Campylobacters and impaired fetal development in mice

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Summary. Intravenous injection of eight human strains of *Campylobacter fetus* ss *fetus* and *Campylobacter jejuni* into mice at various stages of pregnancy demonstrated significant strain differences in ability to affect implantation of the fertilised ovum and to cause resorption of the mouse fetus. Implantation was significantly impaired when *C. fetus* ss *fetus* was injected intravenously on day 2 of pregnancy, but no effect was observed in mice receiving *C. jejuni*. On day 6 of pregnancy, before the development of placental circulation, both *C. fetus* ss *fetus* and *C. jejuni* impaired fetal growth; one strain of *C. jejuni* had a greater effect than others of the same species. In animals inoculated on day 13 of pregnancy, after the development of placental circulation, six of the eight campylobacter strains caused resorption of the mouse embryos. A similar effect on the embryos was observed after injection of heat-killed organisms, and endotoxin-like substances may have been responsible. It is also suggested that factors other than endotoxin-like substances have a deleterious effect on embryonic growth.

Introduction

In the past decade campylobacters have emerged as important pathogens of man. Of the three species known to be associated with human disease, two—*Campylobacter jejuni* and *C. coli*—are among the most common causes of infectious diarrhoea (Skirrow, 1977), whereas *C. fetus* ss *fetus* (henceforth referred to as *C. fetus*) is recognised as a cause of septicaemia in immunocompromised hosts (Guerant *et al.*, 1978). Campylobacters can also cause enteric infections in animals, but infertility and abortion are more common (Coid and Fox, 1983).

Human infection was first described by Vincent *et al.* (1947) in a pregnant woman, who had a bacteraemia caused by *C. fetus*, and who subsequently aborted at 6 months' gestation. Since then, at least 20 cases of abortion and perinatal sepsis associated with campylobacter infection have been reported (Simor *et al.*, 1986), *C. fetus* being identified in ten cases, *C. jejuni* in nine, and *C. coli* in one.

Severe systemic disturbances during pregnancy can be caused by endotoxins from gram-negative bacteria. For example, *Escherichia coli* endotoxin impairs fetal development in the mouse (Coid, 1976), and in many other species endotoxins have an abortifacient effect (Dennis, 1967). Because purified lipopolysaccharide from *C. jejuni* is similar to that from Enterobacteriaceae (Naess and Hof-

stad, 1982), the present investigations were undertaken to determine whether campylobacters have a deleterious effect on fetal development, similar to that produced by endotoxins from other bacterial sources.

Materials and methods

Campylobacter strains

The four *C. fetus* strains were kindly supplied by Dr M. B. Skirrow and the four *C. jejuni* strains by the Microbiology Department of Northwick Park Hospital. All came from human patients and were subcultured three times in 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 faeces of a 77-year-old female with osteomyelitis; no. 43353, from the watery faeces of a 20-year old male; no. 39175, from the blood-stained faeces of a 30-year old female; and no. 40630, from the blood-stained faeces of a 26-year old male. 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 micro-aerophilic environment produced by means of a Campypack (Oxoid). Broth cultures incubated for 30 h were diluted 1 in 4 in sterile medium to give an overall median infecting dose of 1.3×10^9 colony-forming units (cfu)/mouse, the interquartile range (IQR) being $(1.5-1) \times 10^9$. Heat-killed

suspensions of campylobacters were prepared by heating the inocula in a water bath at 63°C for 45 min.

Estimation of the number of campylobacters in mouse tissues

The method (Coid and Nicholson, 1981) was briefly as follows. At the termination of each experiment the mice were killed by dislocation of the cervical vertebrae. The abdomen was opened aseptically and the spleen and liver were removed, dipped in 80% alcohol, and flamed. Each uterus was dissected separately and the placentae and fetuses were removed and transferred to sterile containers. The tissues were homogenised in brucella broth, and the number of campylobacters in each organ was calculated from the number of colonies growing on blood-agar plates after spreading 0.1-ml volumes of appropriate dilutions and incubating for 48 h in a micro-aerophilic environment.

Mice

Female mice of an outbred strain (TO/Crc) aged 10 weeks were obtained from the specific pathogen-free breeding unit at this Centre. The first day of pregnancy was taken to be that on which the vaginal plug was observed. Individual mice were given 0.2 ml of the inoculum intravenously in the tail. The test groups received a *Campylobacter* sp. and the control groups sterile brucella broth.

Estimation of the number of resorptions in mice

All experiments were terminated on the eighteenth day of gestation. Immediately after the mice were killed, their

abdomens were aseptically opened. The uterus was then removed and opened to reveal the number of resorbed and healthy fetuses. The ovaries were also removed from those mice inoculated on day 2 of pregnancy.

To obtain the number of pre-implantation deaths in the group of mice inoculated on day 2 of pregnancy, the differences between the corpora luteal counts and the total numbers of live and dead implants were taken.

Statistical methods

Mann-Whitney U tests were used to compare groups for both the proportion of resorptions (ratio of resorptions to the total number of live and dead fetuses) and the proportion of pre-implantation loss (ratio of pre-implantation deaths to the total number of live and dead implants).

Results

The effect of Campylobacter spp. on 2-day pregnant mice

To determine the effect of *C. fetus* and *C. jejuni* on implantation of the fertilised ovum, 2-day pregnant mice were given inocula of the campylobacters intravenously. Combining results for all *C. fetus* strains, there was a significant effect ($p=0.005$) on pre-implantation loss of the fetus, whereas the *C. jejuni* strains combined had no such effect ($p=0.35$) (table I). No differences could be demonstrated between the effects of individual strains within each species tested.

Table I. The effect of a median dose of 1.6×10^9 campylobacters on the pre-implantation loss of the 2-day mouse fetus

<i>Campylobacter</i> sp. and strain	Origin	Number of mice	Pre-implantation loss in control mice		Number of mice	Pre-implantation loss in infected mice		p Value
			Median	IQR		Median	IQR	
<i>C. fetus</i>								
25320	blood	3	0.08	0-0.20	4	0.16	0.09-0.24	0.22
23907	ovarian abscess	3	0	0-0.14	7	0.18	0-0.33	0.25
3329	blood	4	0.04	0-0.08	5	0.21	0-0.40	0.11
613	wound	6	0	0-0.03	5	0.10	0-0.13	0.32
All <i>C. fetus</i> strains		16	0	0-0.08	21	0.13	0.05-0.27	0.005
<i>C. jejuni</i>								
42734	faeces	5	0.15	0-0.18	4	0	0-0	0.18
43353	faeces	3	0.15	0-0.25	3	0.11	0-0.17	0.83
39175	faeces	4	0	0-0.12	3	0	0-0	0.72
40630	faeces	5	0	0-0.17	3	0.09	0-0.20	0.65
All <i>C. jejuni</i> strains		17	0	0-0.16	13	0	0-0.10	0.35

IQR = interquartile range.

The effect of Campylobacter spp. on 6-day pregnant mice

When 6-day pregnant mice were inoculated with campylobacters, the overall results (table II) showed a significant difference in the proportion of resorbed fetuses between the *C. jejuni* treated group, and the control group ($p=0.02$), and when each strain was considered separately there was a significant difference for strain 42734 ($p=0.01$). *C. fetus* was shown to have impaired the development of the 6-day mouse fetus ($p=0.05$) when all strain data were

combined, but there were no significant differences between individual strains.

The effect of Campylobacter spp. on 13-day pregnant mice

When compared with the control group, animals inoculated with live campylobacters on day 13 of pregnancy showed an increase in the proportion of fetuses resorbed as a result of inoculation with all four strains of *C. fetus* and with two of the four strains of *C. jejuni* (table III).

Table II. The effect of a median dose of 1.2×10^9 campylobacters on the development of the 6-day mouse fetus

Campylobacter sp. and strain	Origin	Number of mice	Proportion of resorptions in control mice		Number of mice	Proportion of resorptions in infected mice		p value
			Median	IQR		Median	IQR	
<i>C. fetus</i>								
25320	blood	9	0	0-0.06	5	0	0-0.25	0.64
23907	ovarian abscess	8	0	0-0	7	0	0-0.25	0.18
3329	blood	10	0	0-0.03	5	0	0-0.67	0.43
613	wound	10	0.06	0-0.13	8	0.10	0-0.16	0.72
All <i>C. fetus</i> strains		37	0	0-0.05	25	0	0-0.25	0.05
<i>C. jejuni</i>								
42734	faeces	10	0	0-0.10	6	0.94	0.56-1.00	0.01
43353	faeces	23	0	0-0.13	11	0.11	0-0.29	0.10
39175	faeces	3	0	0-0.11	5	0	0-0.17	1.00
40630	faeces	12	0	0-0.12	13	0	0-0.21	0.41
All <i>C. jejuni</i> strains		48	0	0-0.11	35	0.11	0-0.30	0.02

Table III. The effect of a median dose of 2×10^9 campylobacters on the development of the 13-day mouse fetus

Campylobacter sp. and strain	Origin	Number of mice	Proportion of resorptions in control mice		Number of mice	Proportion of resorptions in infected mice		p value
			Median	IQR		Median	IQR	
<i>C. fetus</i>								
25320	blood	3	0	0-0	10	0.32	0.11-1.00	0.05
23907	ovarian abscess	7	0	0-0	4	0.63	0.31-0.91	0.01
3329	blood	11	0	0-0	17	1.00	0.62-1.00	0.0002
613	wound	20	0	0-0.08	19	0.17	0-0.70	0.007
All <i>C. fetus</i> strains		41	0	0-0	50	0.52	0.11-1.00	<0.0001
<i>C. jejuni</i>								
42734	faeces	10	0	0-0.03	9	1.00	0.23-1.00	0.002
43353	faeces	9	0	0-0	10	0	0-0.11	0.29
39175	faeces	4	0	0-0	6	0.92	0.43-1.00	0.04
40630	faeces	8	0	0-0.09	8	0	0-0.16	0.60
All <i>C. jejuni</i> strains		31	0	0-0	33	0.13	0-1.00	0.0007

In a separate experiment the effects of heat-killed *C. fetus* and *C. jejuni* strains did not differ significantly from that produced by live bacteria (table IV).

Clinical and bacteriological findings

At no time throughout this series of experiments was any maternal illness noted, even though campylobacters could be isolated from the liver and spleen; in most instances, fewer than 10^3 cfu were recovered from each organ. Campylobacters were readily isolated from the resorbed placentae and resorbed fetuses of animals infected on day 13 of pregnancy. In an animal infected with 4×10^7 cfu of *C. fetus* strain 23907, one fetus alone yielded 4×10^5 cfu, and the corresponding placenta 2×10^6 cfu. However, no evidence of multiplication was noted in any tissue. Not all of the fetuses in the uterus were resorbed; normal, healthy fetuses could be seen interspersed between the resorbed conceptuses (fig. 1). A cross-section of a normal fetus and a resorbed fetus are shown in fig. 2.

Discussion

These experiments demonstrate that resorption of mouse embryos is readily induced by strains of both *C. fetus* and *C. jejuni*. This finding is similar to that made by Coid *et al.* (1978) and Coid (1976), who inoculated pregnant mice with live *E. coli* and with its purified lipopolysaccharide respectively. It seems possible, therefore, that the resorption in mice inoculated with campylobacters is caused by an endotoxin-like substance. Dennis (1959) and Naess and Hofstad (1984) demonstrated that *C. fetus* and *C. jejuni* possess lipopolysaccharide that is pyrogenic, antigenic and toxic for mice. Previously Naess and Hofstad (1982) suggested that the purified lipopolysaccharide of *C. jejuni* was similar to that from the family *Enterobacteriaceae*. Moreover, endotoxin-like activity has been shown in *C. jejuni* by Fumarola *et al.* (1982); this activity was, however, 4 to 8 times lower than that of *E. coli*.

There is a similarity between the effects of campylobacters and *E. coli* on 13-day pregnant mice, but the present experiments also demonstrated that at 6 days' gestation the campylobacters caused resorption of the fetuses, an effect not observed on the embryos of pregnant mice given an injection of *E. coli* endotoxin (Coid, 1976). This lack of effect of *E. coli* endotoxin on the 6-day fetus was attributed to the deficiency of vascular tissue, for which endotoxin appears to have a predilection (Zahl and Bjerknes, 1943), whereas in campylobac-

Uterus from normal
18-day pregnant mouse.

Uteri from infected
18-day pregnant mice.

Fig. 1. Uterus from a normal mouse (18 days pregnant) and uteri from mice (18 days pregnant) given an intravenous injection of campylobacters on day 13 of pregnancy.

ter-infected mice the resorptions may have been a consequence of invasion of the fetus by live bacteria.

Effects similar to those described in this paper have been observed by Gasic and Gasic (1972) in the 6-day mouse embryo after injection of cholera

Table IV. The effect of heat-killed campylobacters on the development of the 13-day mouse fetus

<i>Campylobacter</i> sp.	Proportion of resorptions* produced by				p Value
	living organisms		heat-killed organisms		
	Median	IQR	Median	IQR	
<i>C. fetus</i>	0.14 (33)†	0 to 0.63	0 (35)	0 to 0.25	0.27
<i>C. jejuni</i>	0.25 (35)	0 to 1.00	0.11 (32)	0 to 0.39	0.15

* The results for all strains of each campylobacter species are combined.

† The numbers of mice inoculated are given in parenthesis.

Fig. 2. Transverse section of a normal mouse (18 days pregnant) (C) and a mouse infected on day 13 of pregnancy with campylobacters (I). (Mag. $\times 8$)

enterotoxin. They suggested that the interruption of gestation at this stage was not due to the passage of large amounts of toxin into the circulation, but to intense circulatory and metabolic changes resulting from heavy loss of body fluid from the mother.

In cattle it is well documented that *C. fetus* ss *fetus* causes infertility (Lawson, 1959; Morris and Park, 1971; Morgan and Wrathall, 1977). The present study shows that *C. fetus* also affects fertility in mice, by reducing the proportion of implants after injection into 2-day pregnant females; this

again suggests the presence of a toxic substance having an effect on the pre-implanted embryo or on the uterus.

From these experiments in mice, it is apparent that campylobacters are capable of affecting the development of the fetus, and it seems probable that their pathogenic effects are due to substances similar to *E. coli* endotoxin and cholera enterotoxin.

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