RESEARCH NOTE

Inducing Enterotoxigenic Properties in *Campylobacter jejuni* and *Campylobacter coli* by Serial Intraperitoneal Passage in Mice

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The closely related species *Campylobacter jejuni* and *C. coli* are now recognized as important agents of diarrhea in both developed and developing countries (PL Griffiths & RWA Park 1990 *J Appl Bacteriol 69*: 281-301, H Fernández 1992 *Ciênc Cult 44*: 39-43).

Adhesive capacity, invasiveness, enterotoxin production and cytotoxins elaboration have been described as their potential pathogenicity factors (TM Wassenaar 1997 *Clin Microbiol Rev 10*: 466-476, SA Leach 1997 *Rev Med Microbiol 8*:113-124).

Under unfavorable environmental conditions they become injuried, suffering a morphological transformation from the normal, culturable "s" shaped form to a nonculturable coccoid form, as well as changes in their physiological and pathogenical behaviour (SK Saha et al. 1991 *Appl Environ Microbiol 57*: 3388-3389, Leach *loc. cit.*). Coccoid injuried *Campylobacter* cells can be recovered however, as normal cells after animal passage with restoration of their pathogenical capacities, as it was observed by Saha et al. (1991 *loc. cit*.) by passaging freeze-thaw-injuried strains through rat gut.

Several other biological models, such as intraperitoneal animal passage (SU Kazmi et al. 1984 *Curr Microbiol 11*: 159-164), passage through ligated ileal loops of rats (SK Saha et al. 1988 *J Med Microbiol 26*: 87-91), intragastric passage in chicks (FC Sang et al. 1989 *Av Dis 33*: 425-430) and chick embryo passage (LH Field et al. 1993 *J Med Microbiol 38*: 293-300), have been used to enhance or to restore virulence capacities in *Campylobacter* strains.

The purpose of this study was to investigate the possibility of inducing enterotoxigenic properties in non toxigenic *C. jejuni* and *C. coli* strains.

The strains under study were a *C. jejuni* isolated from human diarrheic stools (strain 1), a *C. jejuni* isolated from fecal material of a sparrow (strain 2) and a *C. coli* isolated from commercial chicken liver (strain 3). All of them were kept at -35°C for several months and after their recovery, they were subcultured several times and characterized as non toxigenic by means of the rat ileal loop test (RILT) (Saha et al. 1988 *loc. cit.*, U Chattopadhyay et al. 1991 *J Diarr Dis J 9*: 20-22, H Fernández et al. 1995 *Mem Inst Oswaldo Cruz 90*: 633-634). Each strain was subjected to five serial intraperitoneal (IP) passages in Rockefeller (3-6 weeks age) mice being the original strain considered as passage zero. Inocula were prepared in Brucella broth (BB) and each animal received 1 ml of 2 x 10^9 CFU/ml bacterial suspension. After 24 hr, they were sacrified by ether overdose and peritoneal content was aseptically removed and seeded on blood agar plates that were incubated during 36 hr at 42°C under microaerobic atmosphere. The resulting pure culture of the isogenic strain, considered as one passage, was then cultured in BB during 72 hr at 42°C under microaerobic atmosphere in order to promote enterotoxin production (H Fernández et al. 1983 *Infect Immun 40*: 429-431).

The toxigenic capacity of each isogenic strain was determined by RILT (Saha et al. 1988 *loc. cit.*, Chattopadhyay et al. *loc. cit.*, Fernández et al. 1995 *loc. cit.*) preparing a cell-free filtrate from each of the 72 hr cultures in BB and inoculating 100 ml individually into two ileal loops (3 to 5 cm lenght) from different adult Wistar rats. An interloop segment of 1 to 2 cm length was left to avoid fluid leaking between loops. Sterile BB and the cell-free filtrate of a toxigenic *C. jejuni* were used as negative and positive controls respectively. After 18 hr the rats were killed by ether overdose and the ileum examined. Distention of the loops with fluid accumulation was considered as a positive result and electrolyte concentrations were measured by standard flame spectrophotometry. The tests were carried out twice in two animals simultaneously.

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The results obtained are shown in the Table. After the third passage, all the three cell-free filtrates began to induce fluid accumulation with higher electrolyte concentrations than that produced by the negative control, the cell-free filtrates obtained from the original and the firsts two isogenic strains. These changes became more evident and drastic in the fourth and fifth passages being, in the latter, similar to that observed in the positive control.

The observations reported here are in agreement with the results obtained by Saha et al. (1991 *loc. cit.*), who reconverted as toxin producers, by passaging through rat gut, freeze-thaw-injuried strains of *C. jejuni* which have been lost their enterotoxigenicity. Previously, the same group (Saha et al. 1988 *loc. cit.*) induced enterotoxin production, by subsequent passage in rat ileal loops, in *C. jejuni* that were negative in the RILT model. Our strains were kept under freezing conditions and suffered several subcultures *in vitro* before they were tested with the RILT model. After the third IP passage in mice, the isogenic variants of each strain appeared as enterotoxin producer. Probably, this capacity was induced or enhanced in a similar way as it occurred with the strains tested by Saha et al. (1991 *loc. cit.*).

Several animal models have been proposed to enhance the virulence of *C. jejuni*, increasing their colonization potential (LH Field et al. 1985 *J Med Microbiol 17*: 59-66, Sang et al. *loc. cit.*, SA Cawthraw et al. 1996 *Epidemiol Infect 117*: 213-215), their resistance to phagocytosis and their survival ability *in vivo* (Field et al. 1993 *loc. cit.*). A mice IP model, using mucin and iron dextran as coadjuvant, enhanced the virulence of *C. jejuni* laboratory-adapted strains, lowering their LD_{50} from 2 x 10^{11} to 2 x 10^{5} CFU (Kazmi et al. *loc. cit.*). We employed the same animal passage model, but without using mucin or iron dextran, to induce successfuly enterotoxin production in three laboratory-adapted strains. The RILT, recognized as a valid method to assess enterotoxigenic properties in *C. jejuni* (Saha et al. 1988 *loc. cit.*, L Maggi et al. 1988 *Rev Méd Chile 116*: 1105-1110, Chattopadhyay et al. *loc. cit.*, A Tresierra et al. 1995 *Arch Med Vet 27*: 53-59), have been also employed as a model to induce or enhance this capacity (Saha et al. 1988, 1991 *loc. cit.*). The IP model used in our work appears to be a suitable method to induce enterotoxin production with the additional advantage that the isogenic variants can be isolated in pure cultures on non selective media due to the absence of contaminant microflora in the IP cavity. In the chick gut or in the rat ileal loop inoculation models, the intestinal microflora can interfere in the obtention of the isogenic strains in pure culture making necessary one or more subcultures as well as the use of selective media.

These results suggest that enterotoxigenicity is a property that could be induced and/or enhanced by IP passages in *C. jejuni* and *C. coli* strains subjected to several subcultures *in vitro* or kept under freezing conditions for long periods. However, further studies are necessary to elucidate the mechanisms involved in this phenomenon.

TABLE

Enterotoxigenic effect in rat ileal loop of *Campylobacter jejuni* and *C. coli* strains subjected to serial intraperitoneal passages in mice

Passage	Strain 1			Strain 2			Strain 3		
	Fluid µl/cm	Na+ µEq/l	Cl- µEq/l	Fluid µl/cm	Na+ µEq/l	Cl- µEq/l	Fluid µl/cm	Na+ µEq/l	Cl- µEq/l
Zero[a]	10[b]	20	30	15	11	30	10	35	26
First	11	20	32	16	12	33	13	38	29
Second	20	19	35	30	18	41	20	40	37
Third	50	108	83	70	70	45	40	63	48
Fourth	80	138	112	100	110	83	90	136	117
Fifth	120	140	120	180	134	125	150	144	110
Positive control	Fluid µl/cm	Na+ µEq/l	Cl- µEq/l			Negative control	Fluid µl/cm	Na+ µEq/l	Cl- µEq/l
	130	175	137				12	35	40

a: original strain; *b*: values are means.