

Experimental colonization of broiler chicks with *Campylobacter jejuni*

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SUMMARY

Minimal colonization inocula for two broiler strains of *Campylobacter jejuni* were determined in broiler chicks aged 2-3 days and 2 weeks. Individually housed chicks were exposed to a single oral or cloacal challenge. Diarrhoeal symptoms were absent in all 380 chicks included in the study. Chick susceptibility to the two *C. jejuni* strains varied. Colonization was effected by $< 10^2$ - 10^4 colony forming units (c.f.u.) via cloacal challenge and 10^4 - 10^6 c.f.u. via the oral route. Colonization inocula for 2-to 3-day and 2-week-old chicks were similar. Treatment of 1-day-old chicks with fresh adult caecal flora or an anaerobic broth culture of adult caecal flora did not inhibit colonization after challenge with low-dose *C. jejuni*. Susceptible chicks were colonized rapidly. *C. jejuni* was detected in 167 of 189 (88%) colonized chicks within 3 days of challenge and persisted during the 2-week monitoring period. Our data suggest that colonization of broiler chicks with *C. jejuni* is effected more easily by the cloacal than the oral route and is independent of age.

INTRODUCTION

Outbreaks of *Campylobacter jejuni* enteritis in humans have frequently been associated with the consumption or handling of chicken (Brouwers *et al.* 1979; Norkrans & Svdehem, 1982; Christenson *et al.* 1983; Hopkins & Scott, 1983; Oosterom *et al.* 1984; Kapperud *et al.* 1984; Rosenfield *et al.* 1985; Harris, Weiss & Nolan, 1986). Studies at broiler processing plants indicate the level of *C. jejuni* contamination of processed broilers varies considerably (Shanker *et al.* 1982; Oosterom *et al.* 1983; Wempe *et al.* 1983; Harris *et al.* 1986). *Campylobacter*-free broiler flocks in grow-out farms are not uncommon (Smitherman, Genigeorgis & Farver, 1984; Neill, Campbell & Green, 1984; Shanker, Lee & Sorrell, 1986; Genigeorgis, Hassuneh & Collins, 1986). However, *C. jejuni* cross-contamination of broilers during processing has been reported (Genigeorgis, Hassuneh & Collins, 1986). We concluded from our earlier findings (Shanker, Lee & Sorrell, 1986) that vertical transmission of *C. jejuni* in commercial broiler production was unlikely.

Data from four farm studies (Smitherman, Genigeorgis & Farver, 1984; Neill, Campbell & Greene, 1984; Genigeorgis, Hassuneh & Collins, 1986; Lindblom, Sjorgren & Kaijser, 1986) have suggested that *C. jejuni* broiler colonization is age-related. Chicks 7 days and younger were campylobacter-free and *C. jejuni* prevalence increased with flock age. These studies did not determine whether the absence of *C. jejuni* in younger flocks was due to husbandry practices or varying susceptibility amongst chicks of different ages.

There is evidence from studies with salmonella that colonization is achieved more readily in young chicks (Brownell, Sadler & Fanelli, 1969) and that endogenous adult caecal flora is protective (Rantala & Nurmi, 1973; Lloyd, Cumming & Kent, 1977; Snoeyenbos, Weinack & Smyser, 1978). Anti-salmonella activity had also been attributed to volatile fatty acids produced by some caecal anaerobes (Meynell, 1963; Barnes, Impey & Stevens, 1979). Oral treatment with fresh caecal contents has been reported to reduce *C. jejuni* colonization (Soerjadi, Snoeyenbos & Weinack, 1982; Soerjadi-Liem, Snoeyenbos & Weinack, 1984). It is not known if this protective effect is due to caecal anaerobes. If measures to maintain grow-out flocks campylobacter-free up to the processing age are to be evaluated, a better understanding of *C. jejuni* epidemiology at the farm level is essential. Fundamental to such measures are data on the minimal colonization inocula for newly hatched chicks and those with a more established gut flora.

In this study, 2- to 3-day and 2-week-old chicks were challenged with two strains of *C. jejuni* to determine the minimal colonization inocula for the two age groups. Chicks 1-day-old were treated orally with fresh adult chicken caecal contents or an anaerobic broth culture of adult caecal flora before similar challenge with *C. jejuni* to determine the protective effect of endogenous flora.

METHOD

Campylobacter challenge strains

Two strains of *C. jejuni* ICP47 and ATT6, isolated from broiler grow-out farms were used for this study. The *C. jejuni* isolates were identified as biotypes 1 and 2 as characterized by Skirrow & Benjamin, (1980). The strain differences were confirmed by serotyping using the Penner scheme (Penner & Hennessy, 1980). The strains belonged to serogroups 3 and 6 respectively.

Experimental colonization studies

Broiler chicks

Broiler eggs were obtained from a commercial hatchery supplying chicks to local grow-out farms. The broiler strain used in this study was a highly cross-bred commercial strain derived from White Leghorn. Eggs were incubated in the laboratory and hatched chicks checked for cloacal carriage of *C. jejuni*. Cloacal swabs were plated on modified Skirrow's medium (Skirrow, 1977) containing Oxoid Blood Agar Base No. 2, 7% lysed horse blood, 0.25 mg/l colistin sulphate, 5 mg/l trimethoprim, 10 mg/l vancomycin, 50 mg/l cefoperazone, 100 mg/l cycloheximide and 5 mg/l amphotericin B. Cloacal specimens were enriched by placement in Oxoid Brucella Broth containing the above antibiotics, 7% lysed horse blood, 0.025% each of ferrous sulphate, sodium metabisulphite and pyruvic

acid; and 0.1% sodium dodecylsulphate. Inoculated plates were incubated in a microaerobic atmosphere (5% O₂, 10% CO₂ and 85% N₂) at 42 °C for 48 h. Enrichment broths were incubated in similar conditions for 72 h before subculture on to *C. jejuni* selective plates. Morphologically characteristic colonies were identified by features previously described (Skirrow & Benjamin, 1980). Hatched chicks were initially housed in modified isolators containing sterilized wood shavings as litter. Mains water and commercial broiler feed similar to that used in grow-out farms containing bacitracin and a coccidiostat were provided *ad libitum*.

Minimal colonization inoculum

Using serial dilutions of *C. jejuni* in saline, 2- to 3-day chicks were challenged with single 0.2 ml inoculations into the crop or cloaca and housed individually. Five chicks were inoculated per treatment. Cloacal swabs were obtained 1, 3, 7 and 14 days post-challenge. On day 14, chicks were killed by CO₂ gas and caecal contents cultured for *C. jejuni*. Oral and cloacal challenge were similarly undertaken using 2-week-old chicks. Cloacal swabs were checked at similar time intervals as above. These chicks were killed at 4 weeks of age and caecal contents checked for *C. jejuni*. Chicks were defined as colonized if the inoculated *C. jejuni*-strain was isolated from cloacal swabs repeatedly or present in the caeca at necropsy.

Treatment with fresh caecal contents

A treatment similar to that described by Soerjadi-Liem, Snoeyenbos & Weinback (1984) was used. Caecal contents were obtained from laboratory housed, freshly killed, 10-week-old broilers. Their salmonella and campylobacter-free status was verified by cloacal culture on hatching, at 6 and 9 weeks and confirmed by caecal culture at necropsy. Fresh caecal contents for chick inoculation were diluted 1/10 in Oxoid Thioglycollate Medium (without Indicator). One-day-old chicks were treated with this broth by inoculating 0.5 ml into the crop. After 24 h, chicks were challenged with *C. jejuni* and housed individually. Cloacal swabs were obtained 1, 3, 7 and 14 days after challenge and cultured for *C. jejuni*. On day 14, caecal contents obtained at necropsy were cultured for *C. jejuni*.

Anaerobic culture treatment

A modified method of Suena, Nagaraja & Pomeroy (1985) was used to prepare a suspension of caecal anaerobes. From freshly killed 10-week-old salmonella- and campylobacter-free broilers, 0.1 gm of caeca was cut into pieces and placed in 200 ml Difco, Veal Infusion Broth (VIB) supplemented with 5 µg/ml haemin and 0.1 µg/ml Vitamin K. After 48 h incubation at 37 °C under anaerobic conditions, 1 ml was subcultured into 200 ml VIB and incubated for 48 h under similar conditions. Aliquots of 0.5 ml of this broth were inoculated into the crops of 1-day-old chicks followed by *C. jejuni* challenge after a 24 h interval. Chicks were housed individually and monitored for *C. jejuni* as previously described.

Table 1. *Experimental C. jejuni* colonization of 2- to 3-day-old broiler chicks via oral and cloacal challenge

<i>C. jejuni</i> strain	Challenge route	Inoculum* (c.f.u. \pm s.d.)	Post-challenge, cloacal isolation of <i>C. jejuni</i> on day			
			1	3	7	14
ICP 47	Oral	$2.6 \times 10^2 \pm 1.2$	0†	0	0	0
		$2.6 \times 10^4 \pm 1.2$	1	2	2	3
		$4.1 \times 10^6 \pm 1.5$	6	8	7/8‡	7/8‡
		$6.9 \times 10^8 \pm 1.6$	8	10	10	10
	Cloacal	$1.2 \times 10^1 \pm 0.6$	3	4	4	4
		$3.0 \times 10^2 \pm 1.8$	8	9	9	9
ATT 6	Oral	$3.4 \times 10^2 \pm 1.4$	0	0	0	0
		$7.3 \times 10^4 \pm 1.3$	0	0	0	0
		$6.1 \times 10^6 \pm 1.3$	1	3	3	3
		$2.3 \times 10^8 \pm 1.9$	3	8	9	10
	Cloacal	$6.1 \times 10^2 \pm 1.3$	0	0	0	0
		$6.1 \times 10^4 \pm 1.3$	3	3	3	4
		$4.8 \times 10^6 \pm 1.7$	8	8	8	8

* Mean of duplicate experiments.

† Total of 10 chicks from duplicate experiments of five chicks in each.

‡ Two lame, culled after day 3.

RESULTS

None of the 380 experimental chicks in this study showed evidence of diarrhoeal illness. Three chicks were culled before the termination of the experimental period due to leg deformity, a condition that occurs in up to 0.75% of this commercial strain of broilers. Caecal culture at necropsy (data not shown) yielded the same number of *C. jejuni*-positive chicks as cloacal culture at day 14. Susceptible chicks were colonized rapidly. Of 189 *C. jejuni*-positive chicks, 117 (62%) were colonized by day 1, 167 (88%) by day 3 and 184 (97%) by day 7. The two *C. jejuni* challenge strains differed in their colonizing ability (Table 1). Strain ICP 47 was more virulent, colonizing with two \log_{10} lower inocula than strain ATT 6. The effect of different routes of challenge were apparent; oral challenge required at least two \log_{10} higher inocula than cloacal. Colonization was effected with $< 10^2$ c.f.u. for cloacal and approximately 10^4 c.f.u. for oral challenge with strain ICP 47. The presence of a more developed endogenous gut flora did not affect colonization. Similar results to those shown in Table 1 were obtained with 130, 2-week-old chicks challenged via oral and cloacal inoculations. There was no significant difference in the number of colonized 2-week-old compared with 2- to 3-day-old chicks challenged with similar sized inocula (χ^2 , $P > 0.1$). These findings were substantiated by the results of adult caecal flora treatment of 1-day-old chicks prior to *C. jejuni* challenge (Table 2). To reflect low environmental load, chicks were challenged with minimal colonization inocula established by our earlier experiments. There was no significant difference in the total number of *C. jejuni*-colonized birds amongst treated and control groups (χ^2 , $P > 0.5$).

Table 2. Experimental *C. jejuni* colonization of treated 2-day-old broiler chicks via oral and cloacal challenge

<i>C. jejuni</i> strain	Challenge route	Inoculum* (c.f.u. \pm s.d.)	Caecal† flora	Post-challenge, cloacal isolation of <i>C. jejuni</i> on day			
				1	3	7	14
ICP 47	Oral	$1.2 \times 10^4 \pm 1.2$	Fresh	2‡	2	2	2
			Anaerobic	6	6	6	6
			Nil	3	3	4	4
	Cloacal	$2.3 \times 10^2 \pm 1.1$	Fresh	8	8	8	8
			Anaerobic	6	7	7	7
			Nil	6	6	6	6
ATT 6	Oral	$2.7 \times 10^6 \pm 0.9$	Fresh	3	6	9	9
			Anaerobic	5	8	10	10
			Nil	5	7	9	9
	Cloacal	$2.3 \times 10^4 \pm 0.9$	Fresh	5	5	5	6
			Anaerobic	5	6	6	6
			Nil	7	8	7/9§	7/9§

* Mean of duplicate experiments;

† Oral treatment 1 day prior to *C. jejuni* challenge;

‡ Total of 10 chicks from duplicate experiments of five chicks in each;

§ One lame, culled after day 3

DISCUSSION

Our data have important implications for husbandry practices in broiler farms. Hatchery chicks are delivered to local grow-out farms within 3 days of hatching. In our study, chicks of this age group could be colonized by $< 10^2$ – 10^4 c.f.u. *C. jejuni* without diarrhoeal illness. These findings contrast with descriptions of diarrhoeal symptoms in chick animal model studies using 1-day and 3-day-old chicks (Ruiz-Pelacios, Escamilla & Torres, 1981; Welkos, 1984). However, asymptomatic colonization in young chicks has also been reported. Conventionally reared and gnotobiotic 3- to 4-day-old chicks challenged orally with approximately 10^8 c.f.u. *C. jejuni* were colonized without diarrhoeal symptoms (Manninen, Prescott & Dohoo, 1982). These reported differences may be due to virulence of *C. jejuni* strains and/or varying susceptibility amongst different breeds of chicken. Sanyal *et al.* (1984) reported that chickens of Starbro strain were more likely to develop *C. jejuni*-induced diarrhoea than White Leghorn. The chicks used in our study were a highly cross-bred broiler strain derived from White Leghorn.

Our data indicate that the presence of endogenous gut flora does not significantly reduce susceptibility to *C. jejuni* colonization. These findings differ from two reports on reduced susceptibility to *C. jejuni* in chicks treated with fresh caecal flora (Soerjadi, Snoeyenbos & Weinack, 1982; Soerjadi-Liem, Snoeyenbos & Weinack, 1984). However, interpretation of their data was complicated by the observation that not all control groups were colonized by low-dose *C. jejuni* inocula. They also reported of one trial in which no protection was afforded in birds treated with caecal flora. It is evident from our data that caecal anaerobes do not have a protective role, in contrast to the reported anti-salmonella activity

of caecal contents (Rantala & Nurmi, 1973; Lloyd, Cumming & Kent, 1977; Snoeyenbos, Weinack & Smyser, 1978). Our studies suggest that husbandry systems aimed at establishing a high environmental bacterial load for young chicks as a means of reducing salmonella infection will not prevent *C. jejuni* colonization. Our data also suggest that *C. jejuni* transmission may result from cloacal contamination. Cloacal challenge resulting in salmonella infection has been reported (Leaney, Cooper & Jackson, 1978). In the chicken intestinal tract, *C. jejuni*, like salmonella, colonize the caeca (Welkos, 1984, Soerjadi, Snoeyenbos & Weinack, 1982). The low colonization inocula for cloacal challenge may be explained by the short distance between the chick caeca and the cloaca. *C. jejuni* colonization of chicks via contaminated litter has been reported (Montrose, Shane & Harrington, 1985), consistent with our findings.

In individually housed birds, challenge with high level inocula resulted in most birds being colonized. Colonization was variable with threshold inocula ie the minimal inocula that resulted in some birds being colonized. However, we did not establish the effect of repeated low level inocula or of introducing *C. jejuni*-positive birds into a group of negative birds. Both are likely to occur within farm sheds. To assess the practical significance of our laboratory findings, further work on the rapidity and extent of horizontal transmission within flocks is being undertaken.

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