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Comparison of agglutination, complement fixation and immunofluorescence tests in *Campylobacter jejuni* infections

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SUMMARY

Good antibody responses usually follow infection with *Campylobacter jejuni*. A comparison of agglutination, complement fixation and immunofluorescence tests was done on 55 sera from 40 sporadic patients with diarrhoeal disease and positive cultures for *C. jejuni*. Results showed 82% positive with immunofluorescence, 62% by complement fixation but only 38% by agglutination, using two reference strains COP and MEL as antigens. Overall 90% of the 40 patients were positive by one or more serological tests. Paired sera from 15 patients showed a fourfold or greater rise in only five, confirming previous observations that antibody formation occurs early in the course of infection. Results also suggest that different test systems may be detecting antibodies of different specificities. Results confirm the value of serological tests, but further information on serotypes is required for selection of suitable reference strains.

INTRODUCTION

Unlike patients infected with shigella, who produce poor systemic antibody responses, humans infected with *Campylobacter jejuni* often manifest high antibody titres. Consequently, serological tests may be of considerable value where culture facilities are limited or in cases where antibiotic therapy has commenced before stool specimens are obtained. Previously we described the value of agglutination tests using three reference antigen preparations (Watson, Kerr & McFadzean, 1979), and a comparative study of agglutination and complement fixation systems (CFT) (Watson, Kerr & Jack, 1981). As part of a study of immunofluorescence (IF) systems we have compared IF, CFT and agglutination tests in patients infected with *C. jejuni* and in control subjects.

MATERIALS AND METHODS

C. jejuni strains. Two reference strains COP and MEL were used, both obtained locally. Strain COP belongs to serotype 3, 25 as designated by the serotyping scheme of Dr S. Lauwers, Free University, Brussels. This strain comprises approximately 9% of isolates found in Belgium. Strain MEL is uncommon and

antisera raised against it by Dr Lauwers agglutinated only three of 50 hitherto untypable Belgian strains.

Preparation of antigens. Formalized agglutination suspensions were prepared as described previously (Watson, Kerr & MacFadzean, 1979). A third reference strain ADA was lost as a result of failure of freeze-drying equipment. Antigens for CFT were prepared as described by Watson, Kerr & Jack (1981). Antigens for IF were made by growing the organisms in nutrient broth at 43 °C for 18 h in an atmosphere of air (34 %), CO_2 (6 %) and hydrogen (60 %). Cultures were then centrifuged and the deposited organisms washed twice with 0·85 % sterile saline and finally resuspended in saline to the original volume. Smears were prepared on 12-well Teflon-coated slides, air dried and gently fixed by heat. The preliminary washing stage was found to be essential in obtaining satisfactory preparations with a clear background.

Sera. Clotted blood samples were obtained from 40 patients with positive stool cultures for *C. jejuni*. In 15 of these a second sample was obtained between 8 and 21 days after the initial sample. All sera were separated and stored at −20 °C till tested. Sera were kept in small aliquot amounts to avoid repeated freezing and thawing.

Two control groups of sera were included. The first consisted of sera from 88 patients with recent histories of diarrhoea but where cultures were negative for *C. jejuni*. The second group consisted of sera from 40 patients with a variety of diseases, but without any diarrhoeal component, and sera from 20 antenatal patients.

Serological tests. Agglutination of formalized suspensions and CFT tests were carried out as described previously (Watson, Kerr & Jack, 1981). IF tests were done on doubling dilutions of patients' sera starting at 1/25, using a rabbit anti-human Ig conjugate reacting with IgG, IgM and IgA (Burroughs Wellcome Ltd). Conjugates were freshly prepared each day at a 1/30 dilution in phosphate buffer pH 7·1 containing 2 % Tween 80. Slides were read by a Leitz incident light u.v. microscope.

Test interpretation. Agglutination tests were considered positive at titres of 160 or greater. Previously we suggested a minimum titre of 320 based on results of culture positive and control groups (Watson, Kerr & MacFadzean, 1979). However, strain COP appears to have lost its initial sensitivity and the figure of 160 appears to be satisfactory. Complement fixation tests were taken as positive at titres of 4 or greater. Immunofluorescent tests were easily read and no difficulty was experienced in determining end-points. Titres of 25 or greater were considered significant and all tests were read by one observer to eliminate subjective errors.

RESULTS

Table 1 details the distribution of positive and negative results for each test system against both reference strains.

Only 38 % were positive by agglutination as compared with 62 % for CFT and 82 % for IF. The low figure of 38 % positive by agglutination contrasts with our previous experience, where 69 % of sera from culture-positive patients reacted with

Table 1. *Summated test results for both antigens*

Possible patterns	No. and (%) positive		
	Agg.	CFT	IF
COP+MEL+	7 (13)	13 (24)	32 (58)
COP+MEL−	6 (11)	17 (31)	2 (4)
COP−MEL+	8 (14)	4 (7)	11 (20)
COP−MEL−	34 (62)	21 (38)	10 (18)

Total positive by agglutination = 21 (38%), CFT = 34 (62%) and IF = 45 (82%).
Group: culture positive.
No. of sera = 55.
Abbreviations for all tables: Agg. = agglutination; CFT = complement fixation test; IF = immunofluorescence test.

Table 2. *Antibody test patterns against COP alone, MEL alone and COP plus MEL*

Possible patterns			No. and (%) for each pattern		
Agg.	CFT	IF	COP alone	MEL alone	COP+MEL*
+	+	+	8 (14)	5 (9)	2 (3·6)
+	+	−	1 (2)	0 (0)	0 (0)
+	−	+	2 (4)	10 (18)	1 (2)
+	−	−	2 (4)	0 (0)	0 (0)
−	+	+	15 (27)	12 (22)	7 (12·7)
−	+	−	6 (11)	0 (0)	0 (0)
−	−	+	9 (16)	16 (29)	4 (7·2)
−	−	−	12 (22)	12 (22)	5 (9)

* No. and (%) of sera giving identical patterns against COP and MEL.
Group: culture positive.
No. of sera = 55.

the same reference antigens. The probable explanation is some antigen loss/variation on repeated subculture, since this has been accompanied by a phenotype change manifested by increasing difficulty in obtaining stable suspensions with strain COP. However, no similar problems have been encountered with strain MEL and the nature of the variation in strain COP is under investigation. IF was superior to both CFT and agglutination tests and 34 (62%) of sera reacted with COP and 43 (78%) reacted with MEL. Overall 45 (82%) reacted with one or both strains. In the CFT system, however, the position was reversed. Here 30 (55%) of sera reacted with COP antigen but only 17 (31%) were positive with MEL. Overall 34 (62%) were positive by CFT.

The relationship of the results in the test systems is set out more fully in Table 2 for each of the two reference strains.

Table 2 shows that 12 (22%) sera were negative in each test system against COP and 12 were also negative with MEL, but only 5 (9%) were negative with both antigens, so that 50 (90%) of the sera were positive by at least one test with one or other antigen. Table 2 also shows that only two sera were positive with all three

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Table 3. *Number of sera negative in each test system where one or both other tests positive against one or both antigens*

Test negative	Agg. and/or CFT positive			Agg. and/or IF positive			CFT and/or IF positive		
	COP alone	MEL alone	COP+MEL*	COP alone	MEL alone	COP+MEL*	COP alone	MEL alone	COP+MEL*
IF	9	0	0	—	—	—	—	—	—
CFT	—	—	—	13	26	5	—	—	—
Agg.	—	—	—	—	—	—	30	28	11

* Both COP and MEL positive with one or both tests.
Group: culture positive.
No. of sera = 55.

tests with both reference antigens. This suggests that there is considerable variation in antibody specificity in different sera from sporadic cases of campylobacter infection.

Table 3 has been obtained by summating the results for two test systems where either or both tests were positive but where the third test system was negative.

The findings in Table 3 show that no serum was positive by IF where one or both of the test antigens gave a positive result in agglutination and/or CFT systems. This compares with 5 that were negative in CFT where one or both of the other two systems were positive, and 11 that were negative by agglutination where one or both of the other two were positive.

Paired sera. Fifteen sets of paired sera were available for study. Initial samples were taken from 4 to 12 days after onset of illness and the intervals between samples varied from 8 to 21 days. Only five sera showed a fourfold or greater rise in antibody titre compared to the initial sample, and these were from patients with clinical histories of less than 7 days. All five fourfold or greater rises were noted in agglutination titres and only one showed a fourfold rise in CFT and another a fourfold rise in IF titres, suggesting that antibody detectable by IF and CFT appears early in the course of illness. Interestingly two patients showed fourfold drops in agglutinating antibody in samples taken 13 and 17 days respectively after the initial samples. No similar drop in titre was noted with CFT and IF tests. One patient failed to produce any antibody detectable in any test system in sera taken 14 days apart and these sera failed to react with his own homologous strain of *C. jejuni*.

Culture-negative group. Table 4 illustrates the findings in a group of 88 patients with recent histories of diarrhoea but who were culture negative for *C. jejuni*.

Of the 12 sera that gave positive results, seven were positive with only one test for one antigen, three reacting with COP and four with MEL. Four sera reacted with two antigens, two by IF only, and one reacted with both agglutinating antigens and with COP in IF test. *Salmonella typhi* was isolated from patient no. 4 but it is not known whether this organism shares any cross-reacting antigen with *C. jejuni*.

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Table 4. *Positive results from 88 culture-negative patients with diarrhoeal episodes*

	Agg.		CFT		IF	
Patient	COP	MEL	COP	MEL	COP	MEL
1	—	—	4	—	50	—
2	—	—	8	—	—	—
3	—	—	4	—	—	—
4	—	—	—	—	—	50
5	80	—	—	—	25	—
6	—	—	—	—	100	100
7	—	—	—	—	—	100
8	80	320	—	—	50	—
9	160	—	—	—	—	—
10	—	—	—	—	50	200
11	—	—	—	—	—	50
12	—	—	—	—	—	50

Group: culture negative.
No. of sera = 88.

In the group of 60 patients with miscellaneous disorders and including 20 antenatal patients only one positive serum was detected. This gave agglutination of COP at 160 titre and positive IF (titres 50 and 100 against COP and MEL respectively).

DISCUSSION

Although numerous outbreaks of infection with *C. jejuni* are recorded, many involving small numbers of individuals, the majority of cases are sporadic and intra-family spread is comparatively rare. The results presented above confirm our experience that serological tests can be of considerable value in diagnosis. They also indicate that it is practicable to use suitable reference strains and that only a small number is required to detect antibody in the vast majority of infected patients. Of the 40 patients in the group that were culture positive, 36 (90%) were positive by one or more tests with at least one of the two reference antigens. Since the two reference strains belong to different serotypes antibody of different specificities can be detected. However, even with one serotype it appears that different test systems may be detecting antibody of different specificities. For example, in spite of the superiority of IF as compared with agglutination, three sera were positive by agglutination against COP that were negative by IF and one of these was positive by CFT against COP but not against MEL. Further analysis of the specificity of antibody response will depend on work now currently being done in a number of laboratories on serotyping of *C. jejuni* isolates. Abbott *et al.* (1980) found that different *C. jejuni* strains can be distinguished on the basis of major heat-stable and heat-labile antigens. Our findings suggest that there may be considerable sharing of common antigens amongst strains or that only a restricted range of serotypes are responsible for infection in this geographical area.

Detection of a fourfold or greater rise in only 5 of 15 pairs of sera is in agreement

with the findings of Skirrow (1977), who showed a similar rise in 10 of 31 patients. Antibody appears early in the course of infection. The paradoxical drop in agglutinating activity noted in the second sample of a pair from each of two patients without a corresponding drop in antibody detectable by CFT or IF suggested the possibility of a switch to production of incomplete antibody of IgG class. However, all attempts to perform antiglobulin tests on these sera were unsuccessful due to clumping of organisms when being washed prior to addition of antiglobulin reagent.

Previously we noted a not uncommon observation that human serum from culture-positive patients may agglutinate reference strains COP and MEL to titres higher than those against the patient's homologous strain (Watson, Kerr & MacFadzean, 1979). Jones (1981) has noted a similar phenomenon. One serum gave a titre of 2560 against strain COP but only a 320 titre against the homologous organism. Whether this represents some form of antigen phase variation, some phenotype antigen loss or some form of blocking of antigen determinant group sites remains unclear.

Positive results in the culture-negative control group of patients with histories of diarrhoea may represent, (*a*) failure to isolate *C. jejuni*, (*b*) previous infection with *C. jejuni*, (*c*) infection with an organism with common antigenic determinant groups. *S. typhi* was isolated from one patient whose serum gave a positive IF titre of 50 against MEL. In general titres in this group were low (Table 4). Only one serum gave an agglutinin titre of 320 against MEL and another an IF titre of 200 also against MEL. It will be noted that the CFT system was more specific than IF, only three sera in this group giving positive reactions. In the second group of 60 control patients with miscellaneous diseases and antenatal patients, only one positive result was obtained, and further enquiry elicited a history of a diarrhoeal episode some six months previously in this antenatal patient. This serum agglutinated COP at 160 and had IF titres of 50 and 100 against COP and MEL respectively.

In attempts to distinguish O and H agglutination we found that alcohol suspensions prepared by standard methods were quite unsuitable for detection of the former. Heated antigens prepared by autoclaving suspension for 2 h were equally unsuitable, but antigens steamed at 100 °C for 60 min reacted with most of our sera at titres between 10 and 80. Jones, Eldridge & Dale (1980) produced an O antigen by heat at 100 °C for 15 min. Our own experience of antigen prepared in this way and reacted against rabbit serum prepared against strain COP was that the agglutinates formed were large and floccular and appeared after incubation at 37 °C after only 4 h, suggesting that 100 °C for 15 min may not be adequate to abolish H-type agglutination. Other workers also appear to have had similar difficulties in preparing satisfactory O antigens (Piemant & Abanamy, 1980).

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