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Isolation and Characterization of *Campylobacter jejuni* and *Campylobacter coli* from Domestic and Wild Mammals in Norway

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A total of 1,262 domestic and wild mammals from Norway were surveyed for fecal carriage of *Campylobacter jejuni* and *Campylobacter coli*. Of the five species of domestic mammals examined, the highest isolation rate was recorded among swine (100.0%), followed by sheep (8.1%) and cows (0.8%). No strains were recovered from horses or goats. Among wild mammals, *C. jejuni* was isolated from 1 of 23 hares, and no isolates were obtained from three species of cervids and three species of rodents. Of the 133 *Campylobacter* strains isolated, 114 were classified as *C. coli*, 18 were *C. jejuni* biotype 1, and 1 belonged to *C. jejuni* biotype 2. All 114 strains from swine were *C. coli*. Milk samples from 113 domestic mammals with clinically diagnosed mastitis (106 cows, 5 sheep, 1 horse, and 1 pig) were negative for campylobacters.

Campylobacter jejuni and *Campylobacter coli* (synonym *Campylobacter fetus* subsp. *jejuni*, *Campylobacter jejuni*, "thermophilic" campylobacters) have been encountered in the intestinal contents of a wide variety of warm-blooded animals, including domestic species and free-living and captive wild animals (3, 5, 9, 13, 16). Although this bacterial group has been convincingly shown to be one of the most important etiological agents of acute enteritis in humans, in other mammalian species the bacteria are present in an apparently healthy carrier state in a majority of cases (3, 14, 22). However, it is becoming increasingly evident that *C. jejuni* and *C. coli* can cause enteritis in several species of animals other than humans (5, 16). Moreover, epidemiological data have provided strong evidence that animals and food products of animal origin are the main reservoirs for human infection (9, 14, 22). Hence, *Campylobacter* enteritis constitutes a zoonosis of major concern in public health and, indeed, has been shown to be a greater problem than salmonellosis in several countries (1, 14, 22, 28).

Among domestic livestock, high fecal carriage rates of *C. jejuni* and *C. coli* have been reported for poultry, pigs, cattle, and sheep (3, 9, 13, 16). Eviscerated carcasses at slaughter are frequently contaminated (2, 6, 9, 26, 30), and it is also evident that both poultry and red-meat products at the retail sale stage may carry campylobacters (21, 22, 29, 30). Raw milk is also a recognized vehicle for contamination (4, 18, 22).

Wild birds constitute an extensive reservoir of *C. jejuni* and *C. coli* (3, 8, 12, 13). However, few attempts have been made to isolate these organisms from nonavian wildlife. An understanding of wildlife diseases is an important aspect of natural resource management programs. Demands for outdoor recreation have focused attention on the epidemiological relationship between humans and wildlife. Moreover, the spreading of infectious diseases between wild animals and domestic livestock may result from fecal contamination of habitats in which both forest and farm animals graze and drink.

In the present study, wild and domestic mammals from Norway were surveyed for fecal carriage of *C. jejuni* and *C. coli*. In addition, the presence of these bacteria in milk from dairy cows and three other species of domestic mammals was investigated.

MATERIALS AND METHODS

Rectal swabs and stool specimens. During February through October 1982, rectal swabs or stool specimens were collected from a total of 1,262 mammals representing twelve species (see Table 1) in Norway. All animals were apparently healthy and showed no signs of enteritis or other illness. Of the total of 1,262, 720 samples were obtained from five species of domestic mammals, whereas the remaining 542 samples originated from seven species of wild mammals.

The domestic animals examined represented more than 60 herds (see Table 1). Pigs and sheep originated from the county of Aust-Agder, and horse samples were obtained from the counties of Oslo and Akers-

hus. Also included in this study were cows representing 36 herds in Aust-Agder, Akershus, and Oslo. Samples were obtained from goats from five different herds in the county of Møre og Romsdal. All wild animals originated from Aust-Agder, except reindeer, which inhabited the mountain areas surrounding the district of Tynset, Hedmark County.

For each of the five sheep herds examined, samples were collected from both ewes and suckling lambs less than 1 month old. With this exception, all wild and domestic mammals incorporated in this study were adults.

Rectal swabs were collected from all swine, sheep, goats, hares, moose, reindeer, roedeer, and from 183 of the cows examined (see Table 1). All wild animals (except small rodents, see below) were killed by shooting, and rectal swabs were taken immediately. All domestic animals were sampled alive, except swine, which were sampled at the slaughterhouse immediately after death. Rectal swabs from both wild and domestic animals were stored on SIFF transport medium (20), brought to the laboratory, and kept at 4°C before cultivation within 1 to 3 days after collection. There was no significant difference in the transport or storage of specimens from wild and domestic animals. The SIFF transport medium is a modification of Stuart's medium (20). When stored on this medium, pure cultures of *C. jejuni* and *C. coli* remained viable for at least 1 week at room temperature and for at least 2 weeks at 4°C (unpublished data).

Fresh stool specimens were collected from all horses and from 71 of the cows. Stools were brought to the laboratory in sterile plastic bags within 1 to 2 h after collection. Samples were taken with swabs which were streaked on a selective agar medium (see below). Small rodents were trapped alive, killed with ether, and kept at −20°C for 1 week. The rodents were subsequently thawed, and 2 cm of rectum and colon was dissected aseptically. Intestinal contents were sampled with swabs and plated immediately.

Milk samples. During May through August 1982, milk samples were collected from 113 domestic mammals (106 dairy cows, 5 sheep, 1 horse, and 1 pig) with clinically diagnosed mastitis in the counties of Akershus and Oslo. The 106 cows under study belonged to 53 different herds. Separate samples from all teats of an infected udder were collected and examined individually. Milk (10 ml) was collected aseptically and stored at 4°C. Cultivation was usually carried out within 20 h, although a few samples were stored for 2 days.

Isolation procedure. Rectal swabs, stool specimens, or loopfuls of milk were plated onto colistin-amphotericin-keflin agar. This selective medium consisted of the gonococcus agar base described previously by Ødegaard (15), defibrinated horse blood (70 ml/liter), IsoVitaleX enrichment (BBL Microbiology Systems, Cockeysville, Md.), and the antimicrobial agents colistin (Colimycin; Lundbeck & Co., Copenhagen, Denmark) (10 IU/ml), amphotericin B (E. R. Squibb & Sons Ltd., Liverpool, England) (1 μg/ml), and cefalotin (Keflin; Eli Lilly France S. A., Fegersheim, France) (15 μg/ml). Plates were incubated at 42 to 43°C in anaerobic jars without catalysts, using gas-generating sachets (no. BR 38; Oxoid Ltd., Basingstoke, Hampshire, England) to achieve the proper microaerobic atmosphere. Plates were read after 24 and 48 h.

Identification of campylobacters. All colonies showing morphology similar to that of *Campylobacter* spp. were examined by phase-contrast microscopy (×1,000). Bacteria exhibiting the typical motility and cell morphology suggestive of *Campylobacter* spp. were subjected to cultural and biochemical examination as follows. The ability to grow under aerobic or anaerobic conditions was assessed after incubation at 37°C for 48 h. Growth at 25°C was tested in a microaerobic atmosphere. Catalase activity was tested on microscope slides by the addition of 1 drop of H_2O_2. Oxidase activity was examined on filter paper with 1% aqueous solution of tetramethyl-*p*-phenylenediamine dihydrochloride. The parameters listed above formed the basis for identification of the isolated strains according to established criteria (25).

Biochemical classification. All isolated strains identified as thermophilic campylobacters were tested for hippurate hydrolysis, H_2S production, and susceptibility to nalidixic acid. These parameters formed the basis for allocation to *C. jejuni* biotype 1 or 2, *C. coli*, or nalidixic acid-resistant thermophilic campylobacters, as proposed by Skirrow and Benjamin (23). Before biochemical examination, all strains were grown on blood agar plates for 18 to 24 h in a microaerobic atmosphere (see above). Hydrolysis of hippurate was tested by the method of Hwang and Ederer (7). H_2S production was examined by using the iron-containing FBP medium described by Skirrow and Benjamin (23). Susceptibility to nalidixic acid was evaluated on blood agar plates with commercial antibiotic disks (Neo-Sensitabs; A/S Rosco, Taastrup, Denmark) containing 130 μg of nalidixic acid. Bacteria showing inhibition zones of ≥28 mm after incubation at 37°C for 24 h under a microaerobic atmosphere were considered susceptible.

RESULTS

Isolation frequencies. *C. jejuni* and *C. coli* were isolated from rectal swabs or stool specimens from 133 (10.5%) of the 1,262 mammals examined (Table 1). Isolates were obtained from 132 (18.3%) of 720 domestic mammals. The highest rate of isolation was recorded among swine (100.0%), followed by sheep (8.1%) and cows (0.8%). No strains were recovered from horses or goats.

The number of herds harboring campylobacters is presented in Table 1. Although campylobacters were obtained from all individuals belonging to 19 different swine herds, considerable variation in the carriage rate was detected among the sheep herds. The carriage rates observed in two of the five sheep herds examined were 30.0% (15 of 50) and 2.0% (1 of 50), whereas no isolates were obtained from the remaining three herds (25, 31, and 41 sheep in each herd). One of the sheep herds showed a significantly higher ($\chi^2 = 15.11$) isolation rate among adults than among suckling lambs. Isolates were obtained from 13 of 25 ewes, whereas only 2 of 25 lambs yielded campylobacters. An insufficient number of isolates was obtained

TABLE 1. Isolation rates of *C. jejuni* and *C. coli* from feces of domestic and wild mammals

Species	Individuals			Herds		
	Total no.	With campylobacters		Total no.	With campylobacters	
		No.	%		No.	%
Domestic mammals						
Swine	114	114	100.0	19	19	100.0
Sheep	197	16	8.1	5	2	40.0
Cows	254	2	0.8	36	2	5.6
Goats	110	0	0	5	0	0
Horses	45	0	0	—[a]	—	—
Wild mammals						
Blue hares (*Lepus timidus*)	23	1	4.3	—	—	—
Moose (*Alces alces*)	372	0	0	—	—	—
Reindeer (*Rangifer tarandus*)	94	0	0	—	—	—
Roedeer (*Capreolus capreolus*)	8	0	0	—	—	—
Bank voles (*Clethrionomys glareolus*)	24	0	0	—	—	—
Wood mice (*Apodemus sylvaticus*)	20	0	0	—	—	—
Beavers (*Castor fiber*)	1	0	0	—	—	—

[a] Data not available.

from the remaining herds to justify conclusions about age distribution.

Fecal samples from a total of 542 wild mammals yielded only one isolate of *C. jejuni* (Table 1). This strain was recovered from 1 of the 23 hares examined, and no campylobacters were isolated from three species of cervids and three species of rodents.

No *Campylobacter* isolates were obtained from milk samples collected from 113 domestic mammals with clinically diagnosed mastitis. The etiological agent was identified in 67 (59.3%) of these cases.

Biochemical characterization. The 133 strains of *C. jejuni* and *C. coli* isolated in this study could be assigned to three biochemically distinct taxa. All 114 strains from swine were classified as *C. coli*. Of the 16 strains isolated from sheep, 15 belonged to *C. jejuni* biotype 1, and 1 strain was *C. jejuni* biotype 2. Two isolates from cows and one from a Blue hare were classified as *C. jejuni* biotype 1. No strains were resistant to nalidixic acid.

DISCUSSION

Published rates of isolation of *C. jejuni* and *C. coli* from the intestinal contents of domestic livestock reveal considerable intra- and interspecific variation. Most workers have reported high carriage rates of *C. coli* among healthy pigs, providing evidence that *C. coli* is a normal component of the intestinal flora of these animals (9, 13, 16, 27). The present results support this conclusion (Table 1). *C. jejuni* is recognized as the most important causal agent of human *Campylobacter* enteritis, whereas *C. coli* is less commonly involved in human infection (11, 22, 24). *C. coli* accounted for 13% of the cases of human infection in Norway (G. Kapperud, J. Lassen, S. Lauwers, and O. Rosef, submitted for publication). The contamination of pig carcasses during the slaughter process represents a potential source of human infection. Rosef (19) isolated *C. coli* from 56% of the cut surfaces and 43% of the liver surfaces of 100 fresh pig carcasses in Norway. However, a causal relationship between this extensive porcine reservoir and the prevalence of *C. coli* among human cases cannot be established at this time. Determination of the relevance of the various animal isolates in terms of their pathogenicity for humans will require further study.

Raw and improperly pasteurized milk from cows has been incriminated as the infection-bearing vehicle in several outbreaks of milk-borne campylobacteriosis (18, 22). Since cows may be intestinal carriers of the bacteria concerned, the fecal contamination of milk represents a potential route leading to human infection. The carriage rate detected among Norwegian cows was low (Table 1). Other workers have reported isolation rates ranging from 0 to 100% (4, 9, 16). In Britain, Robinson (17) found a marked seasonal variation in fecal carriage among lactating cows. *C. jejuni* could be isolated from 10% of each herd in the summer, declining to 0% during the winter, and reemerging in the spring. Since all samples incorporated in our study were collected during May through August, it seems unlikely that the low carriage rate detected represents an underestimation associated with seasonal variation.

Lander and Gill (10) were able to produce fairly severe clinical mastitis in lactating cows

by intramammary inoculation of *C. jejuni*. The syndrome resulted in the shedding of large numbers of campylobacters in milk, enabling effective dissemination of the organism to consumers of unpasteurized milk. However, attempts to isolate campylobacters from milk from cows with mastitis have not been successful. More than 600 foremilk samples collected throughout England from cows with mastitis were negative (31). Our results are similar. In Norway, the distribution of unpasteurized milk to the general public is prohibited, and the pasteurization process is strictly controlled. Furthermore, the absence of reported milkborne outbreaks, together with the low fecal carriage rate among lactating cows, indicates that milk is not an important vehicle of *C. jejuni* and *C. coli* in Norway.

Wild mammals comprise popular game species of considerable economic and recreational value. The handling and consumption of meat from such animals represent potential sources of human infection. In Norway, the number of moose and reindeer consumed each year totals tens of thousands. The present results indicate that *C. jejuni* and *C. coli* were virtually absent in the populations of moose and reindeer examined, in contrast to the high carriage rates among certain domestic mammals. This difference may, at least in part, reflect the environments in which the animals live. Domestic mammals are subjected to crowded conditions and the consequent frequent exposure to fecal excrements, thereby providing an ample opportunity for intra- and interspecific cross contamination. Although the population densities of moose, reindeer, and small rodents may be high, the epidemiological situation cannot be compared with that existing in herds of domestic animals. On the other hand, it is highly probable that mammalian species may differ in their susceptibility to intestinal colonization by *C. jejuni* and *C. coli*, regardless of the degree of exposure to these bacteria. This circumstance may also help to explain the different carriage rates detected in this and similar studies.

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