Incidence of *Listeria monocytogenes* in Wild Animals in Japan

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(Received 11 June 1999/Accepted 22 February 2000)

ABSTRACT. Between 1991 and 1993, the intestinal contents and feces of wild animals in Japan were examined for the presence of *Listeria*. The wild animals examined included 623 mammals (11 species) and 996 birds (18 species). *Listeria* species were isolated from 38 (6.1%) of the 623 mammalian samples and 133 (13.4%) of 996 bird samples. The highest incidence of *Listeria* in the mammals was found in Japanese monkeys (20.0%) and that in birds was found in crows (43.2%). The incidence of *Listeria* in Japanese monkeys varied from 0 to 40.0% depending on the capture area. *L. monocytogenes* was isolated from 11 of these positive samples. Serovars 1/2a and 4b predominated in eight serotyped *L. monocytogenes* isolates.—**KEY WORDS:** incidence, *Listeria*, wild animal.

—J. Vet. Med. Sci. 62(6): 673–675, 2000

Listeria monocytogenes is a gram-positive, rod-shaped and non-spore-forming bacterium [13] and widespread in the environment (i.e., soil, sewage and river water) [1, 7, 14, 16, 17]. It is a zoonosis-causative agent pathogenic to many mammals and bird species [11], and has increasingly been recognized as an important foodborne pathogen. In Japan, reports on isolation of *L. monocytogenes* from wild animals have been limited only to wild rats in urban areas [4–6]. In other countries, however, many investigations have reported *L. monocytogenes* isolation from wild animals other than rats [1, 2, 10, 12, 15]. Therefore, it is very important for public health to understand the incidence of *Listeria* in wild animals in Japan. The purpose of this study was to investigate the incidence of *Listeria* in wild animals other than wild rats in Japan.

During the period from 1991 to 1993, 623 mammals (11 species) and 852 birds (18 species) were captured in 10 prefectures in Japan. Most of them were killed after capture and the intestinal contents were collected. Some crows were caged for a few days before collection of intestinal contents. In addition, fresh feces from 144 rock ptarmigans in Toyama prefecture were collected in Cary-Blair Transport Medium (Nissui, Tokyo) and transported on ice to our laboratory (Table 1).

Listeria was isolated by the method of Perry and Donnelly [9]. Namely, each sample was cultured in nine volumes of UVM Modified *Listeria* Enrichment Broth (Difco, Detroit, MI) at 30°C for 24 hr. Then, 1 ml of each culture was added to 9 ml of modified UVM broth supplemented with acriflavine to a final concentration of 25 mg/l and incubated again at 30°C for 24 hr. After these two-step enrichment, 0.1 ml of the culture was plated on a PALCAM agar (Merck, Darmstadt) plate and incubated at 37°C for 48 hr. Six suspect colonies were picked up from each positive plate and characterized biochemically for *Listeria* by the method of Seeliger and Jones [13].

In mammals, *Listeria* species were detected from 38 (6.1%) of 623 mammals consisting of seven species,

Japanese monkeys (20.0%, 16/80), red foxes (13.3%, 2/15), raccoon dogs (11.5%, 12/104), masked palm civet cats (9.1%, 1/11), Japanese martens (3.9%, 5/127), wild boar (2.7%, 1/37) and “sika” deers (1.1%, 1/95). The *Listeria* species recovered were *L. innocua* (4.7%), *L. monocytogenes* (1.0%), *L. ivanovii* (0.2%), *L. seeligeri* (0.2%) and *L. welshimeri* (0.3%) (Table 1). The highest incidence (20.0%) of *Listeria* was found in Japanese monkeys; *L. monocytogenes* was isolated from one of 16 positive monkeys. Although there was a chimpanzee case of listeriosis [3] so far, there might have been no report of recovery of *Listeria* from wild apes or monkeys. Therefore, we believe that this is the first report of isolation of *Listeria* from wild monkeys. The incidence of *Listeria* in Japanese monkeys varied from troop to troop at different locations (Table 2). The significantly higher incidence of *Listeria* observed in Hachiman-cho troop (40.0%, 12/30, $p < 0.01$) than in other troops. It is suggested that if a troop is once contaminated with *Listeria*, it will spread rapidly among monkeys in the troop. The animal species with the next highest positive rate (13.3%) was red fox; all the two isolates were *L. monocytogenes*.

In birds, *Listeria* species were isolated from 133 (13.4%) of 996 birds consisting of only two species, crows (43.2%, 130/301) and green-winged teals (2.6%, 3/115). The *Listeria* species recovered from crows were *L. innocua* (42.9%, 129/301) and *L. monocytogenes* (1.7%, 5/301) and that recovered from green-winged teals was *L. innocua* (2.6%, 3/115). Fenlon [1] isolated *Listeria* from 16 (13.0%) of 123 feces or intestinal contents of rooks; eight of the *Listeria*-positive specimens yielded *L. monocytogenes*, nine specimens yielded *L. innocua* and one specimen yielded *L. seeligeri*. The incidence of *Listeria* in crows (43.2%) in this study was much higher than that (13.0%) observed by Fenlon [1]. This difference may be due to the capture method; in this study, 121 (47.1%) were positive for *Listeria* of 257 crows captured collectively with a cage-style trap and left for a few days in the cage before collection of intestinal samples, while only nine (20.4%)

Table 1. Isolation of *Listeria* from the intestinal contents or feces of wild animals and birds

	Species	Samples	<i>Listeria</i> (%)	<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. ivanovii</i>	<i>L. seeligeri</i>	<i>L. welsh-imeri</i>
Mammal	Japanese monkey	80	16 (20.0)	1	15			
	Red fox	15	2 (13.3)	2				
	Raccoon dog	104	12 ^{a)} (11.5)	1	10		1	1
	Masked palm civet	11	1 (9.1)		1			
	Japanese marten	127	5 (3.9)	1	2	1		1
	Wild boar	37	1 (2.7)		1			
	Sika deer	95	1 (1.1)	1				
	Japanese weasel	23						
	Japanese hare	62						
	Asiatic black bear	35						
	Nutria	34						
Total		623	38 (6.1)	6	29	1	1	2
Bird	Crow	301	130 ^{b)} (43.2)	5	129			
	Green-winged teal	115	3 (2.6)		3			
	Mallard	72						
	Other duck	42						
	Rock ptarmigan	161						
	Pigeon	135						
	Eastern turtle dove	35						
	Gray starling	60						
	Sparrow	41						
	Green pheasant	16						
	Copper pheasant	8						
	A kind of heron	7						
	Common cormorant	3						
Total		996	133 (13.4)	5	132			

a) One of 12 samples yielded both *L. monocytogenes*- and *L. innocua*-positive.b) Four of 130 samples yielded both *L. monocytogenes*- and *L. innocua*-positive.Table 2. Incidence of *Listeria* in Japanese monkeys at different places of capture

Capture area ^{a)}	Samples	<i>Listeria</i> (%)	<i>L. monocytogenes</i>	<i>L. innocua</i>
Hachiman-cho	30	12 (40.0) ^{b)}		12
Horado-mura	20	2 (10.0)		2
Minami-mura	13	1 (7.7)	1	
Yaotsu-cho	12			
Nannou-cho	4	1 (25.0)		1
Kasuga-mura	1			
Total	80	16 (20.0)	1	15

a) All of the capture areas were located in Gifu prefecture.

b) Incidence of *Listeria* was significantly higher in Hachiman-cho than in the other areas ($p < 0.01$).

were positive of 44 crows captured individually by other methods. Thus, the incidence in the former crows was significantly higher than that in the latter ones ($p < 0.01$). The fact is that *Listeria* species were also recovered from the drinking water in the cage. These results suggest cross contamination of the cage directly or through the drinking water.

All of the pigeons and sparrows were *Listeria*-negative in this study. However, McIlwain *et al.* [8] reported that 12.5% (1/8) of pigeons and 5.3% (2/38) of sparrows

captured in a pasture were *L. monocytogenes*-positive. In addition, Weber *et al.* [15] isolated *Listeria* from 3.4% (12/350) of pigeons. We captured the birds in rural districts, while McIlwain *et al.* [8] captured on a pasture. Therefore, the living environment of the birds might also affect the incidence of this microorganism.

Eight of 11 *L. monocytogenes* isolates from mammals and birds were typed with antisera for serotyping *Listeria* (Denkaseiken, Tokyo). Serovars identified were 1/2a (three isolates), 4b (three isolates), 1/2b (one isolate) and 4c (one

Table 3. Serovars and RAPD types of *L. monocytogenes* isolates

Serovar	Strain No.	Source (Date and location of isolation)	RAPD-type ^{a)}
1/2a	50-1	Japanese marten (Nov. 1991, Gifu prefecture)	III
	2-1	Red fox (Nov. 1991, Shiga prefecture)	II-3
	178-1	Crow (Jul. 1992, Mie prefecture)	II-2
1/2b	152-3	Crow (Jul. 1992, Gifu prefecture)	IV-1
4b	18-1	Sika deer (Feb. 1992, Iwate prefecture)	IV-6
	30-1	Crow (Apr. 1992, Gifu prefecture)	IV-6
	99-7	Crow (Jul. 1992, Gifu prefecture)	IV-7
4c	54-1	Raccoon dog (Dec. 1991, Gifu prefecture)	V

a) The data of RAPD types were retrieved from a previous report [18].

isolate) (Table 3). The three serovars (1/2a, 1/2b and 4b), which have frequently been recovered from human and animals with listeriosis, were found to be predominant in the wild animals in Japan. In addition, eight of these 11 *L. monocytogenes* isolates were typed by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) according to Yoshida *et al.* [18]. Interestingly, two of these 4b isolates showed an identical RAPD profile (type IV-6), in spite of different animal origins, crow and "sika" deer. This suggests that the RAPD type of *L. monocytogenes* might have invaded wild animal populations in Japan.

In conclusion, it is clear that wild animals act as reservoirs or carriers of *L. monocytogenes*. In particular, *Listeria* species were frequently detected in wild animals such as crows that moved broadly and their living areas are overlapped with ours. For public health, it is necessary to clarify the epidemiological relationship between *L. monocytogenes* from wild animals and that from human cases of listeriosis and food.

ACKNOWLEDGMENT. This work was partly supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science, Sports and Culture (No. 03506001).

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