Isolation of *Listeria monocytogenes* from the Skin of Slaughtered Beef Cattle

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(Received 12 March 2007/Accepted 20 June 2007)

**ABSTRACT.** We attempted to isolate *Listeria monocytogenes* from skin, contents of large intestines and carcasses of cattle introduced to a slaughterhouse in order to identify source of contamination for this pathogen. Sixty skin samples, 60 samples of the contents of large intestines and 30 carcass samples were collected in June, August and November 2003 for use in this study. *Listeria* spp. and *L. monocytogenes* were isolated from 30 (50%) and 3 (5%) of the cattle skin samples, respectively. However, no *Listeria* spp., including *L. monocytogenes*, were isolated from intestinal contents or carcasses. Seven isolates were obtained, of which five and two strains were serotypes 1/2a and 1/2b, respectively. Genetic analysis suggested that there was persistent inhabitation of the pathogen around the area investigated in this study.

**KEY WORDS:** cattle, *Listeria monocytogenes*, skin.

*Listeria monocytogenes* is recognized as an important foodborne pathogen [17]. Listeriosis in human is both an invasive disease that manifests in various forms, such as encephalitis and meningitis neurological infections, abortion and septicemia, and a non-invasive gastrointestinal infection [13]. Listeriosis outbreaks have been reported in North America and Europe since the 1980s [2, 5, 12]. In Japan, a foodborne outbreak of listeriosis caused by contaminated cheese was first documented in 2001 [6]. Elucidation of the ecology of *L. monocytogenes* should be useful for control of this bacterial infection in humans.

Previous studies have isolated *L. monocytogenes* from food processing environment [4, 9, 11]. Animal food products, such as milk, dairy and meat products, have been suggested as especially important infectious sources of the pathogen. Isolation of *L. monocytogenes* from foods and animal stool samples has been reported in some countries [1]. A previous study reported isolation of *L. monocytogenes* from cow stools, carcasses and meat products, and suggested that feces was the most likely source of contamination of the carcasses [3]. The pathogen has also been isolated from environments such as slaughterhouses and sewage, sludge and food-processing plants [4, 8, 9]. These reports suggest the presence of various sources of *L. monocytogenes* contamination in the environment. In the present study, we attempted to isolate *L. monocytogenes* from skin samples of beef cattle introduced to a slaughterhouse, which also has the potential to be the source of *L. monocytogenes* contamination in the food processing environment. The serotypes of the isolated strains were determined, and the genetic characteristics of the strains were investigated by determination of partial *iap* nucleotide sequences as described previously [15, 16].

Twenty skin and carcass swabs and the contents of 10 large intestines were collected from beef cattle introduced to a slaughterhouse in Iwate Prefecture, Japan, in June, August and November of 2003. Sections of chest and rump skin and carcass measuring 100 cm² were swabbed with sterile gauze. One g of the intestinal contents was collected and used for isolation of *L. monocytogenes*. The propagation step was performed with UVM broth (Oxoid, Tokyo, Japan) as described by McClain and Lee [7]. The resultant culture was incubated on PALCAM agar (Oxoid) for 48 hr at 35°C. Five to 15 suspicious colonies on the PALCAM agar were subcultured on Tryptic Soy Agar (Nissui, Tokyo, Japan) for 48 hr at 35°C, and then the obtained strains were used for identification of species. Species were identified by gram staining, catalase production and VP tests, umbrella like growth and motility in SIM semiliquid agar (Nissui), utilization of rhamnose, xylose and mannitol and β-hemolysis on Tryptic Soy Agar containing 5% defibrinated sheep blood. Serotyping of the *L. monocytogenes* isolates was performed as described previously [16]. Determination of nucleotide sequences for the partial *iap* was performed as described previously [15].

*Listeria* spp. were isolated from skin swabs of half of the slaughtered beef cattle investigated in the present study (Table 1). The isolation rates were 80, 25 and 45% for June, August and November 2003, respectively, suggesting that there was persistent contamination in the slaughtered beef cattle in this area. Further tests identified *L. monocytogenes* in one and two beef cattle investigated in August and November, respectively. A total of seven isolates were obtained; four strains were isolated from the cattle examined in August, and three strains were isolated from the other two cattle (one from one cow and two from the other) examined in November (Table 2). These results suggest that beef cattle skin plays a role as a source of contamination for this.
pathogen. In contrast, no Listeria spp., including L. monocytogenes, were isolated from any samples of the contents of large intestines. However, previous studies have isolated L. monocytogenes from up to 50% of feces collected from cattle with no clinical symptoms [8, 18]. In farm ruminants, silage is suspected to be an infection source for this pathogen [14, 19]. Beef cattle in Japan, however, are usually fed high-energy diets because of rapid fertilization, and therefore they may have only rare or no opportunity to consume silage. Therefore, different types of feed may lead to the distinct isolation rates for L. monocytogenes from feces or intestinal contents. We failed to isolate the organisms from the carcass swabs investigated in the present study, and this suggests that contamination of the meat and food products derived from livestock may occur in later processes during slaughter. However, the high contamination rate for the cattle skin suggests that it is necessary to examine the carcass for this pathogen.

The strains of L. monocytogenes isolated in the present study were serotypes 1/2a or 1/2b, which are frequently isolated from both human listerioses cases and food processing environments [1]. Out of seven isolates, five and two were serotypes 1/2a and 1/2b, respectively (Table 2). Both of serotypes were isolated in August and November. Both serotypes of L. monocytogenes were isolated from the same beef cattle investigated in August, showing the presence of contamination by multiple serotypes of this pathogen.

The nucleotide sequences for the amplified region of iap were identical among the serotype 1/2a strains isolated in August (1E2, 1E3 and 1E4). The sequences of 3E1 and 3E2, which were serotype 1/2a strains isolated in November, were also identical. In addition, the sequences of the 1/2a strains isolated in August were identical to those isolated in November, except the isolates in November contained a greater number of repeated sequences, composed of ACACAAT or ACGAAT compared with those in August. Although there was also a difference in the numbers of repeated sequences between 1E1 and 2E1, which were serotype 1/2b strains isolated in August and November, respectively, there were only two nucleotide substitutions between these strains. Taken together, comparison of the sequences among the same serotype isolates showed that the differences were mostly limited to the numbers of repeated sequences; this has previously been reported in detail as the polymorphic region of iap [10]. The obtained genetic similarity likely persistent contamination of L. monocytogenes around the area investigated in this study.

In conclusion, Listeria spp., including L. monocytogenes,
was frequently isolated from the skin of slaughtered beef cattle but not from the contents of large intestines and carcasses, suggesting that the skin is an important source of *L. monocytogenes* infection. The isolated strains were serotypes 1/2a or 1/2b. The close genetic relationships among the same serotype isolates likely supports persistent inhabitation of the pathogen around this area.

ACKNOWLEDGMENT. This study was partially supported by a grant from the Ministry of Health, Labor and Welfare of Japan.

REFERENCES