NOTE ***Pathology***

Listeriosis in a Raccoon Dog (*Nyctereutes procyonoides*) Associated with Canine Distemper

Takahiro AOYAGI, Yoshihiko SATO, Shouhei MATSUURA[1)] and Hirohiko WADA[2)]

Matsumoto Livestock Hygiene Service Center, 6931 Shimauchi, Matsumoto, Nagano 390–0851, [1)]*Saku Livestock Hygiene Service Center, 111–179 Setonakaniwa, Saku, Nagano 385–0035 and* [2)]*Nagano Prefectural Office, 692–2 Minaminagano, Nagano 380–8570, Japan*

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ABSTRACT. A wild raccoon dog (*Nyctereutes procyonoides*) that manifested severe illness and died was examined. Necropsy revealed severe emaciation, systemic icterus and petechial hemorrhages on the mucous membranes. Histopathologically, necroses were seen in the liver and brain stem associated with meningitis. Eosinophilic intranuclear inclusion bodies were observed in the spleen and intestinal mucosa, and eosinophilic intracytoplasmic inclusion bodies were seen in transitional epithelium in the bladder. *Listeria monocytogenes* 4b was isolated from the liver, spleen, kidneys and lungs, and the pathogen was also detected in the liver and brain stem immunohistopathologically. The disease was diagnosed as listeriosis associated with canine distemper virus infection in a raccoon dog.—KEY WORDS: canine distemper, listeriosis, raccoon dog.

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Listeriosis is one of the most common zoonotic infections causing meningitis, septicemia and abortion in both human and domestic animals [8]. The infection, especially caused by *Listeria monocytogenes*, has been most frequently reported in cattle, sheep and goat, and less frequently in pigs and horses [8, 15]. The pathogen has been isolated from healthy animals such as rats, cattle, dogs and pigs for reasons of public health concern [2, 3, 5, 6]. There have been also some reports on the infection in wild animals, in over 30 species [4]. However, there have been no report on listeriosis in raccoon dogs and this paper deals with the first report of *Listeria monocytogenes* infection in the animal associated with canine distemper.

A male raccoon dog was captured on a resort area in Nagano Prefecture on April 1995. The animal manifested clinical symptoms such as general weakness, severe emaciation and jaundice, and a watery hemorrhagic discharge was seen around the anus. Though the raccoon dog was medicated immediately in a veterinary clinic with ampicillin and steroid drug, the animal died three hours after capture.

Necropsy was carried out on the dead raccoon dog, which was submitted into our diagnostic laboratory soon after death. Icterus was observed systemically and petechial hemorrhages were seen on the mucous membrane of the oral cavity. The intestines were filled with watery and reddish contents and petechial hemorrhages were seen on the mucous membrane. The liver was slightly swollen and severe ecchymosis was observed on the mucous membrane of the bladder.

For histopathological examination, pieces of the liver, spleen, heart, lungs, kidneys, cerebrum, cerebellum, stomach, intestines and bladder were fixed in 10% formalin. Their paraffin sections were prepared and stained with hematoxylin and eosin (HE). Focal necroses were seen in the liver associated with gram-positive bacilli and a large number of viable-appearing Kupffer cells was observed along the sinusoid (Fig. 1). The biliary canaliculi were dilated with formation of bile thrombus. A large necrotic area infiltrated with neutrophils and foam cells was formed in the brain stem associated with meningitis, and a great number of gram-positive bacilli was detected in these lesions. The white pulp in the spleen was necrotic and eosinophilic intranuclear inclusion bodies were seen in some reticuloendothelial cells. The mucous epithelium of the small intestine was necrotic. Catarrhal enteritis was observed in the large intestine associated with lymphocytic depletion in the lymphoid follicles and haloed eosinophilic intranuclear inclusion bodies were seen in some lymphoid cells. A large number of eosinophilic intracytoplasmic inclusion bodies was found in the transitional epithelium in the bladder (Fig. 2).

For bacteriological examination, the liver, heart, spleen, lungs, kidneys and urine of the necropsied raccoon dog were cultured on plates of blood agar, chocolate agar, heart infusion agar, desoxycholate hydrogen sulfide agar, sabouraud agar, and mannitol salt agar at 37°C for 48 hr. Identification of the species was made by the observation of utilization of rhamnose, xylose and mannitol, β-hemolysis on 5% sheep blood agar plate, and CAMP test with *Staphylococcus aureus* and *Rhodococcus equi* [14]. Serological properties were examined according to the method of Seeliger and Hohne [13]. A large number of *Listeria monocytogenes* was purely isolated from the liver, spleen, kidneys, and lungs. These isolates were serotyped as 4b, which account for 76% of *L. monocytogenes* infection in animals and 60% in humans [8].

After pretreatment with a 0.1% solution of actinase, formalin-fixed, paraffin wax-embedded sections of the liver and brain stem were stained by an avidin-biotin complex immunoperoxidase method with *L. monocytogenes* 1a and 4b antisera and an immunostaining kit (Vector Laboratories Inc., Burlingame) for immunohistochemical examination. The antigen for *L. monocytogenes* 4b was detected in the

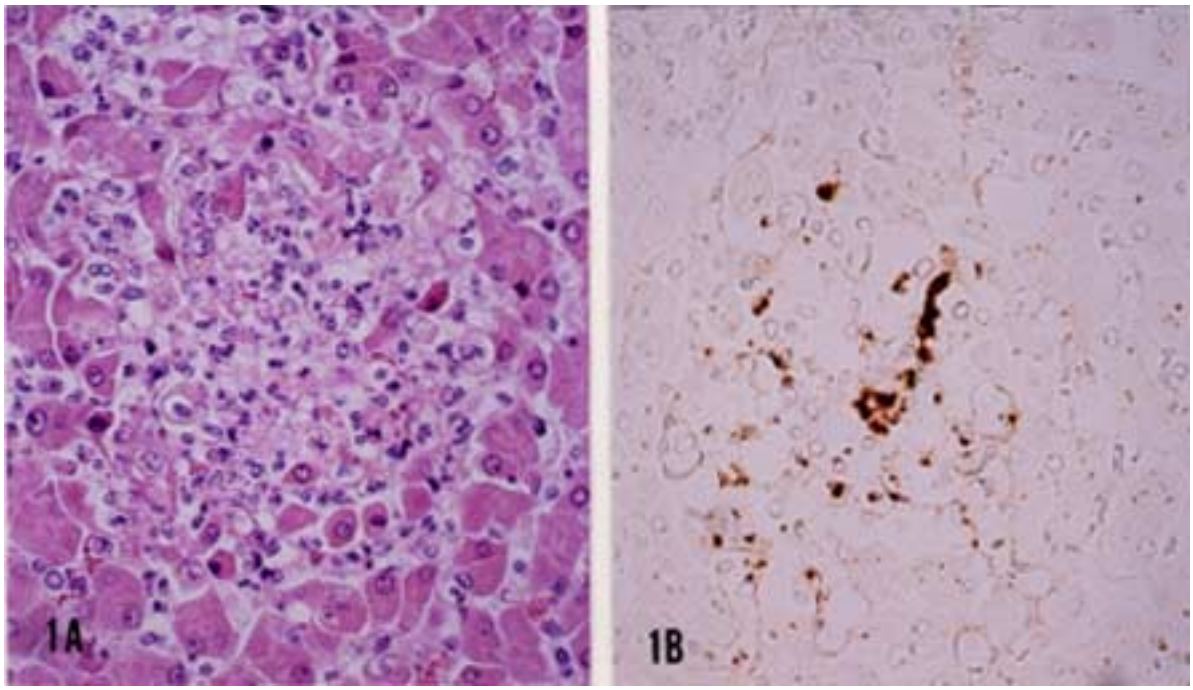

Fig. 1. A focal necrosis seen in the liver. Viable-appearing Kupffer cells were observed along the sinusoid. HE stain, × 290. (A) Avidin-biotin complex immunoperoxidase staining on a formalin-fixed, paraffin-embedded section of the liver. Listeria antigen for 4b was detected in the focal necrosis. × 290. (B)

necrotic lesions of the liver and brain stem (Fig. 1).

Results obtained from the pathological, histopathological and bacteriological examinations indicated that the occurrence of *L. monocytogenes* infection in the raccoon dog was evident.

According to a brief review of listeriosis in wild animals, the first report on the infection was recorded in South African gerbilles in 1925 [4]. Since then there have been many reports on the infection in wild animals including birds throughout the world in over 30 species such as rats, mice, rabbits, hares, guinea pigs, skunks, minks, foxes, raccoons, deer, stag, geese, ducks, capercaillies, blue eagles [4]. As far as we know, this is the first report of *Listeria* infection in a raccoon dog.

The raccoon dog is known to be infected by canine distemper virus [9, 10]. Neagari *et al.* [12] examined the incidence of antibodies against canine distemper in 30 raccoon dogs inhabiting suburban areas and nine of them possessed the antibodies, indicating a higher prevalence of the pathogen in this species. Canine distemper virus infection also occurred in a free-living masked palm civet (*Paguma larvata*) in Japan [11]. Moreover, there are some reports on listeriosis associated with canine distemper in a gray fox [1, 7]. Histopathologically, canine distemper virus infection was characterized by eosinophilic inclusion bodies, both cytoplasmic and intranuclear, which were found in the present case. These findings strongly indicate that the raccoon dog in the present case was also infected with canine distemper virus.

Generally speaking, it is known that canine distemper virus is more susceptible and pathogenic than *Listeria* to Canidae, to which the raccoon dog belongs. Whereas, *Listeria monocytogenes* is often isolated from healthy animals [2, 3] as well as natural environments, listeriosis is regarded as a opportunistic infection which requires certain predisposing factors. In this case, it is suspected that the canine distemper virus infection acted as a predisposing factor for listeriosis.

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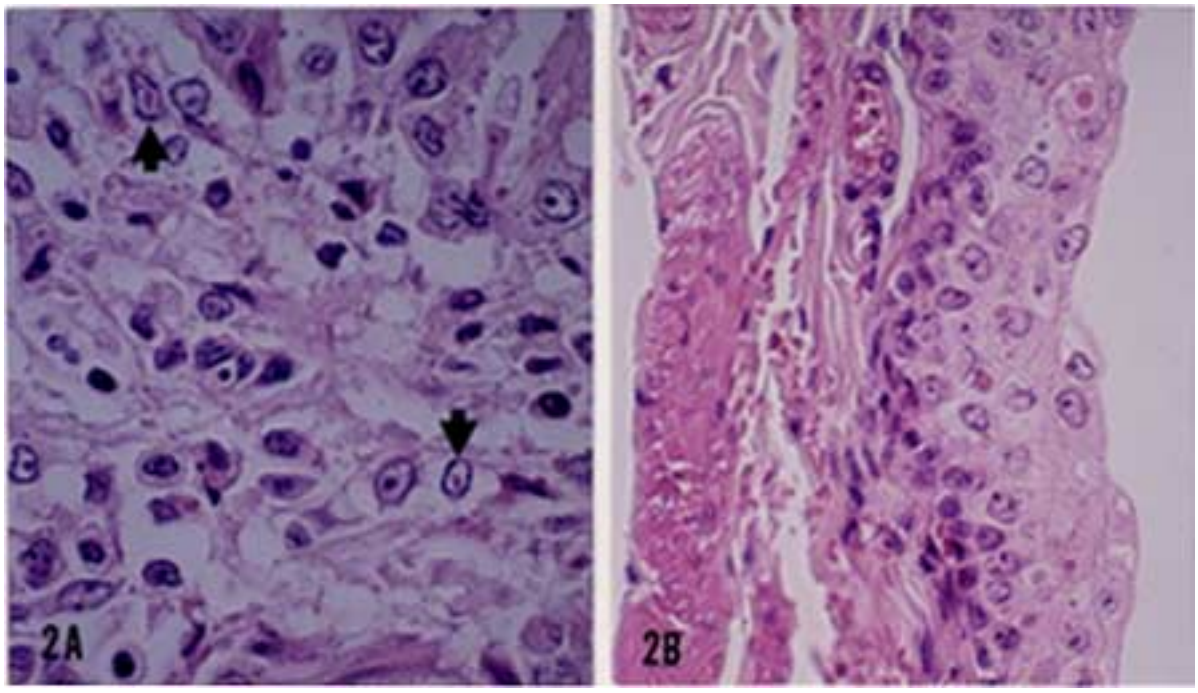

Fig. 2. Haloed eosinophilic intranuclear inclusion bodies (arrows) were seen in some lymphoid cells in the lymphoid follicles in the colon. HE stain, × 580. (A) A large number of eosinophilic intracytoplasmic inclusion bodies was also seen in the transitional epithelium in the bladder. HE stain, × 380. (B)

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