An epidemiological study of campylobacteriosis in Iowa, and the role of unpasteurized milk as a vehicle of infection

by

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GENERAL INTRODUCTION AND LITERATURE REVIEW

Campylobacter jejuni has been incriminated as a leading bacterial etiological agent of human gastroenteritis worldwide. Reports from Indonesia,[1] Belgium,[2] France,[3] Japan,[4] Rwanda (Africa),[5] Bangladesh,[6] Sweden,[7] India,[8] Great Britain,[9] Australia,[10] the United States,[11] and Canada[12] have confirmed the widespread distribution and high incidence of human infections. The first recorded large scale outbreak of campylobacteriosis was in 1938 at 2 neighboring penal institutions in Illinois.[13] However, not until the mid-1970s was C. jejuni recognized as a significant human enteric pathogen.

Early work with enteric campylobacters established them as veterinary pathogens. McFadyean and Stockman cultured vibrios (campylobacters) from aborted lambs, and subsequently they experimentally induced abortion in ewes with the lamb strains in 1909.[14] The natural route of infection of ewes was later determined to be via ingestion of feed or water contaminated with infected placental or fetal materials. From the gut, the organisms entered the bloodstream and were carried to the target organs.

In 1930, Jones and Little isolated vibrios from cows and calves with "winter scours".[15] In 1944, Doyle associated Vibrio coli (C. coli) with dysentery in swine.[16] Currently, neither syndrome is attributed to C. jejuni or C. coli; however, both cattle and swine are recognized as intestinal carriers of the organisms. In the mid-1950s, Hofstad[17] and Peckham[18] independently described vibrios which caused hepatitis in chickens. Although the precise taxonomic classification of these early experimental isolates is obscure, one can be relatively confident that

they were C. jejuni or C. coli based on their morphology and current understanding of the host range and target organs.

Since the early veterinary discoveries of the enteric campylobacters, numerous refinements in cultural techniques have led to the discovery of enteric campylobacteriosis in human beings. In 1957 and 1962, King, at the Centers for Disease Control (CDC), reported cultural differences between Vibrio fetus, which caused multiple systemic effects in compromised individuals, and "related vibrios", which were responsible for gastroenteric symptoms.[19,20] The "related vibrios" (C. jejuni) grew better at 42 C than at 37 C, whereas V. fetus flourished at 37 C, but failed to grow at 42 C. In 1972, Dekeyser et al. reported 2 cases of vibrionic enteritis confirmed by coproculture.[21] The discovery of these cases marked the beginning of a new era in the diagnosis of campylobacter enteritis, since previous cases were only identified by hemoculture.[19] Dekeyser used a 0.65μm filter to selectively culture the feces, eliminating the problem of rapid overgrowth by competing flora. Later in the 1970s, selective agar media became widely used for human coproculture due to the relative simplicity of the technique. Skirrow[9] and Butzler and Skirrow[22] first reported the use of inhibitors in agar plate media to isolate C. jejuni from feces. Shortly thereafter, commercially prepared selective agar plates became available and accepted for diagnostic use.[23]

With the explosion of diagnoses of campylobacter enteritis, many questions arose. C. jejuni and C. coli were already recognized as inhabitants of the intestinal tracts of most domestic animal species. Swine,[24] sheep,[24] cattle,[24] horses,[25] goats,[26] chickens,[27] cats,[28] and dogs[28] had been shown to carry and shed the organisms asymptomatically.

Additionally, dogs and cats had been shown to shed the organism profusely in association with diarrheal illness.[28] Additional work showed that several species of wildlife and laboratory animals may also carry the organism. Laboratory and wild rodents,[29] wild birds,[30] and migratory waterfowl[31] have been shown to carry and shed the organisms asymptomatically. Monkeys may carry C. jejuni asymptomatically or associated with diarrheal illness.[32] Campylobacters shed in the feces of domestic and wild animals can contaminate milk during its collection, surface water via runoff, and meat during processing of animal carcasses. There being no shortage of sources of potential human infection, the logical question is, "Which animal species are important reservoirs for human infections?" To date, consumption of poultry,[9,11,33] beef,[34] and unpasteurized milk[35] have been the mode of exposure for the majority of reported cases of campylobacteriosis; thus chickens and cattle must be considered important reservoirs. Water-borne outbreaks have accounted for a sizable minority of reported human campylobacteriosis cases, but here the animal source has usually been obscure.[7,36,37]

Survival and growth of campylobacters in milk,[38-41] surface water,[42] and meat[43] have been the subjects of research, as they are the most logical vehicles of infection to human beings. The results of these studies have indicated that human campylobacteriosis from these 3 major sources can be prevented by proper pasteurization of milk,[39] boiling of untreated surface water used for consumption,[42] and cooking meat to an internal temperature of 60 C.[43] The use of separate utensils and vessels for cooked and uncooked meat may also be recommended to prevent cross contamination.

Thus, the mode of transmission of C. jejuni has appeared to be fecal to oral, with foods of animal origin and water usually serving as vehicles of exposure. Contact with puppies and kittens with diarrhea has been associated with a few documented cases of campylobacter enteritis.[9,44,45] Secondary transmission has been uncommon, usually limited to cases of vertical transmission from the mother to the neonate,[46,47] and horizontally to people in contact with feces or diapers of children with diarrhea.[48,49]

Although significant reservoirs and modes of transmission have been identified, there are still fundamental problems in epidemiological investigations of campylobacteriosis. Unavailability of suspected vehicles of exposure after outbreaks has been common in campylobacteriosis and other food- or water-borne infections. Even if the suspected vehicles have been available, the organisms may have perished due to their fastidious requirements. Particular difficulty has been encountered in the investigation of unpasteurized milk-associated outbreaks, and to date C. jejuni has not been isolated from milk which has been epidemiologically linked with any published outbreak.

Isolation of C. jejuni from foods and water has been a different type of problem than isolation of the organisms from the feces. While competing flora has been a problem in fecal campylobacter isolation, low numbers of the fastidious organisms complicate recovery from foods and water. A selective enrichment medium appears to be the method of choice for isolation of C. jejuni from foods and water, since it enhances cellular repair and growth of campylobacters, at the same time limiting growth of competing flora.[50] Such media have previously been used successfully to isolate

coliforms,[51] Salmonella spp.,[51] and Vibrio parahemolyticus[52] from foods, and Campylobacter spp. from bovine genital tracts[53] and the gallbladders of slaughtered pigs.[54]

Once C. jejuni has been isolated from a suspected food, animal, or water sample, and a sick patient's feces, the association between the 2 isolates must be examined, since the organisms are ubiquitous. Several biotyping schemes using growth temperatures, and biochemical and tolerance tests have been devised to characterize the enteric campylobacters. One of the early attempts to biotype enteric campylobacters was performed by Lussier, who characterized C. coli from swine.[55] Skirrow and Benjamin devised a scheme using 3 tolerance tests (2,3,5-triphenyltetrazolium chloride, and incubation at 30.5 and 45.5 C) to distinguish C. jejuni from C. coli, but the scheme failed to distinguish these species, due to the existence of strains with intermediate characteristics.[56] In another study, the tolerance of strains to varying concentrations of sodium chloride and bile salts was investigated to distinguish thermophilic campylobacters.[57] Again, the differences among strains were not defined clearly enough to distinguish species. Razi and Park adapted the hippurate hydrolysis test to campylobacters, distinguishing C. jejuni from other enteric campylobacters.[58] Of all the campylobacter species characterized, only C. jejuni hydrolyzes hippurate. The thermophilic campylobacters (C. jejuni, C. coli, and naladixic acid-resistant thermophilic campylobacters [NARTC]) grow at 42 C, whereas C. fetus subsp. fetus does not.[56] Of the 3 thermophilic campylobacters, NARTC is naladixic acid-resistant, while C. jejuni and C. coli are sensitive to naladixic acid.[56]

Generally, these biotyping schemes do not sufficiently distinguish campylobacter isolates to be epidemiologically useful.

Unlike biotyping, serotyping has shown promise in the investigations of C. jejuni enteritis outbreaks. Abbott et al. demonstrated bacteriocidal and agglutinating antibodies to heat-stable and heat-labile antigens in the serum of campylobacter-infected patients.[59] Jones et al. followed with a demonstration of more specific complement-fixing antibodies.[60] Penner and Hennessy have adapted a passive hemagglutination technique to serotyping of C. jejuni and C. coli based on soluble heat-stable antigens.[61] Penner's method of serotyping has been applied to several human and animal isolates in 2 studies, with minor problems of cross reactivity.[62,63] Lior's slide agglutination test based on heat-labile antigens has appeared to be gaining wide use internationally.[64] Additionally, Kosunen has demonstrated strain-specific antigens using immunoelectrophoresis and co-agglutination.[65]

A third method of typing C. jejuni and C. coli has involved the use of lysogenic bacteriophages. Bryner has performed the most extensive work in phage typing of C. jejuni and C. coli; this method may prove to be a useful adjunct to serotyping.[66]

Risk factors will become better defined as typing schemes are used more extensively, and preventive measures will be most effective if they are based on adequate knowledge of the risks.

Having reviewed the epidemiological features of enteric campylobacteriosis, a review of milk-associated campylobacteriosis is in order. The first recorded outbreak of campylobacteriosis associated with unpasteurized milk was reported by Levy in 1945.[13] A dairy that supplied milk to 2

prisons mistakenly delivered unpasteurized milk, and as a result, 355 inmates became ill with campylobacter enteritis. Because specific cultural requirements for campylobacters were not recognized, the isolates were lost for further study.

Beginning in 1979 (34 years after the first reporting of milk-associated campylobacteriosis), reports of milk-associated campylobacterioses have virtually flooded the literature. From the U. S. have come reports of 6 outbreaks involving 438 patients. Potter et al. described an outbreak incriminating certified unpasteurized milk in Atlanta, Georgia.[35] Fifty patients from the community had campylobacter-positive fecal cultures that were diagnosed at hospitals participating in the study. In another certified unpasteurized milk-associated outbreak, Taylor et al. described 3 California patients observed in a hospital medical practice.[67] Only one U.S. outbreak, involving 3 patients, was associated with consumption of non-commercially obtained milk.[68] The number of cases in the U.S. associated with consumption of unpasteurized milk from private sources is probably greatly underestimated. The CDC reported 77 cases associated with unpasteurized milk from an Oregon dairy,[69] 264 cases associated with unpasteurized milk from a Kansas dairy,[70] and 41 cases in school children that consumed unpasteurized milk from a New Mexico dairy.[a]

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[a] M. J. Blaser, unpublished notes on milk-associated outbreaks of C. jejuni enteritis, Centers for Disease Control, Atlanta, GA, 1979.

The occurrence of outbreaks associated with certified unpasteurized milk has called into question the ambiguity of the term "certified". Based on currently available technology, no unpasteurized milk can be certified to be free of pathogenic organisms.

An outbreak among church camp attendees in Canada was associated with unpasteurized milk from a private source.[71] Sixty-four of 111 campers that consumed unpasteurized milk became ill with C. jejuni enteritis. Of 103 campers that did not consume the unpasteurized milk, only 3 became ill ($P < 0.005$ by chi-square test). According to 2 British reviews, 16 milk-associated outbreaks involving 4,054 campylobacter-infected patients occurred in Great Britain from 1978-1981.[72,73] In an unusual outbreak involving 148 patients, inadequate pasteurization was blamed.[74] Apparently, the bypass valve system that recycles milk not reaching pasteurization temperature (72 C) failed. The British also have the dubious distinction of having reported the largest documented outbreak of campylobacteriosis, in which 2500-3500 school children who drank unpasteurized milk became ill.[75] Another noteworthy outbreak in Aberdeen, Scotland resulted when a power failure forced the distribution of unpasteurized milk for 2 days.[76] In all, 616 cases were recorded in this outbreak. The Public Health Laboratory in Manchester, England reported on 2 additional school-related outbreaks involving 111 cases,[77] and 2 outbreaks in which milk from a single farm was associated with 80 cases of C. jejuni enteritis.[78]

Since the recent onslaught of reports of milk-associated campylobacterioses, studies have further defined risk factors and the scope of related problems. Although unpasteurized milk is not considered a growth medium for C. jejuni, the organisms can persist in refrigerated milk for

2-3 weeks.[38] This, coupled with an apparently low infectious dose,[79] may explain the infectivity of unpasteurized milk. In addition to fluid milk, unpasteurized cheese products have been a suggested source of C. jejuni infection.[b] Cheese products should be free of campylobacters, due to the low pH (5.0-5.2) attained in fermentation,[80] and the length of time between manufacture and consumption of the cheese. However, there may be a risk with homemade products, due to lack of batch uniformity and the short length of time between preparation and consumption. Goat milk is occasionally recommended as an alternative to cows milk for infants and other patients, and it should also be considered as a possible source of C. jejuni infection unless it is pasteurized.[b]

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[b] R. W. Currier, from the 1982 infectious disease summary, Iowa State Health Department, Des Moines, IA.

DEVELOPMENT OF THE PROBLEM

During 1981 and 1982, several studies on Campylobacter jejuni were in progress in Iowa. This study has revolved around dairy cows as a reservoir host, and unpasteurized milk as a vehicle of human infection. The impetus for this study arose from the perennial debate over repeal of the pasteurized milk ordinance in Iowa.

Consumption of unpasteurized milk has been reported to be an important mode of C. jejuni exposure for human beings. The number of unpasteurized milk-associated cases found in the literature approaches 5,000[13,35,67-78], and doubtless many additional cases have gone undiagnosed, unreported, or not identified as to source. Although the vast majority of milk-associated campylobacterioses have been due to the consumption of unpasteurized cows' milk, unpasteurized goat milk[b], unpasteurized cheese products[b], and improperly pasteurized cows' milk[74] have been incriminated in a few outbreaks.

Cattle are excellent hosts for C. jejuni, because most harbor the organism asymptomatically. A cow may become chronically infected, with organisms periodically being shed from the gallbladder into bile.[81] The C. jejuni may then enter the milk by fecal contamination or possibly udder infection.[82]

The reported prevalence of C. jejuni in healthy cattle is quite variable, and may reflect differences in herd management, geography,

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[b] R. W. Currier, from the 1982 infectious disease summary, Iowa State Health Department, Des Moines, IA.

laboratory isolation procedures, and other factors. The organism has been isolated from as few as 2.5% and as many as 100% of healthy cattle in several North American studies[38,81,83,84] and one Nigerian study.[85] The prevalence of C. jejuni in a herd as well as the magnitude of fecal shedding and milking parlor hygiene are important factors in determining the risk of C. jejuni infection from unpasteurized milk consumption. However, since C. jejuni is undetected by current standard plate count methods, no unpasteurized milk can be considered free of C. jejuni.

All milk-associated campylobacteriosis is preventable by proper pasteurization (72 C for 15 seconds), as evidenced by the lack of any documented cases associated with the consumption of properly pasteurized products. In addition to this strong circumstantial evidence of the effectiveness of pasteurization, several studies have provided empirical evidence that proper pasteurization eliminates the risk of milk-borne transmission of campylobacters.[38-41,80]

One objective of this study was to determine the carrier rate of thermophilic campylobacters in a sample of Iowa dairy cows by culturing feces of milking cows, the bile of slaughtered cull cows, and milk line sock filters. These prevalence data were obtained to demonstrate the potential risk of campylobacteriosis from consumption of unpasteurized milk in Iowa.

Since the recent discovery of C. jejuni as a leading bacterial cause of human gastroenteritis, there has been an acute need for epidemiological markers to assist in the investigation of outbreaks and to determine the relative importance of different animal species as reservoir hosts. Biotyping has distinguished C. jejuni, C. coli, and naladixic acid-

resistant thermophilic campylobacters (NARTC) from each other; however, none of the thermophilic campylobacter species are host species specific. Serotyping is a more specific method of characterizing zoonotic campylobacter isolates, and its use as an epidemiological tool is already established.[59,61,62,86] In this study, serotyping was used to compare C. jejuni and C. coli isolates of human and domestic animal origin.

Description and analysis of epidemiological variables from campylobacter surveillance data are useful tools for the understanding and prevention of campylobacteriosis. Several variables, including age, gender, geographical distribution, seasonality, and occupation have been examined in relation to campylobacteriosis.[87] An objective of this study was to examine epidemiological variables in relation to unpasteurized milk-associated campylobacteriosis, and to delineate the differences between all reported cases and unpasteurized milk-associated cases. In this manner, additional risk assessment regarding milk-borne campylobacteriosis was provided.

MATERIALS AND METHODS

Two grade A dairy herds and one research herd in central Iowa were selected for collection of prevalence data. One grade A herd and the research herd were parlor-milked; the other grade A herd was milked in a stanchion barn. Each herd was sampled 3 times at monthly intervals, and the first sampling for each herd was staggered one month apart. The sampling periods extended from March through July.

Rectal swabs were obtained at milking time, and placed into 4 ml of Mueller-Hinton broth for transport to the laboratory. Within 4 hours of collection, 0.05 to 0.1 ml of the fecal swab suspension was transferred to 10 ml of selective enrichment broth medium[53] and incubated at 42 C for 24 hours. From the selective medium, 0.1 ml was transferred to a brain heart infusion agar plate containing 10% defibrinated bovine blood. Agar plates were incubated microaerobically at 42 C, observed at 24, 48, and 72 hours postinoculation, and suspected campylobacter isolates were preserved for further testing.

In addition to the local dairy herd isolates, a pool of 88 isolates obtained from the bile of cull cows at a central Iowa packing plant were examined. The 525 animals cultured to obtain the pool of 88 isolates represented numerous herds from throughout the state.

Four milk line sock filters were collected from a grade A dairy at 3 month intervals for one year, in order to detect the prevalence of campylobacters in milk. The filters were collected immediately after milking, or were refrigerated and transported to the laboratory within 12 hours of milking. The filters were washed with and agitated in 100 ml of Mueller-

Hinton broth, and the cultural isolation procedure described for rectal swabs was followed.

In the next portion of the study, thermophilic campylobacter isolates from dairy cattle, chickens, sheep, pigs, and human beings were characterized by biotyping and serotyping. Isolates were tested for catalase, nitrate, and hydrogen sulfide production, tolerance of 0.5% glycine and 3.5% sodium chloride, hippurate hydrolysis, and antibiotic sensitivity with naladixic acid and cephalothin.[24] Antigens were prepared and serotyping performed according to the passive hemagglutination technique of Penner and Hennessy.[61]

Seventy-four thermophilic dairy cow isolates were obtained from the above-mentioned pool of 88 bile isolates. Eighty-six chicken isolates were obtained from body cavities or skin sampled at a poultry processing plant in eastern Iowa. The 16 sheep (reproductive tract) and 19 pig isolates came from the National Animal Disease Center campylobacter collection. Ninety-nine human fecal isolates were provided by the Iowa State Hygienic Laboratory (state reference laboratory).

Serological profiles for the various host species were established, based on the samples of isolates described above. The data from our sampling of human isolates were compared with data published by McMyne et al.[62] Based on surveillance data from the Iowa State Health Department, and laboratory records from Dubuque Mercy Hospital (Dubuque, Iowa) and the Iowa State Hygienic Laboratory, a sub-sample of human isolates containing only unpasteurized milk-associated isolates was established.

Two hundred sixty-three cases of human campylobacteriosis were reported to the Health Department in a recent year (August 1981 to July

1982). Extensive epidemiological data were compiled for 186 of the 263 reported cases, while less complete data were available for the other 77 cases. Data accompanying all cases included date of diagnosis, whether or not the patient was hospitalized, county of residence, urban versus rural residence, gender, and age. Additional data available for some, but not all, cases included occupation, pet ownership, contact with domestic animals, type of drinking water supply, consumption of undercooked chicken or unpasteurized milk products within one week prior to illness, and history of interstate or international travel within one week prior to illness. Unpasteurized milk-associated cases were examined and compared as a group against all reported cases.

RESULTS

Prevalence Findings

The detection of thermophilic campylobacters from the 3 central Iowa dairy herds sampled is shown in Table 1. The prevalence in the research herd was the lowest of the 3 herds at 10% (1/10). The stanchion-milked herd had 35.5% (11/31) campylobacter-infected cows, while the parlor-milked grade A herd had 57.7% (15/26) campylobacter-infected cows. The overall prevalence of thermophilic campylobacters in the 3 herds was 40.3%.

All cows in the research herd were in mid-lactation, and no lactation data were obtained from the grade A parlor-milked herd. Interestingly, in the stanchion-milked herd, all 3 cows that freshened during the sampling period shed campylobacters. Approximately 50% of the cattle demonstrated to be positive (13/27) yielded campylobacters in 2 or all 3 monthly samples.

Results of biotyping of the 26 isolates from 2 herds were unexpected for cattle compared to published reports. All isolates from the stanchion-milked cows were *C. coli*, and all isolates from the grade A parlor-milked herd were NARTC. The single isolate from the research herd was *C. jejuni*, as have been the majority of reported isolates from cattle.[56]

Of the 88 bile isolates of campylobacters from cull cows, 8 were lost during frozen storage, 6 were *C. fetus* subsp. *fetus* (not thermophilic), and 74 were thermophilic. Of the thermophilic isolates, 69 (91%) were *C. jejuni* and 7 (9%) were *C. coli*. Adjusted for the 8 lost isolates, the

detected prevalence of thermophilic campylobacters in the bile of these cull cows was 15.5%.

No campylobacters were isolated from the 16 milk line sock filters cultured, despite the presence of fecal debris on the filters.

Biotyping and Serotyping Findings

Results of biotyping showed that 91% of cattle isolates, 96% of chicken isolates, 100% of sheep reproductive tract isolates, none of the pig isolates, and 94% of human isolates were C. jejuni. The remainder of the isolated were C. coli.

Serological profiles of C. jejuni and C. coli isolated from animal hosts and human beings are shown in Table 2. These profiles were developed by using the 4 most common serological types of campylobacters from each of the host species studied. One group of closely related serotypes (4, 13, 16, 43, and 50) was considered together, because of extensive cross-reactivity. There were 7 (9.5%) cattle isolates, 6 (6.1%) human isolates, and 4 (4.6%) chicken isolates untypable using Penner's antisera 1-55. Thirty-eight (51.4%) of cattle isolates and 50 (50.5%) of human isolates were serotype 2 or were in the group of closely related serotypes. The remaining 29 cattle isolates (39.1%) were widely scattered among the other serotypes, with no single serotype accounting for greater than 4% of the total. Serotypes 5, 10, and the group of closely related serotypes accounted for 45 (52.3%) of chicken isolates and 45 (45.4%) of human isolates. Because of these similarities, cattle, human, and chicken

TABLE 1 Coproculture findings of thermophilic campylobacters in three central Iowa dairy herds

Research Herd				Stanchion-Milked Herd							
Animal No.	Month of Sample			Animal No.	Month of Sample			Animal No.cont.	Month of Sample		
	Mar.	Apr.	May		May	June	July		May	June	July
126	-*		-	-	1	-	-	-	47	-	- -
215	-	-	-	7	-	-	-	52	-	-	-
238	-	-	-	9	Dry†	+‡	+	53	Dry	Dry	+
245	-	-	-	11	-	Dry	Dry	55	-	-	-
258	+	+	-	13	-	-	-	56	+	+	+
265	-	-	-	15	+	-	-	57	-	+	-
1204	-	-	-	19	-	-	-	58	-	-	-
8203	-	-	-	20	-	Dry	Dry	60	-	-	-
8408	-	-	-	22	-	-	-	65	-	-	-
8735	-	-	-	23	-	+	-	80	-	-	-
				25	-	-	-	115	NS§	-	-
				29	Dry	+	-	116	NS	-	-
				31	-	-	-	117	NS	-	-
				32	-	+	+	118	NS	-	-
				33	-	+	+	388	+	Dry	Dry
				37	+	-	+				

* Campylobacter negative culture.

† Dry cow, not sampled.

‡ Campylobacter positive culture.

§ Not sampled.

TABLE 1 (continued)

Parlor-Milked Herd							
Animal	Month of Sample			Animal	Month of Sample		
No.	Apr.	May	June	No.cont.	Apr.	May	June
87	-	NS	-	110	+	-	+
92	+	NS	-	113	-	-	+
93	-	NS	-	115	NS	-	-
94	-	NS	+	116	NS	-	+
95	-	NS	-	117	NS	-	-
99	+	NS	+	118	NS	-	-
101	+	NS	+	119	NS	-	+
102	+	NS	+	120	NS	-	-
104	-	-	-	122	NS	-	+
105	+	+	+	123	NS	-	-
106	-	+	+	124	NS	-	-
107	-	-	+	127	NS	-	-
108	+	+	+	130	NS	-	-

TABLE 2 Serological profiles of C. jejuni and C. coli isolated from human beings and animals (expressed in percent)

	Human Isolates			Animal Isolates			
Penner Serotype	Reported by McMyne[26] (Canada) n=168	Iowa Isolates n=99	Raw Milk-Associated (Iowa) n=17	Bovine (Iowa) n=74	Chicken (Iowa) n=86	Porcine (Iowa) n=19	Ovine* (Iowa) n=16
1	11.6	10.1	none	2.7	1.2	none	100
2	12.5	16.2	17.6	25.5	none	none	none
3	3.6	8.1	5.9	1.4	3.5	none	none
4†	25.3	34.3	41.2	25.7	12.8	10.5	none
5	6.2	6.1	5.9	2.7	20.9	5.3	none
10	1.2	5.0	none	none	18.6	none	none
30	none	none	none	1.4	2.3	15.8	none
44	none	none	none	1.4	20.9	none	none
48	0.6	none	none	2.7	none	26.3	none
Percent of all Isolates	61.0	79.8	70.5	63.7	80.2	57.9	100

* From aborted tissues or reproductive tracts.

† Represents cross-reacting serotypes 4, 13, 16, 43, and 50.

isolates were selected for further comparison. Figure 1 compares the relative serotype distribution of campylobacter isolates among dairy cows, human beings, and chickens. To aid the species comparisons, antisera to serotypes 13, 16, 43, and 50 were absorbed with serotype 4 live whole-cell antigen. This eliminated the cross-reactivity between serotype 4 and related serotypes (13, 16, 43, and 50) without reducing homologous titers greater than one 2-fold dilution. Thus, serotypes 2, 4, and related serotypes (13, 16, 43, and 50) most prevalent among both human and cattle isolates accounted for only 11 (12.8%) of the chicken isolates. The 3 serotypes most prevalent in chickens (5, 44, and 10) accounted for 52 (60.4%) of the chicken isolates, but they only accounted for 11 (11.1%) of the human isolates and 3 (4.1%) of the cattle isolates.

The distribution of serotypes of human isolates in this study paralleled the distribution reported by McMyne et al.[62] (Table 2). The 3 most prevalent serotypes occurred in the same order of prevalence in both studies, and there were several similarities even among the less prevalent serotypes. The small number of unpasteurized milk-associated isolates examined were distributed in a pattern of serotypes similar to that of human isolates from Iowa as a whole (Table 3).

Epidemiological Surveillance Findings

Surveillance data showed that 23% (39/186) of patients had a history of unpasteurized milk consumption during the week prior to diagnosis of campylobacteriosis.

Fig. 1. Comparison of Penner serotype distributions among bovine, human, and chicken isolates

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* Represents cross-reacting serotypes (13, 16, 43, and 50).

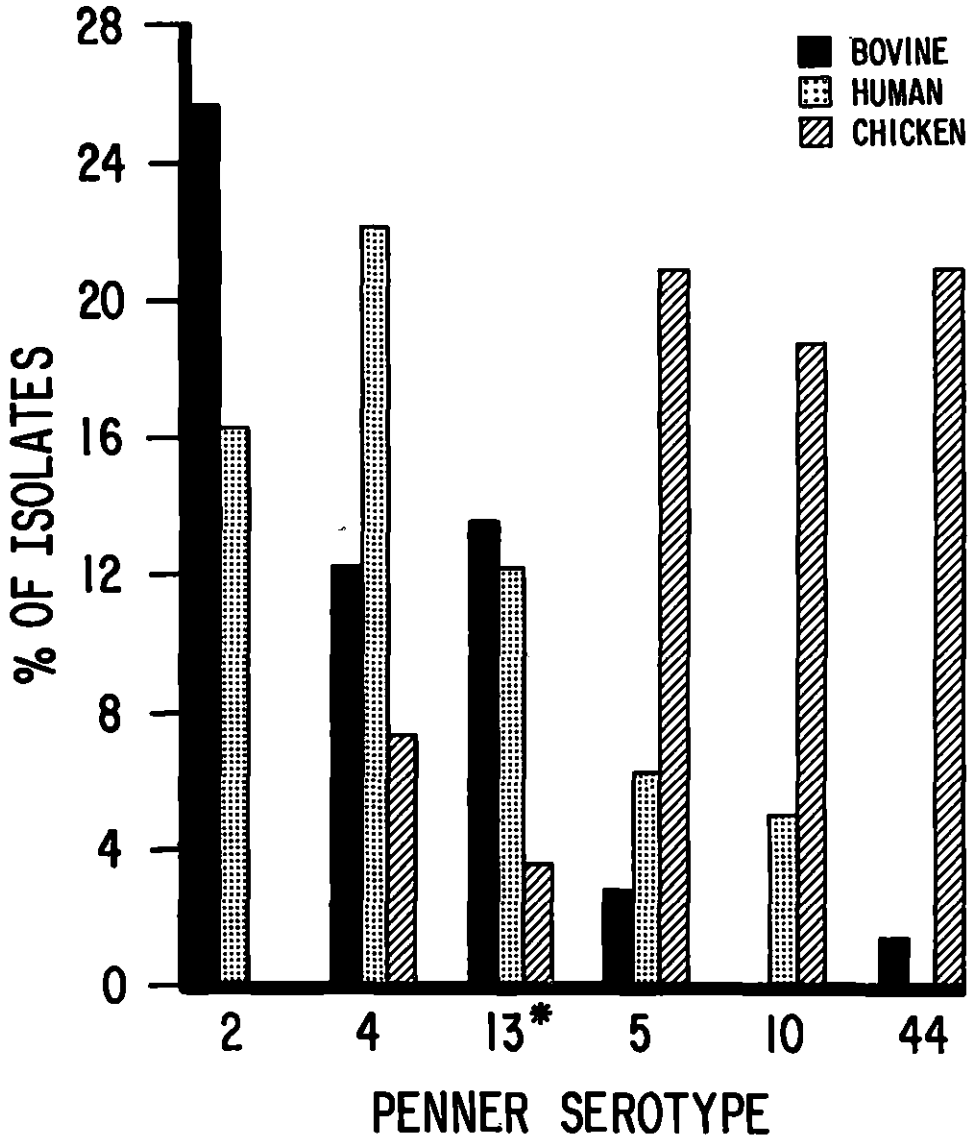

28
24
20
16
12
8
4
0
% OF ISOLATES
BOVINE
HUMAN
CHICKEN
2
4
13*
5
10
44
PENNER SEROTYPE

TABLE 3 Findings of serotyping unpasteurized milk-associated *Campylobacter jejuni* isolates

Month Isolated	Age of Patient	County of Residence	Penner Serotype
January	22	Blackhawk	43*
January	25	Dubuque	4
January	32	Marshall	2
January	11	Dubuque	4
January	1	Dubuque	4
February	20	Blackhawk	11
March	7	Dubuque	4
April	9	Polk	16*
April	<1	Marshall	23
April	9	Dubuque	2
April	1	Dubuque	Untypable
May	5	Dubuque	2
August	38	Tama	Untypable
October	1	Polk	3
November	76	Delaware	43*
December	22	Dubuque	5
December	32	Dubuque	35

* Cross-reacting serotypes (13, 16, 43, 50).

Approximately 50% (126/263) of the human campylobacteriosis patients had onsets in April through July (Fig 2). The peak period of onset of unpasteurized milk-associated cases was January through April (18/39 cases).

Figure 3 depicts the distribution of all reported cases and milk-associated cases of human campylobacterioses in Iowa counties. Interestingly, almost 50% (125/263) of the reported cases occurred in 3 populous counties (Polk, Dubuque, and Linn). However, only 30/263 cases occurred in Blackhawk, Scott, Woodbury, and Pottawattamie counties (also populous counties). Fourteen percent of all reported patients resided in Dubuque County; however, 41% of the unpasteurized milk-associated cases were reported from this county.

Urban residents accounted for 75% (197/263) of all cases, roughly representing the population split in this state (Table 4). Unpasteurized milk-associated cases were more evenly divided between urban (18/39) and rural (21/39) residents.

Distribution of cases by gender is shown in Table 5. Hospitalization status of patients with campylobacteriosis is shown in Table 6.

Figure 4 shows the distribution of cases of campylobacteriosis by age of the patients. This distribution is skewed to the left, with over 50% (23/39) of the unpasteurized milk-associated cases in the category of "birth to 9 years of age".

Fig. 2. Reported cases of campylobacteriosis by month of onset

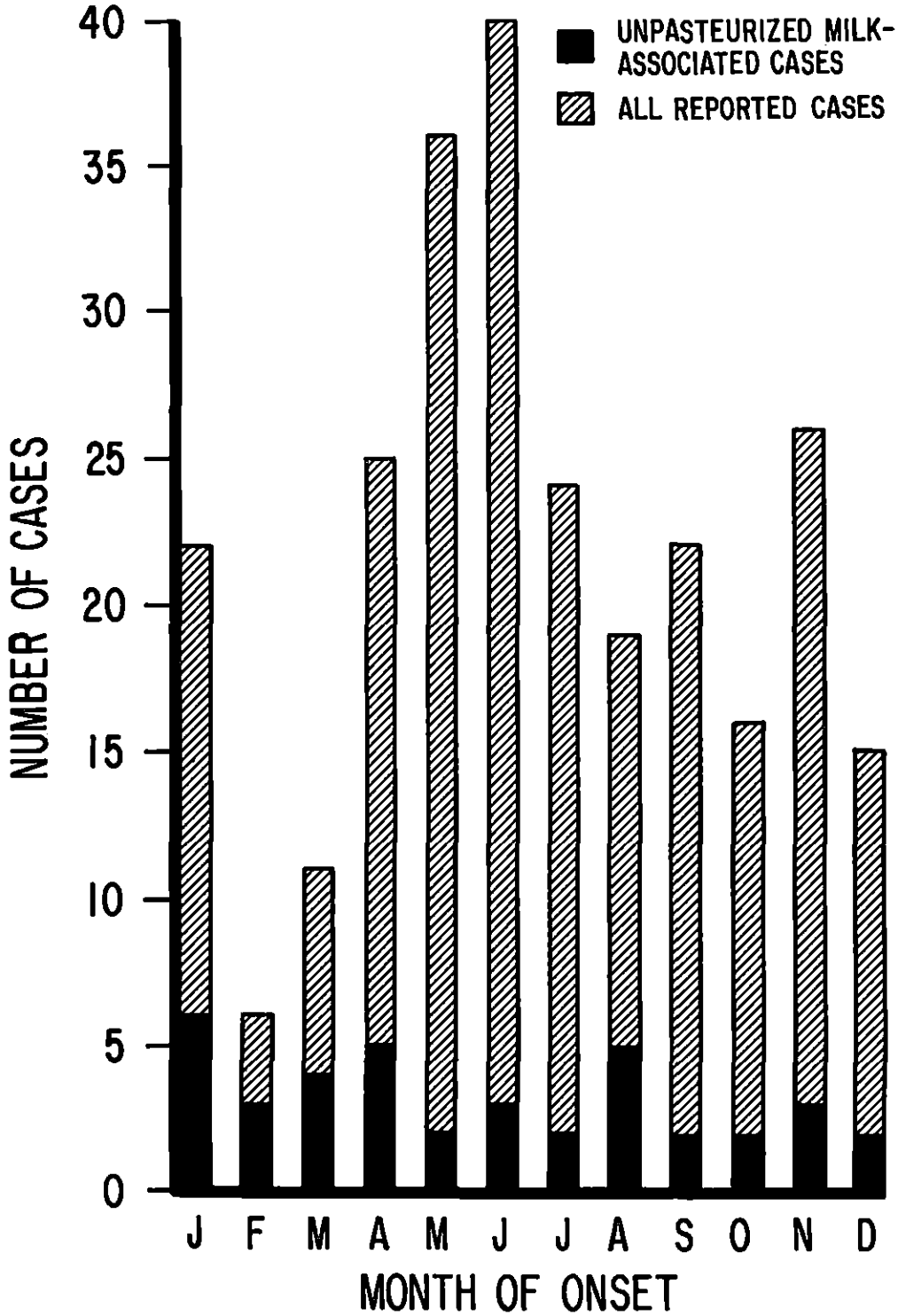

UNPASTEURIZED MILK-ASSOCIATED CASES
ALL REPORTED CASES
NUMBER OF CASES
40
35
30
25
20
15
10
5
0
J F M A M J J A S O N D
MONTH OF ONSET

Fig. 3. Distribution of all reported cases and unpasteurized milk-associated cases ( ) of campylobacteriosis in Iowa by county

Distribution of cases of

Campylobacteriosis by county

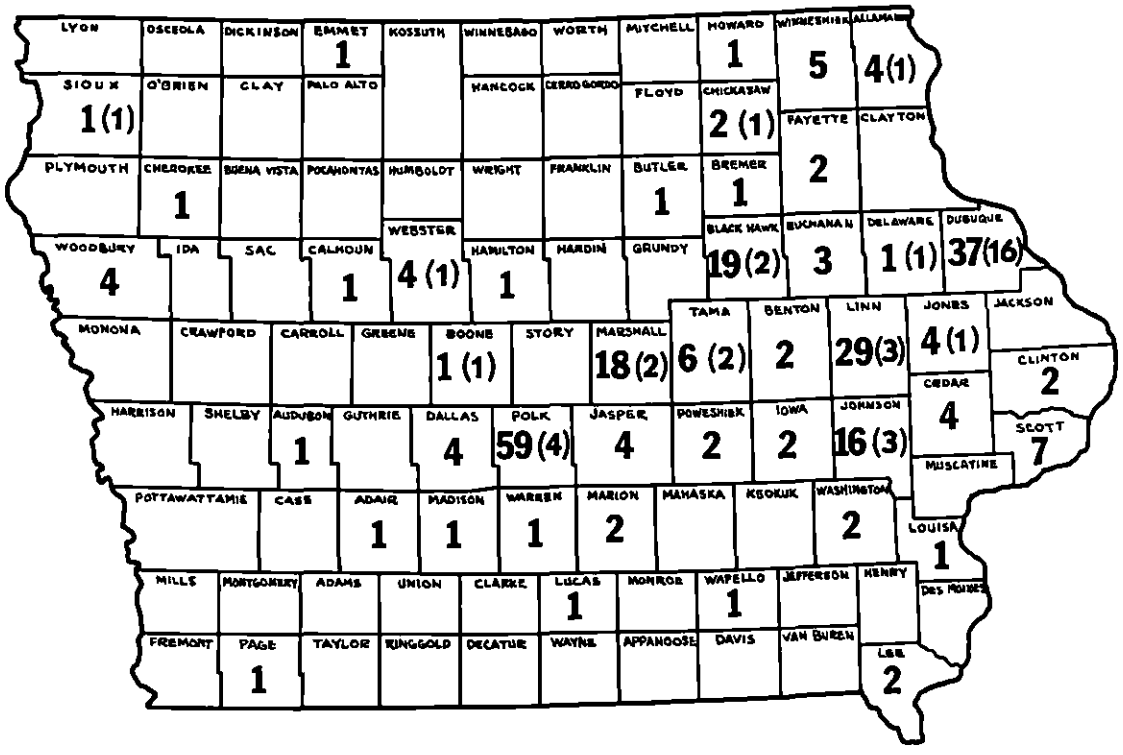


TABLE 4 Distribution of cases of campylobacteriosis by urban versus rural residence

	All Reported Cases	Milk-Associated Cases
Urban	197 (75%)	18 (46%)
Rural	66 (25%)	21 (54%)
Total	263 (100%)	39 (100%)

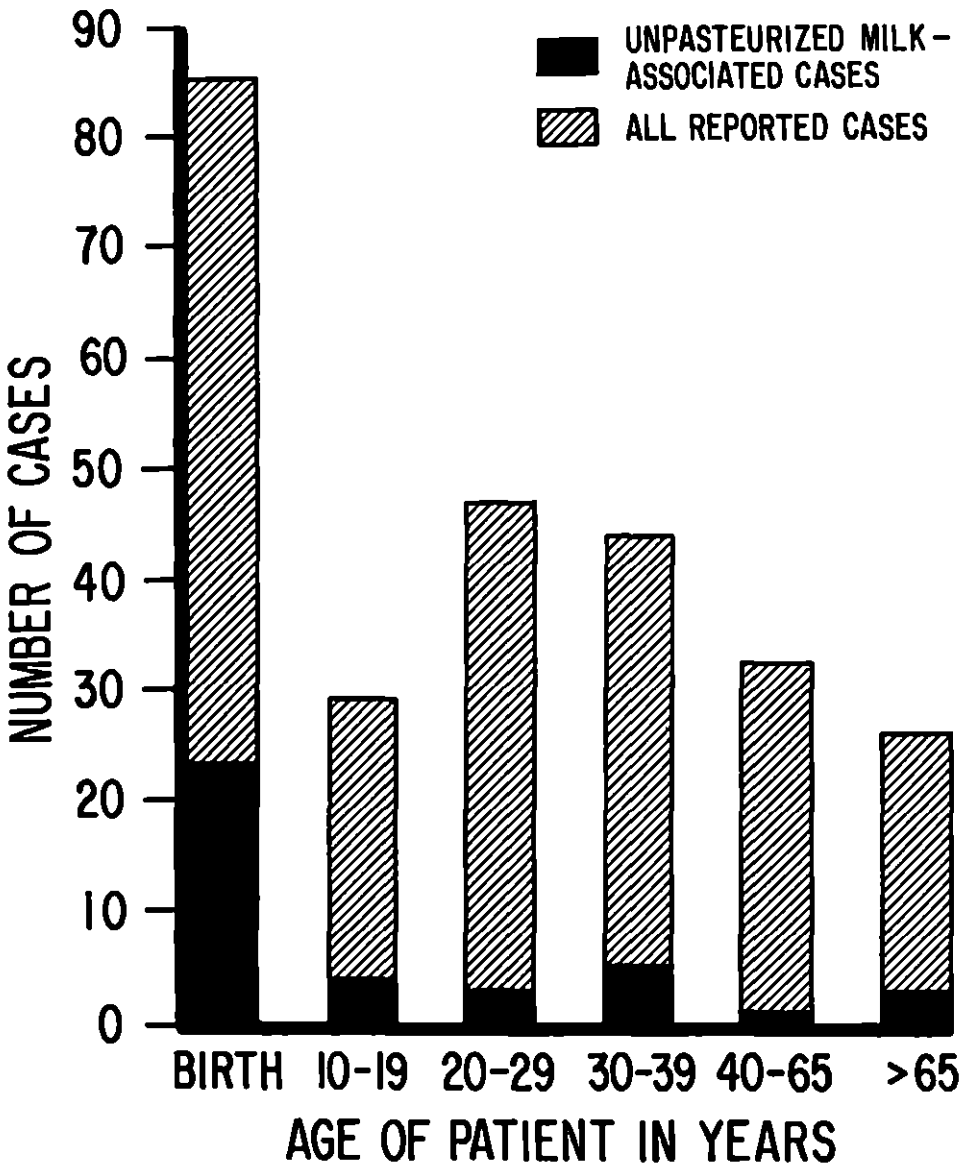
TABLE 5 Distribution of cases of campylobacteriosis by gender

	All Reported Cases	Milk-Associated Cases
Male	128 (49%)	20 (51%)
Female	135 (51%)	19 (49%)
Total	263 (100%)	39 (100%)

TABLE 6 Hospitalization status of campylobacter-infected patients

	All Reported Cases	Milk-Associated Cases
Hospitalized	156 (59%)	22 (56%)
Not Hospitalized	107 (41%)	17 (44%)
Total	263 (100%)	39 (100%)

Fig. 4. Reported cases of campylobacteriosis by age of patient


UNPASTEURIZED MILK-ASSOCIATED CASES
ALL REPORTED CASES
NUMBER OF CASES
90
80
70
60
50
40
30
20
10
0
BIRTH
10-19
20-29
30-39
40-65
>65
AGE OF PATIENT IN YEARS

DISCUSSION

There was considerable variation in the prevalence of campylobacter isolations among the 3 dairy herds and cull cows sampled, although the prevalence values all fell within the range reported in the literature. It appears likely that thermophilic campylobacters could be isolated from nearly any dairy herd, so the risk of milk contamination is ever present. The level of hygiene at the 3 dairy facilities was quite good, and there were no obvious management practices that might explain the differences in prevalence of campylobacter shedding. Although the number was small (3/3), the positive association between freshening and shedding of campylobacters may help to explain differences in the point prevalence of shedding both within a herd and among different herds. More data should be collected to determine the strength of this association.

In milk-borne campylobacteriosis outbreaks reported to date, the milk has been implicated either by isolation of the organisms from milk line sock filters or by statistical associations (food specific attack rates). Since unpasteurized milk is consumed by a very small segment of the milk-drinking population, statistical association has usually been easily demonstrated. Isolation of campylobacters from milk which has been epidemiologically linked to outbreaks has been attempted, but not as yet accomplished. Some success has been met in isolating campylobacters from milk line sock filters, but even this has been difficult, due to dilution and competing flora. Efforts to increase the sensitivity of method of detection should be continued.

Although the isolation of campylobacters from foods (milk in particular) has been difficult, progress has been made in the characterization of those campylobacters isolated. Biotyping of thermophilic campylobacters has been of limited use epidemiologically. Biotyping has provided a basis to reasonably conclude that swine are not a major reservoir host for human campylobacteriosis. Only 6% of the human isolates in this study were C. coli, whereas all the thermophilic campylobacter isolates from pigs were C. coli. Cattle, sheep, and chickens shed a high proportion of C. jejuni relative to C. coli, as do human beings. Differences in results of biochemical and tolerance tests have not been sufficient to distinguish among C. jejuni isolates from different sources. Serotyping is the current method of choice for identifying possible animal sources of infection in human outbreaks of campylobacteriosis.

Chickens and cattle have been suspected to be significant reservoir hosts for human campylobacteriosis, and the host species serologic profiles developed in this study have supported that suspicion. The profiles of sheep and pig isolates likewise indicated that these species were not likely significant reservoir hosts. Also, the similarities between the profiles of the 2 sources of human isolates indicated that the species profile concept is reasonably valid, if the sample of isolates is large enough.

The association of unpasteurized milk consumption and campylobacter enteritis is undeniable. In California, the largest of approximately 13 states which permit the sale of unpasteurized milk, the one large dairy that specializes in selling unpasteurized milk supplies less than 0.5% of all milk marketed in the state. Because a very small segment of the milk-

drinking population consumes unpasteurized milk, the finding in this study that 23% of all cases of campylobacteriosis were associated with consumption of unpasteurized milk is incriminating evidence. Although the specificity of this association was not examined in this study, there is clearly an increased risk of contracting campylobacteriosis among unpasteurized milk consumers.

In this epidemiological study, the unpasteurized milk-associated sample of cases resembled the sample of all reported cases, although seasonally, the peak isolation rate for the unpasteurized milk group was during January through April, unlike the warm weather peak period reported for all cases. Other studies have also reported peak isolation rates during the warmer months of the year.[87] Statistical analysis (chi-square test) indicated that the observed peak isolation period for unpasteurized milk-associated cases could have been due to chance alone. There was no other apparent explanation for this difference, based on examination of the data and knowledge of peak incidence for other milk-borne pathogens.

Although the overall distribution of cases by county was similar between all cases and unpasteurized milk-associated cases, Dubuque county reported a significantly greater proportion of unpasteurized milk-associated cases ($P < .001$ by chi-square test). This may have been due, in part, to the concentration of dairying in this part of the state and home consumption of unpasteurized milk. Not surprisingly, rural residents accounted for most (54%) of the unpasteurized milk-associated cases, whereas they accounted for only 25% of all reported cases. In this regard, Dubuque County is representative of the rest of the state, i.e., 62% of unpasteurized milk-associated patients were rural residents, but rural

residents accounted for only 27% of all reported cases. Of considerable interest was the finding that all 10 rural cases in Dubuque County were unpasteurized milk-associated.

Hospitalization data were only considered to establish points about diagnosis and reporting of campylobacteriosis. First, the most serious cases were much more likely to be diagnosed, and second, undiagnosed cases went unreported. In the context of this study, it is apparent that only a small portion of cases were diagnosed and reported. Further evidence of underreporting was inferred from data from the 7 most populous counties. The total population of Polk, Dubuque, and Linn Counties is equivalent to that of Blackhawk, Scott, Woodbury, and Pottawattamie Counties, yet 125 cases were reported from the first group of counties, and only 30 cases from the latter group.

The age distribution of campylobacteriosis may be explainable by a number of factors. Susceptibility to many infectious agents is higher in infants and young children. Also, young children may be more likely to receive medical attention for mild to moderate ailments than are adults or adolescents. In addition, secondary transmission of fecal-borne infections is most common in pre-school age children. The fact that milk comprises a sizable portion of the infant or toddler diet also supports the finding of over 50% (23/39) of unpasteurized milk-associated cases in the "birth to 9 years of age" category.

In conclusion, this study has demonstrated that consumers of unpasteurized milk are at high risk of becoming infected and ill with C. jejuni. Cows shedding thermophilic campylobacters can likely be found in any milking herd at any time, so the potential risk of milk contamination

is always present. Although campylobacters are difficult to isolate from milk, serological and epidemiological evidences support the conclusion that dairy cows transmit campylobacters to human beings via unpasteurized milk.

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