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Horizontal transmission of *Campylobacter jejuni* amongst broiler chicks: experimental studies

S. SHANKER[1], A. LEE[2] AND T. C. SORRELL[3]

[1] *Bacteriology Unit, Department of Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, NSW 2145, Australia*

[2] *School of Microbiology, University of New South Wales, P.O. Box 1, Kensington, NSW 2033, Australia*

[3] *Department of Infectious Diseases and Microbiology, ICPMR Westmead Hospital and University of Sydney, Westmead, NSW 2145, Australia*

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SUMMARY

Horizontal transmission of *Campylobacter jejuni* was investigated in campylobacter-free broiler chicks. One hundred and twenty chicks housed individually, were provided with water containing 10^2–10^9 c.f.u./ml *C. jejuni*. Colonization was rapid [47 of 73 (64%) positive cloacal cultures within 3 days and 65 of 73 (89%) within 7 days], dependent on *C. jejuni* strain and inoculum size but independent of chick age. Groups of 5–24 chicks in isolators were exposed to *C. jejuni*-contaminated water or colonized seeder chicks. Transmission occurred in 2–7 days concurrent with a gradual increase of *C. jejuni* in litter, water and feed. Environmental samples were culture-negative within 3 days following removal of colonized chicks. Treatment of 1-day-old chicks with adult caecal microbiota did not affect colonization. Treated and control chicks were all *C. jejuni*-positive within 3 days of seeder challenge.

INTRODUCTION

Epidemiological studies and data on *Campylobacter jejuni* serotypes indicate chickens are a significant source of strains pathogenic to humans (1–9). These findings suggest a need for a better understanding of *C. jejuni* epidemiology in broiler chickens. The commercial production of broilers involves several establishments including breeder farms, hatcheries, grow-out farms and processing plants. A reduction of *C. jejuni* contamination of retail chicken would require control measures at one or more of the associated establishments.

Studies of broiler samples from retail outlets and processing plants have shown highly variable *C. jejuni* contamination levels (10–14). It is evident that some broiler flocks remain campylobacter-free up to processing age. However, the likelihood of cross-contamination during processing highlights the difficulties in undertaking intervention at processing plants (15–20).

Farm studies of the progeny of *C. jejuni*-colonized breeder hens and laboratory

investigations of eggs indicate transmission of *C. jejuni* via the egg is unlikely (18–22). These findings suggest control measures should be directed at limiting horizontal transmission and that factors within grow-out farms are responsible for transmission of *C. jejuni*.

Some fundamental aspects of horizontal transmission of *C. jejuni* have already been determined. Investigations on the minimal infective dose for 2- to 3-day-old and 2-week-old chicks showed colonization by *C. jejuni* was dependent on the bacterial strain and inoculum size but independent of chick age. Chicks were colonized by 10^4–10^6 c.f.u. *C. jejuni* via oral inoculation and $< 10^2$–10^4 c.f.u. following cloacal challenge (23). These findings were based on single-dose challenge. The effect of repeated low level inocula or the presence of low numbers of *C. jejuni*-positive chicks, both of which are likely to occur in farm sheds were not assessed. The present study provides additional data on horizontal transmission.

MATERIALS AND METHODS

Broiler chicks

Broiler eggs from a commercial hatchery were incubated in the laboratory and hatched chicks included in the study. The chicks were a highly cross-bred commercial broiler strain derived from White Leghorn.

Organisms

Two local broiler strains of *C. jejuni*, ATT 6 and ICP 47 were used for the studies. The strains were identified as *C. jejuni* biotypes 1 and 2, and Penner serotypes 3 and 6 (24, 25), respectively. Challenge inocula were prepared by culturing the strains on horse blood agar plates at 42 °C for 48 h under microaerobic conditions (5% O_2, 10% CO_2 and 85% N_2) and harvesting with sterile saline.

Isolation of C. jejuni

Primary isolation was undertaken on agar plates 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 cyclohexamide and 5 mg/l amphotericin B (MCTV). Specimens were also inoculated into enrichment broth (EB) containing Oxoid Brucella Broth, 7% lysed horse blood, the antimicrobial agents listed above supplemented with FBP, i.e. 0·025% each of ferrous sulphate, sodium metabisulphite and pyruvic acid. Plates were incubated for 48 h under conditions stated above. EB cultures were incubated for 72 h under similar conditions before subculture on MCTV plates. Morphologically characteristic colonies were identified by methods described previously (24).

Cloacal samples

Broiler cloacal sampling was undertaken using either of two commercial swabs; the small gauge Calgi® Nasopharyngeal Calcium Alginate Applicator (Harwood Products Co., Guildford, Maine, 0443, USA) or the larger gauge Transtube® MW 170 Transport Medium Clear (Medical Wire and Equipment Co. Ltd; Potley, Corsham, Wilts., England). Samples were cultured on MCTV and EB.

Determination of environmental contamination in isolators

Water

C. jejuni contamination of gravity-fed drinkers was determined at fixed intervals. After thorough mixing of contents, a 30 ml sample was removed from the 300 ml-capacity drinker. Serial dilutions were made using 1 ml samples in 9 ml Brucella Broth containing FBP. Triplicate 0·1 ml aliquots were plated on MCTV and colony counts determined. Triplicate 1 ml amounts of undiluted sample were also inoculated into EB and subcultured as described above.

Feed

A composite 25 g sample was suspended in 250 ml sterile saline and shaken vigorously for 5 min. The suspension was filtered through cheese cloth and the filtrate centrifuged at 5000 *g* for 30 min at 4 °C. The sediment was resuspended in 10 ml Brucella Broth containing FBP, serially diluted and triplicate 0·1 ml amounts plated on MCTV. Triplicate 1 ml amounts of undiluted sample were inoculated into EB. Plates and EB were incubated and examined for *C. jejuni*.

Litter

A composite 25 g sample was obtained and treated similarly to feed samples.

Preparation of broiler anaerobic caecal microbiota

A suspension of caecal anaerobes was prepared by a modification of the method of Seuna, Nagaraja and Pomeroy (26). Caeca were removed from freshly killed 10-week-old broilers. These broilers had been housed in the laboratory and their salmonella – and campylobacter-free status verified by cloacal culture on hatching, at 6 and 9 weeks and confirmed by caecal culture at necropsy. Quantities of 0·1 g caeca were cut into pieces and placed in freshly prepared 200 ml Difco, Veal Infusion Broth containing 0·5 mg/ml cysteine, 5 μg/ml haemin and 0·1 μg/ml Vitamin K (VIB). Following 48 h anaerobic incubation at 37 °C, 1 ml was subcultured into 200 ml VIB and incubated under similar conditions. Aliquots of this broth were used for inoculating the crops of 1-day-old chicks.

Experimental transmission of C. jejuni *via contaminated water*

Campylobacter-free, 2-3-day-old chicks were housed individually and provided with water contaminated with varying inocula of *C. jejuni*. The contaminated water was available *ad libitum*. Cloacal samples were obtained 1, 3, 7 and 14 days after placement of *C. jejuni*-contaminated water and cultured for *C. jejuni*. After initial inoculation with *C. jejuni*, the water was replenished with tap water as necessary during the 2-week period. On day 14, caecal samples obtained at necropsy were cultured for *C. jejuni*. Two-week-old campylobacter-free chickens were similarly exposed to *C. jejuni*-contaminated water, housed individually and monitored for cloacal carriage of *C. jejuni*. Isolates from *C. jejuni*-colonized chicks were identified to confirm the strain isolated was the same biotype as the original challenge strain.

In a separate study to determine the *C. jejuni* load on the environment, 1-week-old campylobacter-free chicks in groups of five were placed in isolators and

provided with *C. jejuni*-contaminated water. The litter consisted of sterilized wood shavings. Commercial broiler feed pellets similar to that provided in grow-out farms was provided *ad libitum*. Cloacal, feed, litter and water samples were monitored at regular intervals for 7 weeks. The chicks were than removed and feed, litter and water samples monitored daily for *C. jejuni*. Following a 3-week interval, newly hatched chicks were re-introduced into the isolators and monitored for *C. jejuni*.

Experimental transmission of C. jejuni *via seeder chicks*

Horizontal transmission via seeder chicks was undertaken by placing *C. jejuni*-colonized chicks (seeders) in isolators amongst campylobacter-free chicks of the same age. Cloacal samples from all chicks were cultured for *C. jejuni* 2, 7 and 14 days after placement of seeders. Chicks of varying age groups were subjected to seeder challenge and monitored. *C. jejuni* colonization was confirmed by caecal culture at necropsy on day 14. As controls, chicks hatched from the same batch of eggs as those included in each experimental group were maintained without seeder challenge for their respective monitoring periods.

In a separate study with 1-week-old chicks, samples of feed, litter and water obtained during the period of seeder challenge and after removal of all chicks were cultured for *C. jejuni*.

Seeder challenge of chicks following oral treatment with anaerobic caecal microbiota

Five, 1-day-old campylobacter-free chicks were treated orally with 0·5 ml aliquots of an anaerobic broth culture of caecal microbiota as described above. The treated chicks were placed in isolaters containing sterilized wood shavings as litter. Commercial feed and mains water were provided *ad libitum*. After 24 h, a 2-day-old chick, campylobacter-free and not previously treated with anaerobic caecal microbiota was inoculated orally with *C. jejuni*, and placed immediately amongst the treated chicks. Cloacal swabs were obtained 1, 3, 7 and 14 days after placement of the seeder. On day 14, caecal contents obtained at necropsy were cultured for *C. jejuni*. In control experiments undertaken concurrently, untreated 2-day-old chicks were exposed to seeder challenge and monitored for *C. jejuni*.

RESULTS

C. jejuni *transmission via contaminated water*

A total of 120 individually housed chicks were exposed to varying inocula of *C. jejuni*-contaminated water (Table 1). None of the chicks showed evidence of diarrhoea following exposure to *C. jejuni*. Colonized chicks remained *C. jejuni*-positive throughout the 2-week monitoring period. A similar number of chicks was *C. jejuni*-positive by cloacal culture as caecal culture at necropsy. It was evident the infective dose was strain dependent but independent of chick age. Of a total of 73 colonized chicks, 47 (64%) were *C. jejuni*-positive within 3 days and 65 (89%) within 7 days of exposure to *C. jejuni*-contaminated water. *C. jejuni* isolates obtained from *C. jejuni*-colonized chicks were the same biotype as their respective challenge strain.

The 1-week-old-chicks in isolaters exposed to *C. jejuni*-contaminated water were

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Table 1. *Experimental colonization of campylobacter-free chicks following exposure to* C. jejuni-*contaminated water*

Chick age (days)	*C. jejuni* challenge strain	Level of *C. jejuni* contamination in water (c.f.u./ml ± S.D.)*	Chicks *C. jejuni*-positive† following exposure to contaminated water on day			
			1	3	7	14
2–3	ICP	$1{\cdot}1 \times 10^3 \pm 1{\cdot}5$	0‡	0	0	0
		$2{\cdot}5 \times 10^5 \pm 1{\cdot}8$	4	6	6	6
		$3{\cdot}2 \times 10^7 \pm 1{\cdot}2$	6	10	10	10
	ATT	$5{\cdot}6 \times 10^5 \pm 0{\cdot}9$	0	0	0	0
		$7{\cdot}9 \times 10^7 \pm 1{\cdot}3$	6	7	7	7
		$1{\cdot}2 \times 10^9 \pm 1{\cdot}1$	7	8	10	10
14	ICP	$3{\cdot}0 \times 10^2 \pm 0{\cdot}6$	0	0	0	0
		$7{\cdot}0 \times 10^4 \pm 2{\cdot}0$	0	2	6	10
		$4{\cdot}5 \times 10^6 \pm 1{\cdot}7$	4	10	10	10
	ATT	$1{\cdot}3 \times 10^4 \pm 0{\cdot}4$	0	0	0	0
		$2{\cdot}6 \times 10^6 \pm 1{\cdot}8$	0	2	2	10
		$2{\cdot}9 \times 10^8 \pm 1{\cdot}3$	0	2	10	10

* Mean of duplicate experiments.
† Determined by cloacal swab culture; confirmed by caecal culture on necropsy at day 14.
‡ Total from duplicate experiments of five chicks in each.

Table 2. C. jejuni *contamination of the environment following exposure of 1-week-old broilers* to* C. jejuni-*contaminated water*

Sampling time (days)	*C. jejuni*-colonized chicks	*C. jejuni* contamination of environment*		
		Water (c.f.u./ml)	Feed (c.f.u./g)	Litter (c.f.u./g)
0	0/10†	$1{\cdot}1 \times 10^7 \pm 0{\cdot}1$‡	< 1§	< 5§
2	10/10	$5{\cdot}3 \times 10^2 \pm 0{\cdot}5$	+‖	+‖
14	10/10	$5{\cdot}0 \times 10^4 \pm 0{\cdot}6$	$4{\cdot}3 \times 10^1 \pm 0{\cdot}8$	$8{\cdot}3 \times 10^4 \pm 1{\cdot}0$
21	10/10	$1{\cdot}0 \times 10^4 \pm 1{\cdot}2$	$5{\cdot}6 \times 10^3 \pm 0{\cdot}6$	$1{\cdot}8 \times 10^4 \pm 0{\cdot}9$
28	10/10	$3{\cdot}7 \times 10^5 \pm 0{\cdot}8$	$4{\cdot}1 \times 10^2 \pm 1{\cdot}2$	$1{\cdot}6 \times 10^5 \pm 0{\cdot}6$
35	10/10	$5{\cdot}8 \times 10^6 \pm 1{\cdot}0$	$2{\cdot}0 \times 10^6 \pm 0{\cdot}5$	$4{\cdot}8 \times 10^5 \pm 1{\cdot}5$
42	10/10	$5{\cdot}6 \times 10^7 \pm 1{\cdot}5$	$3{\cdot}5 \times 10^4 \pm 0{\cdot}3$	$9{\cdot}2 \times 10^6 \pm 1{\cdot}4$
49	10/10	$1{\cdot}3 \times 10^7 \pm 0{\cdot}4$	$1{\cdot}5 \times 10^6 \pm 0{\cdot}4$	$2{\cdot}9 \times 10^6 \pm 0{\cdot}3$

* Campylobacter-free broilers housed in 0·9 m^2 isolators
† No. colonized/no. tested for duplicate experiments of five chicks in each.
‡ Initial challenge inoculum. Mean ± S.D.
§ Limit of sensitivity, based on recovery from artificially inoculated specimens.
‖ *C. jejuni* isolated on enrichment.

all colonized within 48 h (Table 2). Environmental monitoring showed a gradual increase in *C. jejuni* contamination of feed and litter. It was evident the feed trough was being contaminated with excreta. Removal of the chicks resulted in a rapid decrease in environmental contamination. Within 24 h, the *C. jejuni* level in feed samples decreased from $1{\cdot}5 \times 10^6 \pm 0{\cdot}4$ (mean ± S.D.) c.f.u./g to below the detectable limit of < 1 c.f.u./g. *C. jejuni* contamination in water decreased from

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Table 3. *Experimental transmission of* C. jejuni *to campylobacter-free broilers* via* C. jejuni*-colonized seeders*

Chick age (days)	*C. jejuni* seeder strain	Broilers exposed to seeders	Newly colonized† broilers following seeder exposure on day		
			2	7	14
2–3‡	ICP	36	8	30	36
	ATT	36	0	6	22
7§	ICP	16	10	16	16
	ATT	16	2	16	16
14‖	ICP	10	10	10	10
	ATT	10	2	8	10

* Campylobacter-free broilers housed in 0·9 m^2 isolators.
† *C. jejuni* status determined by cloacal culture and confirmed by caecal culture on day 14.
‡ Two seeders per group of 18 campylobacter-free broilers; duplicate experiments.
§ One seeder per group of eight campylobacter-free broilers; duplicate experiments.
‖ One seeder per group of five campylobacter-free broilers; duplicate experiments.

$1{\cdot}3 \times 10^7$ c.f.u./ml to $7{\cdot}9 \times 10^2 \pm 0{\cdot}5$ c.f.u./ml in 24 h and to < 1 c.f.u./ml after 48 h. *C. jejuni* in litter decreased from $2{\cdot}9 \times 10^6 \pm 0{\cdot}3$ c.f.u./g to $2{\cdot}8 \times 10^3 \pm 0{\cdot}6$ c.f.u./g, $5{\cdot}3 \times 10^2 \pm 1{\cdot}8$ c.f.u./g and < 5 c.f.u./g after 24, 48 and 72 h, respectively. Duplicate groups of 20 newly hatched chicks were introduced to this environment after a 3-week interval. The chicks remained campylobacter-free during a 2-week monitoring period. The campylobacter-free status was confirmed by caecal culture at necropsy.

C. jejuni *transmission via seeders*

Placement of *C. jejuni*-colonized seeders resulted in rapid transmission of *C. jejuni* to campylobacter-free chicks (Table 3). The rate of transmission, i.e. the number of *C. jejuni*-colonized chicks at each sampling time, varied with the *C. jejuni* challenge strain. This was more apparent with the 2- to 3-day-old chicks, in which 14 days after seeder challenge, there was a significant difference in the number of colonized birds for the two challenge strains ($\chi^2 = 17{\cdot}379$, $P < 0{\cdot}001$).

Seeder challenge resulted in rapid *C. jejuni*-contamination of the environment (Table 4). Within 2 days' exposure to seeders 48% of 48 campylobacter-free chicks were *C. jejuni*-positive and the organism was recovered from water, feed and litter samples. By the seventh day, 47/48 (98%) were colonized and *C. jejuni* environmental contamination had increased to *c.* 10^4 c.f.u. in each of the feed, water and litter samples examined. Following removal of the colonized chicks, feed samples were *C. jejuni* culture-negative within 24 h. Water and litter samples were culture-negative after 48 h.

C. jejuni *transmission amongst treated chicks*

Seeder challenge of 2-day-old campylobacter-free chicks treated orally with anaerobic caecal microbiota resulted in rapid *C. jejuni* colonization of the campylobacter-free chicks irrespective of challenge strain. Seeders were shown to be cloaca-positive within 3 h of oral inoculation with *c.* 10^8 c.f.u. *C. jejuni*. In

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Table 4. C. jejuni *contamination of the environment following exposure of 1-week-old broilers* to* C. jejuni*-colonized seeders*

Sampling time (days)	Newly colonized broilers	*C. jejuni* contamination of environment*		
		Water (c.f.u./ml)	Feed (c.f.u./g)	Litter (c.f.u./g)
0	0	< 1†	< 1†	< 5†
2	23/48‡	+§	+§	+§
3	37/48	$7{\cdot}9 \times 10^3 \pm 1{\cdot}3$‖	$1{\cdot}2 \times 10^2 \pm 1{\cdot}0$	$3{\cdot}5 \times 10^4 \pm 0{\cdot}3$
7	47/48	$3{\cdot}2 \times 10^4 \pm 1{\cdot}0$	$1{\cdot}8 \times 10^4 \pm 1{\cdot}0$	$9{\cdot}8 \times 10^4 \pm 0{\cdot}8$

* Campylobacter-free broilers housed in 0·9 m^2 isolators.
† Limit of sensitivity.
‡ No. colonized/no. exposed to seeders from duplicate experiments of 1 seeder per 24 campylobacter-free broilers.
§ *C. jejuni* isolated on enrichment.
‖ Colony counts represented as mean ± S.D.

duplicate experiments consisting of a total of 20 treated and 20 untreated campylobacter-free chicks exposed to seeders, all 40 were colonized within 3 days and remained *C. jejuni*-positive for the 2-week monitoring period.

DISCUSSION

Data on chicks exposed to varying inocula of *C. jejuni*-contaminated water confirm earlier findings (23) on the oral infective dose for the challenge strains and demonstrate the importance of contaminated water in the rapid colonization of broiler chicks. It is also evident that low numbers of *C. jejuni*-colonized birds effect rapid transmission to campylobacter-free chicks. These findings are consistent with reports of rapid intra-shed transmission in several farm studies in which periodic monitoring of flocks was undertaken (16, 22, 27, 29).

With both modes of experimental transmission, i.e. contaminated water or seeder challenge, there was a gradual increase in the *C. jejuni* load in the environment. The initial decrease in the *C. jejuni* count seen in water samples in the isolator study with contaminated water (Table 2) is consistent with viability data reported previously (30, 31). The subsequent increase in *C. jejuni* water contamination can be attributed to repeated faecal contamination. Similarly, feed samples would have been contaminated with excreta. The isolator study with seeder challenge (Table 4) showed many birds were colonized before the detection of substantial environmental contamination. This may in part be due to sampling error and the non-recovery of sub-lethally injured *C. jejuni*. The susceptibility to lower inocula *per cloacae* (23) and coprophagy could also account for the rapid colonization.

Several investigators have reported the absence of *C. jejuni* in young flocks especially those less than 2 weeks of age. It is evident from the present study that newly hatched chicks are colonized easily and do not suffer morbidity. The absence of *C. jejuni* in young flocks is therefore unlikely to be due to intrinsic protective mechanisms. The data from the present experimental studies indicate

that within a 0·9 m² closed environment, there is a natural delay in the build up of *C. jejuni* in the environment. By analogy with these findings, there could be a corresponding lag period before *C. jejuni* levels within the farm shed environment reach the threshold infective dose.

Importantly, our data indicate the possibility of interrupting transmission of *C. jejuni* to subsequent flocks. *C. jejuni* did not survive in the environment for long periods, consistent with previous findings on the sensitivity of *C. jejuni* to drying (32). *C. jejuni* was not cultured from environmental samples 72 h after removal of chicks from the contaminated environment. Further studies including farm variables have to be undertaken to substantiate our preliminary findings. Our survival studies were based on the recovery of culturable *C. jejuni*. Sub-lethally injured cells may not have been recovered. It has also been demonstrated that non-culturable *C. jejuni* may be detected by a fluorescent antibody test (33). A recent study reported of non-culturable *C. jejuni* from bore water as the source of contamination in a broiler farm (34). Further investigations are required to determine the importance of non-culturable *C. jejuni* in non-aqueous environments. Our preliminary findings show that following a 3-week interval, the placement of newly hatched chicks in a previously contaminated environment did not result in the colonization of these chicks by *C. jejuni*.

The present study confirmed our earlier findings (23) on the ineffectiveness of adult caecal microbiota in protecting young chicks from *C. jejuni* colonization. Our findings are in contrast to the reports of Soerjadi and colleagues (35, 36) but similar to the data reported by Stern and co-workers (37) who included in their study an experimental group treated with the original culture used by Soerjadi and colleagues. Stern and co-workers demonstrated that adult caecal microbiota, whilst useful in reducing salmonella colonization was of no value in the control of *C. jejuni* in chicks. Further work is required to define the caecal microbiota that may be effective in controlling *C. jejuni* colonization.

The inability of *C. jejuni* to multiply in feed, litter or water under normal ambient conditions and the susceptibility to drying provide potential directions for intervention procedures in the farm. A recent study demonstrated that fauna in the confines of broiler farms were carriers of *C. jejuni* serotypes common to the broiler flocks (20). Insects have also been shown to be carriers of *C. jejuni* (38, 39). Husbandry protocols should therefore be revised to include measures to dissuade entry of potential carriers of *C. jejuni* as well as controls preventing intra-shed transmission by proper litter and water management. We conclude that re-assessment of husbandry practices is required to maintain campylobacter-free grow-out flocks.

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