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

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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 monocytognes*, *Salmonella*, STEC O157.

Campylobacter spp., Shiga toxin-producing *Escherichia coli* (STEC) O157 and O26, *Salmonella* spp. and *Listeria monocytogenes* are important bacterial agents of foodborne disease in humans. These bacteria occasionally colonize the intestinal tracts of animals and birds and are excreted in the feces. Consumption of contaminated beef and dairy products can cause foodborne disease [12, 13]. The beef of dairy cows accounted for approximately 24% of beef production in Japan from 2006 to 2009 [23]. Moreover, dairy cows bear both dairy and beef calves. If the latter is infected with foodborne pathogens, the pathogens are carried to beef farms. Therefore, the prevalence and characteristics of these bacteria in dairy cows are essential background information for the application of food safety measures along the food chain. Therefore, we investigated the prevalence and characterization of these foodborne pathogens in dairy cattle.

Owners of 25 dairy farms in five prefectures in eastern Japan voluntarily participated in this study. None of the cattle in these farms were vaccinated against salmonellosis. The average number of dairy cows raised on each farm was 128 (minimum = 30; maximum = 600). On each farm, 10 healthy lactating dairy cows were selected. All lactating dairy cows investigated in this study were Holstein-Friesian (HF) with average age 47.2 months (minimum = 25; maximum = 156). From each animal, rectal content grab samples of approximately 100 g were collected between December 2010 and February 2011. The samples were sent to the Research Institute for Animal Science in Biochemistry and Toxicology by express delivery under

refrigeration and examined within 72 hr of collection.

Isolation of *Campylobacter* was carried out as previously described [20]. A *Campylobacter* polymerase chain reaction (PCR) detection kit [*Campylobacter* (cdt gene) PCR Detection and Typing Kit, Takara Bio Inc., Tokyo, Japan] was used to identify *Campylobacter jejuni* and *C. coli*. In addition, the minimum inhibitory concentration (MIC) of eight antimicrobial agents [ampicillin, dihydrostreptomycin, gentamicin (GM), oxytetracycline (OTC), chloramphenicol (CP), erythromycin (EM), nalidixic acid (NA) and enrofloxacin (ERFX)] was determined using the agar dilution method of the Clinical and Laboratory Standards Institute [3]. *Campylobacter jejuni* ATCC33560 was used as quality control strains. Except for GM, resistance breakpoints of ≥2 mg/l, as specified by the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme [4] and adopted in previous reports [11, 16], were used in this study.

Isolation of *E. coli* O157 and STEC O26 was carried out as previously described [19]. *E. coli* O157 isolates were tested by tube agglutination with H antisera (Denka Seiken Co. Ltd., Tokyo, Japan). The types of the Stx genes (stx_{1a}, stx_{2a}, stx_{2c}, stx_{2d}, stx_{2e} and stx_{2f}) and enterohaemorrhagic *E. coli* (EHEC) *hlyA*, *eae*, $rfbE_{O157}$ and $fliC_{H7}$ were investigated by PCR analysis using primers reported by Wang *et al.* [27]. The production of Stx1 and/or Stx2 was confirmed by reverse passive latex agglutination with a Shiga toxin detection kit (VTEC-RPLA SEIKEN, Denka Seiken).

For detecting *L. monoctogenes*, 25 g of each sample was incubated in 225

ml of Half-Fraser broth (Oxoid, Hampshire, UK) at 30ºC for 24 hr with shaking for enrichment. After incubation, a loopful of each broth was streaked on CHROMagar Listeria (CHROMagar, Paris, France) and PALCAM agar (Oxoid) and incubated at 37 ºC for 48 hr. In addition, 0.1 ml of the remaining Half-Fraser broth was enriched in 10 ml of Fraser broth (Oxoid) and incubated at 37ºC for 48 hr. Then, a loopful of each broth was streaked on CHROMagar Listeria and PALCAM agar and incubated at 37ºC for 48 hr. Five suspected colonies were subjected to Gram staining, motility test, catalase test, CAMP (Christie, Atkins, Munch-Peterson) test, carbohydrate utilization and biochemical identification by the BBL CRYSTAL Identification Gram-Positive ID kit (Becton Dickinson, Sparks, MD, U.S.A.). *L. monocytogenes* isolates were tested by 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 stx_{1a}, EHEC *hlyA, eae* and $rfbE_{O157}$ 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.

L. monocytogenes was isolated from three (1.2%) cows from three dairy farms (12%). The serovars of the *L. monocytogenes* isolates were 1/2b, 1/2a and 4b. All the *L. monocytogenes* isolates carried all of the virulence-associated genes (*actA*, *hly*, *iap* and *prfA*) tested in the study and gave positive results by CAMP test. The serovars (1/2a, 1/2b and 4b) of *L. monocytogenes* isolates in this study are common serovars in human

listeriosis [26]. The only reported foodborne listeriosis 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 three (5%) 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.

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Table 1. Isolation of foodborne pathogens from dairy cows

		No. of positive case (%)			
	n	*Campylobacter* spp.	STEC O157	*L. monocytogenes*	*Salmonella* spp.
Farm	25	23 (92)	1 (4)	3 (12)	1 (4)
Dairy cow	250	106(42.4)	3 (1.2)	3 (1.2)	8 (3.2)

Table 2. Antimicrobial resistance profiles of *Campylobacter* isolates.

Antimicrobial resistance profile	n
Susceptible	59
Resistant against	
DSM	1
OTC	15
NA-ERFX	16
DSM-OTC	1
OTC-NA-ERFX	12
ABPC-OTC-NA-ERFX	2
Total	106

DSM: dihydrostreptomycin, OTC: oxytetracycline, NA: nalidic acid, ERFX: enrofloxacin, ABPC: ampicillin.

Table 3. *Campylobacter* prevalence among age groups.

	Age group (months of age)				
	< 30	30 - 35	36 - 47	48 - 59	> 60
Percentage of *Campylobacter-positive*	50 (18/36)	38 (17/45)	47 (36/77)	43 (17/40)	35 (18/52)