JOURNAL OF CLINICAL MICROBIOLOGY, May 1982, p. 761-768
0095-1137/82/050761-08$02.00/0

Vol. 15, No. 5

Serotyping of *Campylobacter jejuni* by Slide Agglutination Based on Heat-Labile Antigenic Factors

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Received 29 October 1981/Accepted 12 January 1982

A serotyping scheme for *Campylobacter jejuni* was developed based on slide agglutination of live bacteria with whole cell antisera absorbed with homologous heated and heterologous unheated cross-reactive antigens. Among 815 isolates from human and nonhuman sources, 21 serogroups were recognized. Of the 615 isolates from human cases of gastroenteritis, 529 (86%) were typable; 455 strains agglutinated in 20 single antisera, whereas 74 isolates agglutinated in various pairs of antisera, allowing subdivision of some main serogroups into subserogroups. Of the 200 isolates of *C. jejuni* from nonhuman sources (chicken, swine, etc.), 166 (83%) were typable, 145 cultures agglutinated in various single antisera, and 21 strains agglutinated with different pairs of antisera. Among isolates from all sources, 8 serogroups (1, 2, 4, 5, 7, 8, 9, and 11) were encountered most frequently. Serogroups 1, 2, 4, 5, 7, 9, and 11 were most common among human isolates; the majority of the chicken and all of the swine isolates belonged to the same serogroups identified from human cases. Very good serological correlation was obtained in 20 family outbreaks and 4 community outbreaks.

With the development of new selective stool culture techniques by Dekeyser et al. (12), Butzler et al. (8), and Skirrow (20), the recognition of *Campylobacter jejuni* (formerly "related vibrios" or *Campylobacter fetus* subsp. *jejuni*) as important agents in human gastroenteritis has highlighted the need for a better understanding of the epidemiology of these organisms. In the absence of a serological typing scheme, differentiation of the strains has been described by Bokkenheuser et al. (6), using bacteriophages, and more recently by Skirrow and Benjamin, who used a biotyping scheme based on the rapid hippurate hydrolysis test and the rapid H_2S test (21).

Early attempts to determine the serological identity of these organisms were made in 1957 by King (14), who has shown that the "related vibrio" organisms and *Vibrio fetus* isolated from human disease were antigenically different. In 1971, Berg et al. (3) divided the *Campylobacter* group (*Vibrio fetus*) into three serotypes based on heat-stable antigens. Butzler and Skirrow (9) have reported on the serotyping of *C. jejuni* isolated from human disease with a slide agglutination technique. Recently Penner and Hennessy (18) and Lauwers et al. (16) have reported on the serotyping of *C. jejuni* by a passive hemagglutination technique with heat-stable soluble antigens.

The purpose of this paper is to report on the development of a serotyping scheme for *C. jejuni* based on a rapid slide agglutination technique with live bacteria and absorbed antisera for the detection of heat-labile antigenic factors.

MATERIALS AND METHODS

Bacteria. Reference strains 1 to 16 and 22 were isolated for the first time from humans; reference strains 17, 18, 19, and 21 were isolated from chickens; and reference strain 20 was isolated from swine. *C. jejuni* strain NCTC 11168 was used as reference strain 4. A total of 815 cultures of *C. jejuni* (615 isolated from human cases of gastroenteritis and 200 strains isolated from nonhuman sources) were obtained from various provincial laboratories of public health, hospitals, universities, and federal agencies.

Ten strains of *C. fetus* subsp. *fetus* (*C. fetus* subsp. *intestinalis*) were isolated from human sources. Ten strains of *C. fetus* subsp. *venerealis* isolated from bovine were received from the Animal Disease Research Institute, Agriculture Canada, Ottawa, Ontario.

All cultures were grown on Mueller-Hinton agar (Oxoid Ltd., London, England) containing 5% sheep blood and incubated at 37°C in the gas mixture recommended by Skirrow (20). Since preliminary investigations indicated that the antigenic factors may be poorly defined after 24 h of incubation, all cultures were tested after 48 h of incubation.

Antisera. For the preparation of antisera, smooth strains were inoculated on five petri plates (150 by 15 mm) containing Mueller-Hinton broth (Oxoid) with 1% agar (Difco Laboratories, Detroit, Mich.) and incubated for 48 h at 37°C in the appropriate gas mixture. The bacterial growth was transferred to 10 to 12 ml of phosphate-buffered saline (PBS, pH 7.2) containing

0.5% formaldehyde, washed three times, and resuspended in the PBS containing formaldehyde to approximately 10^{10} bacteria per ml.

After preimmune bleeding, New Zealand white rabbits (2.7 to 3.0 kg) were injected intravenously at 4- to 5-day intervals for 4 weeks with increasing doses of bacterial suspension (0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, and 2.5 ml). The rabbits were bled by cardiac puncture 7 to 10 days after the last injection, and the sera were separated and preserved with 1:10,000 Merthiolate at 4°C.

Homologous and heterologous titers of the antisera were determined by tube titration. Washed (three times) and formalinized bacterial suspensions diluted to an optical density of 0.45 at 530 nm in a Beckman 25 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) were mixed with equal amounts of antisera serially diluted twofold in PBS and incubated for 4 h in a 50°C water bath. The initial serum dilution was 1:200 and the end titer was the reciprocal of the highest dilution of antisera showing a partial (2+) agglutination. Heterologous and homologous reactions were also determined by slide agglutination with crude antisera diluted 1:5 in PBS.

Absorptions. Antisera were absorbed with homologous heat-stable and heterologous, unheated cross-reactive antigen suspensions. For the removal of homologous antibodies to heat-stable antigenic factors, growth obtained from 10 to 15 large plates (150 by 15 mm) was heated for 2 h at 100°C and washed three times with PBS. Subsequently, 5 ml of antisera diluted 1:2 with PBS was added to the packed cells and mixed well. The mixture was incubated for 4 h at 37°C and then incubated overnight at 4°C and centrifuged for 15 min at 5,000 × *g*. Cross-reactive antibodies to heat-labile antigens were removed in the same manner, except that the washed bacterial suspensions were not heated. Single or double absorptions were performed as follows: antiserum 1 absorbed with reference strain 2; antiserum 5 absorbed with reference strain 6; antiserum 6 absorbed with reference strains 4 and 5; antiserum 7 absorbed with reference strain 1; antiserum 11 absorbed with reference strain 2; antiserum 12 absorbed with reference strain 5; antiserum 17 absorbed with reference strains 4 and 9; antiserum 19 absorbed with reference strain 18; antiserum 20 absorbed with reference strain 5; and antiserum 22 absorbed with reference strains 11 and 14.

Slide agglutination. Slide agglutinations were performed on glass slides with crude and absorbed antisera diluted 1:5 with PBS. Presence or lack of agglutination was determined 30 to 45 s after a small loopful of bacteria was mixed with 1 drop (50 μl) of antiserum. Autoagglutinability was determined by mixing a small loopful of bacteria with 1 drop of PBS.

DNase treatment. Strains of *C. jejuni* showing a rubbery, sticky consistency were emulsified on the glass slide before the addition of antisera in a small droplet (10 μl) of a nuclease solution containing 0.1% DNase (bovine pancreas DNase; Boehringer Mannheim Canada, Dorval, Quebec).

RESULTS

Antisera. The results of homologous and heterologous tube titrations with crude antisera and Formalin-treated antigens are shown in Table 1. A positive reaction occurred within 4 h as floccular aggregates, typical of flagellar agglutination. Further incubation of the tubes did not produce alterations in the titers. Titers of up to 1:800 were seen with unilateral (one-way) reactions of 14 antisera, with most sera showing agglutination with two to three reference strains. Furthermore, antiserum 16 showed cross-reactivity with six reference strains, antiserum 17 showed cross-reactivity with four reference strains, and antiserum 18 showed cross-reactivity with five reference strains.

Slide agglutination results of the 21 antisera with the homologous and heterologous live *C. jejuni* strains are shown in Tables 2 and 3. Of the crude antisera, 16 were found to cross-react with live reference strains before the removal of homologous heated antigens (Table 2). Agglutination with single reference strains was seen between antiserum 5 and reference strain 6, antiserum 7 and reference strain 1, antiserum 9 and reference strain 13, antiserum 13 and reference strain 11, antiserum 15 and reference strain 13, and antiserum 19 and reference strain 18. Agglutination with two to four reference strains was seen with antisera 1, 2, 6, 12, 16, 18, 20, and 22 (Table 2). Antisera 11 and 17 showed the most heterologous reactions, each agglutinating five and seven of the reference strains, respectively. Antisera 4, 8, 10, 14, and 21 were highly specific and did not cross-react with any of the reference strains.

Upon absorption of the antibodies to homologous heated antigens, many of the heterologous reactions observed with unabsorbed sera were no longer detectable (Table 3); antisera 2, 9, 13, 15, 16, and 18 did not require further absorptions. The remaining antisera that showed cross-reactivity with the live reference strains were absorbed according to the reactions obtained on slide agglutination (Table 3).

No reactions were observed in the antisera with 10 *C. fetus* subsp. *fetus* (*C. fetus* subsp. *intestinalis*) and 10 *C. fetus* subsp. *venerealis* strains (Table 2).

DNase treatment. Serotyping of mucoid, sticky *C. jejuni* strains was made possible by the use of DNase. A homogenous bacterial suspension was obtained with all mucoid strains tested within 5 s after the bacteria was mixed with the DNase solution. The serological specificities of the strains were not affected by this treatment, although the agglutination reactions were somewhat slower to appear (45 to 60 s).

Serotyping of isolates. Of the 815 isolates from all sources, 695 or 85% were typable. Eight serogroups (1, 2, 4, 5, 7, 8, 9, and 11), each comprising 20 or more isolates, were encountered most frequently and represented 72% (501 isolates) of all typable strains. Of the strains,

TABLE 1. Homologous and heterologous titers[a] of *C. jejuni* antisera

Reference[b] strains	Unabsorbed antisera																				
	1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	**12,800**[c]	–[d]	–	–	–	–	–	–	–	–	–	–	–	–	200	–	200	–	–	–	–
2	800	**6,400**	–	–	–	–	–	–	–	800	–	–	–	–	–	200	–	–	–	200	–
4	–	–	**3,200**	–	400	–	–	–	–	–	–	–	–	200	800	–	800	–	–	–	–
5	800	–	–	**12,800**	800	–	–	–	–	200	–	–	–	–	400	–	800	200	–	200	200
6	–	–	–	800	**1,600**	–	–	–	–	–	–	–	–	–	–	–	400	–	–	–	–
7	–	–	–	200	–	**1,600**	–	–	–	–	–	–	–	–	400	–	800	–	–	–	–
8	–	–	–	–	–	–	**12,800**	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	–	–	–	–	–	–	–	**12,800**	–	–	–	–	–	–	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–	–	**800**	–	–	–	–	–	–	–	–	–	–	–	–
11	400	–	–	–	–	–	–	–	–	**3,200**	–	–	–	–	–	200	–	–	–	–	–
12	–	–	–	–	–	–	–	–	–	–	**3,200**	–	–	–	–	–	–	–	200	200	–
13	–	–	–	–	–	–	–	–	–	–	–	**12,800**	–	400	–	200	–	–	–	–	200
14	–	–	–	–	–	–	–	–	–	–	–	–	**12,800**	–	–	–	–	–	–	–	–
15	–	–	–	–	–	–	–	–	–	–	–	–	–	**25,600**	–	400	–	–	–	–	–
16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**3,200**	–	–	–	–	–	–
17	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**1,600**	–	–	–	–	–
18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	200	–	**204,800**	6,400	–	–	–
19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**1,600**	–	–	–
20	–	–	–	–	–	–	–	–	–	–	200	–	–	–	200	–	–	–	**12,800**	200	200
21	–	–	–	–	–	–	–	–	–	–	200	–	200	–	–	–	–	–	–	**3,200**	–
22	–	–	–	–	–	–	–	–	–	–	–	–	400	–	–	–	–	–	–	–	**12,800**

[a] Tube titrations incubated at 50°C for 4 h.
[b] Formalinized antigens.
[c] Reciprocal titer.
[d] Negative, <200.

TABLE 2. Homologous and heterologous slide agglutination reactions of *C. jejuni* unabsorbed antisera[a]

Reference live strains	Antisera																				
	1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	**4+**	3+	–	–	–	4+	–	–	–	2+	–	–	–	–	–	–	3+	–	–	–	–
2	3+	**4+**	–	–	–	–	–	–	–	3+	–	–	–	–	–	2+	–	–	–	–	–
4	–	–	**4+**	–	4+	–	–	–	–	–	–	–	–	–	–	2+	–	–	–	–	–
5	–	–	–	**4+**	4+	–	–	–	–	–	2+	–	–	–	–	–	3+	–	3+	–	–
6	–	–	–	4+	**4+**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	–	–	4+	2+	–	–	–	–
8	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	–	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	3+	3+	–	–	–	–	–
10	–	2+	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	–	–	–	–	–
11	2+	–	–	–	–	–	–	–	–	**4+**	–	2+	–	–	–	3+	–	–	–	–	2+
12	–	–	–	–	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	3+	–	–
13	–	–	–	–	–	–	–	4+	–	3+	–	**4+**	–	2+	2+	3+	–	–	–	–	–
14	–	–	–	–	–	–	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	3+
15	–	–	–	–	–	–	–	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–
16	–	–	–	–	–	–	–	–	–	2+	–	–	–	–	**4+**	–	–	–	–	–	–
17	3+	3+	–	–	–	–	–	–	–	2+	–	–	–	–	–	**4+**	–	–	–	–	–
18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**4+**	3+	–	–	–
19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**4+**	–	–	–
20	–	–	–	–	–	–	–	–	–	–	3+	–	–	–	–	–	–	–	**4+**	–	–
21	3+	–	–	–	–	–	–	–	–	–	–	–	–	–	2+	2+	–	–	–	**4+**	–
22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**4+**
C. fetus subsp. *fetus* (*C. fetus* subsp. *intestinalis*), 10 strains	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C. fetus subsp. *venerealis*, 10 strains	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] Antisera diluted 1:5 in PBS.

61% (424) belonged to five common serogroups (1, 2, 4, 7, and 11), each comprised of more than 50 isolates. Eleven percent of the strains from all sources (65 human and 25 nonhuman isolates) were untypable and are under continued investigation. About 4%, or 30 cultures (21 human and 9 nonhuman isolates), were rough and autoagglutinable in saline (Table 4).

Of the human isolates, 529 (86%) were typable; 455 isolates belonged to 20 serogroups (Table 4), and 74 isolates showed the presence of two antigenic factors belonging to 18 subserogroups (Table 5). Seven common serogroups (1, 2, 4, 5, 7, 9, and 11), each comprised of 20 or more isolates, represented 72% (381) of the human typable strains. Two hundred and forty-five strains (46%) belonged to the three most common serogroups, each comprised of 50 or more isolates. Serogroup 1, with 98 isolates (18.5%), was most common among *C. jejuni* isolated from human cases of gastroenteritis, followed by serogroup 4 (96 isolates, 18%), serogroup 2 (51 isolates, 9.6%), serogroup 7 (49 isolates, 9.2%), serogroup 11 (46 isolates, 8.6%), and serogroups 9 and 5 with 21 and 20 isolates, respectively. Very good serological correlation was observed in 20 family outbreaks and 4 community outbreaks (Table 6). Six isolates from a waterborne outbreak were found to belong to subserogroup 1,2, whereas 13 isolates from a probable milkborne outbreak were found to belong to serogroup 1.

In the nonhuman group, 84% of the chicken isolates were typable and belonged to 13 serogroups (Table 4) and 7 subserogroups (Table 5). Of the isolates, 27 (32%) belonged to serogroup 4, followed by serogroup 2 with 11 isolates (13%). Seven isolates from turkeys belonged to serogroups 2, 12, and 21, also found among isolates from chickens. Reference strain 19 was isolated for the first time from chickens.

Of the 73 swine isolates studied, 55 (76%) were typable, 17 were untypable, and 1 was rough; 43 of the typable strains belonged to 12 serogroups (Table 4) and 12 strains belonged to 9 subserogroups (Table 5). Nineteen cultures (34.5%) belonged to common serogroups 1, 2, 4, 5, 7, and 11. Eleven cultures (20%) agglutinated in antiserum 20 prepared from a reference strain isolated for the first time from swine.

DISCUSSION

The antigenic study of campylobacters (*V. fetus*) of animal origin initiated by von Mitscherlich and Liess (26) and Morgan (17) has been advanced by Berg et al. (3), who have further classified campylobacters into three groups (A, B, and C) on the basis of heat-stable antigens. Using antisera against whole cells absorbed with homologous boiled and heterologous live cross-reactive antigens, they have identified seven thermolabile antigenic factors. Ten *C. jejuni* strains examined were found to belong to serogroup C, and five of them that were studied further were found to possess thermolabile antigen 1.

Butzler and Skirrow (9) have reported earlier work on the serotyping of *C. jejuni* by slide agglutination with live bacteria. With unabsorbed antisera produced in response to isolates from human and nonhuman sources, 84 of 90 isolates from human cases of gastroenteritis were agglutinated by either one or both groups of antisera. Penner and Hennessy (18) and Lauwers et al. (16) have described serotyping systems that use passive hemagglutination techniques with heat-stable soluble antigens and crude, unabsorbed antisera produced against whole cells. Abbott et al. (1), using absorbed antisera against heat-stable and heat-labile factors, have been able to show the relationships of the strains isolated from outbreaks.

The serotyping scheme we developed is based on an easily performed slide agglutination technique using live bacteria and specific absorbed antisera for the detection of heat-labile antigens. One major improvement in the slide agglutination technique is the treatment of the rubbery, sticky strains of *C. jejuni* with DNase. Observations made with some halophilic organisms (23) and *Neisseria meningitidis* (11) showed that extracellular DNA may be associated with the slime responsible for the rubbery, sticky appearance of the organisms. A similar characteristic which may result in autoagglutinability has been described for *Neisseria gonorrhoeae* and has been eliminated by treatment of the cells with nucleases (2). The addition of DNase, which did not affect the serological specificities of the *C. jejuni*, did allow the serotyping of otherwise untypable strains.

As to the autoagglutinability in saline observed in other studies (1, 9), we reduced the number of rough strains by repeated subculturing of colonies on fresh, moist media incubated at 37°C. Only about 30 stains (21 from human sources and 9 from nonhuman sources) were found untypable due to roughness (autoagglutinable in saline).

Marked heterologous reactions observed in other studies (1, 3, 9, 15) were also seen in our study. Some of the cross-reactivities (Tables 2 and 3) have been attributed to the presence of antibodies to homologous heat-stable factors. Therefore, all antisera were completely absorbed to ensure the specificity of the serotyping scheme with respect to heat-labile factors.

This simple, rapid serotyping method so far recognizes 21 serogroups. With this scheme, 455 (86%) of the typable human isolates and 145

TABLE 3. Homologous and heterologous slide agglutination reactions of *C. jejuni* antisera[a] absorbed with homologous heated antigens

Reference live strains	Antisera																				
	1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	**4+**	–	–	–	–	4+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	2+	**3+**	–	–	–	–	–	–	–	3+	–	–	–	–	–	–	–	–	–	–	–
4	–	–	**4+**	–	4+	–	–	–	–	–	–	–	–	–	–	2+	–	–	–	–	–
5	–	–	–	**4+**	4+	–	–	–	–	–	2+	–	–	–	–	–	–	–	2+	–	–
6	–	–	–	3+	**4+**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	–	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	2+	–	–	–	–	–
10	–	–	–	–	–	–	–	–	**3+**	–	–	–	–	–	–	–	–	–	–	–	–
11	–	–	–	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	–	–	–	2+
12	–	–	–	–	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	–	–	–
13	–	–	–	–	–	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	–	–
14	–	–	–	–	–	–	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–	2+
15	–	–	–	–	–	–	–	–	–	–	–	–	–	**4+**	–	–	–	–	–	–	–
16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**3+**	–	–	–	–	–	–
17	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**4+**	–	–	–	–	–
18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**4+**	3+	–	–	–
19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**4+**	–	–	–
20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**4+**	–	–
21	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**3+**	–
22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**4+**

[a] Antisera diluted 1:5 in PBS.

TABLE 4. Serogroups[a] of *C. jejuni* isolated from human and nonhuman sources

Serogroup	Human sources	Nonhuman sources			
		Chickens	Turkeys	Swine	Others
1	98	5		4	
2	51	11	3	6	
4	96	27		3	4[b]
5	20			3	
6	8	3			
7	49	4		1	2[c]
8	18	4		1	4[d]
9	21	5			1[e]
10	8			1	
11	46	8		2	4[f]
12	4	1	1	5	2[g]
13	3			4	
14	1				
15	7				
16	1				2[h]
17	15	1		2	1[i]
18	3	3			
19		1			
20	1			11	
21	4	3	2		
22	1				
UT[j]	65	8		17	
Rough	21	8		1	

[a] Agglutination in single antiserum.
[b] 3 cattle, 1 dog
[c] 1 sheep, 1 dog
[d] 3 dogs, 1 cat
[e] 1 sheep
[f] 1 sheep, 3 rabbits
[g] 2 ducks
[h] 2 pigeons
[i] 1 pigeon
[j] UT, Untypable, under investigation.

TABLE 5. Subserogroups[a] of *C. jejuni* isolated from human and nonhuman sources

Subserogroups	Human sources	Nonhuman sources			
		Chickens	Turkeys	Swine	Others
1, 2	21		1	2	
1, 4	13	1		1	
1, 9	5				
1, 10	5			1	
1, 11				2	
1, 12				1	
1, 17					1[b]
1, 18	1				
2, 4	2				
2, 8	1				
2, 11	3	1		1	
2, 18	1				
4, 7	2				
4, 8	1	1			
4, 10	2				
4, 11		1			
5, 6	7	1		1	
5, 7				2	
5, 10	1				
7, 12	1				
8, 9		1		1	
8, 10	1				
8, 11		1			
8, 12	1				
9, 11	6				

[a] Agglutination in pairs of antisera.
[b] 1 pigeon

TABLE 6. Serotyping of *C. jejuni* isolated from outbreaks and related cases

No. of foci	No. of cases	Serogroup
4	2 Each	1
4	2 Each	1, 2
2	2 Each	2
1	3	4
2	2 Each	4
2	2 Each	7
2	2 Each	8
1	2	11
2	2 Each	UT[a]
2	2 Each	17
1	3/?	1
1	3/?	1
1	13/27	1
1	6	1, 2
	1 Child, family dog	8
	1 Adult, 2 pet ducks	12

[a] UT, Untypable.

(87%) of the typable nonhuman isolates were agglutinated by one or another of the antisera (Table 4). Of the remaining typable strains, 74 (14%) of the human isolates and 21 (13%) of the nonhuman isolates were typable with various pairs of absorbed antisera (Table 5). The latter observation indicates the presence of two antigenic factors. Strains of *C. jejuni* possessing multiple antigenic factors were previously described (3). Ninety untypable strains that showed reactions in three or more antisera are under investigation to establish the serological significance of additional factors.

Of the 20 serogroups found among isolates from human cases of gastroenteritis, 16 were also seen among isolates from poultry and swine (Tables 4 and 5). The seven most common serogroups seen among human isolates were also commonly seen among isolates from chicken and swine. Of the chicken isolates, 66% belong to five serogroups (1, 2, 4, 7, and 11) also common among human isolates. Serogroups 6, 9, and 18 were found among human and chicken isolates but not among swine isolates. All swine isolates belong to the same serogroups seen with human isolates. Serogroups 5, 10, 13, and 20, identified with human and swine isolates, have not been seen with chicken isolates. Reference

strain 19, isolated for the first time from chickens, has not yet been identified among human or swine isolates. Only one human isolate agglutinated in antiserum 20 prepared from a reference strain isolated for the first time from swine. All of these findings lend further support to the view that poultry and swine may play a significant role in human campylobacteriosis. Other studies (7, 16) have shown that isolates from chickens, swine, sheep, dogs, and cows were identical serologically with strains isolated from human patients.

Serogroups 14, 15, and 22, presently found only among human isolates, are consistent with person-to-person spread reported by some workers (7, 10, 19). Recently, Blaser et al. (5) have brought additional evidence of person-to-person transmission of *C. jejuni*. In our study, very good serological correlation was observed in 20 family outbreaks and 4 community outbreaks.

Transmission from pets to humans has been previously documented (4, 13, 22, 24, 25, 27). In this study *C. jejuni* isolated from both a sick child and a pet dog belonged to serogroup 8, and three other isolates from an adult and two pet ducks belonged to serogroup 12 (Table 6).

The serological specificity of this serotyping scheme and its obvious practicability should make it a welcome tool in diagnostic laboratories for the rapid epidemiological analysis of suspected outbreaks. Studies are currently under way to establish the precise nature and role of heat-labile surface determinants, particularly the flagellar and capsular antigens.

ACKNOWLEDGMENTS

We thank W. M. Johnson for assistance with the manuscript, W. P. Winter for providing culture media, and M. M. Garcia (Animal Disease Research Institute, Ottawa, Ontario) for kindly providing the *C. fetus* subsp. *venerealis* cultures.

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