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Human Serum Antibody Response to *Campylobacter jejuni* Infection as Measured in an Enzyme-Linked Immunosorbent Assay

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An enzyme-linked immunosorbent assay was adapted to measure immunoglobulin A (IgA), IgG, and IgM classes of human serum antibody to *Campylobacter jejuni*. Sera were tested from healthy controls, from ill persons at various intervals after exposure to an epidemiologically implicated vehicle for *Campylobacter* sp. enteritis, from persons exposed to these same vehicles who remained well, and from persons who chronically drank raw milk. The major antigens in the *C. jejuni* acid-washed antigen preparations from three different strains all migrated at about 30,000 and 63,000. Persons with *Campylobacter* enteritis developed rising serum IgA, IgG, and IgM antibodies during the second week after infection; IgG and IgM elevations persisted longer than did IgA. Exposed persons who remained well showed similar, but lower, antibody rises. Chronic raw milk drinkers had elevated IgG levels, but not IgM or IgA levels, whether or not they were acutely exposed to an implicated vehicle.

Campylobacter jejuni is the most common bacterial cause for acute diarrheal illnesses in developed countries (6). Despite significant advances in our knowledge of the microbial characteristics and transmission of this organism, many aspects of the host response to infection have not been fully studied. Of particular importance is defining the serum antibody response of infected hosts to this organism, for several reasons. Most evidence suggests that invasiveness is one of the primary pathogenetic mechanisms for *C. jejuni* (6), and transient bacteremia may be common. Specific serum antibodies may thus have a protective role. That a broad diversity of serotypes of *C. jejuni* is common (17, 18) raises the question of whether homologous or heterologous reinfection immunity occurs. The ability to measure class-specific, *Campylobacter*-specific serum antibodies may permit a partial answer to this question. In certain high-risk populations, vaccination to protect against *C. jejuni* infection may be useful. As such the ability to measure production of specific antibodies after candidate vaccines will be critical.

Several assays including agglutination (22, 23), immunofluorescence (2), complement fixation (9, 10), and bactericidal (8) tests have been used for serological diagnosis of *C. jejuni* infection, but these have been limited by low sensitivity or specificity or the need to use homologous isolates. Agglutination, complement fixation, and bactericidal tests chiefly measure immunoglobulin M (IgM), another limitation. Enzyme-linked immunosorbent assays (ELISAs) have high sensitivity and with proper selection of antigen have high specificity and have been successfully used for serodiagnosis of many other infections (7, 10, 13). Recently, Kaldor and colleagues (11), Walder and Forsgren (21), and Svedhem and colleagues (20) have reported on the development of enzyme immunoassays for detecting antibody response to *C. jejuni*.

Based on their work and with our accumulated sets of epidemiologically relevant sera and past interest in *Campylobacter* serology (2), we have established ELISAs to understand the characteristics of human serum antibody response to this infection and to determine whether such assays also could be of value in diagnosing acute infections. The materials selected for use as antigens in these ELISAs were the surface components of *C. jejuni* cells that had been harvested by an acid wash, as described by McCoy et al. (16). Svedhem and colleagues used the materials in a diffusion-in-gel ELISA for *C. jejuni* (20), as did Rautelin and Kosunen (19) in an ELISA examining rabbit sera, and Logan and Trust (14) have initiated an analysis of its antigens. Using a standard antigen is valuable in developing a serological assay.

MATERIALS AND METHODS

Bacterial strains and antigen preparation. We used *C. jejuni* strains PEN1, PEN2, and PEN3, the type strains of the Penner serotyping system based on passive hemagglutination by heat-stable antigens (18). Strains of these serotypes are among the most common *C. jejuni* isolates from humans (17), and antigens were prepared as described by McCoy et al. (16). In brief, strains were grown on brucella agar (BBL Microbiology Systems, Cockeysville, Md.) for 24 h at 42°C in a microaerobic atmosphere. Cells were harvested in sterile distilled water, washed twice in sterile distilled water, and suspended in 0.2 M glycine-hydrochloride buffer (pH 2.2) at a concentration of 0.1 g (wet weight) of cells to 2.5 ml of buffer. Suspensions were stirred at 25°C for 15 min and then centrifuged at 11,000 × *g* for 15 min, the supernatant was retained, and the pH was neutralized with sodium hydroxide. The supernatant was dialyzed against sterile distilled water for 24 h at 4°C. Protein concentrations were determined by the Markwell et al. modification of the Lowry assay (15), and the preparations were stored at −70°C until used. Protein profiles of each of the acid wash preparations were examined by discontinuous sodium dodecyl sulfate-polyacrylamide electrophoresis as previously described (4). In brief, 1.0 μg of the preparation from each strain was boiled in sample buffer at 100°C for 3 min, loaded onto a 4.5% stacking gel, and electrophoresed at 35 mA through a 10% separating gel. After electrophoresis, gels were fixed, and proteins were resolved by using a modified silver stain. The molecular weights of peptides resolved were calculated on the basis of a calibration curve of marker proteins. Immunoblotting of unstained gels was done as previously

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described (5). Test sera were from normal rabbits and those immunized with one of the three *C. jejuni* strains (5) and from a healthy human (sample 82-67) and one convalescent from *Campylobacter* enteritis (sample 82-432). Sera were diluted 1:100, and the capture antibody was horseradish peroxidase-conjugated *Staphylococcus aureus* protein A or horseradish peroxidase-conjugated rabbit or goat anti-human IgA, IgG, or IgM.

Sera. Serum specimens from 461 persons were studied. Sera had been stored at −20°C for 3 months to 5 years. Sera were from the following groups: (i) healthy hospital personnel or medical students and healthy controls at the time of an outbreak who were not exposed to the implicated vehicle; (ii) persons with *Campylobacter* enteritis during milk-borne outbreaks in Colorado (2), Minnesota (2), and Oregon, food-borne outbreaks in Colorado and Minnesota, and volunteers experimentally infected at the University of Maryland (1); (iii) patients who were exposed to the implicated vehicle in these outbreaks and who did not become ill; (iv) persons who were chronic raw milk drinkers whose sera were taken at the time of milk-borne outbreaks (chronic drinkers, exposed); (v) persons who were chronic raw milk drinkers not exposed to an outbreak-associated source (n = 9) or who had been exposed several months previously (n = 10).

ELISA procedure. For the ELISA, optimal dilutions of all reagents were determined by checkerboard titration, and optimal times and temperatures for incubation were determined before assays were carried out.

Equal amounts of each of the three antigen preparations were diluted in 0.5 M carbonate buffer (pH 9.6) and combined to yield a total protein concentration of 2.5 μg/ml. A 0.2-ml sample of this solution was added to each well of a flat-bottom polystyrene microtiter plate (Flow Laboratories, Inc., Hampden, Conn.), covered and incubated for 24 h at 4°C. Each well was aspirated dry and refilled with 0.3 ml of phosphate-buffered saline (PBS) at pH 7.2 containing 0.001 mg of thimersol per ml, 1.0 mg of gelatin per ml, and 0.05% Tween 80. Plates were stored wet for a minimum of 16 h at 4°C until used. The wells were emptied and washed twice with PBS–thimersol–Tween 80. Each test serum was diluted in PBS–thimersol–Tween 80 containing 5 mg of bovine gamma globulin per ml and 1.0 mg of gelatin per ml. The screening serum dilutions were 1:50 for IgM and IgA and 1:100 for IgG determinations. A 0.2-ml sample of diluted serum was added to each well and then incubated for at least 90 min at 25°C or overnight at 4°C. The wells were aspirated and washed three times with 0.3 ml of PBS–thimersol–Tween 80. Peroxidase conjugates (and use dilutions) were as follows: goat anti-human IgG (1:3,000), goat anti-human IgM (1:2,000), and goat anti-human IgA (1:2,000). All were from Tago Inc., Burlingame, Calif. Specific antibodies had been isolated from the goat sera by affinity chromatography and showed 1% reactivity to heterologous human myeloma proteins. Conjugates were appropriately diluted in PBS–thimersol–Tween 80 containing 1.0 mg of bovine gamma globulin per ml and 20 mg of bovine serum albumin per ml, and 0.2 ml was added to each well and incubated for 90 min at 25°C. Wells were washed three times with PBS-T-Tween and then washed twice with PBS-thimersol. A 0.2-ml sample of developing solution consisting of 1.0 mg of 2,2′-azino-di-(3-ethylbenzthiasoline sulfonic acid) per ml in McIlvain's buffer (pH 4.6) with 0.005% hydrogen peroxide was added to each well and incubated at 25°C for 30 min. Each microtiter plate was read immediately on a Titertek Multiscan (Dynatech Laboratories, Inc., Alexandria, Va.) at 414 nm. A control well on each plate was processed in an identical fashion, except that diluent rather than test serum was added. Results were expressed as an optical density reading on each well at 30 min.

Inhibition assay. To assess the specificity of the *C. jejuni* ELISAs, high-titer pooled serum from patients convalescent from *Campylobacter* sp. enteritis were incubated at 37°C for 30 min with 10 μg of other proteins. Proteins used were bovine serum albumin and soluble antigens of *C. jejuni*, *Campylobacter fetus* subsp. *fetus*, *Shigella sonnei*, *Yersinia enterocolitica*, and *Brucella abortus* used in standardized complement fixation serological assays (10). After incubation, 200-μl samples of the pooled serum diluted 1:50, 1:100, 1:200, and 1:400 were placed in triplicate wells, and the IgA, IgG, and IgM ELISAs were performed as described above.

Analysis of data. Eight reference sera were included in each day's ELISA run, and optical density values for the unknown sera were corrected based on the variation between that day's reference serum results and the mean value for all refrence serum results. For sera for which the optical density measurement at the screening dilution was greater than 1.500, we performed serial twofold dilutions until the determination was in the readable range. The optical density value assigned was this readable value multiplied by the dilution factor so that all values were standardized for the screening dilutions. For statistical analysis of data, we used unpaired (one-way) analysis of variance testing.

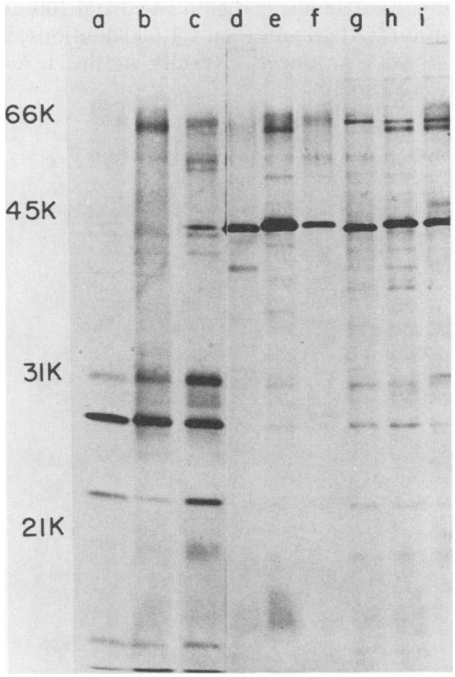

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic profiles on 12% polyacrylamide gels of the acid-washed preparations (lanes a through c) used in the ELISA. Preparations were harvested as described in the text (3), 1.0 mg of each was applied to the stacking gel in sodium dodecyl sulfate-buffer and electrophoresed at 35 mA for about 2 h. Gels were silver stained by the method of Oakley et al. (17a). The profiles of the preparations from PEN1 (lane a), PEN2 (lane b), and PEN3 (lane c) appear virtually identical with the major protein migrating at 29K. Other bands also are visible at 31K, 23K, and 63K. For comparison, bleb (lanes d through f) and whole cell (lanes g through i) preparations are shown. The 29K band is absent in the bleb preparation and present in very low concentration in the whole cell preparation.

RESULTS

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of antigens used. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of the acid-washed material from the three strains used were similar (Fig. 1). The major band for each migrated at 29K, and a second band was seen at 31K. More minor bands migrated at 23K for all three strains, at 63K for the PEN2 strain, and at 43K for the PEN3 strain. The bands in the 29K to 31K region were not present in the outer-membrane enriched (bleb) fractions (4) of these same three strains and represented only a small proportion of the proteins resolved in the whole cell preparation.

Western blots of the acid-washed material. Sera from rabbits immunized to one of the three strains reacted to each of the three preparations with nearly identical results, whereas normal serum showed no reactivity. For each of the three strains, the two major antigens migrated at 31K and 63K, and less consistently identified minor antigens migrated at 23K, 29K, 44K, and 75K (Fig. 2). Differences in stain intensity between lanes reflect differences in protein concentrations of the preparations loaded. The results with convalescent human serum were similar, whereas normal serum showed no reactivity. Use of the anti-IgA, -IgG, and -IgM conjugates demonstrated that for each, the major antigens migrated at about 63K and 30K.

ELISA results. We found that a nearly linear relationship existed between reciprocal dilution of serum from patients with *Campylobacter* sp. enteritis and absorbance in an IgG ELISA (Fig. 3). The linear region was from 100- to 800-fold serum dilutions. At greater than 1:1,600 dilutions, an asymptotic curve was produced. Results in the IgA and IgM ELISAs were similar.

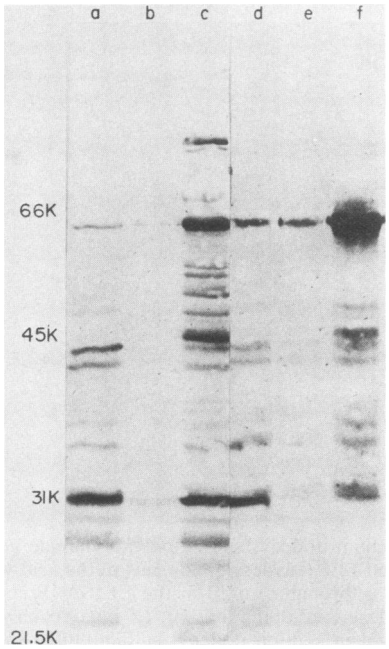

FIG. 2. Western blots of 12% polyacrylamide gels of acid-washed preparations with PEN1 immune rabbit serum (lanes a through c) and convalescent human serum (lanes d through f). Preparations are from PEN1 (lanes a and d), PEN2 (lanes b and c), and PEN3 (lanes c and f). Sera were diluted 1:100, and the capture antibody is horseradish peroxidase-labeled *S. aureus* protein A (1:1,000; lanes a through c) and horseradish peroxidase-conjugated rabbit-antihuman IgG (1:800; lanes d through f).

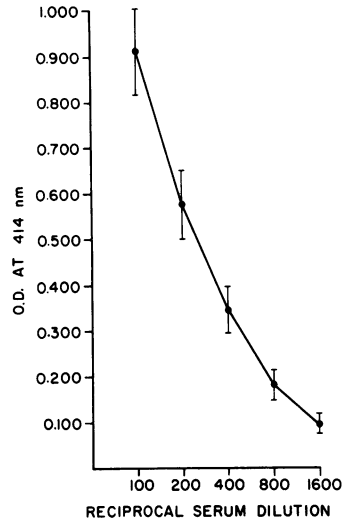

FIG. 3. Relation of optical density to dilution of serum from 17 patients tested for antibodies to *C. jejuni* in the IgG ELISA.

The mean optical density for sera from 182 healthy persons in the IgG ELISA was 0.381 (Fig. 4); however, values ranged from 0.021 to 2.100. Among persons who developed *Campylobacter* sp. enteritis, the mean optical density level of sera obtained less than 1 week after exposure (0.183) was lower than in the controls, a difference that was not statistically significant. However, IgG levels were significantly higher in sera obtained 8 to 14 days postexposure and remained elevated in groups of sera obtained up to 45 days after exposure. By 90 days postexposure, values had returned to the level observed from control sera. Among persons exposed to an implicated vehicle who did not become ill, a similar phenomenon was seen. During the first week after exposures, levels were low and then rose during the second week, remaining elevated for 30 days. The rise in mean optical density was much less than the rise observed among sera from exposed persons who became ill. Among chronic raw milk drinkers, whether or not serum was obtained in relation to an outbreak of *Campylobacter* enteritis, the mean optical density values were elevated. Compared with healthy controls, differences in distribution of optical density readings were significant ($P < 0.05$) for ill 8 to 14 days ($P < 0.0005$), 15 to 21 days ($P < 0.0005$), 22 to 30 days ($P < 0.0005$), 31 to 45 days ($P < 0.01$), exposed, well 15 to 21 days ($P < 0.025$), 22 to 30 days ($P < 0.01$), chronic exposure outbreak associated ($P < 0.001$), and chronic exposure postoutbreak ($P < 0.0005$).

The results from the serum IgM ELISA are similar in most respects to those obtained in the IgG ELISA (Fig. 5). During the first week postexposure, serum IgM levels were low in relation to those of healthy controls in sera from persons exposed who became ill and those remaining well. Subsequently the rise and fall in optical density values closely paralleled that observed in the IgG assay. However, among sera from chronic raw milk drinkers, whether or not they were obtained in relation to an outbreak, mean IgM levels were similar to those in normal persons, and the distribution of values fell in a narrow range. Compared with healthy controls, differences in distribution of optical density were significant for all groups of sera obtained from those ill 8 to 45 days (all $P < 0.0005$), and from those exposed and well for less than 7 to 21 days (all $P < 0.05$).

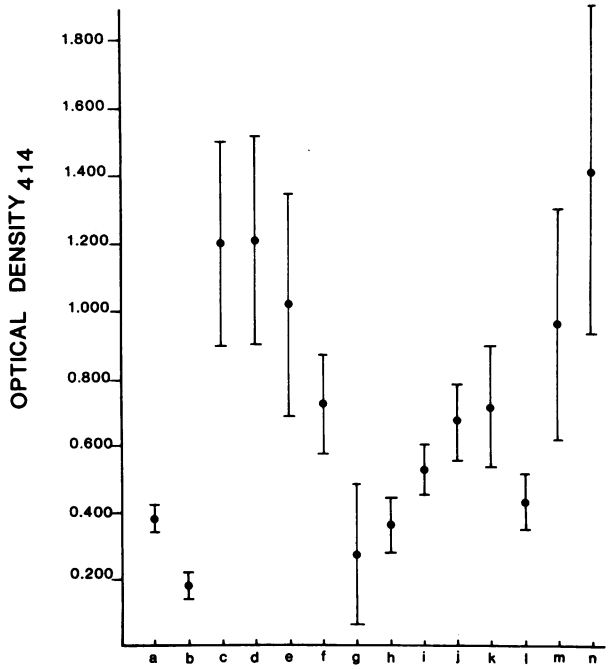

FIG. 4. *C. jejuni* serum IgG ELISA showing mean (± standard error) of optical density values in 461 sera from 14 population groups. Groups: (a) healthy controls (n = 182), (b) persons ill with *Campylobacter* enteritis, less than 1 week after exposure (n = 17), (c) 8 to 14 days (n = 26), (d) 15 to 21 days (n = 25), (e) 22 to 30 days (n = 24), (f) 31 to 45 days (n = 27), (g) 90 days (n = 6), (h) persons exposed to an implicated vehicle who remained well after exposure, less than 1 week after exposure (n = 12), (i) 8 to 14 days (n = 27), (j) 15 to 21 days (n = 28), (k) 22 to 30 days (n = 28), (l) 31 to 45 days (n = 16), (m) persons with chronic exposure to raw milk evaluated in association with an outbreak of *Campylobacter* sp. enteritis, (n = 24), (n) persons with chronic exposure to raw milk evaluated in nonoutbreak settings (n = 19).

Results from the serum IgA ELISA were somewhat different from those in the two previous assays (Fig. 6). During the first week postexposure, mean serum IgA levels were low in both ill and well exposed persons. During the second week after exposure, mean optical densities rose in sera from both exposed groups, but then quickly fell toward normal values. Among both groups of chronic raw milk drinkers mean optical density values approximated those observed in the healthy controls. Compared with healthy controls, differences in distribution of optical densities were significant for sera from those ill less than 7 days ($P < 0.025$), 8 to 30 days (all $P < 0.0005$), and exposed, well 8 to 14 days ($P < 0.0025$).

Inhibition assay. Incubating soluble *C. jejuni* antigens with known positive serum reduced the optical density 33% in the IgA assay, 72% in the IgG assay, and 25% in the IgM assay at the screening dilutions. In contrast, all of the five other antigens inhibited the assay by less than 5% at the screening dilutions in each of the three assays. The data from the IgA inhibition assay are shown in Fig. 7; the results from the other assays were similar. These observations support the conclusion that these ELISAs detect specific anti-*C. jejuni* antibodies rather than nonspecific components of immune serum.

Sensitivity and specificity of the ELISAs for serodiagnosis. To calculate the sensitivity and specificity of these assays for serodiagnosis of acute *C. jejuni* infection, we compared several optical density values as cutoffs. We found that using an optical density value equal to the mean plus two intervals of standard error for the control serum provided the best discrimination. As shown in Table 1, use of this value as a cutoff in the IgG ELISA showed the test to be 73.6% specific and, for those exposed and ill from 8 to 21 days after exposure, 58.8% sensitive. For the same groups in the IgM ELISA, the specificity was 68.4%, and the sensitivity was 74.0%. For the IgA ELISA, the specificity was 81.4%, and the sensitivity was 76.1%. Thus the serum IgA ELISA was the most specific and most sensitive for detecting an acute *C. jejuni* infection on the basis of a single convalescent serum specimen.

For the 43 chronic raw milk drinkers, 65.1% had IgG values above the cutoff versus 11.6% with elevated IgM values and 23.8% with elevated IgA values. By chi-square analysis, the difference of between the proportion of these persons with elevated IgG levels was significantly greater than the proportion with elevated IgA or IgM levels ($P <$ 0.001).

DISCUSSION

The development of standardized assays for detecting human antibody response to *C. jejuni* infection has several values. First, serological assays have been used to aid in the diagnosis of infections due to other agents. Such assays could be of value in trying to determine retrospectively whether a patient or group of patients was infected with *C. jejuni* or related agents since these may not be isolated from all those who are ill. Second, serological assays are useful for epidemiological purposes in defining populations at risk for *Campylobacter* infection. Third, such assays can be

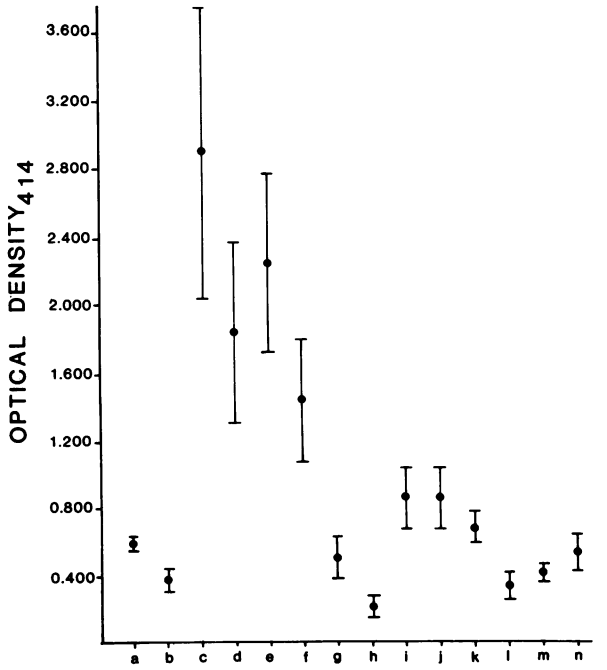

FIG. 5. *C. jejuni* serum IgM ELISA showing mean (± standard error) of optical density values in 452 sera from 14 population groups. Groups represent the same populations as indicated in Fig. 4 with the following numbers: (a) (n = 177); (b) (n = 17); (c) (n = 26); (d) (n = 24); (e) (n = 24); (f) (n = 27); (g) (n = 6); (h) (n = 12); (i) (n = 27); (j) (n = 26); (k) (n = 28); (l) (n = 15); (m) (n = 24); (n) (n = 19).

used to help define the pathogenesis of infection, specifically the role of serum antibodies in the host response.

Because of a diversity of *C. jejuni* serotypes, we used strains with one of three different hemagglutinating antigens (18) to provide the antigen for this assay. The use of pools has been a standard practice for the development of broadly specific serological assays for other bacterial species with considerable serotypic heterogeneity (13). However, recently Svedhem and colleagues produced the acid-wash extract from 11 *C. jejuni* strains from different parts of the world. Immune rabbit serum to these preparations examined in double diffusion in gel against various preparations showed a major line of identity and several minor precipitates (20). Immunoblotting and radioimmunoprecipitation studies by Logan and Trust suggest that the 31K protein band is a common antigen (14). Rautelin and Kosunen found that rabbits immunized with one of 21 Formalin-treated or 9 boiled *C. jejuni* cell types all developed antibodies to the same acid extract that we used (19). Because the bacterial strains had different serotypic characteristics and originated from diverse locales, the acid extract was believed to include common antigens. Our data support this conjuncture in several ways: (i) the major proteins present in the acid wash preparation of three different strains had identical migration characteristics in sodium dodecyl sulfate-polyacrylamide gel electrophoresis; (ii) by immunoblot analysis using both rabbit and human serum, the two major antigens of three different strains migrated at 30K and 61K; (iii) by diffusion in gel and immunoelectrophoresis with immune rabbit serum, lines of identity for the three preparations were seen (M. J. Blaser and H. A. Cody, unpublished data); and (iv) humans infected with a diversity of *C. jejuni* strains under field or laboratory conditions mounted antibody responses to this antigen preparation. In future assays it may not be necessary to use more than one strain as the source for the acid-labile antigens.

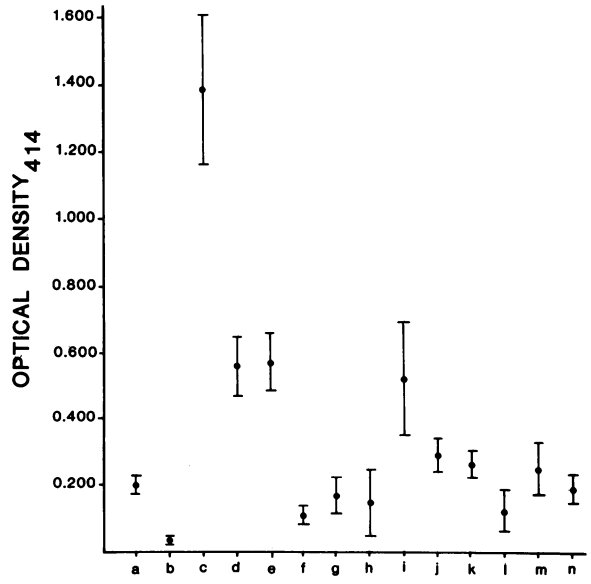

FIG. 6. *C. jejuni* serum IgA ELISA showing mean (± standard error) of optical density values in 382 sera from 14 population groups. Groups represent the same populations as indicated in Fig. 4 with the following numbers: (a) (n = 113); (b) (n = 26); (c) (n = 20); (d) (n = 20); (e) (n = 24); (f) (n = 26); (g) (n = 6); (h) (n = 12); (i) (n = 27); (j) (n = 26); (k) (n = 28); (l) (n = 15); (m) (n = 23); (n) (n = 19).

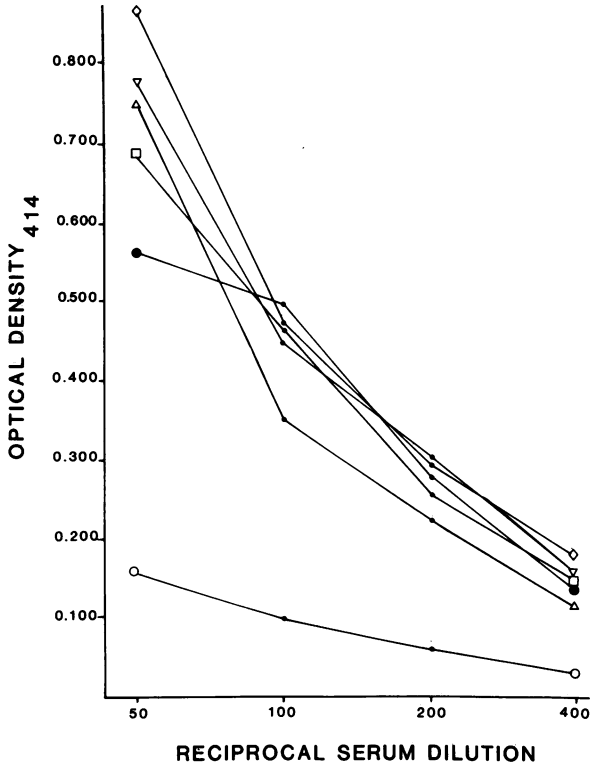

FIG. 7. Inhibition assay of IgA ELISA. Pooled high-titer human *C. jejuni* antisera were incubated with soluble protein antigens of *C. jejuni* (○), *C. fetus* subsp. *fetus* (□), *S. sonnei* (▽), *Y. enterocolitica* (◇), or *B. abortus* (●) or incubated alone (control) (△). Serum dilutions as shown were plated in triplicate in the IgA ELISA.

It is interesting to note that, whereas our bleb and whole cell preparations showed that little or none of this surface material was present, immunization of rabbits with boiled cells still provoked an antibody response, suggesting that small amounts may be immunogenic in rabbits. Previously reported ELISAs used whole cell sonicates (11) or lipopolysaccharide (21) as antigens.

Our results indicate that serum IgG and IgM levels are elevated for less than 3 months in most persons after acute exposure. As also observed by Walder and Forsgren (21), we found that IgG and IgM values rose simultaneously. However, in nonoutbreak groups of patients seeking medical attention because of acute diarrheal disease (Blaser, unpublished data), IgM titers became elevated before IgG titers. It is possible that differences in the severity of illness or preinfection immunity could explain the relative response of IgG and IgM. In contrast to the IgG and IgM results, serum IgA levels were elevated for 1 month or less. This phenomenon, early and transient specific serum IgA elevation, has been observed after exposure to other enteric pathogens and may reflect specific antibody levels in the gut (12). The data from the inhibition assays demonstrate the specificity of the IgA, IgG, and IgM ELISAs. However, none of the ELISAs is sufficiently specific or sensitive to permit diagnosis of acute *C. jejuni* infection on the basis of a single serum specimen. Nonetheless, the serum IgA ELISA had the best discriminating power.

The rise in serum IgG, IgM, and IgA titers in persons who were exposed to an implicated vehicle, but remained well, was less than that among persons who became ill. These phenomenon may be due to a heterogeneity in the exposed.

TABLE 1. Sensitivity and specificity of *C. jejuni* ELISAs

Group[a]	% of Sera tested with optical density (mean + 2 SE of control value)		
	IgG (0.461)	IgM (0.679)	IgA (0.253)
Controls	26.4	31.6	18.6
Ill			
<7 days	5.9	17.7	0
8 to 14 days	57.7	65.4	80.8
15 to 21 days	60.0	83.3	75.0
22 to 30 days	54.2	79.2	66.7
31 to 45 days	44.4	51.9	7.7
90 days	16.7	16.7	33.3
Well			
<7 days	33.3	8.3	8.3
8 to 14 days	48.2	40.7	55.6
15 to 21 days	57.1	53.9	38.5
22 to 30 days	60.7	39.3	39.3
31 to 45 days	42.9	13.3	6.7
Chronic			
Outbreak	58.3	12.5	26.1
Nonoutbreak	73.7	10.5	21.2

[a] For the number of sera tested in each group, see the legends to Fig. 3 through 5.

well group, i.e., not everyone exposed was actually infected. An alternative explanation is that the exposed, well group had asymptomatic infections resulting in less tissue invasion and damage with concomitantly less serum antibody production. A similar difference in serum antibody levels has been observed in persons with symptomatic and asymptomatic brucella infections (7).

Persons who are chronic drinkers of raw milk, who presumably have repeated exposure to *C. jejuni* and related organisms, had elevated serum IgG levels. However, in the same serum samples, mean IgM and IgA levels were within the normal range. These results confirm our previous observations on IgG and IgM levels in chronic raw milk drinkers when other serological assays were used (2). Included among the sera we tested were those assayed in our previous report and sera from other settings. We thus believe that the discordance between the IgG and the IgM and IgA antibody titers represents a real phenomenon.

Svedhem and colleagues (20) examined sera from slaughterhouse workers who also presumably had frequent exposure to *C. jejuni* and related organisms. They found that IgG levels were elevated, but that IgM levels were significantly above those in controls. It is unlikely that differing techniques were responsible for these discrepant observations since in other respects our results are similar. One explanation may be that the exposure to *C. jejuni* in the raw milk drinkers differed from that in slaughterhouse workers. Factors that could account for such differences include dose, interval and number of exposures, overall length of period of exposure, and the nature of the vehicles and mode of transmission. Among the slaughterhouse workers, there was no relation between length of employment and increase in either IgG or IgM levels. Milk is a good buffer and may permit the gastric acid-sensitive *C. jejuni* (3) to readily pass through the stomach in greater number than after ingestion after contamination of hands. The dichotomy between our results and the Swedish study results deserves further analysis.

Analysis of data obtained from our serological studies indicates that with all three assays we can now differentiate between nonexposure, chronic exposure, and recent exposure to *C. jejuni* antigens. In addition to being of use for determining serum antibody response to *C. jejuni* infections, these assays also can be used for seroepidemiological studies.

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