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Serotyping and Serology Studies of Campylobacteriosis Associated with Consumption of Raw Milk

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A community outbreak of 15 cases of gastroenteritis was traced to consumption of unpasteurized milk produced at one commercial dairy. Using two different testing schemes, we found that a *Campylobacter jejuni* isolate from an ill patient and an isolate from a sick cow were the same serotype. Bacteriological studies suggested that a single epidemic strain of *Campylobacter jejuni* caused this outbreak.

Campylobacter jejuni has previously been epidemiologically linked to ingestion of raw milk (3, 9, 13). Other known modes of transmission include infection through ingestion of municipal and raw water, undercooked chicken, processed turkey, raw clams, and raw hamburger; person-to-person transmission and transmission from ill animals have also been reported (5).

The Vermont Department of Health, Division of Epidemiology, received a call from a local pathologist at a regional medical center concerning an increase of *Campylobacter jejuni*-positive stool specimens. The two largest laboratories in Chittenden County routinely culture stools from persons with diarrhea for *Campylobacter* spp. Each of the two laboratories provided a list of names of patients with stools positive for *Campylobacter* spp. within a 3-week period. Nine cases of *Campylobacter*-positive diarrhea were identified in Chittenden County residents from stool samples received between 22 September 1982 and 12 October 1982. An epidemiological and environmental investigation was undertaken to identify the possible sources of infection.

A matched-pair, case-control study individually matched nine culture-positive *Campylobacter* cases in separate households with their nearest geographic friend. Cases were matched by sex and age within 5 years. All who were selected participated in the study.

In all cases of gastroenteritis and in matched controls, persons were asked about the onset, duration, and symptoms of illness and about their home water supplies. Questions were also asked about other possible sources of *Campylobacter* spp., including consumption of water, ice, raw milk, raw cheese, clams, undercooked chicken, undercooked hamburger, or processed turkey. In both groups, cases and controls, individuals were queried about exposure to pet dogs, cats, and other ill persons with diarrhea and about restaurant patronage and attendance at large gatherings, including a large gathering at a small commercial dairy farm, designated here as dairy A, on 25 September 1982. Family members living in the households of the cases were questioned similarly.

Milk at dairy A was pooled from all cows at the dairy farm and then was packaged for retail sale. In our investigation, samples were taken from cows and milk-processing and -packaging equipment. Fecal samples from one cow that had had diarrhea on 11 September 1982 and from 14 other cows randomly selected at dairy A were obtained. Milk from the ill cow was used for raw-milk production during and after the illness of the animal. Samples were also taken from the milk sock filters (five samples) and milk bulk tanks (six samples). Using Centers for Disease Control methods (6), we analyzed stool samples for *Campylobacter* spp. Samples from the milk bulk tanks were inoculated directly onto Skirrow agar. Samples of solids from the milk sock filter were suspended in *Brucella* broth before they were inoculated onto Skirrow agar.

No milk produced during the time of the outbreak was available for analysis. Milk samples from two cartons of recently packaged unpasteurized milk from dairy A were cultured for *Campylobacter* spp. by incubation in Oosterom enrichment broth (11) before inoculation to selective plating medium.

Two *Campylobacter* isolates, one from the cow with diarrhea and the other from an ill patient (designated here as patient X) who consumed raw milk from dairy A were characterized by biochemical tests, the serotyping procedures of Penner and Hennessey (12) and Lior et al. (7), antimicrobial susceptibility patterns from a microdilution MIC procedure with plates incubated for 48 h in a microaerophilic atmosphere (14), and plasmid analysis with a modification of the Birnboim and Doly method (1). The method for plasmid analysis was modified by treating the cleared lysates with RNase and then extracting the protein with phenol and ethanol precipitating the DNA for a second time. Two plasmid preparations from each strain were combined for electrophoresis.

Sera from seven case patients and seven controls identified in the case-control study were tested for *Campylobacter* antibodies by a modified indirect fluorescent-antibody technique described by Blaser et al. (3). Formalinized whole cells, instead of boiled cells, were used as antigen. The sera were tested against three antigens, one prepared from the autologous strain (isolate from patient X) and two prepared from reference strains (Penner serotypes 2 and 3).

In the matched-pair, case-control study, no illness was identified in any control selected for analysis. All nine case patients and three of nine controls had consumed unpasteurized, commercial milk from dairy A that had been purchased in a variety of stores. Consumption of this raw milk was associated with development of *Campylobacter* gastroenteritis (relative risk = ∞; $P < 0.05$ by McNemar matched-pair analysis). No other modes of exposure were significantly

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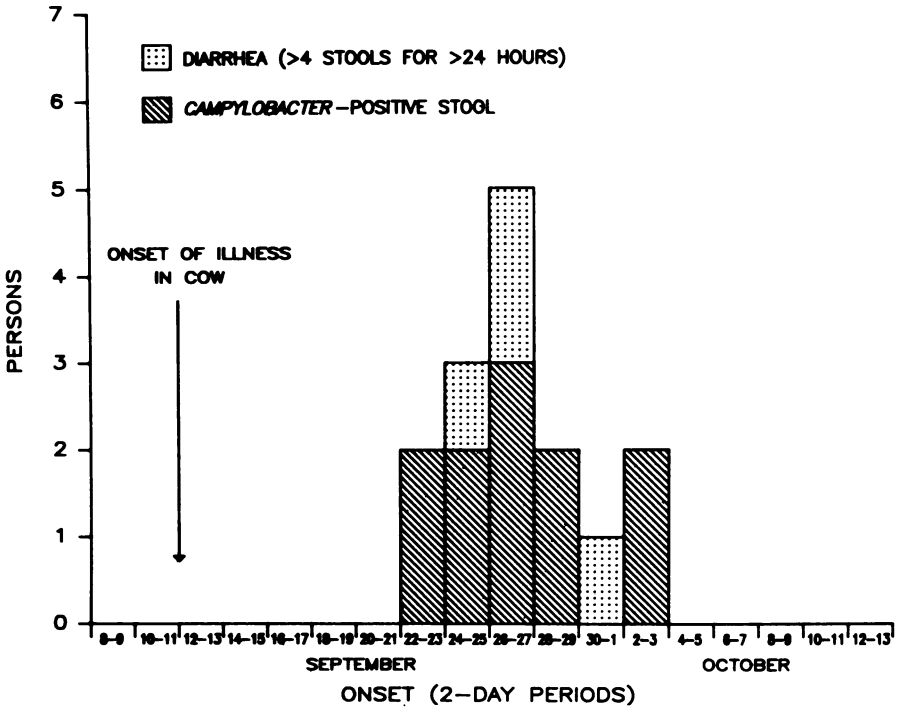

FIG. 1. Dates of onset of gastroenteritis in 15 persons who drank raw milk from dairy A, Vermont, 1982.

more common in cases than in controls, including attending the large gathering at dairy A on 25 September 1982.

Two additional symptomatic persons with stools positive for *Campylobacter* isolates and four other persons with diarrheal illness (defined as ≥4 stools per day for ≥24 h) were identified in case households. Of 10 family members of the 9 case patients who drank raw milk, 6 developed diarrhea compared with none of 5 family members who did not drink raw milk ($P = 0.044$ by Fisher exact test, two-tailed). The dates of onset of illness for the 15 ill persons in the nine affected households are shown in Fig. 1.

Fecal specimens from two cows, including one healthy cow and one cow with diarrhea, yielded *Campylobacter jejuni*. *Campylobacter jejuni* was not isolated from pooled milk or from other environmental samples.

The isolates from patient X and from the cow with diarrhea were indistinguishable in laboratory tests. These were the only isolates available for further laboratory analysis; the isolate from the healthy cow died before further testing could be done. Morphological and biochemical characterization indicated that each of the two strains was typical *C. jejuni*. Both isolates were Penner serotype 2 based on heat-stable antigens and were Lior serotype 4 based on heat-labile antigens. The MICs of 37 antimicrobial agents were determined for the human and cow strains, and no significant differences were noted in the profiles of the two strains. Plasmid DNA was not detected in either isolate.

The reciprocal geometric mean immunoglobulin G titer of sera from cases was 311.9 compared with 29.0 for the sera from controls ($P = 0.001$ by the Wilcoxon rank-sum test) when tested against the autologous antigen (Fig. 2). Similar titers were obtained against two *Campylobacter* reference strains (Penner serotypes 2 and 3).

The epidemiological evidence implicated raw milk produced at dairy A as the cause of the outbreak of *C. jejuni* infection. There was a highly significant association between consumption of unpasteurized milk from dairy A and illness, and there was no significant association with exposure to other modes of transmission. Milk taken from an ill cow was the most likely source of the infection, although other cows may have been infected with this epidemic strain, causing subsequent milk contamination. Even though *Campylobacter* spp. were not isolated from raw milk or raw milk products, bacteriological findings were the same for an isolate from the ill cow and an isolate from a patient who drank raw milk. Both isolates showed similar biochemical-test results, were the same serotype by two typing systems, and had almost identical antimicrobial-susceptibility profiles. Plasmid DNA could not be demonstrated in either strain. These laboratory findings are consistent with the hypothesis that there was a single epidemic *Campylobacter*

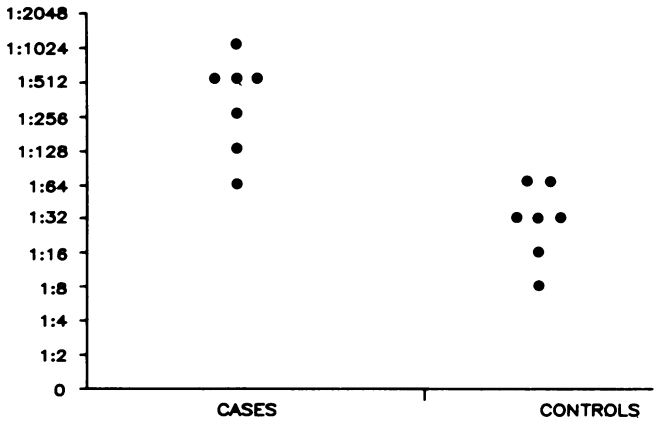

FIG. 2. Indirect fluorescent-antibody serological response to the autologous *Campylobacter* antigen in seven cases and seven controls.

strain in this outbreak. Penner serotype 2-Lior serotype 4, the serotype found in this outbreak, has been previously associated with *C. jejuni* outbreaks, related cases (7, 8), or both. This serotype has also been isolated from cattle and dairy cows with diarrhea in Canada (10). However, the number of isolates serotyped from cattle and dairy cows in the Canadian study was too small to determine the most frequently occurring serotypes. Common serotypes in dairy cows in the United States have yet to be determined.

Serotyping systems and serological studies have shown *Campylobacter* strains to be antigenically diverse. Cases in this outbreak showed similar serological responses to the epidemic strain and to two reference strains, suggesting that common antigens may exist in *Campylobacter* strains of different serotypes.

In this outbreak the isolate from the cow was indistinguishable from the isolate from the human by four separate epidemiological marker systems. Although several reports of outbreaks of campylobacteriosis have been traced to raw-milk consumption, few reports have so conclusively matched a *Campylobacter* isolate recovered from an epidemiologically implicated environmental source with one or more isolates from humans infected during the outbreak (4).

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