common incidental finding in aged captive animals,⁹ was not seen in any of the current raccoons. This result may reflect differences in the nutritional status (maintained on commercial dry dog food compared with waste from meat food factories) or quality of life (one was allowed to breed, whereas the other group was not) between the 2 studies. Given that free-ranging raccoons are distributed over wide geographic areas, their local environment may have considerable influence on the range of spontaneous lesions observed. Therefore, depending on the region from which an animal is obtained, the lesions observed may differ more or less from those recorded from raccoons from central Iowa.

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