NOTE *Wildlife Science*

Histopathological findings in wild Nutrias (*Myocastor coypus*) with *Capillaria hepatica* infection

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ABSTRACT. *Capillaria hepatica* is a zoonotic nematode that uses rodents and other mammals as hosts, especially rats and mice, and causes hepatic granuloma and eventually fibrosis/cirrhosis. However, *C. hepatica* infection in nutria, a large semiaquatic rodent, has rarely been reported, and histopathologic features of the infection have not been described in detail. We conducted necropsy on 36 wild nutrias. Some animals were found to have milky spots, parasitic eggs and worms within hepatic microgranuloma involving central calcification with cell debris, macrophages, eosinophils and multinucleated giant cells (MGCs). Interestingly, the eggs were closely surrounded by MGCs and appeared to be destroyed without inducing further chronic changes. Based on microscopical examination, *C. hepatica* infection was diagnosed, and we describe its histopathological characteristics in wild nutrias.

KEY WORDS: *Capillaria hepatica*, milky spot, multinucleated giant cell, *Myocastor coypus*, nutria

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The nutria (*Myocastor coypus*), which is also known as the coypu, is a large rodent native to South America. This semiaquatic herbivore has been introduced to many countries including Korea for meat and fur production [4]. The failure of nutria farms in Korea has led to their release into the wild, and they now inhabit an area near the Nakdong River (southeastern Korea). As in other countries, nutria has become problematic in Korea, because of its high reproductivity, lack of enemies, and propensity to damage crops and wetland plants. As a result, Korean Ministry of the Environment has designated nutria as a pest and is conducting an eradication campaign utilizing wire cage traps with vegetable bait.

Nutrias have been reported as reservoirs or carriers of a variety of pathogens and parasites in many countries, such as *Fasciola hepatica*, *Strongyloids myopotami*, *Toxoplasma gondii*, Coccidia, *Leptospira spp*., *Giardia* spp., encephalomyocarditis, *Chlamydia psittaci* and *Francisella tularensis* [2, 5, 6, 11, 15, 17, 23]. However, *Capillaria hepatica* infection in nutrias is relatively very rare, and its histopathological characteristics have not been reported in detail [15, 16, 19]. Therefore, we describe macro- and microscopic characteristics of *C. hepatica* infection of wild nutrias which were different from those observed in rats and mice.

Thirty-six nutrias were captured by wire cage traps from their main habitats of southeastern areas (Gyeongsangnam-do province) in Republic of Korea from September 2014 to March 2015. These areas were cities located near small or large branches of The Nakdong River. The captured nutrias were transferred alive to our laboratory, euthanized by carbon dioxide (CO_2) inhalation and submitted to necropsy. For microscopic examination, the tissues were processed routinely for embedding in paraffin wax and then cut into 4 μm thick sections, which were subsequently stained with hematoxylin and eosin (H&E) and examined microscopically.

Most nutrias were in healthy condition, although a few had injured or broken tails which were presumed to have been caused by cannibalism when they were transported in the same cage. Upon necropsy, no significant lesions were observed in organs except for the livers, although 19 nutrias had hepatic milky spots as microgranulomas (Fig. 1). Most milky spots were very small and focally distributed and could therefore be easily disregarded. Only one of the nutrias had randomly distributed multifocal milky spots in the liver (Fig. 1D). Microscopically, all milk spots showed focal granulomatous inflammation involving central calcification with cell debris, infiltration of macrophages, eosinophils and a few multinucleated giant cells (MGCs) (foreign body type) (Fig. 2A and 2B). However, no specific causative organisms were detected in 14 of the nutrias with hepatic milky spots, while the other five had numerous parasitic eggs or adult worms within the same granulomatous inflammation. The eggs were lemon-shaped and had striated double shells with

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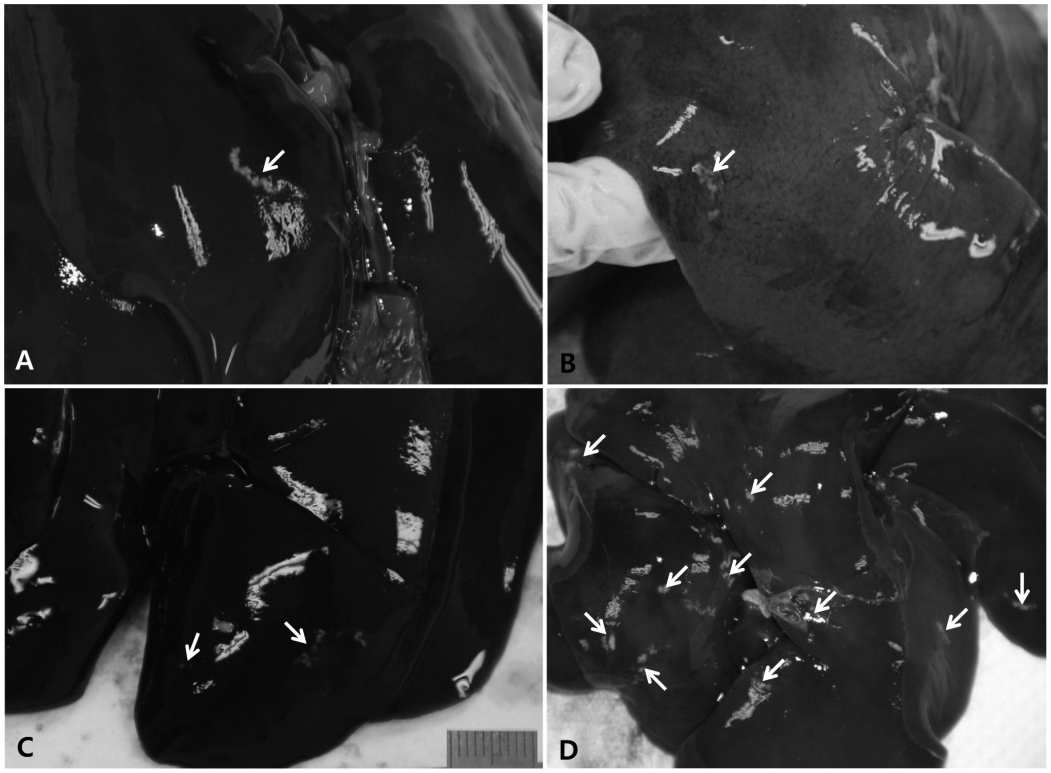

Fig. 1. Hepatic milky spots in wild nutrias. (A−C) Milky spots (arrows) are small and focally distributed. (D) One nutria has milky spots multifocally (arrows).

polar plugs at either end (Fig. 2C). Numerous adult nematodes were surrounded by macrophages and eosinophils, as well (Fig. 2D). Interestingly, MGCs were usually seen close to the eggs, and sometimes, they seemed to enclose and destroy the eggs (Fig. 2E and 2F). Consequently, we concluded that nutrias were infected by *C. hepatica* based on the shape of the eggs and adult worms. Information describing individual nutrias that had milky spots, granulomatous lesions, eggs or adult worms in the livers is provided in Table 1. Thus, 52.8% of nutrias had milk spots as hepatic microgranulomas, and the infection rate was 13.9% based on the number of individuals containing eggs or adult worms. The prevalence rate of *C. hepatica* infection and milky spots was the highest in Changwon-si and Haman-gun.

C. hepatica is a nematode that afflicts a wide range of mammalian species worldwide. This organism parasitizes the host's liver after the infection of embryonated eggs, and female worms lay eggs after mating. The eggs in the livers can only be released into the environment upon the death and decomposition of the host, at which time they can be transferred to other hosts [8]. The life span of adult worms is relatively short in mice (18–60 days) [13], however, no studies have reported the exact life span of the eggs within the liver. Nevertheless, the parasitized eggs are presumed to live until death of the host, because the eggs within the liver have been shown to cause focal necrosis, granuloma and eventually fibrosis/cirrhosis in rats and mice, which are the most susceptible hosts for *C. hepatica* [1, 9, 12, 20]. In the present study, 19 nutrias had hepatic granulomas, however, the eggs were found in only four of these, and there were no fibrotic/cirrhotic changes observed. Moreover, many MGCs closely surrounded the eggs and appeared to be destroying them. The physiological function of MGCs in granulomatous inflammation has not been clearly elucidated to date. However, MGCs have generally been considered the final differentiated form of macrophages, showing decreased phagocytosis but the ability to produce important pro-inflammatory and anti-inflammatory cytokines [3, 10]. It has been suggested that during infection by *Schistosoma (S.) mansoni,* which is a helminth that causes formation of hepatic granuloma and fibrosis in mice and humans, MGCs help disintegrate the eggs by making shell fenestrations, which might facilitate diffusion of antigenic materials from the eggs to the surrounding phagocytes, and subsequent resolution of the egg granuloma [22]. Furthermore, the resolution of granuloma by *S. mansoni* has been shown to differ depending on host's immunity [7]. Therefore, we speculate that even though nutrias belong to the rodent, their immune-response to *C. hepatica* is different from that of mice and rats; however, future studies to elucidate the life-cycle of *C. hepatica* in nutrias in greater detail are needed.

C. hepatica infection of house rats in Korea used to be extremely common, with a prevalence of 38–88% according to surveys conducted in 1956–1964 and 1978 [18, 21]. Al-

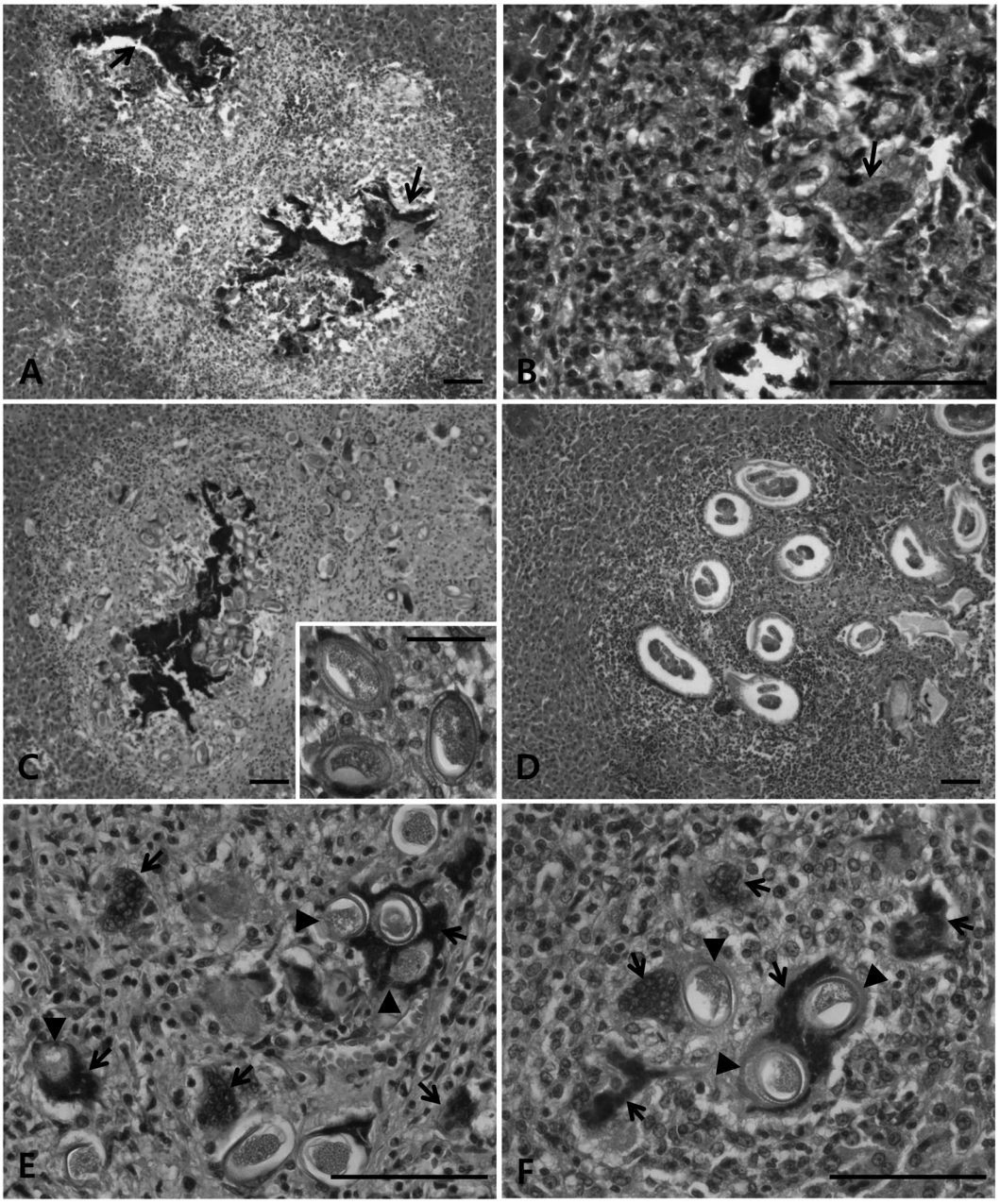

Fig. 2. Microscopic examination of milky spots, eggs and adult worms of *C. hepatica* in hepatic granulomas of wild nutrias. (A) Typical granulomatous inflammation involving central calcification (arrow) with cell debris and inflammatory cells. (B) Infiltration of macrophages, eosinophils and multinucleated giant cells (foreign body type) (arrow). (C) Numerous eggs showing lemon-shaped and double shells with bipolar plugs surrounded by inflammatory cells. (D) Numerous cross-sectioned adult worms in granulomatous inflammation. (E, F) Disintegrating eggs (arrowhead) closely surrounded by multinucleated giant cells (arrow). H&E (A–F). Scale=100 μm (A–F), 50 μm (inset of C).

though there have been no regular investigations of parasitic diseases in animals since then, we believe that the distribution of *C. hepatica* in our environment has decreased as has that of other parasites. However, there are still occasional reports of their occurrence in wildlife, such as a raccoon dog [14] and nutria [19]. *C. hepatica* infection of nutrias in other countries has been very rare compared to other parasite infections, with 3.6% and 1.9% infection rates were reported for Argentina and Japan, respectively [15, 16]. While these low infection rates may be due to low distribution of *C. hepatica* in the environment, we speculate that they may be related to possible resolution of the eggs within the hepatic

Table 1. Macro and microscopic examinations of milky spots in the livers of nutrias

No. of nutrias	Sex	City name of captured area	Milky spots	Microscopic examination		
				Granulomas	Eggs of parasite	Adult worms
1	M[a)]	Busan-si (N35.10877, E128.884866)				
2	M					
3	F[b)]	Gimhae-si (N35.198553, E128.859866)				
4	F					
5	M					
6	M					
7	M					
8	M					
9	F		○	○		
10	M		○	○		
11	M					
12	F		○	○		
13	F					
14	F					
15	F		○	○		
16	F					
17	F					
18	F					
19	M	Changwon-si (N35.300113, E128.685683)	○	○		
20	F		○	○	○	
21	M		○	○	○	
22	F		○	○		
23	F		○	○		
24	F	Haman-gun (N35.314052, E128.380811)	○	○		
25	M		○	○		
26	M		○	○		○
27	M		○	○	○	○
28	F		○	○		
29	F		○	○		
30	F		○	○	○	
31	M					
32	M	Jinju-si (N35.207885, E128.152157)				
33	F					
34	M		○	○		
35	M	Yangsan-si (N35.290061, E129.013379)	○	○		
36	F		○	○		

a) M: male, b) F: female.

granuloma of nutrias.

Unexpectedly, 36 wild nutrias had rare pathological injuries or infectious lesions other than hepatic granulomas at necropsy. Overall, 52.8% of nutrias had hepatic granulomas, and 13.9% had *C. hepatica* eggs or worms within the granulomas. Our study is the first report of *C. hepatica* infection from high numbers of wild nutrias and to describe histopathological features of the infection in detail.

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