SHORT ARTICLE

Variations in the virulence, for pregnant guinea pigs, of campylobacters isolated from man

C. R. COID, A. M. O’SULLIVAN and C. J. DORÉ*

Divisions of Comparative Medicine and *Medical Statistics, Clinical Research Centre, Harrow, HA1 3UJ

Summary. Pregnant guinea pigs were used to compare the virulence of four human isolates of Campylobacter fetus ss. fetus and four of C. jejuni on the basis of their ability to cause abortion and bacteraemia. Of the four strains of C. fetus ss. fetus two produced abortion readily after intramuscular injection. The four C. jejuni isolates were, however, of comparatively low virulence and no differences between them were demonstrated. Some of the isolates differed in their ability to survive in vitro in human and guinea-pig serum. It is suggested that campylobacters vary in their virulence for man and that this may influence the outcome of infections. Guinea pigs may prove useful in studying the pathogenesis of systemic campylobacter infections.

Introduction

Infections caused by organisms of the genus Campylobacter are associated with various clinical abnormalities, principally acute enterocolitis, but occasionally meningitis, abortion, arthritis, endocarditis and other systemic infections (Skirrow, 1984). Infertility and abortion are the main features of the infections in ruminant animals.

In determining the virulence of micro-organisms isolated from man there is usually no alternative to the use of laboratory animals. Few animal models are available for the study of campylobacters. Newell et al. (1985) showed, however, that isolates associated with diarrhoea in man differed from those isolated from water in their ability to colonise the intestines of infant mice and in their cytotoxic activity for HeLa cells. Moreover, abortion in pregnant guinea pigs has been used as an indicator of the pathogenicity of campylobacters from various sources (Sultan Dosa et al., 1983; Taylor and Bryner, 1984).

For the study reported here, pregnant guinea pigs were used to compare the virulence of four strains of Campylobacter fetus ss. fetus (Veron and Chatelain, 1973; referred to hereafter as C. fetus) and four of C. jejuni isolated from human patients.

Materials and methods

Guinea pigs

Guinea pigs, aged 12–15 weeks, of the Dunkin-Hartley strain were used during their sixth week (range 42–49 days) of pregnancy. The campylobacters were injected intramuscularly or intraperitoneally or were administered orally by means of a syringe without a needle. Twelve days later, or as soon as abortion occurred, the animals were killed by exposing them gradually to an increasing concentration of carbon dioxide.

Campylobacter strains

The four C. fetus isolates were kindly supplied by Dr M.B. Skirrow and the four C. jejuni isolates by the Microbiology Department of Northwick Park Hospital. All came from human patients and were subcultured twice upon arrival at this laboratory before use. Stock cultures were maintained at −70°C. The C. fetus strains comprised: nos. 3329 and 25320, isolated from blood; no. 23907, from an ovarian abscess; and no. 613, from a wound. The C. jejuni strains comprised: no. 42734, isolated from the watery motions of a 77-year-old female with osteomyelitis; no. 43353, from the watery motions of a 20-year-old male; no. 39175, from the blood-stained stool of a 30-year-old female; and no. 40630, from the blood-stained stool of a 26-year-old male.

Preparation of inocula

Each suspension for injection was prepared by inoculating 10 ml of Brucella Broth (Difco) with 0·1 ml of stock culture and incubating at 37°C in a microaerophilic environment produced by means of a Campypack (Oxoid). After 30 h the broth was found to contain a median value of 10^8 cfu/ml (interquartile range 8·4 × 10^7 to 2·6 × 10^8). The bacterial suspension was then appropriately diluted in brucella broth for administration.

Estimation of the number of campylobacters in guinea-pig tissue

The method was that described by Coid and Nicholson (1981) with the modifications that the tissues were...
homogenised in brucella broth and the blood-agar plates incubated in a microaerophilic environment for 48 h. Bacteraemia was detected by plating blood from an ear vein on blood agar by means of a 0.01 ml loop (Gibco).

Serum

Pooled human serum was prepared from the blood of three healthy volunteers. Complement, as assayed by lysis of antibody-sensitised erythrocytes, was within the normal limits. Pooled guinea-pig serum was prepared from three pregnant animals, which were deeply anaesthetised (Sagatal; May and Baker Ltd) before exsanguination.

Serum inactivation of campylobacters

The susceptibility of each isolate to the bactericidal activity of human and guinea-pig serum was determined as described by Johnson et al. (1978). The incubation period was 1 h.

Statistical methods

Fisher's exact test was used.

Results

Abortion and bacteraemia in infected guinea-pigs

The table gives the results of two experiments, one in which pregnant guinea pigs received $10^9$ cfu by the oral, intramuscular or intraperitoneal route, and the other in which $10^5$ cfu were given intraperitoneally.

In the animals inoculated intramuscularly, the abortifacient activity of *C. fetus* strain 23907 was significantly greater than that of strains 3329 ($p=0.008$) and 613 ($p=0.01$) but similar to that of strain 25320. This finding was supported by the results in animals given $10^5$ cfu intraperitoneally. Bacteraemia was detected at 24 h in a greater number of animals given $10^5$ cfu intraperitoneally than in those given *C. fetus* strain 613.

<table>
<thead>
<tr>
<th>Campylobacter species, strain, and origin</th>
<th>10^9 cfu orally</th>
<th>10^5 cfu intramuscularly</th>
<th>10^5 cfu intraperitoneally</th>
<th>10^9 cfu intraperitoneally</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. fetus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25320 Blood</td>
<td>0/4*</td>
<td>3/4</td>
<td>5/9</td>
<td>6/7</td>
</tr>
<tr>
<td>23907 Ovarian abscess</td>
<td>0/3</td>
<td>0/3</td>
<td>9/12</td>
<td>8/12</td>
</tr>
<tr>
<td>3329 Blood</td>
<td>0/3</td>
<td>0/3</td>
<td>1/9</td>
<td>5/8</td>
</tr>
<tr>
<td>613 Wound</td>
<td>0/3</td>
<td>0/3</td>
<td>2/12</td>
<td>1/12</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42734 Faeces</td>
<td>0/3</td>
<td>0/3</td>
<td>1/9</td>
<td>1/9</td>
</tr>
<tr>
<td>43353 Faeces</td>
<td>0/3</td>
<td>0/3</td>
<td>1/9</td>
<td>1/9</td>
</tr>
<tr>
<td>39175 Faeces</td>
<td>0/3*</td>
<td>0/3</td>
<td>0/9*</td>
<td>0/9</td>
</tr>
<tr>
<td>40630 Faeces</td>
<td>0/3*</td>
<td>0/3</td>
<td>0/9</td>
<td>1/9</td>
</tr>
</tbody>
</table>

ND = Not done.

* = One animal died in these groups.

† = Campylobacters not isolated from any tissues.

Table. Abortion and bacteraemia 24 h after the administration of *C. fetus* and *C. jejuni* to pregnant guinea pigs
or the four C. jejuni isolates. No difference between the
four C. jejuni isolates was demonstrated in terms of the
abortions produced by parenteral inoculation.

After the oral administration of the eight isolates, no
abortions occurred, but bacteraemia was detected at 24 h
in guinea pigs given C. fetus strain 25320. In a subsequent
experiment (not shown in the table), 5 x 10^9 cfu of strain
25320 given orally produced abortion in two of three
guinea pigs, and one of three animals given the same dose
of C. fetus strain 23907 also aborted; in none of these
animals was bacteraemia detected.

Bacteriological examination of guinea-pig tissues

Evidence that certain strains of C. fetus multiply
profusely in guinea-pig tissues was shown by isolate
25320. Thus, one placenta from a guinea pig that aborted
three conceptuses after intramuscular inoculation (10^9
cfu) contained 1.5 x 10^9 cfu. Moreover, one of three
placentas from a guinea pig that aborted after intraperito-
neal inoculation (10^9 cfu) contained 7.2 x 10^9 cfu.
Similarly, one of two aborted placenta from a guinea pig
given C. jejuni strain 42734 (10^5 cfu) by intraperitoneal
inoculation contained 8.9 x 10^7 cfu.

At the end of each experiment, the liver, spleen, kidneys
and gallbladder of each animal were examined for
campylobacters but the numbers present were usually
fewer than 10^2 cfu/organ. Infection of the fetus, as
determined by examination of the liver, spleen and fetal
stomach contents, was not a regular occurrence even
when the corresponding placenta was infected.

Survival of campylobacters in serum

Human and guinea-pig serum gave similar results. C.
fetus strains 25320 and 23907 and C. jejuni strain 42734
showed a survival rate in excess of 95%. Survival of <1%
was shown, however, by C. fetus strains 613 and 3329
and C. jejuni strains 39175, 43353 and 40630.

Discussion

In an analysis of non-intestinal (systemic) human
infections with campylobacters, Rettig (1979) observed
that most patients had pre-existing debilitating disorders.
Little evidence is available, however, of variations in the
virulence of campylobacters that might influence the
course of infections in man. The experiments described
here demonstrate that different isolates of C. fetus from
human patients can vary significantly in their virulence
for guinea pigs as determined by their abortifacient
activity and ability to cause bacteraemia. Virulence for
experimental animals may or may not truly reflect
virulence for man. No differences in virulence for guinea
pigs could be demonstrated in four C. jejuni isolates. Both
C. fetus and C. jejuni showed striking strain variations in
susceptibility to the bactericidal effect of serum. The
observations on C. jejuni are similar to those reported by
Blaser et al. (1983). However, C. fetus strains used in the
experiments by these workers were found to be highly
serum resistant, whereas two out of four strains used in
our experiments were readily killed by both human and
guinea-pig serum.

Infection was established experimentally by the oral
administration of two C. fetus strains. Presumably,
therefore, adherence to and penetration of the gut wall,
together with the ability to multiply in body tissues, are
likely to play a significant role in the pathogenicity and
virulence of campylobacters.

Previously, Sultan Dosa et al. (1983) demonstrated
that, in pregnant guinea pigs, the virulence of campylo-
bacters appeared to be related to their host origin. In this
report, guinea pigs were shown also to be capable of
demonstrating variations in the virulence of isolates from
the same host. These animals seem likely to be useful in
studying other aspects of the pathogenesis of campylo-
bacter infections.

We wish to thank members of the Division of Immunological
Medicine for performing the serum complement assays and Dr
A.P. Johnson for helpful comments during the course of this
work.

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