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Campylobacter jejuni and *Campylobacter coli* Serotypes Isolated from Chickens, Cattle, and Pigs

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A total of 191 *Campylobacter jejuni* and 125 *Campylobacter coli* were isolated from the intestinal content of 398 chickens, 421 cattle, and 203 pigs. All 108 chicken isolates and 73 of 80 cattle isolates were *C. jejuni*, but 115 of the 118 pig isolates were *C. coli*. A total of 84% of the *C. jejuni* and 64% of the *C. coli* isolates were typed on the basis of thermostable antigens with 20 antisera prepared against frequently occurring serotypes in *Campylobacter* enteritis in man (15 *C. jejuni*, 6 *C. coli* serotypes). A total of 96% of the chicken isolates and 67% of the cattle isolates belonged to 11 *C. jejuni* serotypes that occur most frequently in human cases of enteritis (serotypes 1, 2, 3, 4, 5, 13/16, 18, 21, 23, 31, and 36). Serotype 8, a relatively common human isolate, was not recovered. The *C. coli* isolates from pigs belonged to serotypes uncommon among human isolates.

Campylobacter jejuni is a common and important cause of bacterial diarrhea in humans, equalling or exceeding *Salmonella* and *Shigella* spp. in prevalence. Since the first report in 1977 of the frequency of the disease in humans (21), there has been an explosion of information on all aspects of the organism and the disease it causes. Several excellent reviews are available (3, 7). *Campylobacter coli* is less frequent than *C. jejuni* as a cause of diarrheic disease in humans (2, 3). The source of infection for humans in developed countries is thought to be the massive reservoir of *C. jejuni* and to a lesser extent *C. coli* in the animal population (2, 3, 7, 20). Many studies, reviewed elsewhere (19), have recorded the common occurrence of *C. jejuni* or *C. coli* in the intestines of domesticated animals and in wild animals and birds (12, 13). The contribution of each animal species to human infection is not known. This problem can now be investigated with the use of recently developed serotyping methods (1, 5, 9, 10, 17, 20). The purpose of the work described in this report was to determine the serotypes of *C. jejuni* or *C. coli* from a limited number of animal species by using one of the recently developed serotyping methods.

MATERIALS AND METHODS

Source of animal isolates. Fecal specimens from diarrheic cattle and pigs submitted to the Diagnostic Microbiology Laboratory of the Ontario Veterinary College (OVC) were plated directly onto Campy-BAP agar. Swabs of feces or the intestinal contents of diarrheic animals submitted to the Veterinary Laboratory Services, Ontario Ministry of Agriculture and Food were placed in Cary-Blair transport medium at room temperature (14) and inoculated onto Campy-BAP agar within 8 h. Swabs of the rectal or colonic contents of healthy pigs and chickens were taken immediately after slaughter from local abattoirs, transported to the laboratory in Cary-Blair medium, and inoculated onto Campy-BAP agar within 4 h of sampling. Fecal samples from pigs and chickens were obtained at the slaughterhouse on 10 and 26 occasions, respectively. Usually 15 animals were sampled on each occasion. On any one occasion, the pigs sampled were from many sources, whereas the chickens were from the same farm. Fecal samples were also obtained from 10 dairy herds, and from each herd 10 cows were sampled.

Isolation and identification. Fecal or intestinal contents were examined for *C. jejuni* or *C. coli* with Campy-BAP medium (2), modified by the use of 5% (vol/vol) calf blood rather than sheep blood and by the use of 5,000 IU/liter of polymyxin B (6). Healthy dairy cow isolates were made on Campy-BAP and by a broth enrichment process (D. L. Munroe, unpublished data). Plates were incubated at 42°C in an atmosphere of 5% oxygen, 10% carbon dioxide, and 85% nitrogen in standard evacuation-replacement anaerobe jars and were examined after 48 h for *C. jejuni* and *C. coli*. These were identified on the criteria of catalase and cytochrome *c* production, growth in 1% glycine, growth at 42°C but not at 25°C, hydrogen sulfide production with 1% lead acetate strips, sensitivity to nalidixic acid and resistance to cephalothin, and presence or absence of hippurate hydrolysis (6, 11, 15, 22). Hippurate-positive strains were *C. jejuni*, and negative strains were *C. coli* (22). Isolates were stored in the

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frozen or lyophilized state. Isolates were passaged generally only once or twice before lyophilization.

Serotyping. Antisera were prepared at OVC in rabbits against 20 serotype reference strains of the scheme under development at the Department of Medical Microbiology at the University of Toronto (UOT) (17). Twenty strains (*C. jejuni* serotypes 1, 2, 3, 4, 5, 8, 10, 11, 16, 18, 19, 21, 23, 31, 37, and *C. coli* serotypes 24, 28, 30, 34, and 38) were selected from the 59 defined serotypes because they were found in a previous prevalence study (17) to be the most frequently occurring. Previously described methods for antiserum production and titration by passive hemagglutination were followed (17). Antigen was prepared by washing confluent 48-h bacterial cultures from four blood agar plates with 7.5 ml of saline (0.85% NaCl) and heating at 100°C for 1 h. The supernatant from the centrifuged cells was used to sensitize sheep erythrocytes. The isolate under examination was assigned to the serotype corresponding to the antiserum or antisera in which agglutination of the erythrocytes occurred to within one or two dilutions of homologous titer. To show that the 20 OVC antisera had the same specificities as the corresponding UOT antisera, each was cross-titrated against antigens extracted from the other 19 strains, once at the OVC laboratory and again at the UOT laboratory. As a further test of the efficacy of the OVC antisera, 32 randomly selected isolates were serotyped at both laboratories, and the results were compared. Strains of *C. jejuni* or *C. coli* which could not be typed at OVC were sent to UOT for typing with a wider range of antisera.

RESULTS

Reproducibility studies. The 20 antisera produced at OVC were cross-titrated both at OVC and UOT, and the results were compared. Except for occasional differences in titer that could reflect minor differences in technical manipulations, the results were essentially the same. Moreover, the antisera had essentially the same specificities as 20 antisera prepared against the same strains in the UOT laboratory 2 years earlier. However, differences in homologous titers were noted—in some cases, higher titers were seen in OVC antisera, and higher titers, in other cases, occurred in the UOT antisera.

A comparison of results of serotyping 32 randomly selected isolates with the 20 OVC antisera and the 59 UOT antisera showed that the results were the same for 17 isolates. Four isolates reacted in only one or another of the OVC antisera but were found to be multiply agglutinating in the UOT antisera, reacting in one or more of the antisera against the 39 serotypes not included in the OVC antisera. Two isolates that were untypable in the OVC antisera were found to be serotype 2 in the UOT antisera. The higher titrated UOT antiserum 2 was assumed to account for this difference. One isolate identified as serotype 4 at OVC was typed at UOT as 13/16. Eight isolates not typable in OVC antisera were typable in antisera against 39 other serotypes of the complete scheme. This suggested that approximately 75% of the isolates to be encountered in the study would be typable in the 20 antisera, and thus only 25% would have to be sent to the UOT for serotyping.

Isolations of *C. jejuni* and *C. coli* from animals. Fecal samples from 398 healthy chickens, 314 diarrheic and 107 healthy cattle, and 59 diarrheic and 144 healthy pigs were cultured. The intestinal specimens from the chickens were acquired during 26 visits to a chicken slaughterhouse; *C. jejuni* were recovered on only 7 visits, but on these visits most birds sampled harbored the organism (Table 1). A total of 68 isolates, randomly selected from each visit, were serotyped. The results of the serotyping revealed a distinct pattern of serotype distribution (Table 1) and suggested that flocks, in most cases, were carriers of one or only a limited number of serotypes.

Isolates of *C. jejuni* were recovered from 53 (17%) of the 314 diarrheic cattle and from 27 (25%) of the healthy dairy cows. *C. coli* were recovered from 7 (2%) of the diarrheic cattle but not from healthy cows. The details of the results on the isolations and serotyping are shown in Table 2. Two-thirds (67%) of *C. jejuni* from cattle belonged to 11 serotypes most common among human isolates (8, 15). The numbers of

TABLE 1. *C. jejuni* serotypes isolated from the large bowel of healthy chickens at slaughter

Visit no.	No. of chickens sampled (116)[a]	No. of isolates (108)	No. of isolates[b] of serotype:						
			1	2	3	4	5	31	NT[c]
1	26	20		9		1			
2	15	15					10		
3	15	15			9				1
4	15	13	5		3				2
13	15	15						10	
18	15	15	10						
21	15	15	10						

[a] Totals in parentheses.
[b] Ten isolates from each visit were serotyped.
[c] NT, Not typable.

TABLE 2. *C. jejuni* serotypes isolated from feces of healthy and diarrheic cattle

Source of isolates	No. of animals sampled	No. of isolates obtained	No. of isolates of serotype:												
			1	2	4	11	18	21	23	23/36	36	37	50	Other[a]	NT[b]
Diarrheic cattle	314	53	1	19	9	1			1		1		5	3	13
Healthy cows	107	27	3	3	7		1	1	1	2		3			6
Farm distribution of isolates from healthy cows															
1[c]	17	4		1								3			
2	10	8		1	4			1							2
3	10	2		1											1
4	10	1													1
5	10	0													
6	10	0													
7	10	2	2												
8	10	2	1				1								
9	10	4			1				1						2
10	10	4			2					2					

[a] See text.
[b] NT, Not typable.
[c] Farm number.

isolates from healthy and diarrheic cattle were too small for significant differences in serotypes to be detected between the groups. The seven diarrheic cattle from which *C. coli* were isolated were from two different farms; all seven isolates were serotype 30, a common serotype in pigs.

The differences in results obtained for pigs and for the animals described above were striking. In examining 59 specimens from diarrheic pigs, 18 (30%) of the animals yielded isolates of *C. coli; C. jejuni* was recovered only from 2 animals. Of 144 healthy pigs, 100 (69%) yielded isolates of *C. coli* but *C. jejuni* was obtained from only one animal. All isolates from diarrheic pigs and most from the healthy pigs were serotyped (Table 3). The isolates from diarrheic pigs were too small in number for valid comparison, but wide differences from the healthy pigs in serotype distribution were not apparent (Table 3). The *C. jejuni* isolate found in a healthy pig was serotype 23/36 and the two in diarrheic pigs were not typable.

TABLE 3. *C. coli* serotypes isolated from feces or large bowel of healthy and diarrheic pigs

Source of isolates	No. of pigs sampled	No. of *C. coli* isolates	No. of isolates serotyped	No. of isolates of serotypes:										
				5	24	25	28	30	34	39	46	48	Other[a]	NT[b]
Diarrheic pigs	59	18	18		2	1	1	1	1		5	2	3	2
Healthy pigs	144	100	86	15	6	2	2	8	1	7	7	19	8	11
Distribution of isolates according to visits to slaughter house														
1[c]	15	5	5					1		1		2		1
2	15	10	10	4				1		1		3		1
3	15	13	10	1	2		1					1	4	1
4	9	8	8			1				1	3	2		1
5	15	13	10	3	2		1	2					2	
6	15	14	10	2	1	1				1	3		2	
7	15	12	10					1		1	1	3		4
8	15	12	10		1			2	1	2		4		
9	15	7	7	3				1				1		2
10	15	6	6	2								3		1

[a] See text.
[b] NT, Not typable.
[c] Visit number.

In this study, a total of 171 *C. jejuni* and 111 *C. coli* were serotyped. Eleven multiply agglutinating isolates were encountered. In addition to the two *C. jejuni* isolates of serotype 23/36 in Table 2, three others of the same species were 4/13, 13/16, and 5/13/16/43. These were included under "other serotypes" in Table 2. Six multiply agglutinating *C. coli* (5/48, 25/48, 28/59, 28/56/59, 39/39, and 49/54/56) were included under "other" serotypes in Table 3. However, 22 (12.9%) of the *C. jejuni* and 13 (11.7%) *C. coli* were not typable. This indicated the existence of serotypes not yet defined by the serotyping scheme. Except for isolates that remain untypable, the use of the 20 OVC antisera enabled the identification of *C. jejuni* from all chickens and from all but nine animals in the cattle study. However, 32 (31%) of the typable *C. coli* isolates from pigs could not be identified with the OVC set of antisera. Six other UOT antisera (against serotypes 25, 35, 49, 46, 56, and 59) were needed to serotype these 32 isolates. Nevertheless, 84% of the *C. jejuni* and 64% of the *C. coli* could be serotyped with the set of 20 antisera, supporting the assumption made at the outset of the study that epidemiological investigations could be undertaken with a limited number of typing antisera provided that their selection was based on the most frequently occurring serotypes.

DISCUSSION

One objective of this study was to determine whether the serotyping scheme under development in one laboratory could be reproduced in another. Evidence that this was the case was established through a comparison of the specificities of the newly produced antisera with those of the original scheme and by comparing the results of serotyping a group of isolates with the two sets of antisera. The reproducibility of the scheme was confirmed. In the case of one isolate, an apparent discrepancy was noted in that it was identified as serotype 4 in one scheme and as 13/16 in the other. This was, however, not unexpected as serotypes 4, 13, 16, and 50 are a related group, and isolates have been found previously to react in only one of these antisera on first isolation but in two or more if they are serotyped after maintenance or passaged in the laboratory (J. L. Penner, unpublished data). Our isolates were passaged once or twice before lyophilization. This suggested antigenic variation is currently under investigation.

However, the major purpose of this study was to isolate and serotype *C. jejuni* and *C. coli* from animal feces so as to contribute to our understanding of the epidemiology of *Campylobacter* human infections. Two recent studies of *C. jejuni* from human cases of enteritis from widely separated areas of Canada showed that the most frequently isolated serotypes were, in order, 2, 4, 3, 1, 8, and 13/16 (60.6% of isolates) (15) and 4, 2, 1, 5, 8, and 13/16 (50.7% of isolates) (8). Other relatively frequent but less commonly isolated serotypes were 18, 31, and 45 (15) and 5, 18, 21, 23, and 36 (8). In the present study, 96% of the isolates from chickens, 57% of diarrheic cattle isolates, and 60% of the isolates from healthy cows belonged to these common serotypes. In an earlier study, 25 *C. jejuni* isolated from zoo animals were also reported to belong to these serotypes (13). In contrast, the pigs examined in our study commonly and almost exclusively harbored *C. coli*. In this respect, the work of other investigators was confirmed (24–26) but, in our study, it was also shown that the *C. coli* isolates were different from the *C. jejuni* serotypes found in chickens and cattle except for one case (serotype 5). The seven *C. coli* isolates found in seven diarrheic cattle were also found to belong to a serotype of *C. coli* that occurred in nine pigs but only rarely among isolates from people.

It has been well established that chickens may carry *C. jejuni* in their intestinal tracts (4, 19, 26). The carcasses of chickens at retail are commonly contaminated with *C. jejuni* (17, 23, 27) and present a potentially important source of infection for humans. However, in our study it became clear that some flocks (19 of 26) were evidently free of the organisms, but other flocks (7 of 26) harbored the organisms and within the flock many birds were carriers. This finding is important since it suggests that *Campylobacter* infection in chicken flocks might be controlled by husbandry methods.

At least two serotypes were isolated from the majority of the healthy cow herds yielding *Campylobacter*, which is in contrast to chicken flocks in which one serotype usually predominated. The *C. jejuni* in healthy cows and diarrheic cattle were, in a number of cases, the same with respect to serotype. The factors governing virulence of *C. jejuni* have not been elucidated, but the occurrence of the same serotypes in both ill and healthy animals suggested that the thermostable antigens, on which the serotyping scheme is based, could not be directly implicated as significant in pathogenesis. However, *C. jejuni* has been reported to be isolated from beef at retail sale (27, 28), and our results therefore suggest that cattle, like chickens, could serve as a source of serotypes commonly found in cases of human enteritis.

Since pigs commonly harbor *C. coli*, evidently at a frequency greater than cows harbor *C. jejuni, C. coli* would be expected to be found as contaminants of pork at retailing, and this has been shown to be the case (25). However, only 3% of the human *Campylobacter* cases of enteri-

tis have been reported to be due to *C. coli* (8). This points to a difference between *C. jejuni* and *C. coli* in epidemiology or perhaps pathogenesis and merits further investigation. The large numbers of different serotypes of *C. coli* in groups of pigs at slaughter probably reflects the practice of mixing pigs from many different farms before slaughter.

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