<table>
<thead>
<tr>
<th>Studies of the outer membrane proteins of Campylobacter jejuni for vaccine development (U) Colorado Univ Health Sciences Center Denver M J Blaser Apr 84</th>
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<tr>
<td>UNCLASSIFIED</td>
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<tr>
<td>DAMD17-82-C-2227</td>
</tr>
<tr>
<td>F/G 6/5</td>
</tr>
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<td>NL</td>
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STUDIES OF THE OUTER MEMBRANE PROTEINS OF CAMPYLOBACTER JEJUNI FOR VACCINE DEVELOPMENT

Final Report

by

Martin J. Blaser, M.D.

April 1984

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-82-C-2227

University of Colorado
Health Sciences Center
Denver, Colorado 80262

Approved for public release; distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.
MAY 1984

19 ABSTRACT

The major objective of our fifteen months of study was to develop methods for isolating outer membrane-enriched fractions of Campylobacter jejuni. Using standard disruption and solubilization techniques, we have prepared outer membrane-enriched fractions of C. jejuni and have characterized the proteins resolved. Subsequently, we began studies to determine which of the antigens are authentic and thus could have potential as vaccine candidates. Using both immunization, precipitation, and immunoblot procedures, we have acquired preliminary data on the usefulness of these proteins. Currently, we have continued to explore the possibility of using mice for an animal model of Campylobacter infection in which to test vaccine candidates. After initial characterization of the consequences of oral challenge, we have focused on determining the kinetics of the bacteremia produced, and the mechanisms for clearance. We have also developed an enzyme-linked immunosorbent assay (ELISA) for determining human serum IgA, IgM, and IgG responses to C. jejuni surface antigens. This technology will be of value at the time human subjects are challenged with candidate vaccines.
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In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).
Summary

The major objectives of our fifteen months of study was to develop methods for isolating outer membrane-enriched fractions of Campylobacter jejuni. Using standard disruption and solubilization techniques, we have prepared outer membrane-enriched fractions of C. jejuni and have characterized the proteins resolved. Subsequently, we began studies to determine which are antigenic and thus could have potential as vaccine candidates. Using both radioimmunoprecipitation and immunoblot procedures, we have acquired preliminary data on the antigenicity of these proteins.

Currently, we have continued to explore the feasibility of using mice for an animal model of Campylobacter infection in which to test vaccine candidates. After original characterization of the consequences of oral challenge, we have focused on determining the kinetics of the bacteremia produced, and the mechanisms for clearance.

We have also developed an enzyme-linked immunosorbent assay (ELISA) for determining human serum (IgA, IgG, and IgM responses to C. jejuni surface antigens. This technique will be of value at the time human subjects are challenged with candidate vaccines.
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## III. Progress in development of a standardized serologic assay for *C. jejuni* infections in humans: 43-52

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Final report

The final report will be divided into three sections as follows:

I. Progress in characterizing outer membrane proteins of *Campylobacter jejuni* and defining antigenicity.

II. Progress in development of an animal model of *Campylobacter* infection.

III. Progress in development of a standardized serologic assay for *C. jejuni* infections in humans.

IV. References

I. Progress in characterizing outer membrane proteins of *Campylobacter jejuni* and defining antigenicity.

The work in this section is that which is being funded under the first 15-month segment of the contract. The aim of our initial studies was to develop a standardized method for fractionating *C. jejuni* cells to produce outer membrane-enriched preparations. We have developed such a method, demonstrated that the fractions produced are outer membrane enriched, and have characterized the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles. The description of the studies done follows immediately in the attached manuscript which appeared in the October issue of *Infection and Immunity* (Footnote 1).

Our major findings were as follows:

a. Sonication of cells and incubation of crude membranes with sarcosyl produced an outer membrane-enriched fraction, based on small number of major bands, increase in ketodeoxyoctonate concentrations, presence of surface-exposed \(^{125}\)I-labeled proteins that are hydrophobic and similarity of protein profiles to that seen in blebs by SDS-PAGE.

b. Most isolates contained a single major band with molecular weight of 41,000 to 45,000, and other bands were resolved in the 30,000 to 70,000 range.

c. The SDS-PAGE profiles of \textit{C. jejuni} and \textit{C. coli} were indistinguishable from one another but could easily be differentiated from those of \textit{C. fetus} and \textit{C. faecalis}.

d. The profiles seen were stable for cultures incubated for 24 to 120 hours, incubated at 37°C and 42°C, grown in a variety of broths and agar plates, and passaged 10 times on a plate medium.

e. One hundred and ten isolates from patients with \textit{Campylobacter} enteritis were classified into one of nine different subtypes based on SDS-PAGE profiles. Two subtypes accounted for 76% of the isolates.

f. Epidemiologically-related strains showed complete concordance of SDS-PAGE profiles.
g. A plasmid encoding for tetracycline-resistance did not alter the outer membrane protein of a recipient \( C. \text{jejuni} \) strain.

A. **Defining Antigenicity**

Based on these studies, we have begun work on characterizing the antigenicity of the proteins resolved. The methods used for these studies were the radioimmunoprecipitation and Western blot techniques.

Our findings, represented as a series of figures are shown on the following pages and may be summarized as follows:

1. Using the radioimmunoprecipitation method, sera from homologously and heterologously immunized rabbits recognized several of the surface-exposed outer membrane proteins (Figure B-1). The most radiolabel was detected at 44k, or at the bottom of the gel representing degraded peptides. No radiolabeled protein bands were detected when normal rabbit serum or phosphate-buffered saline were incubated with the outer membrane preparations.

2. Using the radioimmunoprecipitation method, sera from humans convalescent from *Campylobacter* enteritis and from healthy controls were compared (Figure B-2). Although not as clear-cut as in the rabbit studies, a difference in the amount of radiolabel visualized in these two groups was discerned. Again, most activity occurred in the protein band migrating at about 44k.
3. We then used the same rabbit sera described in Figure B-1 to establish a Western blot system. The *Staph aureus* protein A conjugate captured specific antibody to *C. jejuni* outer membrane proteins that was in the serum of the immune rabbit but not in the normal rabbit (Figure B-3). Based on these findings the use dilution for *Staph aureus* protein A in future assays was 1:1000. Similarly, using serum from a normal human and a human convalescent from *Campylobacter* enteritis we determined the use dilution of a rabbit-antihuman IgG was 1:800. Using these same human sera, we determined the use dilutions of a goat-antihuman IgA conjugate (1:1000; Figure B-4) and a goat-antihuman IgM conjugate (1:800; Figure B-5).

4. Using the Western blot method with *Staph aureus* protein A-conjugate we showed that immune rabbit serum recognizes a wide variety of *C. jejuni* outer membrane proteins whereas normal serum does not (Figure B-6). Immune serum diluted 1:1000 still produced strong reactions. The range if proteins recognized was from 15k to 92k. Similar to the findings in the radioimmunoprecipitation procedure, both homologously and heterologously immunized rabbits had IgG antibodies (Figure B-7).

5. In contrast, among sera from mice that had been experimentally infected with *C. jejuni*, only that raised after intraperitoneal challenge had significant antibody (Figure B-8), and only at a low (1:20) serum dilution.

6. We then turned to serum from humans. As shown in Figure B-9, both persons convalescent from *Campylobacter* enteritis and healthy persons have IgA antibodies to the *C. jejuni* outer membrane proteins (OMPs) resolved using the Western bloy
procedure. The major band is the most antigenic both for ills and wells but the serum from ill persons had a stronger response. Several minor bands were antigenic for the serum from ills much more than the serum from wells, especially a band migrating at 62k.

7. IgG antibody to the major band was just as pronounced in the serum from wells as in the serum from ills (Figure B-10). The reasons why uninfected persons show such antibodies are not clear, but possibly the C. jejuni major outer membrane protein shares antigenic determinants with those of other gram-negative organisms to which normal persons are usually exposed. Another possibility is that the denaturing of proteins required for solubility in the PAGE system may uncover core antigens. Again, differences in serum response of ill and well persons to the minor protein bands are seen. Most prominent are proteins migrating at 62k, 54k, and 30k.

One explanation for the apparent recognition of the major OMP by normal serum is that the second antibody source, the rabbit anti-human IgG, has activity against the OMP. To test this we did several experiments using the second antibody alone (without pre-incubation of the nitrocellulose paper (NCP) with first antibody). As shown in Figure B-11, in the absence of human serum there is no recognition of the major OMP by the rabbit serum.

8. For IgM, the picture is similar to that for IgA and IgG (Figure B-12). There is differentiation between the amount of recognition by serum from ill and well persons of the OMP but no all-or-none phenomenon. Several minor bands appear to provide better differentiation.
9. To summarize these findings, as illustrated in Figure B-13, both the major and several minor OMPs are better recognized by immune human serum than by non-immune serum. Better characterization of the antigenicity of the peptides especially under non-denaturing conditions will permit further evaluation of their role as vaccine candidates.

10. Since normal human serum appears to have IgG to the major OMP, we were interested in whether these specific antibodies were transferred to the fetus. Not surprisingly, cord blood contained IgG but not IgA or IgM antibodies (Figure B-14). This observation may have importance in suggesting immunization strategies for children in developing countries. Immunization of mothers may protect their infants for their first several months through passive IgG antibody transfer.

11. Similarly, we examined breast milk from women from Bangladesh, Mexico and the United States. Up to a 1:100 dilution, all three women had IgA antibody to several OMPs, especially the major band (Figure B-15). By visual inspection, concentration of antibody was highest in the milk from the Bangladeshi woman and lowest in the milk from the American woman.

12. Bile obtained from three healthy persons in the United States was assessed for IgA antibody to the OMPs (Figure B-16). Two of the three persons had antibody demonstrated at 1:10 dilutions but further ten-fold dilutions were largely negative. The breast milk and bile studies show that antibodies are naturally present to C. jejuni OMPs in two body fluids that are of physiologic significance in relation to enteric infections. Immunization with specific antigens could enhance antibody secretion in these fluids.
13. We then began to assess serum antibody response to OMPs from two other \textit{C. jejuni} and one \textit{C. fetus} isolate. Convalescent serum from a patient with \textit{Campylobacter} enteritis showed strong IgA response to all three \textit{C. jejuni} preparations, and a weaker response to the \textit{C. fetus} strain (Figure B-17). Convalescent serum from a patient with \textit{C. fetus} systemic infection (bacteremia and meningitis) had antibody to \textit{C. fetus} OMPs, but the antigenic proteins were poorly resolved; in contrast, the \textit{C. jejuni} OMP was well resolved. Serum from a healthy person had slight antibody to the \textit{C. fetus} major bands. For serum IgG (Figure B-18) and IgM (Figure B-19), similar phenomena were observed. These studies suggest that among \textit{C. jejuni} strains antigenic OMPs are conserved and are cross-reactive, and that there is little cross-reaction between \textit{C. fetus} and \textit{C. jejuni} OMP antigens. The level of specific antibody to \textit{C. jejuni} antigens present in serum from the patient post-\textit{C. fetus} infection is similar to that in serum from normals.
Figure B-1: Autoradiograph of SDS-PAGE after radioimmunoprecipitation of *C. jejuni* outer membrane proteins by rabbit serum. Preparations are: outer membrane fraction of $^{125}$I-extrinsically-labelled *C. jejuni* PEN 1 whole cells (lane a); preparation in lane a incubated with phosphate buffered saline (lane b); preparation in lane a incubated with convalescent serum from rabbit immunized with PEN 1 *C. jejuni* (lane c); same as lane c except serum from rabbit immunized with PEN 2 (lane d); same as lane c except normal rabbit serum (lane e).
Figure B-2: Autoradiograph of SDS-PAGE after radioimmunoprecipitation of C. jejuni outer membrane proteins by human serum. Preparations are: outer membrane fraction of $^{125}$I-extrinsically labelled C. jejuni PEN 1 whole cells (lane a); outer membrane fraction of $^{125}$I-extrinsically labelled C. jejuni with serum from patients convalescent from Campylobacter enteritis (next 10 lanes); and healthy controls (next 5 lanes).
Figure B-3: Determination of use dilutions for rabbit anti-human IgG and Staph aureus protein A conjugates in a Western blot procedure. The outer membrane preparations is from C. jejuni PEN 1. Plus indicates convalescent serum 81-90, from a patient with Campylobacter enteritis, minus indicates serum 82-70 from a healthy control. Both sera are diluted 1:100. Numbers indicate dilutions of the conjugates used.
**Figure B-4:** Determination of use dilution for goat anti-human IgA conjugate in a Western blot procedure. The antigen and first antibodies are the same as in Figure B-3.
Figure B-5: Determination of use dilution of goat anti-human IgM conjugate in a Western blot procedure. The antigen and first antibodies are the same as in Figure B-3.
Figure B-6: Western blot of rabbit serum against *C. jejuni* outer membrane preparations. First antibody is serum diluted 1:100, 1:500, 1:1000 from rabbit immunized with PEN 1, and pooled normal rabbit serum diluted 1:100. Second antibody is swine anti-rabbit immunoglobulin (1:200) conjugated with horseradish peroxidase (HRPO).
Figure B-7: Western blot of rabbit serum against PEN 1 C. jejuni outer membrane preparations. Sera are from rabbit immunized with PEN 1 (lane a); immunized with PEN 2 (lane b); immunized with PEN 3 (lane c); and unimmunized (lane d). All sera are diluted 1:100. Second antibody is swine anti-rabbit immunoglobulin-HRPO conjugate (1:200).
Figure B-8: Western blot of mouse serum against PEN 1 C. jejuni outer membrane preparations. Sera are from: normal mice (lane a); mice 1 week after intraperitoneal infection with PEN 1 (lane b); mice 1 week after oral infection (lane c); mice 1 week after intravenous infection (lane d); mice 2 weeks after oral infection (lane e); mice 2 weeks after intravenous infection (lane f); human 2 weeks after oral infection (lane g). Mice sera are diluted 1:20 and conjugate is Staph aureus protein A-HRPO (1:1000). The human serum is diluted 1:100.
Figure B-9: Western blot of human serum IgA against PEN 1 C. jejuni outer membrane preparations. Sera, all diluted 1:100, are from five patients after C. jejuni infection (sera 82-439, 82-442, 82-445, 82-32, and 82-37; lanes a-e) and from healthy controls (sera 82-422, 82-424, 82-427, 82-364, 82-367; lanes f-j). Second antibody is goat-antihuman IgA-HRPO conjugate (1:1000).
Figure B-10: Western blot of human serum IgG against *C. jejuni* outer membrane preparations. Sera are the same as in Figure B-9. Second antibody is rabbit-antihuman IgG-HRPO conjugate (1:800).
Figure B-1: Western blot of rabbit-antihuman IgG conjugate against PEN 1 C. jejuni outer membrane preparations. The first antibody is from serum (1:100) from a patient with Campylobacter enteritis (lane a), from a healthy control (8-382, lanes b, d, and f), and borate buffer alone without any serum (lanes c, e, and g). The second antibody is rabbit-antihuman IgG-HRPO conjugate (1:800) without normal rabbit serum (NRS) (lanes a-c), with 10% NRS (lanes d and e), with 20% NRS (lanes f and g).
Figure B-12: Western blot of human serum IgM against PEN 1 C. jejuni outer membrane preparations. Sera are the same as in Figure B-9. Second antibody is goat-antihuman IgM-HRPO conjugate (1:800).
Figure B-13: Western blot of human serum immunoglobulin against PEN 1 C. jejuni outer membrane preparations. The first antibody is from serum (1:100) from a patient convalescent with *Campylobacter* enteritis (82-454; lane a) and from a healthy control (82-67; lane b). Second antibody conjugates are as specified in Figures B-9, B-10 and B-12.
Figure B-14: Western blot of human cord blood against *C. jejuni* outer membrane preparations. The first antibody is from cord blood from healthy control mothers and infants as follows: 82-134 (lanes a, f, k); 82-135 (lanes b, g, l); 82-136 (lanes c, h, m); 82-137 (lanes d, i, n); 82-138 (lanes e, j, o). The second antibody is goat-antihuman IgA-HRPO conjugate (lanes a-e), rabbit anti-human IgG-HRPO conjugate (lanes f-j), and goat anti-human IgM-HRPO conjugate (lanes k-o).
Figure B-15: Western blot of human breast milk IgA against PEN 1 C. jejuni outer membrane preparations. The first antibody is from breast milk from an American woman (lanes a, d, g), a Mexican woman (lanes b, e, h), and a Bangladeshi woman (lanes c, f, i). Breast milk is diluted 1:10 in lanes a-c, 1:100 in lanes d-f, 1:1000 in lanes g-i. Second antibody is goat antihuman IgA-HRP conjugate.
Figure B-16: Western blot of human bile IgA against \textit{PEN} \textit{C. jejuni} outer membrane preparations. The first antibody is from bile obtained from three healthy persons at the time of cholecystectomy (patient 1, lanes a–c; patient 2, lanes d–f; patient 3, lanes g–i). Bile is diluted 1:10 (lanes a, d, g), 1:100 (lanes b, e, h), 1:1000 (lanes c, f, i). Second antibody is goat anti-human IgA-HRPO conjugate.
Figure B-17: Western blot of human serum IgA against *C. fetus* and *C. jejuni* outer membrane preparations. Antigens are *C. fetus* (lanes a, e, i), *C. jejuni* PEN 3 (lanes b, f, j), PEN 2 (lanes c, g, k) and PEN 1 (lanes d, h, l). Sera are from a patient convalescent after *Campylobacter* enteritis (82-442), lanes a-d), a patient convalescent after *C. fetus* meningitis (81-167, lanes e-h), and a healthy person (82-424, lanes i-l). Second antibody is goat anti-human IgA HRPO conjugate.
Figure B-18: Western blot of human serum IgG against *C. fetus* and *C. jejuni* outer membrane preparations. Antigens and first antibody are as specified in Figure B-17. Second antibody is rabbit antihuman IgG-HRPO conjugate.
Figure B-19: Western blot of human serum IgM against *C. fetus* and *C. jejuni* outer membrane preparations. Antigens and first antibody are as specified in Figure B-17. Second antibody is goat antihuman IgM-HRPO conjugate.
II. Progress in development of an animal model of *Campylobacter* infection

Our initial studies in this area were done prior to receiving funding from the USAMR DC. We have described the characteristics of experimental oral infection of adult HA-ICR mice with *C. jejuni*. This manuscript appeared in the February 1983 issue of *Infection and Immunity* (Footnote 2). Several of the most significant findings are as follows:

1. Oral infection did not produce overt clinical symptoms.
2. Infected mice became long-term intestinal carriers.
3. Mice produced specific serum IgG antibodies which peaked at one week.
4. Transient bacteremia was observed.

Because bacteremia has also been reported in humans during *C. jejuni* infection, and because occurrence and clearance of bacteremia may be affected by organism virulence and host immunity, we sought to characterize this phenomenon in mice. Our major findings have been as follows:

1. Systemic infection is nearly universal after atraumatic oral challenge with *C. jejuni* (Table C-1). Portal drainage of the intestine results in clearance by the liver rather than the spleen (Figure C-1). Bacteremia is not detectable by 24 hours after dosage.

2. Radiolabeled studies show that organisms that are cleared in the reticuloendothelial system are mostly dead by one hour after dosing (Table C-2).
3. After intravenous infection clearance also is rapid (Figure C-2), and occurs in both liver and spleen (Table C-3) with the liver being twice as efficient as the spleen per gram of tissue.

4. After intravenous challenge, fecal excretion occurred in 87% of mice receiving \(10^7\) cfu initially, and fecal excretion was associated with biliary carriage (Table C-4).

5. Treatment of mice with silica before intravenous challenge resulted in an early (within 60 minutes) decrease in the clearance rate (Figure C-3). After intraperitoneal challenge, a similar decrease in clearance rate in silica-pretreated mice occurred, lasting until 12 hours.

6. A *C. jejuni* isolate susceptible to the bactericidal activity in normal human serum was cleared from the circulation more rapidly than was a strain that was relatively serum-resistant (Figure C-4).

7. However, the strain that was sensitive to human serum was resistant *in vitro* to immune mouse serum (Figure C-5). Similarly, a very serum-sensitive isolate was also resistant to mouse serum (Figure C-6). One explanation for the increased clearance *in vivo* but resistance *in vitro* is that mouse serum opsonizes the organism for reticuloendothelial clearance rather than being bactericidal. Failure to bind complement could be responsible for this phenomenon. Pretreatment with cobra venom factor which complement-depleted mice did not result in slowed clearance, further supporting this hypothesis.

Table C-1

Systemic infection after oral challenge of groups of adult mice

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Site</th>
<th>( \text{Log}_{10} ) cfu/ml or g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Dose</td>
<td>8.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.18</td>
</tr>
<tr>
<td>10</td>
<td>Blood</td>
<td>2.00 ±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.44 ±0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.03 ±0.50</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>3.21 ±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.00 ±0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.76 ±0.58</td>
</tr>
<tr>
<td>60</td>
<td>Blood</td>
<td>2.33 ±0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.56 ±0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.48 ±0.64</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>3.04 ±0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.37 ±0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.16 ±0.54</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each number represents the mean of five determinations and the standard error of the mean.
Table C-2
Clearance of $^{32}$P-labeled *C. jejuni* from bloodstream and livers of orally infected mice

<table>
<thead>
<tr>
<th></th>
<th>Cells per ml or g</th>
<th>Counts per ml or g</th>
<th>Cells per counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood - 10 minutes</td>
<td>2.00 ± 0.34$^a$</td>
<td>2.47 ± 0.15$^{c,e}$</td>
<td>3.13 ± 1.5</td>
</tr>
<tr>
<td>Liver - 10 minutes</td>
<td>3.21 ± 0.12$^a$</td>
<td>3.00 ± 0.16$^{c,f}$</td>
<td>3.11 ± 1.6</td>
</tr>
<tr>
<td>Blood - 60 minutes</td>
<td>2.33 ± 0.05$^b$</td>
<td>3.13 ± 0.07$^{a,e}$</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>Liver - 60 minutes</td>
<td>3.04 ± 0.07$^b$</td>
<td>3.87 ± 0.07$^{d,f}$</td>
<td>0.14 ± 0.02</td>
</tr>
</tbody>
</table>

$^a$ p < .005  
$^b$ p < .0005  
$^c$ p < .001  
$^d$ p < .0005  
$^e$ p < .001  
$^f$ p < .0005
Table C-3
Clearance of *C. jejuni* strains by liver and spleen 10 minutes after intravenous challenge

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. injected</th>
<th>Liver</th>
<th>% dose</th>
<th>Spleen</th>
<th>% dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No./g</td>
<td></td>
<td></td>
<td>No./g</td>
<td></td>
</tr>
<tr>
<td>PEN 1</td>
<td>1.4x10^8</td>
<td>1.8x10^7</td>
<td>18.5</td>
<td>5.9x10^6</td>
<td>0.7</td>
</tr>
<tr>
<td>PEN 2</td>
<td>4.3x10^7</td>
<td>1.1x10^7</td>
<td>38.5</td>
<td>4.3x10^6</td>
<td>1.7</td>
</tr>
<tr>
<td>PEN 3</td>
<td>4.2x10^7</td>
<td>5.9x10^6</td>
<td>22.3</td>
<td>5.9x10^6</td>
<td>2.6</td>
</tr>
<tr>
<td>MEAN</td>
<td>7.5x10^7</td>
<td>1.2x10^7</td>
<td>26.4</td>
<td>5.4x10^6</td>
<td>1.7</td>
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<td>3.2x10^7</td>
<td>3.5x10^6</td>
<td>6.1</td>
<td>5.3x10^5</td>
<td>0.6</td>
</tr>
</tbody>
</table>
Table C-4

PEN 1 C. _jejuni_ biliary carriage and fecal excretion after intravenous dose

<table>
<thead>
<tr>
<th>Biliary</th>
<th>Percentage</th>
</tr>
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<tr>
<td>Carrier</td>
<td>Non-carrier</td>
</tr>
<tr>
<td>Fecal excretor</td>
<td>13</td>
</tr>
<tr>
<td>Non-excretor</td>
<td>8</td>
</tr>
</tbody>
</table>

\[\chi^2 = 3.97, \ p < 0.05\]
Figure C-1: Isolation of C. jejuni from systemic sites in groups of 5 adult HA-ICR mice.
Figure C-2: Clearance from blood of *C. jejuni* given intravenously to adult HA-ICR mice.
Figure C-3: Clearance of *C. jejuni* from the blood of adult mice after intravenous challenge by whether they were or were not pretreated with silica.
Figure C-4: Clearance of serum-sensitive (PEN 1) and serum-resistant (Moore) strains of *C. jejuni* from the bloodstream of orally-infected adult HA-ICR mice.
Figure C-5: Incubation of C. jejuni PEN 1 with serum. C' = serum from a human with hypogammaglobulinemia (complement source); HIIMS = heat-inactivated (56°C x 30 minutes) serum from immune mice (2 weeks post oral infection). HINHS = heat inactivated serum from normal humans.
Figure C-6: Incubation of C. jejuni 79-193 with serum. Abbreviations are as in Figure C-5. HINMS = heat inactivated serum from normal (uninfected) mice.
III. Progress in development of a standardized serologic assay for *C. jejuni* infections in humans.

Based on initial studies of antibody responses to *C. jejuni* among patients with inflammatory bowel disease, we now have an ELISA developed for this organism. The antigen used consists of microcapsule prepared from the Penner type strains 1, 2, and 3, extracted at low pH using the method of McCoy. The major findings in this section are as follows:

1. The optimal concentrations of antigen and conjugates used in the ELISA were determined by checkerboard titrations. We have shown that a nearly linear relationship exists between reciprocal dilution of serum from patients with *Campylobacter* enteritis and optical density in an IgG ELISA (Figure D-1). Examining sera from known healthy controls and patients convalescent from *Campylobacter* enteritis enabled us to calculate optimal use dilutions for screening unknown sera. The screening dilutions were 1:100 for the IgG, and 1:50 for the IgA and IgM assays.

2. We first examined sera from a small milkborne outbreak of *Campylobacter* enteritis in Minnesota (Table D-1). We found that even with a small number of sera, these assays could distinguish between ill persons and unexposed controls. The few sera examined from persons who remained well after exposure to the implicated vehicle showed IgA and IgG values intermediate between the two other groups.
3. Investigation of a milkborne outbreak of *Campylobacter* enteritis in Oregon in 1982 yielded new serologic information (Table D-2). Sera were obtained acutely and 25–42 days later from 17 persons who drank the implicated milk and became ill, 12 persons who drank the milk but who remained well, 11 persons who had no exposure to the milk, and 10 from farmers in the area who had drank raw milk for many years. Study of sera from this outbreak demonstrated the following points:

a. Convalescent sera from ill persons showed significantly more *C. jejuni*-specific IgA, IgG, and IgM antibody than did their acute sera.

b. The IgA, IgG, and IgM *C. jejuni* ELISA differentiated between unexposed controls and patients convalescing from *Campylobacter* enteritis.

c. Persons who were exposed to the vehicle but who did not drink the milk showed no change in IgA, IgG, and IgM titers suggesting that their exposures did not lead to infection.

d. These persons who were exposed but remained well had significantly higher serum IgA levels compared with unexposed persons and the acute sera of the exposed but ill persons. Possibly, the presence of *C. jejuni*-specific pre-existing IgA (or s-IgA) antibodies may have protected against infection.

e. Persons with chronic exposures had elevated IgA and IgG antibody levels, and although some had drank the implicated milk, none became ill. These data also suggest that elevated serum IgA and IgG *C. jejuni*-antibody levels may reflect protective phenomena.
f. IgM levels in chronically infected persons were only slightly elevated, corroborating findings in another outbreak (Footnote 3), and using a different method (ELISA vs. IFA).

4. We have recently obtained from Dr. Robert Black sera from volunteers dosed with *C. jejuni* at the Center for Vaccine Development at the University of Maryland. In these trials, volunteers were given low doses (10^2 - 10^4) of *C. jejuni* in milk and not all those exposed were infected. From 3 trials, 9 became ill after infection, 21 had asymptomatic infections, and 14 had no detectable infection.

The results of the IgG, IgM, and IgA ELISA's are shown in Figures D-2 - D-4. Among the volunteers who became ill, the differences in optical density between the acute and the 11 and 21 day sera were significant (p < .05) in the IgM and IgA assays. Among the infected-well volunteers, the rise in IgG antibody was significant at 11, 21 and 28 days. At 11 days after the dose, IgG titers were significantly higher in the infected-well group than in the uninfected group and IgM and IgA titers were higher as well. At 11 and 21 days after dosing, the uninfected group had significantly lower IgG titers than did the infected-well group and lower IgM than did the infected-ill group.

The results of these titrations in a highly controlled setting confirms our earlier studies that the *C. jejuni* ELISA's developed for specific human IgA, IgG, and IgM can be used as specific and sensitive indicators of *Campylobacter* infection. Analysis of data obtained from these serologic
studies should differentiate between non-exposure, chronic exposure and recent exposure to *C. jejuni* antigens. These assays can now be used for seroepidemiologic studies, as well as for assessing serologic responses to vaccine trials.

Table D-1

*C. jejuni* serum antibodies by an ELISA during a 1982 Minnesota outbreak of *Campylobacter enteritis*

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed, ill (convalescent)</td>
<td>7</td>
<td>0.799±0.206*</td>
<td>2.379±0.907*</td>
<td>4.060±1.933**</td>
</tr>
<tr>
<td>Exposed, well (convalescent)</td>
<td>4</td>
<td>0.333±0.194</td>
<td>0.725±0.615</td>
<td>0.484±0.208</td>
</tr>
<tr>
<td>Unexposed matched controls</td>
<td>7</td>
<td>0.157±0.063</td>
<td>0.341±0.075</td>
<td>0.521±0.049</td>
</tr>
</tbody>
</table>

*p < .05, **p < .07 compared with unexposed matched controls.*
Table D-2

*C. jejuni* serum antibodies by an ELISA during a 1982 Oregon milkborne outbreak of *Campylobacter enteritis*

<table>
<thead>
<tr>
<th>O.D. 414 in ELISA for</th>
<th>Exposed, ill (n=17)</th>
<th>Exposed, well (n=12)</th>
<th>Unexposed (n=11)</th>
<th>Chronic exposure (n=10)</th>
</tr>
</thead>
</table>

**Serum 1 (10/22/82)**

<table>
<thead>
<tr>
<th></th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.027±0.009</td>
<td>0.182±0.036</td>
<td>0.378±0.066</td>
</tr>
<tr>
<td></td>
<td>0.150±0.100</td>
<td>0.360±0.080</td>
<td>0.277±0.060</td>
</tr>
<tr>
<td></td>
<td>0.057±0.017</td>
<td>0.383±0.098</td>
<td>0.318±0.035</td>
</tr>
<tr>
<td></td>
<td>0.364±0.168</td>
<td>1.618±0.791</td>
<td>0.548±0.094</td>
</tr>
</tbody>
</table>

**Serum 2 (11/16/82 - 12/3/82)**

<table>
<thead>
<tr>
<th></th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.123±0.038</td>
<td>0.835±0.227</td>
<td>1.871±0.544</td>
</tr>
<tr>
<td></td>
<td>0.144±0.073</td>
<td>0.408±0.091</td>
<td>0.375±0.080</td>
</tr>
<tr>
<td></td>
<td>0.058±0.014</td>
<td>0.353±0.075</td>
<td>0.392±0.023</td>
</tr>
<tr>
<td></td>
<td>0.208±0.047</td>
<td>2.215±0.864</td>
<td>0.656±0.169</td>
</tr>
</tbody>
</table>
Figure D-1: Relation of optical density to serum dilution in 17 patients tested for anti-Campylobacter antibodies in an ELISA using low pH-extractable antigen
Figure D-2: *Campylobacter jejuni* IgM ELISA of serum from 44 volunteers. Sera were taken just before oral dosing and at days 11, 21 and 28 after dosing. Points represent means ± standard error of the mean for each group. For the infected-ill group, significant differences in distribution of optical densities occur between the control and 11 day (p < .01), 21 day (p < .005) groups. At 11 days, there are significant differences in optical density distribution between the uninfected group and the infected-ill group (p < .05) and at 21 days between the uninfected group and the infected-ill group (p < .05).
C. JEJUNI IgG ELISA OF SERUM FROM 44 VOLUNTEERS

Figure D-3: Campylobacter jejuni IgG ELISA of serum from 44 volunteers. For the infected-well group, significant differences in optical density distribution occur between the control and 11 day (p < .0025), 21 day (p < .005), and 28 day (p < .05) groups. At 11 days there are significant differences in optical density distribution between the uninfected group and the infected-well group (< .025) and at 21 days between the uninfected group and the infected-well group (p < .025).
Figure D-4: Campylobacter jejuni IgA ELISA of serum from 44 volunteers. At 11 days, there were significant differences in distribution of optical densities between the infected-ill group and the infected-well group (p < .025) and the uninfected group (p < .0005). At 21 days these differences remained significant (p < .05 for both comparisons). For the infected ill group, significant differences in distribution of optical density occur between the control and 11 day (p < .005) and the 21 day group (p < .05).
References


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