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* (Véron 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 10^9 cfu/ml (interquartile range 8.4×10^8 to 2.6×10^9). 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 intramuscular inocula of three *C. fetus* strains than in those given *C. fetus* strain 613

Table. Abortion and bacteraemia 24 h after the administration of *C. fetus* and *C. jejuni* to pregnant guinea pigs

Campylobacter species, strain, and origin	Abortion (A) and bacteraemia (B) in groups of animals given							
	10 ⁹ cfu orally		10 ⁹ cfu intra-muscularly		10 ⁵ cfu intra-peritoneally		10 ⁹ cfu intraperitoneally	
	A	B	A	B	A	B	A	B
<i>C. fetus</i>								
25320								
Blood	0/4*	3/4	5/9	6/7	3/3	2/2	ND	ND
23907								
Ovarian abscess	0/3	0/3	9/12	8/12	3/3	2/3	ND	ND
3329								
Blood	0/3	0/3	1/9	5/8	0/3	1/3	0/4	0/4
613								
Wound	0/3	0/3	2/12	1/12	0/3	0/3	0/4	0/3
<i>C. jejuni</i>								
42734								
Faeces	0/3	0/3	1†/9*	1/9	2/3	0/3	ND	ND
43353								
Faeces	0/3	0/3	1†/9*	1/9	2/3	0/3	ND	ND
39175								
Faeces	0/3*	0/3	0/9*	1/9	0/3	0/3	2/3	1/3
40630								
Faeces	0/3*	0/3	0/9	1/9	0/3	1/3	1/3	1/3

ND = Not done.

* = One animal died in these groups.

† = Campylobacters not isolated from any tissues.

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), 5×10^9 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 1.5×10^9 cfu. Moreover, one of three placentas from a guinea pig that aborted after intraperitoneal inoculation (10^5 cfu) contained 7.2×10^7 cfu. Similarly, one of two aborted placentas from a guinea pig given *C. jejuni* strain 42734 (10^5 cfu) by intraperitoneal inoculation contained 8.9×10^7 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 10^3 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.* (1985). 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 campylobacters 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 campylobacter 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.

REFERENCES

- Blaser M J, Smith P F, Kohler P F 1985 Susceptibility of *Campylobacter* isolates to the bactericidal activity of human serum. *Journal of Infectious Diseases* **151**:227-235.
- Coid C R, Nicholson J 1981 The multiplication of three different isolates of Group B streptococci in pregnant mice. *Placenta* **2**:187-92.
- Johnson A P, Osborn M F, Taylor-Robinson D 1978 Iron-mediated protection of *Neisseria gonorrhoeae* against killing by normal human serum. *FEMS Microbiology Letters* **4**:225-227.
- Newell D G, McBride H, Saunders F, Dehele Y, Pearson A D 1985 The virulence of clinical and environmental isolates of *Campylobacter jejuni*. *Journal of Hygiene* **94**:45-54.
- Rettig P J 1979 Campylobacter infections in human beings. *Journal of Pediatrics* **94**:855-864.
- Skirrow M B 1984 Campylobacter infections of man. In: Easmon, C S F and Jeljaszewicz, J (eds): *Medical Microbiology* 4. Academic Press, London p 105.
- Sultan Dosa A B, Bryner J H, Foley J W 1983 Pathogenicity of *Campylobacter jejuni* and *Campylobacter coli* strains in the pregnant guinea pig model. *American Journal of Veterinary Research* **44**:2175-2178.
- Taylor D E, Bryner J H 1984 Plasmid content and pathogenicity of *Campylobacter jejuni* and *Campylobacter coli* strains in the pregnant guinea pig model. *American Journal of Veterinary Research* **45**:2201-2202.
- Véron M, Chatelain R 1973 Taxonomic study of the genus *Campylobacter* Sebald and Véron and designation of the neotype strain for the type species, *Campylobacter fetus* (Smith and Taylor) Sebald and Véron. *International Journal of Systematic Bacteriology* **23**:122-134.