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Campylobacter jejuni in broilers: the role of vertical transmission

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SUMMARY

The role of broiler eggs in the transmission of *Campylobacter jejuni* to broiler grow-out flocks was investigated. Six breeder flocks supplying broiler eggs to hatcheries were examined for cloacal carriage of *C. jejuni*. Of 240 birds tested, 178 (74%) were *C. jejuni*-positive. Eggs from these birds examined for *C. jejuni* penetration of the egg shell indicated that 185 of 187 were campylobacter-free. Eggs from breeder flocks of unknown *C. jejuni* status were also examined for *C. jejuni* shell penetration. *C. jejuni* was not isolated from 142 eggs examined. A further 193 hatchery eggs incubated and hatched in the laboratory were campylobacter-free. Six farms containing the progeny of *C. jejuni*-positive breeder flocks were monitored. Eight hundred and forty birds from 14 flocks in these grow-out farms were campylobacter-free during their 6-week grow-out period. Experimental egg-penetration studies indicated that *C. jejuni* transmission via the egg is not easily effected. Of 257 eggs surface-challenged with *C. jejuni*, 162 hatched; all were campylobacter-free. Of 167 eggs injected with *C. jejuni*, 12 hatched; 2 of these were colonized with *C. jejuni*. Our data do not support a role for vertical transmission of *C. jejuni* in commercial broiler production.

INTRODUCTION

Campylobacter jejuni contamination of commercially produced chickens has been widely reported (Smith & Muldoon, 1974; Simmons & Gibbs, 1979; Park *et al.* 1981; Shanker *et al.* 1982). Serotyping of human *C. jejuni* isolates has shown that many chicken and human strains are of the same serotype (Lior *et al.* 1981; McMyne *et al.* 1982; Munroe, Prescott & Penner, 1983; Abbott *et al.* 1983). These findings support circumstantial evidence (Brower *et al.* 1979; Oosterom *et al.* 1983; Hopkins & Scott, 1983) linking the occurrence of *C. jejuni* enteritis to the consumption or handling of chickens. An outbreak of *C. jejuni* enteritis amongst workers at a

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chicken processing plant in Sweden, emphasizes the occupational hazard of handling contaminated chicken (Christenson *et al.* 1983).

In assessing the practicability of raising campylobacter-free flocks, the relative contribution of vertical and horizontal transmission of *C. jejuni* amongst broiler flocks has to be established. Epidemiological studies of *C. jejuni* in broiler flocks have been limited mainly to surveys of broiler farms (Cruickshank *et al.* 1982; Mehle, Gubina & Gliha, 1982; Smitherman, Genigeorgis & Farver, 1984). In a study evaluating evidence for vertical transmission (Doyle, 1984), *C. jejuni* penetration of the egg shell of layer eggs was demonstrated but the organism was not isolated from egg contents. The present study was undertaken to determine the role of vertical transmission in *C. jejuni* colonization of broiler flocks. Over a 2-year period, breeder flocks, eggs and grow-out flocks were examined for *C. jejuni*. Experimental laboratory studies were also undertaken to effect *C. jejuni* entry of broiler eggs.

MATERIALS AND METHODS

I. Farm studies

(*a*) Two breeder farms from separate districts (A,B) supplying broiler eggs to separate hatcheries were investigated. Cloacal samples from six flocks in lay, aged 34–45 weeks were examined for *C. jejuni*. Cloacal swabs were placed in transport media, (Medical Wire Equipment Co Ltd, Corsham, Wilts, England) stored at 5 °C and cultured within 4 h of collection. Samples were plated on modified Skirrow's medium (1977) containing Oxoid Blood Agar Base No. 2, 7% lysed horse blood, 0·25 mg/l colistin sulphate, 5 mg/l trimethoprim and 10 mg/l vancomycin (CTV medium). Inoculated plates were incubated at 42 °C for 48 h under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2). Morphologically characteristic colonies were identified by methods previously described (Skirrow & Benjamin, 1980).

(*b*) Six grow-out farms containing the progeny of known *C. jejuni*-colonized parent breeder flocks were monitored for *C. jejuni*. Cloacal swabs and pooled samples of feed, litter and water from sheds housing separate flocks were tested at weekly intervals from the day of placement till the processing day. Visceral samples obtained at the processing plant were checked for *C. jejuni*.

II. Egg studies

(*a*) *Eggs from* C. jejuni-*colonized breeder flocks*

Eggs from known *C. jejuni*-colonized breeder flocks from two separate farms were obtained at the first collection of each day. These eggs were no more than 14 h old. *C. jejuni* penetration of the egg shell was determined using a modified method of Board & Board (1967). The shell above the air-sac was cut out, the egg contents removed and replaced with Oxoid Brucella Broth containing 2% Oxoid Bacteriological Agar; MCTV antibiotics i.e. 0·25 mg/l colistin sulphate, 5 mg/l trimethoprim, 10 mg/l vancomycin, 50 mg/l cefoperazone, 100 mg/l cyclohexamide, 5 mg/l amphotericin B; 0·025% each of ferrous sulphate, sodium metabisulphite and pyruvic acid (FBP); and 0·001% triphenyltetrazolium chloride (Sigma Chemicals). The air-sac area was sealed with tape and the eggs incubated under microaerobic conditions for 3 days at 42 °C. The eggs were then sliced in half and the shell separated from the agar. Areas of the inner surface of the shell

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or, on the agar, showing pink colouration were swabbed with sterile, moist cotton wool. The swabs were inoculated onto *C. jejuni* selective agar plates containing 5% lysed horse blood and MCTV antibiotics. After 48 h incubation under microaerobic conditions at 42 °C suspect colonies were identified.

(*b*) *Hatchery eggs*

To assess if the hatchery is a possible disseminator of *C. jejuni*, eggs from a hatchery supplying chicks to broiler grow-out farms were obtained. *C. jejuni* penetration of the egg shell was determined as described above. Separate batches of eggs obtained from the hatchery were incubated in the laboratory and the hatched chicks monitored for *C. jejuni*. All hatchery eggs were from breeder flocks whose *C. jejuni* status was not established.

(c) *Experimental egg penetration studies*

Egg penetration was undertaken by varying modifications of the method of Haines & Moran (1940) who demonstrated that a warm egg contracts on cooling and this process aids microbial penetration of the shell.

(*i*) *Shell surface swabbing.* Fresh broiler eggs obtained from the hatchery (2–3 days old) were surface sterilized with 70% alcohol and equilibrated at 37 °C for 1 h. Using a 25 °C saline suspension of *C. jejuni* (strain ICP47, a chicken isolate) the eggs were swabbed thoroughly at room temperature (23 °C), air-dried under u.v. light and placed in an egg incubator. Control eggs were treated similarly with sterile saline. Hatched chicks were checked for cloacal carriage of *C. jejuni*. After 28 days of incubation, unhatched eggs were checked for *C. jejuni*. The contents of each unhatched egg were placed in a stomacher bag and agitated for 15 sec. Samples of 1 ml of fluid egg were placed in 10 ml of enrichment broth containing MCTV antibiotics, FBP and 0·1% laurylsulphate (Sigma Chemicals). The inoculated broths were incubated for 3 days at 42 °C under microaerobic conditions, sub-cultured on to *C. jejuni* selective plates and suspect colonies identified.

(*ii*) *Vacuum treatment.* A separate batch of fresh broiler eggs from the hatchery were equilibrated and swabbed as described above. Using a modified method of Alls *et al.* (1963), the moist eggs were placed in a vacuum jar and subjected to three rapid evacuations to −60 kPa. The eggs were then air-dried and treated as above. Control eggs were similarly treated with sterile saline. After incubation hatched chicks and unhatched eggs were examined for *C. jejuni*.

(*iii*) *Immersion.* Fresh broiler eggs were equilibrated at 37 °C for 1 h then immersed for 1 h in 5 °C saline suspensions of varying concentrations of *C. jejuni*. After immersion, the eggs were removed, air-dried under UV light and placed in the 37 °C egg incubator. A separate batch of eggs were equilibrated at 43 °C before immersion in a 5 °C saline suspension of *C. jejuni* for 1 h. Control eggs were treated similarly and immersed in sterile saline at 5 °C. After incubation, hatched chicks and unhatched eggs were checked for *C. jejuni*.

(*iv*) *Injection.* Fresh broiler eggs were surface sterilized with 70% alcohol and air-dried under u.v. light. Using a 19G needle, 0·1 ml aliquots of varying concentrations of *C. jejuni* in saline were injected into the albumin. Control eggs were injected with sterile saline. The injection hole was sealed with sterile, porous tape

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Table 1. C. jejuni *colonization of broilers in two breeder farms*

Farm	Age (weeks)	Cloacal isolation of *C. jejuni*
District A		
Flock I	43	8/10*
Flock II	39	33/40
Flock III	34	27/40
District B		
Flock IV	45	37/50
Flock V	45	35/50
Flock VI	45	38/50

* No. of positive isolations/No. of birds sampled.
Flock size, 2500–3500 birds.

Table 2. *Survey of six grow-out farms containing progeny of* C. jejuni-*colonized parent flocks*

Farm	Flocks	No. samples	Monitored period	*C. jejuni* status
(i)	4	240	2 days–6 weeks	-ve*
(ii)	1	60	3 days–6 weeks	—
(iii)	4	240	2 days–6 weeks	—
(iv)	2	120	2 days–6 weeks	—
(v)	1	60	1 day–6 weeks	—
(vi)	2	120	3 days–6 weeks	—

* *C. jejuni* not isolated from cloacae and composite samples of feed, litter, water.
10 birds per flock sampled each week.
Flock size, 9000–15000 birds.

and the eggs incubated. Hatched chicks and unhatched eggs were checked for *C. jejuni*.

RESULTS

Farm studies. All six established breeder flocks in the two separate farms were in lay and heavily colonized (Table 1). Fourteen grow-out flocks were monitored in the six farms containing progeny of *C. jejuni*-colonized breeder flocks (Table 2). Despite the small sample size relative to flock populations, *C. jejuni* was not isolated from a total of 840 cloacal swabs and 36 separate pooled samples of feed, litter and water obtained during the 6-week monitoring period. Ten visceral samples from one of the monitored flocks, obtained at the processing plant and examined for *C. jejuni* were campylobacter-free.

Egg studies

Breeder farms. The 187 freshly laid eggs obtained from *C. jejuni* colonized breeder flocks included 85 nest and 102 floor eggs. In two samples, *C. jejuni* penetration of the egg shell was evident. Both were floor eggs from District A breeder farm. *C. jejuni* was not isolated from District B breeder farm eggs.

Hatchery. C. jejuni penetration of the egg shell was not evident in 142 hatchery eggs examined. A further 193 eggs incubated and hatched in the laboratory were also campylobacter-free.

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Table 3. *Isolation of* C. jejuni *from experimentally treated broiler eggs*

Method	Inoculum c.f.u./ml	No. tested	No. hatched (%)	*C. jejuni* isolation: Unhatched eggs	*C. jejuni* isolation: hatched chicks
1. Swabbing	$5{\cdot}3 \times 10^9$	100	58 (58)	0	0
Control	0	100	44 (44)	0	0
2. Swab + vacuum	$3{\cdot}7 \times 10^8$	28	19 (68)	0	0
Control	0	28	20 (71)	0	0
3. Immersion (a)*	$1{\cdot}3 \times 10^8$	89	54 (61)	0	0
Control	0	30	14 (47)	0	0
Immersion (b)†	$5{\cdot}5 \times 10^3$	20	17 (85)	0	0
	$5{\cdot}5 \times 10^2$	20	14 (70)	0	0
Control	0	20	17 (85)	0	0
4. Injection	$6{\cdot}5 \times 10^5$	60	2 (3)	58/58‡	0
	$1{\cdot}3 \times 10^5$	20	2 (10)	18/18	0
	$1{\cdot}3 \times 10^4$	54	2 (4)	52/52	0
	$1{\cdot}3 \times 10^3$	20	4 (20)	16/16	0
	$1{\cdot}3 \times 10^2$	13	2 (15)	11/11	2/2‡
Control	0	70	39 (56)	0	0

* Eggs equilibrated at 42 °C prior to immersion.
† Eggs equilibrated at 37 °C prior to immersion.
‡ No. of isolations/No. of specimens tested.

As the results indicated that natural transmission via the egg was unlikely, laboratory studies with broiler eggs were undertaken to see if colonization is possible under defined conditions.

Experimental egg-penetration studies (Table 3). Of 257 eggs surface-challenged by swabbing, vacuum treatment or immersion, 162 (63%) hatched, compared with 95/178 (53%) control eggs. All hatched chicks were campylobacter-free and *C. jejuni* was not isolated from unhatched eggs. When *C. jejuni* was injected into the albumin, 12/167 (7%) hatched compared with 39/70 (56%) of control eggs. These differences are significant ($P<0{\cdot}001$, Chi square analysis). Two of the 12 chicks which hatched following injection of *C. jejuni* into the egg albumin were colonized with *C. jejuni*. One was grossly stunted and was culled. The other was of below average birth weight but was otherwise normal. *C. jejuni* was isolated from the contents of all inoculated unhatched eggs. Control unhatched eggs and hatched chicks were campylobacter-free.

DISCUSSION

Our data suggest that vertical transmission in the natural state is not easily effected. Although the breeder flocks in lay were colonized, *C. jejuni* penetration of the egg shell was not evident in 185/187 eggs examined. The two ' positive ' eggs were soiled, floor eggs. Husbandry practices in this farm prevent soiled, floor eggs from reaching the hatchery thereby minimizing potential transmission. In our study, the hatchery was not a source for *C. jejuni* transmission. In addition, *C. jejuni* was not isolated in grow-out farms containing progeny of colonized breeder flocks. The campylobacter-free status during their 6-week grow-out period indicates that delayed establishment of *C. jejuni* acquired via the egg is unlikely.

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Our laboratory treatment of broiler eggs indicates that egg shell surface challenge with *C. jejuni* does not result in colonized chicks. Colonization was possible only by injecting the organism into the albumin. In a recent study (Clarke & Bueschkens, 1985) colonization was effected by temperature and pressure differentials with immersed eggs. However, successful colonization was effected only in the presence of iron in the challenge broth. In our comparable immersion studies, i.e. in the absence of iron, despite a greater temperature differential, a longer immersion period and a heavier inoculum, *C. jejuni* was not present in any of the hatched chicks. This may be due to differences in the *C. jejuni* strains used as well as the physical status of the egg shell characteristic of the breed of laying hen. Clark & Bueschkens (1985), by injecting *C. jejuni* into the egg were able to monitor the viability of the organism in the egg. However, they did not extend their study to examine if any of the injected eggs resulted in hatched colonized chicks. In our study, injection affected hatchability but was not inoculum dependent. Colonization however was inoculum dependent. As the sample size was small, further work needs to be carried out to verify this observation. Our recovery of *C. jejuni* from inoculated eggs held at 37 °C for 28 days extends the previously reported viability in liquid egg at 37 °C of up to 48 h (Hanninen, Korkeala & Pakkala, 1984).

Our data suggest that vertical transmission of *C. jejuni* does not occur in the natural state. These results are of considerable significance in poultry management if the goal of campylobacter-free flocks is considered desirable. Further studies should be directed toward assessing the relative importance of horizontal transmission in *C. jejuni* colonization of broiler flocks.

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