Prevalence and Characterization of Foodborne Pathogens in Dairy Cattle in the Eastern Part of Japan

Yoshimasa Sasaki1)*, Mariko Murakami1), Mika Haruna1), Noriko Maruyama2), Tetsuya Mori3), Kazuo Ito1) and Yukiko Yamada1)

1)Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries, 1–2–1 Kasumigaseki, Chiyoda-ku, Tokyo 100–8950, Japan
2)Research Institute for Animal Science in Biochemistry and Toxicology, 3–7–11 Hashimotodai, Midori-ku, Sagamihara, Kanagawa 252–0132, Japan
3)Institute for Food and Environmental Sciences, Tokyo Kenbikyo-in Foundation, 5–1 Toyomi-cho, Chuo-ku, Tokyo 104–0055, Japan

NOTE

Public Health

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(Received 23 July 2012/Accepted 19 November 2012/Published online in J-STAGE 3 December 2012)

ABSTRACT. To investigate the prevalence and characterization of foodborne pathogens [Campylobacter spp., Shiga toxin-producing Escherichia coli (STEC), Listeria monocytogenes and Salmonella spp.] in dairy cows, rectal content grab samples were collected from 250 dairy cows reared on 25 dairy farms in eastern Japan from December 2010 through February 2011. Campylobacter jejuni was isolated from 106 (42%) cows on 23 (92%) farms, STEC O157 from three cows on one farm, L. monocytogenes from three cows on another three farms and Salmonella enterica subsp. enterica serovar Typhimurium from eight cows on another farm. STEC O26 was not isolated from any of the dairy farms investigated. The results suggest that C. jejuni is widespread in dairy farms in eastern Japan.

KEY WORDS: Campylobacter, dairy cattle, Listeria monocytogenes, Salmonella, STEC O157.

After incubation, a loopful of each broth was streaked on CHROMAgar Listeria (CHROMagar, Paris, France) and incubated at 37°C for 48 hr. Five suspected colonies were subjected to slide agglutination with O and H antisera (Denka Seiken). Four virulence-associated genes (actA, hly, iap and prfA) were previously investigated by PCR analysis [2, 28].

Isolation of Salmonella was carried out as previously described [21]. Salmonella isolates were tested by slide agglutination with O antisera (Denka Seiken) and tube agglutination with H antisera (Denka Seiken). Serovars were determined on the basis of reaction with O- and H-group antigens according to the Kauffmann-White scheme [18].

Campylobacter was isolated from 106 (42.4%) dairy cows from 23 (92%) farms (Table 1). All Campylobacter isolates were C. jejuni. This result suggests that C. jejuni is widespread in dairy farms in eastern Japan. The prevalence of Campylobacter in dairy cattle in the present study (42.4%) was considerably higher than that in Japanese beef cattle reported by Ishihara et al. [11] (17.8%). In the 8 years since that study, several studies conducted outside of Japan showed prevalence rates higher than 50% for Campylobacter in dairy and beef cattle [7–9, 15]. Because these results suggest an increase in the prevalence of Campylobacter in beef cattle in recent years, we propose that studies on the current prevalence of Campylobacter in beef cattle are needed. With regard to the antimicrobial susceptibility of Campylobacter, all the isolates were susceptible to GM (MIC≤0.5 mg/l), CP (MIC≤4 mg/l) and EM (MIC≤4 mg/l). High isolation rates of resistance bacteria were observed against OTC (28%, 30/106; MIC≥16 mg/l) and ERFX (28%, 30/106; MIC≥2 mg/l) (Table 2). Thirty isolates from 15 farms (60%, 15/25) were resistant to ERFX and 14 isolates from 9 farms (36%, 9/25) were resistant to both OTC and ERFX. This result suggests that C. jejuni resistant to ERFX has already spread to more than half of the dairy farms in eastern Japan. Although erythromycin is considered to be the first-line drug for treatment of human campylobacteriosis, fluoroquinolones are often used for treatment of human enteritis when no microbiological diagnosis is available [6]. ERFX should be used for the treatment of diseases in dairy cows after confirming the absence of ERFX-resistant C. jejuni in the isolates obtained from the farms.

Campylobacter prevalence between age groups ranged from 35% in animals above 60 months of age to 50% in those below 30 months (Table 3). The difference in prevalence between age groups was not significant (P>0.15, chi-square test). This result suggests that the susceptibility of lactating dairy cows to Campylobacter is not influenced by age.

Three E. coli O157:H7 isolates were obtained from three (1.2%) dairy cows on one farm, whereas no E. coli O26 isolates were obtained on any of the tested farms. All three E. coli O157:H7 isolates were positive for stx1, EHEC-hlyA, eae and rfbE and produced Stx1. The prevalence of STEC O157 in lactating cows (1.2%) was lower than that in beef cattle (8.9%, 218/2436) reported in our previous study [19]. We propose that this difference in the prevalence of STEC O157 between dairy and beef cattle reflects the entirely different feeding and hygiene management on beef and dairy farms rather than the breed difference, because it has been reported that there was no association between the prevalence of STEC O157 and the cattle breed [Japanese Black (JB), HF and the first-generation hybrid of JB and HF] [19]. The identification of risk factors for prevalence of STEC O157 on dairy farms may be helpful for the identification of the risk factors on beef farms.

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Table 1. Isolation of foodborne pathogens from dairy cows

<table>
<thead>
<tr>
<th></th>
<th>Campylobacter spp.</th>
<th>STEC O157</th>
<th>L. monocytogenes</th>
<th>Salmonella spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm</td>
<td>No. of positive case (%)</td>
<td>23 (92)</td>
<td>1 (4)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Dairy cow</td>
<td>250</td>
<td>106 (42.4)</td>
<td>3 (1.2)</td>
<td>3 (1.2)</td>
</tr>
</tbody>
</table>

Table 2. Antimicrobial resistance profiles of Campylobacter isolates

<table>
<thead>
<tr>
<th>Antimicrobial resistance profile</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>59</td>
</tr>
<tr>
<td>Resistant against</td>
<td></td>
</tr>
<tr>
<td>DSM</td>
<td>1</td>
</tr>
<tr>
<td>OTC</td>
<td>15</td>
</tr>
<tr>
<td>NA-ERFX</td>
<td>16</td>
</tr>
<tr>
<td>DSM-OTC</td>
<td>1</td>
</tr>
<tr>
<td>OTC-NA-ERFX</td>
<td>12</td>
</tr>
<tr>
<td>ABPC-OTC-NA-ERFX</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
</tr>
</tbody>
</table>

Table 3. Campylobacter prevalence among age groups

<table>
<thead>
<tr>
<th>Age group (months of age)</th>
<th>&lt;30</th>
<th>30–35</th>
<th>36–47</th>
<th>48–59</th>
<th>&gt;60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of Campylobacter-positive</td>
<td>50</td>
<td>38</td>
<td>47</td>
<td>43</td>
<td>35</td>
</tr>
</tbody>
</table>

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from 6 to 44%. The prevalence of *S. Typhimurium* in dairy farms varying widely in Japan occurred in 2001, and *L. monocytogenes* serovar 1/2b was isolated from not only feces of patients but also cheese and cow barn samples from the contaminated factory [12]. *L. monocytogenes* in dairy farms could thus cause foodborne listeriosis. *L. monocytogenes* was obtained from only one animal of each of the *L. monocytogenes*-positive farms, whereas the other pathogenic bacteria investigated tended to be isolated from two or more dairy cows in each target-positive farm. The result suggests that the within-farm prevalence of *L. monocytogenes* is very low and that samples of more than 10 animals are needed to determine the presence of *L. monocytogenes* on a given dairy farm. Takahashi et al. [22] reported that *L. monocytogenes* was isolated from the skins of cows (3%) of 60 beef cattle sampled at an abattoir, although *L. monocytogenes* isolates were not obtained from large intestinal contents. Ochiai et al. [17] reported that *L. monocytogenes* was isolated from 15.5% (17/110) of retailed beef samples and that the serovars of the isolates were 1/2a (three isolates), 1/2b (two isolates), 1/2c (eight isolates) and 4b (five isolates). It is possible that beef is contaminated with *L. monocytogenes* hiding in the skins of cattle at abattoirs. Therefore, environmental samples of cattle farms, including skins, should be taken to detect *L. monocytogenes* in dairy farms. *Salmonella* was isolated from eight dairy cows (3.2%) on one farm, and all eight isolates were *Salmonella enterica* subsp. *enterica* serovar Typhimurium. Asai et al. [1] reported similar results in that the prevalence of *Salmonella* in beef cattle was 2.5% (16/650) and the predominant serovar was *S. Typhimurium*, suggesting that the results of the present study are compatible with those of Asai et al. [1]. In the present study, although samples were obtained from 10 healthy dairy cows without clinical signs of salmonellosis, eight of the animals tested on one farm were positive for *S. Typhimurium*. Van Schaik et al. [25] reported a within-herd prevalence of *S. Typhimurium* in dairy farms varying widely from 6 to 44%.

This survey was conducted only in eastern Japan and only between December 2010 and February 2011. Therefore, there may be geographical and seasonal variation in the prevalence of foodborne pathogens in dairy cattle. For example, a seasonal variation in prevalence of *E. coli* O157 in dairy farms of several countries has been reported with a peak in summer [5, 10, 14, 24], suggesting that continuous and nationwide surveys of the prevalence of foodborne pathogens in dairy cattle in Japan are essential.

ACKNOWLEDGMENT. This study was funded by the Ministry of Agriculture, Forestry and Fisheries of Japan.

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