Sources and Routes of Contamination of Raw Milk with *Listeria monocytogenes* and Its Control

Tetsuya YOSHIDA, Yumiko KATO¹, Moritoshi SATO and Katsuya HIRAI²

Division of Food Hygiene, Nagano Research Institute for Health and Pollution, 1978 Komemura, Amori, Nagano 380–0944, ¹Matsumoto Meat Inspection Center of Nagano Prefecture, 9839 Shimauchi, Matsumoto 390–0851, and ²Department of Veterinary Microbiology, Faculty of Agriculture, Gifu University, 1–1 Yanagido, Gifu 501–1112, Japan

(Received 16 December 1997/Accepted 5 June 1998)

ABSTRACT. To identify the source of contamination of raw bulk milk with *Listeria*, we attempted to isolate the bacteria from various samples such as cattle-related samples, bulk storage tanks and the environments on three farms. On farms A and B, *Listeria monocytogenes* was repeatedly isolated from raw milk, while on farm C, it was scarcely isolated from it. On the former farms, *Listeria* was detected in cattle-related samples and the environments. On the other hand, only one fecal sample on the latter farm was *Listeria*-positive. Especially, we demonstrated that the bulk tank on farm A was contaminated with *L. monocytogenes*. Then, *L. monocytogenes* was controlled by continuously washing the bulk tank on farm A with alkaline detergent. — KEY WORDS: contamination route and source, *Listeria monocytogenes*, raw milk.

After outbreaks of food-borne listeriosis in Western countries [7, 14, 19], there have been several reports of isolation of *Listeria monocytogenes* (*L. monocytogenes*) from various food items including raw milk [3–5, 10, 16]. We previously conducted a survey in Nagano Prefecture to study the prevalence of *Listeria* in bulk tank raw milk and found that the incidence differed from region to region [24]. A follow-up study was also made one year later for bulk tank raw milk on 56 farms with a high frequency of contamination and there being a seasonal variation in the incidence of *Listeria*. The recovery of the organisms differed among the farms investigated.

Two possible routes of contamination into bulk tank raw milk have been generally suggested; by direct contamination from listeric infections or mastitis and by secondary contamination from feces or the environment. To improve milk safety, it is important to determine the sources and the routes of contamination with *L. monocytogenes*, but there have been only a few epidemiological studies on this subject [21].

The purpose of this study was to identify the source of contamination among farms that had shown a high frequency of detection of *L. monocytogenes* in the bulk tank raw milk in the previous study. The effectiveness of a method controlling listeric contamination was also investigated.

The study was conducted from February 1991 through January 1994 on three farms. The three were located in the southern area of Nagano Prefecture and small-scale farms keeping three to 11 milking cows. One of the three farms (farm A) was practicing milking with a bucket milker; the other two farms B and C were practicing milking with a pipeline milker.

Cattle-related samples were obtained in the following manners: Each dropping of feces was collected in a sterilized plastic bag. The surface (100 cm² area) of the udders was wiped with sterilized gauze tampon swabs. Milk from each udder quarter immediately before milking was collected into sterilized plastic bags.

Bulk tank raw milk samples and samples from environments, cattle feed, milkers and bulk tanks were obtained in the following way: Bulk tank milk, sawdust bedding, farmyard manure, soil in which fodder plants were grown, and cattle feed such as silage, hay cubes, beet pulp and wheat lees were collected in sterilized plastic bags. Well water, which was being used on farm A for cattle drinking, was collected in a sterilized plastic bag. Rubber liners of milkers, which are highly subject to contamination and the insides of the bucket milkers that were used only on farm A were sampled. Various parts of bulk tank surfaces (100 cm² area) such as inner and outer walls, agitator, discharging valve and funnel were wiped with sterilized gauze tampon swabs moistened with sterilized saline on farms A and B.

Bulk tank raw milk and milk from udder quarters were enriched and cultured as described previously [24]. The isolation method used for the other samples was a two-step enrichment procedure [18]. In this procedure, gauze samples were suspended in 9 ml of UVM broth (Difco, Detroit), and 25 ml of a liquid sample or 25 g of a solid one was added to 225 ml of UVM broth, which was incubated at 30°C for 24 hr. After incubation, 1 ml of the culture was added to 9 ml of secondary enrichment broth, which was incubated at 30°C for 24 hr. The secondary enrichment broth was UVM broth supplemented with acriflavine to a final concentration of 25 mg/l.

An aliquot (0.1 ml) of the secondary enrichment broth culture was plated on a PALCAM agar (Merck, Darmstadt) plate, as previously described [24]. Five to 10 suspect colonies of all positive samples were first tested for biochemical characteristics of *L. monocytogenes* by the method of Seeliger and Jones [20] and then confirmed by the CAMP-test. Serovars of the isolates were identified with antisera for serotyping *Listeria* (Denkaseiken, Tokyo).

In this study period, *L. monocytogenes* was repeatedly
isolated from bulk tank raw milk on farms A and B, and only once from the milk on farm C (Table 1). Farm A gave positive isolation of \textit{L. monocytogenes} at all seventeen sampling times, and farm B also indicated comparatively high frequency (47.1%).

The prevalence of \textit{Listeria} among the cattle on the three farms investigated is shown in Table 2. The fecal samples as well as the bulk tank milk samples from farms A and B showed high rates of \textit{Listeria} contamination (63.6% on farm A and 50.0% on farm B). There were a total of 18 \textit{Listeria}-positive animals on these two farms and 15 of them had \textit{L. innocua}, other two \textit{L. ivanovii}, and the other one \textit{L. welshimeri}. Animals on farm A had higher positive rates (75.0% in February and 57.1% in July) in the udder surface samples, all of which contained \textit{L. innocua}.

The milk samples from udder quarters had a lower rate of \textit{Listeria} contamination (0.0%–16.7%) on both farms A and B. On farm B, \textit{L. innocua} was found in milk samples from three udder quarters of one animal, while on farm A, \textit{Listeria} was not found in milk from more than one udder quarter of a single animal. Among six \textit{Listeria}-positive udder quarters on farms A and B, five were posterior udder quarters, which are easily defiled with droppings. The animals whose milk was contaminated with \textit{Listeria} also had the organisms on the surface of the udder. These results indicate that the posterior udder quarters could be one of the sources of contamination of the bulk tank milk. The differences in the isolation rate by different sampling dates were scarcely shown on farm A or B. On farm A, \textit{L. monocytogenes} serovar 4b only was detected in milk from two udder quarters in July 1993. In June 1993, only fecal samples yielded \textit{Listeria} on farm B. On farm C, only one fecal sample yielded \textit{Listeria} (\textit{L. innocua}).

The prevalence of \textit{Listeria} in the farm environments, cattle feed, milkers and bulk tanks is shown in Table 3. Sawdust bedding, farmyard manure and soil on two farms A and B with high detection frequency of \textit{L. monocytogenes} in bulk tank milk yielded \textit{L. innocua} (66.7%) and thus these sites could be important contamination sources. No \textit{Listeria} was found in the feed or milkers on farm A or B, or the environmental samples on farm C. The pH values of silage samples measured as described previously [18] were 3.78–4.62, and their qualities, as determined by color and smell, were good. Listeriosis of cattle is considered to be strongly associated with silage [8] and has been called “silage disease”. In spite of reports on the isolation of \textit{Listeria} from silage with a low pH, lower than 4.0 [6, 18], we did not find \textit{L. monocytogenes} nor any other \textit{Listeria} in

Table 1. Prevalence of \textit{Listeria monocytogenes} in bulk tank raw milk on three farms\(^a\)

<table>
<thead>
<tr>
<th>Farm</th>
<th>1991</th>
<th>1992</th>
<th>1993</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+(^b)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>+(^c)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^a\) The data between February 1991 to January 1992 overlap with those in the previous report [24]. \(^b\) \textit{L. monocytogenes} serovar 4b was isolated. \(^c\) Not isolated.

Table 2. Incidence of \textit{Listeria} in cattle-related samples on three farms

<table>
<thead>
<tr>
<th>Farm</th>
<th>Examined month and year</th>
<th>Number of positive samples/number of samples examined (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feces(^a)</td>
<td>Surface of udder(^b)</td>
</tr>
<tr>
<td></td>
<td>All \textit{Listeria} spp.</td>
<td>All \textit{Listeria} spp.</td>
</tr>
<tr>
<td>A</td>
<td>Feb. ‘92</td>
<td>4/4 (100.0)</td>
</tr>
<tr>
<td></td>
<td>Jul. ‘93</td>
<td>3/7 (42.9)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>7/11 (63.6)</td>
</tr>
<tr>
<td>B</td>
<td>Jun. ‘92</td>
<td>7/11 (63.6)</td>
</tr>
<tr>
<td></td>
<td>Jun. ‘93</td>
<td>4/11 (36.4)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>11/22 (50.0)</td>
</tr>
<tr>
<td>C</td>
<td>Jun. ‘92</td>
<td>1/5 (20.0)</td>
</tr>
</tbody>
</table>

\(^a\) \textit{L. monocytogenes} was not isolated from these samples. \textit{L. innocua} was isolated from the other positive samples. \(^b\) \textit{L. monocytogenes} serovar 4b was isolated from the two samples. \(^c\) Three positive udder quarters were yielded from different three cows. \(^d\) One cattle had three positive udder quarters. \(^e\) \textit{L. ivanovii} was isolated from two samples. \textit{L. innocua} and \textit{L. welshimeri} were isolated from each of the remaining two samples.
Sources of contamination of raw milk with Listeria

The bulk tank on farm A was found to be contaminated with L. monocytogenes. We assumed that if the tank was the primary source of contamination, the concentration of L. monocytogenes in the milk in the tank would decrease dramatically as fresh, relatively uncontaminated milk flows into the tank. We conducted examinations twice to substantiate this assumption in October and November 1993.

The milk samples (500 µl) of a dilution series of 10^0, 10^-1 and 10^-2 were each spread onto each of three PALCAM agar plates and typical colonies on the plates were counted and the number of the organisms in whole bulk tank milk was calculated. The results proved that our assumption was true, that is, the number of L. monocytogenes (cfu/ml) showed a sharp decrease during an hour period from the start to the end of milking (Fig. 1).

Further, we attempted to control L. monocytogenes by washing the bulk tank with alkaline detergent (CleanA, Clean Kagaku Kougyou, Hokkaido) each time the tank was emptied every other day to load the milk into a road tanker. The detergent consisted mainly of 5% of NaOH and 12% of NaOCl, diluted 200-fold with water (pH=11.7 ± 0.2). The number of L. monocytogenes in the bulk tank raw milk enumerated as described above was determined to evaluate the effectiveness of the practice. At the beginning of this practice, L. monocytogenes was not recovered from the surface of stainless bulk tank steel such as the inner wall, outer wall and agitator, and the number of organisms in the bulk tank milk reduced dramatically to 87 cfu/ml. One month later of the continuous practice, the number of organisms in the bulk tank milk decreased to less than 1 cfu/ml.

Frequent cleaning of the empty tank on farm A was shown to be effective in preventing contamination with L. monocytogenes. The constituents of the detergent used were NaOH and NaOCl. The former eliminated milk fat on the tank surface and the latter, acting as a germicide, killed L. monocytogenes. Lopes [15] and Brackett [2] reported that the concentration of available chlorine over 50 ppm was effective in destroying L. monocytogenes. The concentration...
of NaOCl in the detergent used in this study was 600 ppm and considered to be sufficient for this purpose.

Two publications [11, 17] reported that _L. monocytogenes_ sticks to the surface of stainless steel with vast amounts of polymeric materials. Hood and Zottola [12] showed that a level of about 10³ _L. monocytogenes_ cells/cm² adhered to the surface of stainless steel chips in reconstituted skim milk at 23°C for 1 hr. As the tank of farm A was not thoroughly cleaned with detergent, _L. monocytogenes_ on the surface was probably protected by a layer of milk fat, protein or saccharide. The remaining organisms would strongly adhere with their excreted polymers to the residual milk on the surface of the empty tank, and then start to grow again. As a result, the raw milk poured into the tank was contaminated continuously to a concentration of approximately 1,000–3,000 cfu/ml (Fig. 1).

Based on our results, we suggested that the bulk storage tank on farm A was a very important source of secondary contamination of raw milk with _L. monocytogenes_ beside the previously recognized sources such as the environment [6, 22, 23], feed [6, 18], cattle feces [13] and wild life [1, 6, 9]. It is not possible to eliminate _L. monocytogenes_ in the cattle intestines or from the environment. However, effective control could be achieved by avoiding secondary contamination due to improper milking procedures and by washing _L. monocytogenes_ adhering to the surfaces of bulk tanks and instruments by continuously using an alkaline detergent.

ACKNOWLEDGEMENTS. We thank the Milk Testing Association in Nagano Prefecture for providing raw milk samples used in this study. We are also indebted to Mr. Y. Imamura, Imamura Animal Clinic, for his invaluable help in sampling on the dairy farms. This work was supported partially by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture (No. 03919008).

REFERENCES