

Isolation of *Yersinia* spp. from Bovine Feces

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Yersinia spp. were sought in 618 fecal samples from cows. Four strains of *Yersinia enterocolitica*, serotype O:12,26 (one), O:13,7 (two), and O:18 (one); seven strains of *Yersinia kristensenii*, serotype O:11,24 (five) and O:12,26 (two); and one strain of *Yersinia pseudotuberculosis* serotype IIB, were isolated. This is the first time that *Y. pseudotuberculosis* has been isolated from cows in Japan, and the isolation of serotype IIB of this organism from cows seems to be the first in the world.

Numerous *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* have been isolated from various species of animals (3, 7). However, there are few data on the isolation of these organisms from cows. In Japan, Inoue and Kurose (4) reported that *Y. enterocolitica* could be isolated with a rather high frequency (7.9%) from bovine intestinal contents, whereas Zen-Yoji et al. (13) recovered neither *Y. enterocolitica* nor *Y. pseudotuberculosis* from cecal contents of cattle. On the other hand, *Y. enterocolitica* was isolated from cattle imported from the United States and Canada (8; Morita, personal communication).

We isolated *Y. enterocolitica* and *Yersinia intermedia* from raw milk (Fukushima et al., submitted for publication). We looked for *Yersinia* spp. in milking cows to determine whether or not cows are carriers of these organisms and whether their feces may be a source of milk contamination.

Fecal samples were collected directly from the rectum of 618 cows from 87 farms in Shimane Prefecture. Isolations were made after cold enrichment culture. About 1 g of the sample was suspended in 10 ml of 0.067 M phosphate buffer solution (pH 7.6) and was kept at 5°C for 3 weeks. Then, a suspension was subcultured on salmonella-shigella agar (Difco Laboratories) and MacConkey agar plates at 25°C for about 40 h. The isolates were identified by conventional methods. Serological typing was performed by slide agglutination tests with O-antisera prepared in our laboratory.

Four strains of *Y. enterocolitica* biotype 1, seven strains of *Yersinia kristensenii*, and one strain of *Y. pseudotuberculosis* were isolated. Their biochemical reactions and serotypes are shown in Table 1. The cultures were serotyped

as *Y. enterocolitica* O:12,26, O:13,7, and O:18 and *Y. kristensenii* O:11,24 and O:12,26. *Y. pseudotuberculosis* was serotyped as IIB.

The frequency of isolation of these organisms differed among farms, and the organisms were detected in cows from four different farms. Seven strains, two *Y. enterocolitica* (serotypes O:12,26 and O:13,7) and five *Y. kristensenii* (all serotype O:11,24), were isolated from cows at the same farm.

It is well known that pigs are major carriers of *Yersinia* spp.; however, the frequency of detection of these organisms in cows differed in different studies (4, 8, 13). All bovine strains in the above reports were so-called "environmental strains," except one strain of serotype O:3, biotype 4, isolated from imported cattle (8). In the present study, the isolates of *Y. enterocolitica* and *Y. kristensenii* were thought to be environmental strains. These strains have not yet been detected in humans in Japan. Thus, cows may not be important carriers of so-called "clinical strains" of *Y. enterocolitica*, as compared with pigs. Nevertheless, the fact that cows have been the source of milk contaminated with *Yersinia* spp. cannot be ignored. It is especially significant that *Y. pseudotuberculosis* was detected from bovine feces. Most recently, a community outbreak of *Y. enterocolitica* enteritis in the United States was linked to milk, and the serotypes of the isolates concerned were 13 and 18 (6).

Three strains of *Y. pseudotuberculosis* serotype IA were isolated from bovine fetuses in Great Britain (7), and one strain of serotype III was detected in calves in the United States (2). Our isolation of *Y. pseudotuberculosis* from cows is the first such report in Japan, and the isolation of *Y. pseudotuberculosis* serotype IIB

TABLE 1. Biochemical reactions and serotypes of *Yersinia* spp. isolated from 618 bovine fecal samples

Biochemical test ^a	<i>Yersinia</i> spp. (no. of strains) tested		
	<i>Y. enterocolitica</i> (4)	<i>Y. kristensenii</i> (7)	<i>Y. pseudotuberculosis</i> (1)
Nitrate reduction	+	+	+
Indole (30°C)	+	-	-
Voges-Proskauer	+	-	-
Lecithinase	+	+ ~ - (2) ^b	-
Ornithine decarboxylase	+	+	-
Lysine decarboxylase	-	-	-
Citrate (Simmons)	-	-	-
Cellobiose	+	+	-
Maltose	+	+	+
Melibiose	-	-	+
Raffinose	-	-	-
Rhamnose	-	-	+
Salicin	+	-	+
Sorbitol	+	+	-
Sorbose	+	+	-
Sucrose	+	-	-
Trehalose	+	+	+
Esculin	+	- ~ + (1)	+
α-Methyl glucoside	-	-	-
Serotype	12, 26 (1); 13, 7 (2); 18 (1)	11, 24 (5); 12, 26 (2)	IIB (1)

^a All biochemical tests were conducted at 25°C unless otherwise noted.

^b Number of strains are indicated in parentheses.

from cows seems to be the first in the world.

In Japan, *Y. pseudotuberculosis* was isolated from eight types of animals: monkeys (1, 9), pigs (9, 10, 13), goats (9), dogs (12), cats (12), rabbits (9), guinea pigs (9), and rats (5); and our finding of this organism in cows makes nine animal types. Strains of serotype II have been isolated from humans (11), pigs (9), rabbits (9), and dogs and cats (Fukushima, unpublished data). All eight human strains were serotype IIA, whereas all seven strains (including the one strain documented here) of animal origin were classified into serotype IIB.

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