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Oral administration of antibodies as prophylaxis and therapy in *Campylobacter jejuni*-infected chickens

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SUMMARY

Passive immunity against gastrointestinal infections has recently been successfully applied as prophylaxis and therapy in patients in a variety of virally and bacterially induced infections. *Campylobacter jejuni* is frequently associated with acute diarrhoea in humans, and several species of animals have been shown to transmit the disease, although birds have been implicated as the main source of infection. We used bovine and chicken immunoglobulin preparations from the milk and eggs, respectively, of immunized animals for prophylactic and therapeutic treatment of chickens infected with *C. jejuni*. A marked prophylactic effect (a >99% decrease in the number of bacteria) was noted using either antibody preparation, whereas the therapeutic efficacy, i.e. when antibodies were given after the infection was established, was distinctly lower (80–95%) as judged by faecal bacterial counts. These observations may serve as a starting point for experiments aimed at elimination of the infection in an industrial or farm setting. It may also encourage future attempts to treat, prophylactically or therapeutically, patients with Campylobacter-induced diarrhoea.

Keywords passive immunity immunoprophylaxis Campylobacter

INTRODUCTION

Campylobacter jejuni is a Gram-negative rod which is associated with acute gastrointestinal infection in humans. It is one of the most common causes of enterocolitis world-wide. Several species of animals have been shown to transmit the infection to man. Although the disease is usually self-limiting, severe sequels may be seen in selected patients, and immunodeficient patients may become chronic carriers [1].

Campylobacter jejuni is a normal commensal in the chicken gut. Many of the cases of Campylobacter-associated diarrhoea in patients have been suggested to be linked to the ingestion of raw or poorly cooked poultry products [2], although there are a number of additional contaminating sources.

Human breast milk, which contains secretory IgA, has previously been shown to protect against Campylobacter-induced diarrhoea in infants [3]. Oral administration of purified human immunoglobulin has also previously been suggested to exhibit a prophylactic effect against development of necrotizing enterocolitis in prematurely born children [4], and a therapeutic effect in *C. jejuni*- [1] and *Clostridium difficile*- [5] induced diarrhoea in immunodeficient patients. Due to the high costs involved, attempts have recently been made to use alternative sources of antibodies, and bovine immunoglobulins from immunized animals have recently been introduced successfully for human therapy (for review, see [6]).

Oral administration of bovine or chicken antibodies against different gastrointestinal pathogens has also been shown to be effective both prophylactically and therapeutically in a variety of animal species (for review, see [6]). Currently, bovine and chicken immunoglobulin preparations against rotavirus are both commercially available for prophylaxis in calves and piglets.

Recently, oral administration of chicken antibodies against *C. jejuni* was shown to inhibit bacterial colonization of chickens and also to be effective as therapeutic agents [7]. However, as these antibodies were derived from the bile of immunized birds, the availability of material is severely limited. In this study, we describe the successful application of IgY from egg yolk and bovine IgG from milk in immunoprophylaxis of *C. jejuni* infection in chickens.

MATERIALS AND METHODS

Animals

The broiler chickens were purchased from Kronfågel AB (Väderstad, Sweden) and kept under standard conditions in individual

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cages before and during the experimental period. Food and water were not restricted in the prophylactic experiments and the initial therapeutic experiments. However, in the final therapeutic experiment, food and water were removed during the final 24 h of the test in order to simulate conditions for broilers immediately before slaughter. The project was approved by the local animal ethical committee.

Antibodies against C. jejuni

Bovine antibodies were prepared from milk day 8–40 post-parturition [8] from cows immunized with a mixture of two reference strains (*C. jejuni* NCTC 11168 and 11322) and 12 clinical *C. jejuni* and one *C. coli* isolates from Europe and South America. The cows were repeatedly immunized with formaldehyde-inactivated bacteria as described previously [8]. Briefly, after 48 h of culture, the bacteria were harvested and inactivated with 0·5% formaldehyde overnight. The cows were immunized altogether 10 times with varying doses (20–100 ml) of a suspension containing 5×10^8 bacteria, starting 42 days before parturition with intramuscular injections (where alum was added as adjuvant) and subsequently with subcutaneous and intra-cisternal injections.

Egg yolk antibodies were prepared from chickens immunized with a mixture of reference strains (*C. jejuni* CCUG 12074, 12070, 12066, 12067, 15036 and 19506 and *C. coli* CCUG 12080) according to previously described methods [9]. Briefly, after 48 h of culture, the bacteria were harvested and inactivated overnight and used five times for immunization at a dose of 10^9 bacteria. The injections were given intramuscularly in both legs at weekly intervals and eggs were collected for a period of 4 weeks after finalization of the immunization schedule.

Treatment protocol

Campylobacter jejuni CCUG 12070 and 19506 were used as challenge strains in all experiments. In the first prophylactic experiment, 1500 *C. jejuni* bacteria were administered orally to 22-day-old chickens with ($n = 5$) or without ($n = 10$) addition of 2·5 g of the bovine immunoglobulin preparation (20% purity; mixed with the bacterial suspension 1 h before administration to the chickens). No additional antibodies were given to the animals and the result was followed by daily collection of faecal samples for 5 days. Faeces (1 g) from each individual animal was suspended in 9 ml of 0·85% sodium chloride, serially diluted in saline and plated on Preston agar plates [10] and incubated microaerobically at 42°C for 48 h, after which the number of colonies was counted and the number of bacteria per g faeces calculated. Bacterial cultures were performed daily on all animals during the 5 days of the experiment.

In the second prophylactic experiment, 10^6 bacteria, preincubated either with bovine (2·5 g immunoglobulin preparation, 20% purity, $n = 5$) or chicken (0·5 g immunoglobulin preparation, 95% purity, $n = 5$) antibodies, were administered to 14-day-old chickens. Bacterial cultures were performed on days 1, 3, 5 and 7 after infection.

In the first therapeutic experiment, 30-day-old chickens (8 days after the initial infection) were treated daily with an oral administration of bovine antibodies (0·5 g immunoglobulin preparation, 20% purity, $n = 5$) or left untreated ($n = 5$). Bacterial cultures were performed daily during the treatment phase (days 1–5) and at day 8 (3 days after termination of treatment).

In a subsequent therapeutic experiment, the efficacies of bovine and chicken antibodies were compared in 18-day-old chickens (4 days after initial infection) where the animals were given either bovine (1 g immunoglobulin preparation, 20% purity, $n = 5$) or chicken (0·2 g immunoglobulin preparation, 95% purity, $n = 5$) antibodies as a single dose. Bacterial cultures were performed before treatment and at 12, 24, 48 and 72 h after administration of the antibodies.

In a third and final set of therapeutic experiments, aimed at reflecting a treatment strategy suitable for the poultry industry, stably infected 34-day-old chickens (6 days after initial infection) were given two oral doses (spaced at 8 h) of bovine (altogether 1·25 g immunoglobulin preparation, 20% purity, $n = 10$) antibodies or left untreated ($n = 5$). Four hours after the final treatment, the chickens were killed and quantitative faecal bacterial cultures performed.

Statistical analysis

Both the *t*-test and Mann–Whitney test were employed for statistical calculations.

RESULTS

Prophylaxis

Prophylactic administration of bovine antibodies resulted in a very marked reduction in the mean number of bacteria (> 99%) throughout the duration of the experiment (Fig. 1 and Table 1), and at day 5, three of the five treated animals still remained culture-negative, whereas all control animals ($n = 10$) were infected. The decrease in the number of bacteria was highly significant at day 5 ($P < 0·001$). After an additional 3 days, bacteria could be recovered from all treated animals, possibly reflecting the coprophagic nature of chickens. The number of bacteria was not determined at this time.

A comparison of the efficacy of bovine and chicken antibodies showed that there were no major differences in their respective prophylactic properties (data not shown).

Therapy

When bovine anti-Campylobacter antibodies were given to infected animals with a mean number of 17×10^6 bacteria per gram faeces, a 50–80% reduction in the number of bacteria in the faecal samples was observed during the treatment phase of the first experiment (Table 2) compared with control animals. However, 3 days after discontinuation of antibody administration (day 8), the mean numbers of bacteria were similar in both groups ($5·1 \times 10^7$ compared with $4·8 \times 10^7$ bacteria per g of faeces). However, due to the large variation in bacterial counts in the individual animals, these differences were not statistically significant.

A comparison between the therapeutic efficacy of bovine and chicken antibodies showed no major difference in biological effect, with a mean starting concentration of 10^7 bacteria per g of faeces in infected animals, which was reduced to 2×10^5 and 5×10^4 12 h after treatment with chicken and bovine antibodies, respectively ($n = 5$ in both groups). The concentration of bacteria returned to pretreatment levels in both groups with an identical kinetics, with 3×10^5 bacteria per g faeces after 24 h and 48 h, and 10^6 at 72 h after treatment.

In the final therapeutic experiment, 34-day-old animals were treated with bovine antibodies. There was a marked (62%) and statistically significant ($P < 0·01$) reduction in the mean number of Campylobacter after administration of antibodies (Fig. 2), and a >90% reduction in the number of bacteria in a majority (six out of 10) of the treated animals. In the control group, the mean number of bacteria increased during the 1 day course of the experiment,

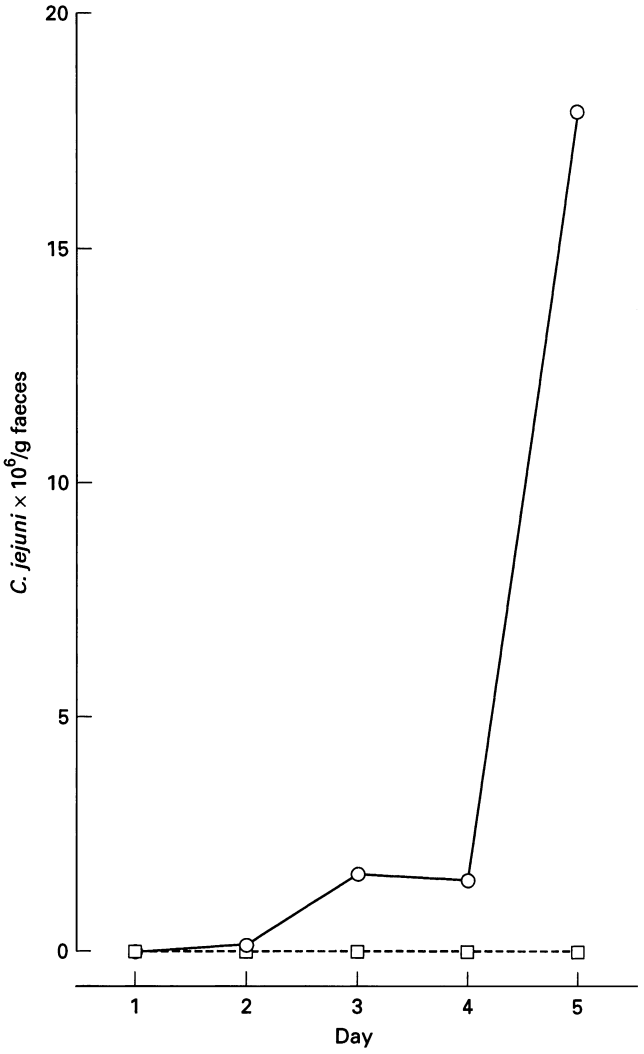

Fig. 1. Prophylactic effect of bovine antibodies against *Campylobacter jejuni* infection in untreated (○) and treated (□) chickens. Results are given as mean number of bacteria/g faeces of five treated and 10 control animals. The number of bacteria in the treated chickens ranged from < 10 to 160 000 bacteria/g faeces and from 189 500 to 94 000 000 bacteria/g faeces in untreated animals.

resulting in a 82% overall reduction in the number of bacteria in the treatment group at slaughter.

DISCUSSION

Oral administration of both human and bovine immunoglobulins appears to be effective in prophylaxis, and may also be used in treatment of a number of gastrointestinal infections in man. In most cases, human IgG has been employed [6], but during the past few years a number of studies have been published, utilizing human IgA [6]. The IgA in this preparation (IgAbulin), is derived from plasma and thus mainly monomeric, and although perhaps theoretically more appropriate, there have in fact been no differences in clinical efficacy noted to date. This form of passive immunotherapy is not restricted in terms of antibody source and immunoglobulins from a vast number of species have previously been used in experimental animal models of passive immunity [6]. This also includes egg yolk IgY from immunized hens, which has also been successfully employed as prophylaxis against infections in a number of animal species (including chickens), and recently in man [11].

In our experiments, a marked prophylactic effect on bacterial numbers could be demonstrated by oral administration of immunoglobulin preparations from both cows and hens. In previous prophylactic experiments against *Streptococcus mutans*, employing an intact MoAb or Fab fragments, it was clearly shown that $F(ab)_2$ fragments were effective in protecting against colonization, whereas Fab fragments were not [12]. This clearly argues against any major effect by phagocytosing cells which requires an intact Fc portion of the molecule. It also rules out that the complement system would play a decisive role in this model. The antibody used did not possess any bactericidal or bacteriostatic effect *in vitro* [13], but led to altered growth characteristics with clumping of the bacteria, which may affect the degree of colonization. Similar data, i.e. suggestions of immune exclusion at the mucosal surface by monoclonal IgA antibodies, have also been recorded in model systems of gastrointestinal disease [14]. It is therefore likely that one of the major mechanisms of action in our experiments could be

Table 1. Prophylactic effect of bovine antibodies in Campylobacter-infected chickens*

Animal no.	Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
1	+	<0·001	<0·001	<0·001	<0·001	<0·001
2	+	<0·001	<0·001	<0·001	<0·001	0·1
3	+	<0·001	<0·001	<0·001	<0·001	<0·001
4	+	<0·001	<0·001	<0·001	<0·001	<0·001
5	+	<0·001	0·001	0·012	0·027	0·16
6	–	<0·001	<0·001	<0·001	1·56	0·95
7	–	<0·001	0·13	7·0	3·3	7·1
8	–	<0·001	<0·001	<0·001	0·36	6·3
9	–	<0·001	0·38	5·8	2·0	94·0
10	–	<0·001	<0·001	<0·001	0·53	3·8
11	–	<0·001	<0·001	0·47	0·42	9·4
12	–	<0·001	<0·001	<0·001	0·003	0·19
13	–	<0·001	<0·001	<0·001	4·8	32·0
14	–	<0·001	<0·001	0·01	2·4	23·0
15	–	<0·01	<0·001	<0·001	0·005	2·8

*Results are given as mean number of bacteria $\times 10^6$/g faeces.

Table 2. Therapeutic effect of bovine antibodies in Campylobacter-infected chickens*

Treatment	*n*	Day 1	Day 2	Day 3	Day 4	Day 5
+	5	20·2	8·6	10·1	11·4	10·6
–	5	13·2	20·5	28·2	5·9†	52·5

* Results are given as mean number of bacteria $\times 10^6$/g faeces.

† The levels of bacteria were markedly reduced in one animal on this day ($0{\cdot}16 \times 10^6$), as chicken no. 1 in the treatment group showed approximately 20×10^6 bacteria per g faeces on the preceding day and 48×10^6 on the following day, thus suggesting a sampling or cultivating error.

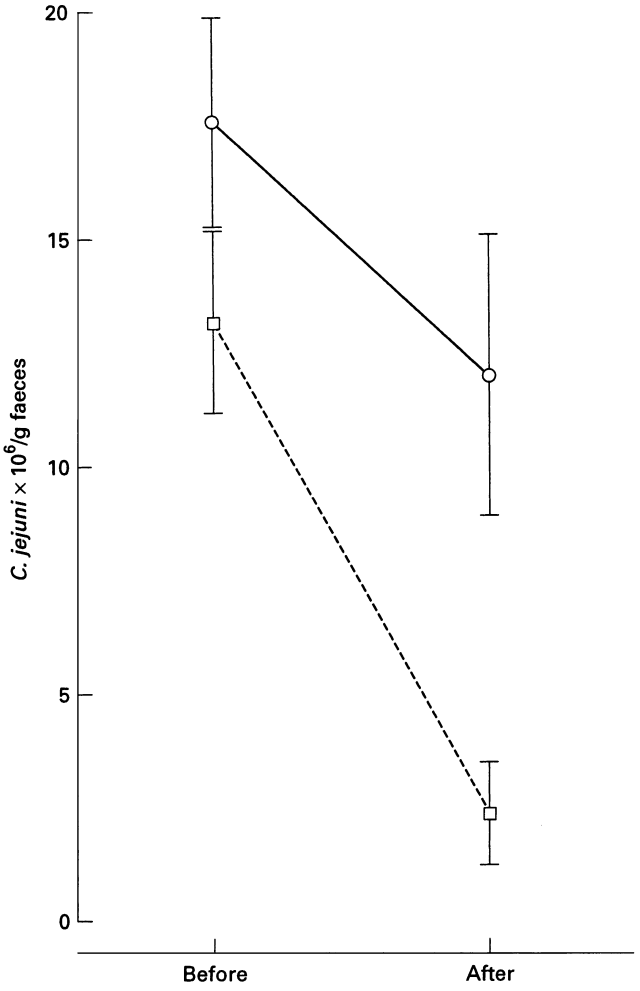

Fig. 2. Therapeutic effect of bovine antibodies against *Campylobacter jejuni* infection in untreated (○) and treated (□) 34-day-old chickens before slaughter. Results are given as mean number (±s.e.m.) of bacteria/g faeces of 10 treated and five control animals.

the blocking of attachment of bacteria to the intestinal wall, thus preventing colonization.

Although we could not completely eliminate Campylobacter bacteria from the chickens with the therapeutic regime used, the 1–2 log reduction observed may serve as a starting point for refinement of our current strategy. It is still unclear to what extent the lowered number of colonies observed reflects a true reduction of bacteria in the intestine of the treated birds, or whether part of our findings is due to agglutination of bacteria which may give a 'false' low number after treatment. However, the complete clearance of the infection in one of our immunodeficient patients [1] suggests that a true reduction/elimination of the Campylobacter can actually be achieved.

The coprophagic nature of the chickens naturally constitutes a complicating factor, and may necessitate the development of preparations containing high titres of antibodies, and may also require prolonged administration, as suggested by our previous clinical observation [1]. Higher doses of antibodies may also aid in the optimization of future attempts to eliminate the bacteria, and as there are limited possibilities for feeding the animals more antibodies, i.e. a larger volume, a heightened titre would be desirable. However, recent advances in the development of chicken hybridomas [15] suggest that application of MoAbs resulting from this technology might be advantageous in therapeutic applications in gastrointestinal infections, and we have recently successfully been applying hybridoma-derived anti-cholera toxin IgY both *in vitro* and *in vivo* in animal experiments [16]. If the preparation of antibodies can be improved in biological efficacy and problems with administration can be solved, this form of therapy might thus be applied to cleansing infected chickens immediately before slaughter.

The prophylactic potential of orally administered antibodies against *C. jejuni* has hitherto not been explored in man. One possible indication would be as short-term prophylaxis against traveller's diarrhoea, where recent estimates suggest that up to 50% of all cases may be associated with Campylobacter infection [17]. Furthermore, workers in the poultry industry might benefit from prophylactic treatment in order to prevent or mitigate disease during the initial phases of occupational exposure. It may also be applied in immunodeficient patients who are especially prone to infection with *C. jejuni*.

The concentration of immunoglobulins in the intestine of normal healthy adults is in the order of 10–100 mg/*l*. The proportion of specific anti-Campylobacter antibodies would be expected to constitute a very small fraction of this (less than one per thousand) even in immune individuals, and in previous experiments, using polyclonal bovine antibodies, the doses employed successfully in clinical trials have been in the order of 0·6–1·5 g of immunoglobulin preparation (for review see [18]). If MoAbs were to be applied therapeutically, the daily doses needed would probably be in the μg range. As chicken antibodies may constitute a cost-effective alternative source of antibodies for oral administration, clinical trials on the potential use of polyclonal or monoclonal IgY in patients with Campylobacter-induced diarrhoea or other gastrointestinal disorders may thus be warranted.

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