Prevalence of *Listeria monocytogenes* in Intestinal Contents of Healthy Animals in Japan

Takashi IIDA, Masako KANZAKI, Tsutomu MARUYAMA, Satoshi INOUE, and Choji KANEUCHI

The Tokyo Metropolitan Research Laboratory of Public Health, 24-1, Hyakunin-cho 3 chome, Shinjuku-ku, Tokyo 169, The National Institute of Health, 2 chome, Kamiosaki, Shinagawa-ku, Tokyo 141, and School of Veterinary Medicine, Azabu University, Sagamihara, Kanagawa 229, Japan

(Received 17 December 1990/Accepted 3 July 1991)

**ABSTRACT.** A total of 1,705 fecal specimens or ileo-cecal contents of cattle, pigs, dogs, cats, chicken and rats were submitted for the isolation of *Listeria monocytogenes* by the use of the combination of Oxford-LPM agar plates after the cold enrichment in PBS at 4°C for 4–6 weeks. Prevalence of *L. monocytogenes* was found to be 1.9% in cattle, 0.6% in pigs, 0.9% in dogs and 6.5% in rats. However, none of *L. monocytogenes* was isolated from chicken or cats. Among 26 isolates of *L. monocytogenes*, 13 strains (50%) were classified into types 1/2a (3 strains), 1/2b (5 strains) and 4b (5 strains) and were often associated with human listeriosis. The majority of the *Listeria* spp. other than *L. monocytogenes* isolated from these animals was found to be *L. innocua.*—**KEY WORDS:** animal feces, *Listeria monocytogenes*, Oxford medium, prevalence.

---

*L. monocytogenes* is known to be an etiologic agent of zoonosis, and to cause premature birth meningitis and septicaemia in both man and animals [3, 18]. Recently, however, a series of mass outbreaks of human listeriosis due to the intake of the food contaminated with *L. monocytogenes* have been reported in U.S.A., Canada and European countries, and a part of human listeriosis was proved to be a food-borne infection [2, 9, 15]. In these countries, evidences have been accumulated on the contamination of food by *L. monocytogenes*, especially milk and meat and their products [5, 11, 20].

On the other hand, in Japan, hitherto more than 600 sporadic human listeriosis have been reported, but little is known about concerning their epidemiological background [14]. Thus, in this survey, isolation of *L. monocytogenes* from animals, which are closely related with human living environment of man, was attempted, and serotyping of the isolates was subjected to clarifying the source of human infections.

**MATERIALS AND METHODS**

*Specimens:* During the period from April 1989 to March 1990, a total of 1,705 specimens were subjected to the presence of *L. monocytogenes*. Namely, ileo-cecal contents of food animals, which consisted of 312 cattle and 343 pigs and were obtained at 2 abattoirs in Tokyo, fresh fecal specimens from 540 dogs and 161 cats obtained at the stray animal pound in Tokyo, ileo-cecal contents of 199 rats (*Rattus rattus*) caught in the buildings in Tokyo, and 150 fresh chicken droppings obtained at 4 chicken farms in the suburbs of Tokyo.

*Media:* As for the cold enrichment, 10 mM PBS, pH 7.4 was used. A combination of Listeria selective agar base (Oxford: Oxoid) and Listeria agar base (LPM: Difco) was used for the isolation.

*Isolation:* One gram of the specimen was placed into 9 ml of enrichment medium, and incubated at 4°C for 4–6 weeks. Then, it was plated onto Oxford agar plate, and incubated at 30°C for 48 hours. After the incubation, black-brown colonies with esculin utilization were subcultured onto LPM agar plate, and incubated at 30°C for 24 hours. Then, colonies appeared were observed under the low-power microscope with obliquely reflected light, and typical blue-greenish colonies were fished, and subcultured onto Tryptose agar (Difco) plate.

*Identification:* Identification of the genus *Listeria* was made by Gram staining, catalase test, VP reaction, and observation of umbrella like growth and motility in semisolid agar [16]. 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* [17].

*Serological typing:* Antisera were prepared from hyperimmune sera obtained by immunizing rabbits with known reference strains for the serotyping of the isolates. Descriptions of the serogroup of the isolates were followed after Seeliger [17].
RESULTS

Prevalence of L. monocytogenes in the 6 animal species: Prevalence of Listeria spp. organisms and L. monocytogenes in the 6 species of animals is shown in Table 1. In the ileo-cecal contents of cattle, 16 (5.1%) out of 312 specimens harbored Listeria spp., and 6 of them were identified as L. monocytogenes. Prevalence of Listeria spp. was 12.2% in pigs, 2.0% in dogs and 1.7% in rats. Among them, 0.6% of pig isolates, 0.9% of dog isolates, and 6.5% of rat isolates were found to be L. monocytogenes. In other words, rats showed the highest positive rate among the animals.

In the chicken droppings, 4.7% of them had harbored Listeria spp., but none of them was identified as L. monocytogenes. None of the fecal specimens from cats harbored Listeria spp. organisms. Throughout the investigation period, it was found that most of Listeria spp. isolates other than L. monocytogenes were identified as L. innocua.

Serological typing of Listeria organisms: It was found that 26 out of 1,705 specimens harbored L. monocytogenes, and each positive specimen harbored a single serotype of the organisms (Table 1). Six isolates from cattle were identified as serotypes 1/2a and 1/2c, 2 isolates from pigs as serotypes 1/2b and 4b, 5 isolates from dogs as serotypes 1/2c, 4b, and 4 with unidentified subtype antigens and 13 isolates from rats as serotypes 1/2a, 1/2b, 1/2c, 4ab and 4b.

DISCUSSION

Little is known about the prevalence of Listeria spp. and L. monocytogenes in animals in Japan since there have been few reports on animal listeriosis, especially on its epidemiological study in this region [13].

Prevalence of L. monocytogenes in cattle and pigs studied at 2 abattoirs in Tokyo was relatively low (1.9% in cattle and 0.6% in pig), compared with the results reported in foreign countries (9.2% in cattle and 1.7% in pig) [4, 5, 19]. However, it is of interest that the prevalence of L. monocytogenes in cattle was comparatively higher than that of in pigs, although the prevalence of Listeria spp. in pigs was higher than that of in cattle.

None of the chicken droppings from 4 chicken farms harbored L. monocytogenes, however, further studies will be necessary to support the results since it is well known that the prevalence of certain pathogens such as Salmonella varied according to the farms and/or flocks [1, 19].

As for the prevalence of Listeria spp. in the specimens of pet animals, it was found that the specimens from 11 (2%) out of 540 dogs harbored the organisms, and that 5 of them were identified as L. monocytogenes. Furthermore, 2 out of these 5 isolates were classified into type 4b which were often associated with human listeriosis [12], suggesting that these pet dogs can be a source of human infection. However, none of the organisms was isolated from cats.

L. monocytogenes is known as a pathogen for rodent, and it is well known in many countries that rats are often carriers of the organisms [10, 16]. Quite recently, Inoue et al. [6] have reported the higher prevalence of L. monocytogenes in Rattus rattus in Tokyo. In this survey, it was also clarified that 13 (6.5%) out of 199 specimens from Rattus rattus contained L. monocytogenes and 2 of them

<table>
<thead>
<tr>
<th>Animals</th>
<th>Number of specimens</th>
<th>Number of positive Listeria spp. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serotype</td>
<td>L. monocytogenes</td>
</tr>
<tr>
<td></td>
<td>1/2a</td>
<td>1/2b</td>
</tr>
<tr>
<td>Cattle</td>
<td>312</td>
<td>6(1.9)</td>
</tr>
<tr>
<td>Pig</td>
<td>343</td>
<td>2(0.6)</td>
</tr>
<tr>
<td>Chicken</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>Dog</td>
<td>540</td>
<td>5(0.9)</td>
</tr>
<tr>
<td>Cat</td>
<td>161</td>
<td>0</td>
</tr>
<tr>
<td>Rat</td>
<td>199</td>
<td>13(6.5)</td>
</tr>
<tr>
<td>Total</td>
<td>1,705</td>
<td>26(1.5)</td>
</tr>
</tbody>
</table>
were type 4b. This suggests that rats sharing the human living environment are dominant sources of the organisms in the natural environment.

High incidence of *L. monocytogenes* in commercial meat (29.3–39.9%) [7, 8] and its related materials such as a knife (26.9%) and a table (31.0%) [13] was reported in Japan. They consisted of the same serotypes of organisms isolated from carrier food animals, although the prevalence of the organisms in food animals and pet animals were relatively low. However, such carrier food animals are likely to play an important role in the contamination of abattoirs or meat processing plant.

ACKNOWLEDGEMENTS. The authors are greatful to the staffs of both Shibaura and Tama Meat Inspection Laboratories for their assistance of collecting samples.

REFERENCES