

# Comparison of Fecal *Campylobacter* in Calves and Cattle of Different Ages and Areas in Japan

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**ABSTRACT.** To define the species of *Campylobacter* harboring in the intestine of calves and adult cattle and to examine their distribution among different age groups and farms, 34 calves and 60 adult cattle raised in 6 farms in 3 different areas were studied. *Campylobacter* were detected in the feces obtained from healthy calves (97.1%) less than 1-year-old but were much less frequently found in adult cattle (46.7%). Young calves harbored more species of *Campylobacter* than adult cattle. The isolation rate of *Campylobacter* in adult cattle differed depending upon the farms examined. *C. jejuni*, *C. hyointestinalis* and *C. fetus* subsp. *fetus* were isolated from 61.8, 26.5, and 26.5% of calves, respectively. However, these 3 species were detected at the lower rates of 11.7 to 15.0% in adult cattle. *C. coli*, *C. lari*, *C. fetus* subsp. *venerialis*, *C. fecalis*, and other *Campylobacter* spp. were also detected in a few calves and adults. These results indicate that *Campylobacter* colonization in the intestine is very common in young calves but not in adult cattle.—**KEY WORDS:** age, calf, *Campylobacter*, cattle, feces.

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*Campylobacter* are important pathogens of man and animals. *C. jejuni* and *C. coli* are the most common causative agents of human gastroenteritis and also induce enteritis in animals [30]. *C. jejuni* also causes infertility and abortion in cattle, sheep [10, 30, 37], and other animals [6, 19, 39] and has been isolated from lesions of avian hepatitis of laying hens [13]. *C. hyointestinalis* is not only a causative agent of proliferative ileitis in pigs [15, 38] but also a minor cause of human gastroenteritis [8] and bovine diarrhea [9]. *C. mucosalis* and *C. fecalis* are thought to be associated with porcine intestinal adenomatosis [38] and enteritis in calves [2], respectively. *C. fetus* subsp. *fetus* causes sporadic abortion in cattle and sheep and *C. fetus* subsp. *venerialis* is a major cause of bovine genital campylobacteriosis [13].

*Campylobacter* are normal inhabitants of the intestines of several domestic and pet animals [7, 17, 21, 23, 29, 33] and frequently occur in various environments [7, 14, 34]. However, the ecological status of *Campylobacter* in healthy animals is still unknown in detail.

Final goal of our studies is to prevent the colonization of *Campylobacter* in the intestine of animals. As the first step the present study was carried out to define *Campylobacter* species and to examine their distribution in the intestinal tracts of calves and adult cattle in 6 farms with different age groups in 3 different areas.

Table 1. Farms, breeds, and ages of calves and adult cattle tested

Farm <sup>a)</sup>	Breed	Groups	Age <sup>b)</sup>	No. tested
O-I	Angus	1	>2Ys (A)	11
		2	4–6Ms (Y)	9
O-II	Holstein	1	>2Ys (A)	10
		2	3–4Ms (Y)	10
I-I	Holstein	1	>2Ys (A)	5
		2	8–1Ms (Y)	10
K-I	Japanese black	1	>2Ys (A)	5
		2	14–20Ms (A)	4
		3	14–20Ms (A)	5
		4	4–5Ms (Y)	5
K-II	Japanese black	1	21–22Ms (A)	5
		2	12Ms (Y)	5
K-III	Japanese black	1	21–22Ms (A)	10

a) O-I and -II, farms in the outskirts of Obihiro city in Hokkaido, the most northern part of Japan; I-I, a farm in the outskirts of Mito city in the middle part of Japan; and K-I, -II, and -III, farms in the outskirts of Kagoshima city in the southern part of Japan.

b) Ys, years; Ms, months; (A), adults older than 1 year of age; and (Y), calves younger than 1 year of age.

## MATERIALS AND METHODS

**Animals:** Rectal contents were obtained from apparently healthy calves and cattle. Farms, ages and breeds of the animals examined in this study are summarized in Table 1. In this report we described the animals less than 1 year of age as “young” (Y) and those older than 1 year as “adult” (A).

**Sampling:** About 1 g of the rectal contents was placed directly in 9 ml of anaerobic transport medium [24]. The atmosphere of the medium in glass tubes was replaced

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with CO<sub>2</sub> gas. The samples were kept at 4°C until cultivation.

**Isolation techniques:** Preston broth [Nutrient broth No. 2 (OXOID) supplemented with F.B.P. (OXOID), Preston selective supplement (OXOID), and 5% defibrinated horse blood] was used as the enrichment medium. One loopful of the suspension of the rectal content in anaerobic transport medium was directly inoculated onto Skirrow agar plates (OXOID) and 1 ml of the suspension was also inoculated into Preston enrichment broth. The Skirrow agar plates were cultured at 37°C for 48 hr in a microaerophilic atmosphere of 78% N<sub>2</sub>, 10% CO<sub>2</sub>, 5% O<sub>2</sub>, and 7% H<sub>2</sub>. Preston broths were incubated at 37°C for 48 hr using Campy Pak (BBL). One loopful of the cultured Preston broth was inoculated onto selective media, Skirrow and Butzler agar plates (OXOID) and they were cultured microaerophilically at 37°C for 48 hr as described above.

**Identification of *Campylobacter*:** Colonies grown on Skirrow and Butzler agar plates were presumptively identified as *Campylobacter* according to their colony form, Gram stain, cell form, motility, oxidase reaction, and fermentation of glucose. The species were identified by the following tests; catalase, nitrate reduction, H<sub>2</sub>S production (TSI and lead acetate strips), hippurate hydrolysis, growth at 25°C and 42°C, and growth in 1% glycine and in 0.04% TTC, and sensitivity to 30 µg of nalidixic acid and cephalothin disks. All tests were carried out as described by Skirrow and Benjamin [32] and Itoh *et al.* [20]. Suspected strains as *C. fetus* and *C. hyointestinalis* were examined by slide agglutination tests using anti-*C. hyointestinalis* whole rabbit serum produced by injecting formalin-killed bacteria of the strain ATCC 35217. This anti-serum was kindly supplied from Dr. T. Ohya, Kyushu Branch Laboratory, National Institute of Animal Health, Kagoshima, Japan.

## RESULTS

Ninety-four rectal contents obtained from 34 calves and 60 cattle were examined. A total of 121 of *Campylobacter* strains were isolated from 33 calves (97.1%) and 28 cattle (46.7%). Among those isolates, 115 were identified as 6 species and the remaining 6 were unidentifiable (Table 2). Table 3 shows the species and the numbers of isolates identified by the different isolation methods. *C. jejuni* was the most dominant species, followed by *C. fetus* subsp. *fetus* and *C. hyointestinalis*. Although *C. jejuni* and *C. fetus* subsp. *fetus* were isolated by all the 3 isolation methods, almost all strains of *C. hyointestinalis* were isolated only by the Preston-Skirrow combination method.

Table 4 shows the isolation of campylobacters from calves and adult cattle in 6 farms of 3 different areas. Campylobacters were detected in almost all calves (97.1%). However, the detection rate of campylobacters from adults varied depending upon the farms. Campylobacters were isolated from 17 (89.5%) out of 19 adults in

Table 2. Characteristics of unidentified *Campylobacter* strains isolated from feces of calves and cattle

Characteristics	Groups				
	C-1	C-2	C-3	C-4	C-5
No. of isolates	1	2	1	1	1
Oxidase test	+	+	+	+	+
Catalase test	+	+	+	+	+
Glucose fermentation	-	-	-	-	-
Nitrite reduction	+	+	+	+	-
Growth at:					
25°C	+	+	+	+	W
42°C	+	+	+	+	+
Growth in presence of:					
1% Glycine	+	+	+	+	+
0.04% TTC	+	+	+	+	±
Susceptibility (30 µg/disk):					
Nalidixic acid	R	R	R	R	R
Cephalothin	S	S	S	S	R
Hippurate hydrolysis	+	+	+	+	+
H <sub>2</sub> S production					
TSI	-	-	+	±	-
Lead acetate strip	-	+	+	+	+

Symbols: +, positive reaction; -, negative reaction; W, weak reaction; ±, very weak reaction; R, resistance; and S, sensitive.

Table 3. *Campylobacter* species and numbers of strains isolated from feces of calves and cattle by the three culture methods

Species	No. of isolates			
	Total isolates	Direct Skirrow	Preston to Skirrow	Preston to Butzler
<i>C. jejuni</i>	62	15	21	26
<i>C. coli</i>	1	0	0	1
<i>C. lari</i>	2	0	1	1
<i>C. fetus</i> subsp. <i>fetus</i>	24	5	8	11
<i>C. fetus</i> subsp. <i>venerialis</i>	3	0	1	2
<i>C. hyointestinalis</i>	22	0	21	1
<i>C. fecalis</i>	1	0	1	0
<i>Campylobacter</i> spp.	6	0	4	2

farms K-I and K-II but from 10.0% to 50.0% of adults in other farms. Two or more species were isolated from 32.4% of all the calves tested but from only 10.0% of the adults.

As shown in Table 5, *C. jejuni* was isolated most frequently from the rectal contents of calves at the rate of 61.8% and *C. fetus* subsp. *fetus* and *C. hyointestinalis* were also dominant species at the rate of 26.5%. Though these 3 species were also dominant in adult cattle, the isolation rates were lower than in the calves. More than 2 species of *Campylobacter* were detected from all the groups except the adults in farms I-I and K-III. The calves in farm O-II harbored the greatest variety of species, 6 different species. *C. fetus* subsp. *venerialis* was detected only from adult cattle of farm K-I. Although 22 isolates were identified as *C. hyointestinalis* by biological and biochemical tests, 3 of them were negative in the slide agglutina-

tion test using anti-*C. hyointestinalis* serum. All of strains identified as *C. fetus* subsp. *fetus* were negative in this test.

#### DISCUSSION

Although campylobacters have been isolated from the intestines of both healthy calves and adult cattle and diseased animals exhibit enteritis [3, 5, 7, 17, 26, 29, 33], almost all the reports focused on 1 or a few species. There is no available reports indicating that many species of

*Campylobacter* are harbored in the intestines of healthy calves and cattle.

In the present study, *C. jejuni*, *C. hyointestinalis*, and *C. fetus* subsp. *fetus* were major species of *Campylobacter* in the feces of healthy calves and adult cattle. Some reports [14, 17, 26] showed that *C. jejuni* and *C. hyointestinalis* were detected in the intestines of healthy calves and cattle. It was reported that isolation of *C. fetus* subsp. *fetus* from the intestines of healthy adult cattle was not uncommon [13], while Uematsu *et al.* [35] and AL-Mashat *et al.* [4] reported that *C. fetus* subsp. *fetus* was isolated from the feces of cattle associated with diarrhea but not from normal cattle.

The numbers of *C. hyointestinalis* in feces of calves and adult cattle in the present study were very few because the species were detected only after enrichment culture, although Grau [17] reported that *C. hyointestinalis* was often present at a level of  $10^5$ /g in feces of calves and cattle in Australis. The present results revealed that *C. jejuni* was the predominant species in calves, of which isolation rate was about 60.0%, and that the detection rate of *C. fetus* subsp. *fetus* and *C. hyointestinalis* were 26.5%. In adult cattle, however, *C. jejuni*, *C. fetus* subsp. *fetus*, and *C. hyointestinalis* were detected at the similar rate (12% to 15%). The frequency of occurrence of *C. jejuni* in both calves and cattle in this study agreed with that found in Australia [17]. The differences in the number and the isolation rate of *C. hyointestinalis* between Australia and Japan might be due to differences in environmental and breeding conditions of the cattle. It was shown that the frequency of occurrence of campylobacters in adult cattle differed depending upon farms examined in this study. This might also be attributable to the same reasons.

The detection rate of campylobacters, the number of *Campylobacter* species isolated, and the percentage of

Table 4. Isolation of *Campylobacter* from calves and cattle of different farms

Farm <sup>a)</sup>	Groups	Age <sup>b)</sup>	No. of animals tested	No. positive (%)		
				Total	1 species <sup>c)</sup>	2 or more species <sup>d)</sup>
O-I	1	A	11	3(27.2)	2(18.2)	1( 9.1)
	2	Y	9	9(100)	8(88.9)	1(11.1)
O-II	1	A	10	5( 50)	5( 50)	0( 0)
	2	Y	10	10(100)	3( 30)	7( 70)
I-I	1	A	10	1( 10)	1( 10)	0( 0)
	2	Y	5	4( 80)	4( 80)	0( 0)
K-I	1	A	5	4( 80)	2( 40)	2( 40)
	2	A	4	4(100)	4(100)	0( 0)
	3	A	5	5(100)	4( 80)	1( 20)
	4	Y	5	5(100)	3( 60)	2( 40)
K-II	1	A	5	4( 80)	2( 40)	2( 40)
	2	Y	5	5(100)	4( 80)	1( 20)
K-III	1	A	10	2( 20)	2( 20)	0( 0)
Total		A	60	28(46.7)	22(36.7)	6( 10)
		Y	34	33(97.1)	22(64.7)	11(32.4)
		A+Y	94	61(64.9)	44(46.8)	17(18.1)

a) and b) See Table 1.

c) Number of animals harboring 1 species.

d) Number of animals harboring 2 or more species.

Table 5. *Campylobacter* species in calves and cattle of different farms

Farm <sup>a)</sup>	Groups	Age <sup>b)</sup>	No. of animals tested	No. of animals positive (%)							
				<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>	<i>C. fetus</i> subsp. <i>fetus</i>	<i>C. fetus</i> subsp. <i>venerialis</i>	<i>C. hyo-intestinalis</i>	<i>C. fecalis</i>	<i>Campylo-bacter</i> spp.
O-I	1	A	11	2		1			1		
	2	Y	9	8			1				1
O-II	1	A	10			1	1		3		
	2	Y	10	4	1		6		4	1	3
I-I	1	A	10	1							
	2	Y	5	3					1		
K-I	1	A	5				3	2	1		
	2	A	4				2		2		
	3	A	5	2				1	1		1
	4	Y	5	1			2		3		
K-II	1	A	5	3			1		2		
	2	Y	5	5					1		
K-III	1	A	10						2		
Total		A	60	8(13.3)	0( 0)	2(3.3)	7(11.7)	3(5.0)	9(15.0)	0( 0)	1( 1.7)
		Y	34	21(61.8)	1(2.9)	0( 0)	9(26.5)	0( 0)	9(26.5)	1(2.9)	5(14.7)
		A+Y	94	29(30.9)	1(1.1)	2(2.1)	16(17.0)	3(3.2)	18(19.1)	1(1.1)	5( 5.3)

a) and b) See Table 1.

animals with 2 or more species were higher in young calves than in adult cattle. More *Campylobacter* species were isolated from the young of farm O-II compared with the others. This might be due to the fact that almost all the calves in the farm O-II were younger (under 3 months old) than the calves in the other farms. These results suggest that the intestine of young calves are more susceptible to colonization with campylobacter than that of adult cattle. The reduction in infectious rates by enteric pathogens with aging is closely related to the development of the intestinal flora [27, 28]. Further studies are required to analyze the role of intestinal flora against colonization with campylobacters in the intestines of calves and cattle.

*C. hyointestinalis* and *C. fetus* subsp. *fetus* were distinguished by the production of H<sub>2</sub>S in TSI. However, H<sub>2</sub>S production of *C. hyointestinalis* isolated in this study was very weak. The serological test using antiserum of *C. hyointestinalis* was therefore carried out to confirm the identification of *C. hyointestinalis*. The test was very useful in distinguishing *C. hyointestinalis* from *C. fetus* subsp. *fetus*. However, 3 out of 22 *C. hyointestinalis* strains showed a negative reaction in the serological test, though they produced H<sub>2</sub>S in TSI. These serologically negative strains will be a subject for the further bacteriological investigation.

The distinction between *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* was made by 1% glycine resistance. In this study, 3 *C. fetus* subsp. *venerealis* strains were isolated from adult cattle. However, the judgement whether these strains were tolerant against 1% glycine or not was sometimes hard. Harvey and Greenwood [18] suggested that the glycine tolerance test was quite unreliable and both subspecies were closely related in the DNA-DNA-hybridization test. Although we identified these 3 strains as *C. fetus* subsp. *venerealis* from the results of biological and biochemical tests, further studies including nucleic acid analysis [1, 16, 31, 40] concerning the distinction between *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* should be done.

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