The extent of surface contamination of retailed chickens with *Campylobacter jejuni* serogroups

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SUMMARY

Eighty-two chickens purchased at 11 retailers (supplied by 12 wholesalers) in the south of England were cultured for *Campylobacter jejuni* by a method involving total immersion. The organism was isolated from 22 (48%) of 46 fresh birds, 12 of 12 uneviscerated (New York dressed) birds, but only 1 of 24 frozen birds. Viable counts of up to 1.5×10^6/chicken were obtained from fresh birds and 2.4×10^7/chicken from uneviscerated birds. Surface swabbing of breasts, thighs, wings and vents of fresh chickens showed that contamination was generally distributed over the carcasses. Salmonellas were found in only 2 of 69 of the fresh chickens.

The prevalence of several Lior and Penner *C. jejuni* serogroups was similar in chickens and sporadic human cases of enteritis. Penner serogroup 4 (mostly of Lior serogroup 1) was found in 26% of human isolates and 14% of chicken isolates.

The rising incidence of campylobacter enteritis during the last 6 years could well be a reflection of the increasing proportion of fresh chickens consumed over that period (32% higher in 1986 than in 1981).

INTRODUCTION

Campylobacter jejuni commonly inhabit the caeca of apparently healthy chickens and survive a variety of processing procedures to which chickens are subjected before being presented for sale in retail outlets (Simmons & Gibbs, 1979; Park *et al.* 1981; Smith & Muldoon, 1974).

In England and Wales reported enteritis in man due to infection with *C. jejuni* has increased significantly over the past few years. It is now the principal cause of acute bacterial diarrhoea. Almost twice as many campylobacter infections as salmonella infections were reported in 1986 (personal communication, PHLS Communicable Disease Centre, 1987).

More than three hundred million chickens are now purchased annually for consumption in the UK (Poultry World, 1986) and it would thus seem probable that *C. jejuni* contaminated chickens could well be responsible, directly or indirectly, for many of these human infections. There is an obvious direct potential hazard to the chicken handler before cooking and an indirect hazard to consumers through contamination of other cooked foods awaiting consumption in

the kitchen or refrigerator. The chances of infection would depend upon the site and degree of contamination on the outer surface of the chicken and on the magnitude of the oral ID_{50} for man. The latter has not been well established, but the indication from a deliberate consumption of contaminated milk is that it could be as low as 500 organisms (Robinson, 1981).

The present investigation was undertaken in an attempt to assess the infective potential from locally purchased chickens and to determine whether there was any correlation between the serogroups of *C. jejuni* from chicken and sporadic human cases of enteritis.

MATERIALS AND METHODS

Outer surface contamination.

Eighteen 'fresh' oven-ready chickens were purchased between July and November 1985 from 15 city retailers, including national supermarkets and small butchers supplied by 13 wholesalers. They were sold in wrapped polythene covers. After carefully peeling off the polythene, each of the following surfaces was swabbed separately with a swab moistened in phosphate buffered saline (Oxoid Br.14a; PBS): breasts, under wings, inner thighs and vent (cavity). Each swab was used to inoculate a vancomycin, polymycin, trimethoprim (VPT) agar plate (Skirrow, 1977) which was then incubated at 43 °C in a reduced oxygen atmosphere for 24–48 h. The swab was also used to inoculate a selenite broth to be incubated at 43 °C and subcultured to desoxycholate citrate agar (Oxoid CM227) to be examined for *Salmonella* spp. Growth of *C. jejuni* on VPT agar was determined by direct visual inspection followed by confirmation microscopically of Gram stain and morphology and subsequent serogrouping, by two methods: Penner & Hennessy (1981) and Lior *et al.* (1982). The latter was undertaken by the PHLS laboratory, Manchester, with the kind permission of the Director, Dr D. M. Jones.

Whole chicken contamination

Eighty-two 'whole' chickens weighing between 1 and 2 kg (46 fresh, 24 frozen and 12 uneviscerated (New York dressed) birds) were purchased locally in March and April 1986 in batches of 3, 6 or 12 from 11 retailers. They were supplied by 12 wholesalers from chicken farms in 12 counties. Vent swabs were taken from all but the frozen birds and inoculated as above. Each chicken was then totally immersed in PBS, at room temperature, contained in a boilable plastic bag (high density polyethylene, Transatlantic Plastics Ltd.) supported in a plastic beaker. Volumes of 1·5 or 2 l of PBS were required for total immersion. After immersion for at least 1 h (see later) the PBS was stirred, either with a pipette or simply by rotating the chicken about 360°. A 100 ml portion of the PBS was removed and used for the isolation and enumeration of viable *C. jejuni*: 10, 50 and 250 μl volumes were applied and spread with a glass spreader to each of four well dried (3 h at 37 °C) VPT plates. Volumes of 10 and 50 ml were also filtered through 0·2 μm pore sized cellulose nitrate membranes which were applied to VPT agar. Cellulose nitrate membranes were used because previously unpublished investigations showed an apparently high (> 90 %) loss of *C. jejuni* when using cellulose acetate membranes. Plates were incubated as above for 1–3 days and

Table 1. *Removal of* C. jejuni *from fresh chickens by immersion in phosphate buffered saline: effect of immersion time*

Total viable *C. jejuni* isolated/chicken × 10^5

Immersion time (h)	Chicken 1	Chicken 2	Chicken 3	Chicken 4	Chicken 5	Chicken 6
< 0·1	4·0	0·8	3·6	0·9	0·6 ·	0·2
1	3·8	0·6	4·0	3·6	3·6	—
2	4·2	—	—	—	—	—
4	13·0	—	—	—	—	—
6	12·0	0·4	5·2	2·7	3·2	0·2

Table 2. *Efficiency of* C. jejuni *removal from fresh chicken by immersion in PBS*

Total viable campylobacter/chicken

1st immersion	2nd immersion*	Recovery from 1st immersion
1·5 × 10^3	< 30	> 99%
7·5 × 10^3	< 30	> 99%
1·3 × 10^5	1·2 × 10^3	> 99%
3·7 × 10^4	< 30	> 99%
1·5 × 10^6	5·3 × 10^5	65%
1·3 × 10^5	1·5 × 10^4	89%

* Of drained chicken from 1st immersion.

examined daily for growth of *C. jejuni*. Total viable counts per chicken were calculated from weighted colony counts/ml of immersion fluid. A negative result was given a probable value from the highest volume assessed showing the absence of *C. jejuni*, e.g. no growth from a 50 ml sample of 1·5 l immersion fluid would be recorded as < 30.

RESULTS

Surface contamination: 'fresh' oven-ready chickens

C. jejuni was isolated from 61% (11/18) of the fresh chickens. The organism was found on 6 (34%) breasts, 8 (44%) wings, 9 (50%) thighs and 10 (55%) vent cavities which clearly indicated a general surface distribution of *C. jejuni* on contaminated birds.

Whole chicken contamination

Assessment of immersion method. Preliminary tests were made with six fresh chickens immersed for up to 6 h and sampled (10 ml) hourly and with six immersed chickens drained of excess PBS and given a second immersion in fresh PBS. The results established that immersion for 1–4 h removed nearly all readily displaced surface *C. jejuni* (Tables 1 & 2).

All subsequent total viable *C. jejuni*/chicken estimates were derived from viable counts made after 3–4 h immersions except for frozen chickens. These required an extended time of about 8 h at room temperature or 3–4 h following an overnight immersion at 4 °C to equilibrate to room temperature.

Table 3. *Fresh chickens: isolation of* Campylobacter jejuni *from PBS immersion fluid*

No. of chickens	Producer	No. positive	No. *C. jejuni*/chicken	
			Mean	Range
1	1618	1	6.1×10^4	—
11	S	5	1.2×10^4	< 30–8.2×10^4
2	D	0	< 200	—
4	E	4	4.6×10^5	3.7×10^4–1.5×10^6
6	J	2	8.1×10^3	< 30–4.0×10^4
12	R	0	< 150	—
1	A	1	3.7×10^4	—
3	N	3	4.7×10^4	3.4×10^4–5.7×10^4
6	V	6	3.8×10^5	3.3×10^4–4.9×10^5

Table 4. *Uneviscerated chickens: isolation of* Campylobacter jejuni *from PBS immersion fluid*

Chicken no.	Retailer	Producer	Vent. camp	Immersion total camp
1	B	W	+	1.6×10^5
2	B	W	+	3.7×10^5
3	O	K	+	2.4×10^7
4	O	K	+	2.3×10^7
5	M	H	+	2.8×10^5
6	M	H	+	1.6×10^6
7	M	I	+	4.7×10^6
8	M	I	+	4.7×10^5
9	M	I	+	2.4×10^6
10	M	I	+	1.2×10^6
11	B	W	+ + +	1.5×10^6
12	B	W	+ + +	6.2×10^5

Total 6.0×10^7
Mean 5.0×10^6
Range 1.6×10^3–2.4×10^7

Fresh chickens

C. jejuni was isolated from 48% (22/46) of vent cavity swabs from the fresh chickens and viable counts were obtained from 48% (22/46) of their immersion fluids. The counts of *C. jejuni* per chicken varied widely ranging from 1.5×10^3 to 1.5×10^6. This variation occurred between retailers and also within the purchases from each retailer supplied by the same producer with one important exception. *C. jejuni* was not isolated (< 150/chicken) from immersion fluids or vent cavity swabs of 12 chickens from one producer, R, who supplied one national retailer (Table 3).

Uneviscerated chickens

A 100% (12/12) isolation rate from both vent swabs and immersion counts was obtained from uneviscerated (New York) chickens from four retailers. The counts of *C. jejuni* per chicken ranged from 1.6×10^3 to 2.4×10^7 with a mean of 5×10^6 (Table 4).

Table 5. *Campylobacter serogroups from chicken, 1985–1986*

Penner	Lior 1	2	6	7	8	9	11	17	21	NT	Total	(%)
1	—	—	—	1	—	—	—	—	—	—	1	2
2	1	—	—	—	—	—	—	—	—	2	3	6·1
4	4	—	—	—	—	—	—	2	—	1	7	14
5	—	—	—	—	—	1	—	—	—	—	1	2
6	1	—	—	—	—	—	1	—	—	—	2	4·1
6,7	—	—	1	—	—	—	—	—	—	—	1	2
9	—	—	—	—	—	3	1	—	—	—	4	8
9,37	—	—	—	1	—	1	—	—	—	—	2	4·1
11	—	—	—	—	—	—	—	—	1	—	1	2
21	1	1	—	—	—	—	—	—	—	3	5	10·2
31	1	—	—	—	—	—	—	—	—	3	4	8·2
35	—	—	—	—	—	—	—	—	—	1	1	2
40	1	—	—	—	—	—	—	—	—	1	2	4·1
55	—	—	—	—	1	—	—	—	—	2	3	6·1
60	—	—	—	—	—	—	—	—	—	1	1	2
NT	1	1	—	—	—	1	5	—	—	3	11	22·4
Total	10	2	1	2	1	6	7	2	1	17	49	
(%)	20·4	4·1	2	4·1	2	12·2	14·2	4·1	2	34·6		

Frozen chickens

Only 1 out of 24 (4%) frozen chickens from 2 retailers representing 6 producers yielded a countable number of viable *C. jejuni* and this was low at 350 per whole chicken.

Serogrouping

The serogrouping results, excluding minor cross-reactions, of 49 strains isolated from chickens during the 1985–6 trial periods and of 108 strains from sporadic human cases of enteritis occurring during these periods are shown in Tables 5 & 6. A comparison of the rate of isolation of the various serogroups from both sources showed clearly that Lior serogroup 1 occurred most frequently in human (31%) and chicken (20·4%) and usually exhibited an antigenic association with Penner serogroup 4. Lior serogroup 11 was the second most frequent type from chickens (14·2%), but this was not similarly reflected from human cases, in which no other distinct Lior serogroup predominated. After a 26% incidence of Penner 4 in humans, Penner serogroups 1 and 2, both having a 14% incidence, were followed by similar lower rates of isolation for the remaining 15 serogroups found. In contrast although Penner 4 (14%) was also the most frequent to occur in chickens three others, 9 (8·2%), 21 (10·2%) and 31 (8%) were also well represented.

Salmonellas

Salmonella species were isolated from less than 3% (2/69) of the fresh chickens examined.

Table 6. *Campylobacter serogroups from Southampton human cases, 1985–1986*

Penner	|— Lior —| 1	2	5	6	7	8	9	11	17	20	21	23	NT	Totals	(%)	UK National* human strains (%)	Southampton:† chicken strains (present study)
1	10	—	—	—	—	—	—	—	—	—	—	—	1	15	14	5·3	2
2	—	4	—	—	—	1	—	—	—	—	—	2	11	15	14	13·4	6·1
4	20	—	—	—	1	—	—	—	1	—	—	—	6	28	26	13·5	14
5	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0	5·8	2
6	—	—	—	2	—	—	—	—	—	—	—	—	—	2	2	—	4·1
6,7	—	—	—	—	—	—	—	—	—	1	—	—	—	0	0	3·7	2
8	—	—	—	—	1	1	—	—	—	—	—	—	2	4	4	7·7	—
9	1	—	—	—	1	1	—	—	—	—	—	—	2	5	5	3·0	8
9,37	1	—	—	—	—	—	—	1	—	—	—	—	1	3	3	—	—
10	—	—	—	1	—	—	—	—	—	—	—	—	—	1	1	1·0	—
11	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	3·5	2
16	—	—	—	—	—	—	—	—	1	—	—	—	—	1	1	3·2	—
18	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	1·0	—
19	1	—	1	1	1	—	—	1	1	—	—	—	2	5	5	1·1	—
21	—	1	—	—	—	—	—	—	—	—	—	—	3	7	6	1·4	10·2
23	—	—	—	—	—	—	—	—	—	—	1	—	1	2	2	1·4	—
24	—	—	—	—	—	—	—	—	—	—	1	—	1	2	2	1·0	—
27	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1·4	—
31	—	—	—	—	—	—	—	1	—	—	—	—	1	2	2	1·8	8·2
35	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
39	—	1	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—
57	—	—	—	—	—	—	—	—	1	—	—	—	—	1	1	—	—
NT	—	—	—	—	—	2	—	—	1	3	1	—	4	11	10	6·4	22·4
Totals	33	7	1	3	4	6	0	2	4	4	3	2	39	108			
(%)	31	6	1	3	4	6	0	2	4	4	3	2	36				
*	28·8	8·4	1·2	3·7	4·2	2·8	2·1	8·1	1·9	—	—	—	35				
†	20·4	4·1	—	2	4·1	2	—	14·2	4·1	—	—	—	34·6				

* Jones, Sutcliffe & Abbott (1985).

DISCUSSION

Viable *C. jejuni* have previously been reported in the caeca of 72% of chicken retailed in the UK (Simmons & Gibbs, 1979). The present investigation was undertaken to establish the potential threat of infection by *C. jejuni* posed by its presence on retailed chickens purchased in the Southampton area.

We showed extensive contamination with *C. jejuni* in about half of the 'fresh' chickens for sale in large and small retailers. The organism was not confined to the cloacal region of the chickens, but was well dispersed over the surface. The surface contamination varied from about 10^3 to 10^7 organisms/chicken. Thus with a typical 1·5 kg chicken having a surface area of about 1000 cm², a two-handed grasp could result in a hand pick-up of a large porportion of the total viable *C. jejuni* available. Further distribution of the organism would depend mainly upon the standard of hygiene adopted by the food handler. Contamination of the water tap knob is almost inevitable and could provide a source of infection after hand washing.

It is of interest to note the absence or low carriage of *C. jejuni* in the frozen chickens examined. Whole chicken consumption per annum has remained at about 250 000 tons for the last 6 years, 1981–6, but the proportion of fresh whole chicken consumed has steadily increased to about 107 000 tons – 32% higher than it was in 1981 (British Chicken Information Service, Bury House, 126–128 Cromwell Rd, London SW7 4ET). The rising incidence of enteritis due to campylobacter during this period (PHLS Communicable Disease Centre, 1987) could well be a reflection of the increasing proportion of fresh chickens, especially uneviscerated ones, consumed and handled (Fig. 1).

The frequency distributions of Penner serogroups previously found in chicken strains of *C. jejuni* from the UK (Jones *et al.* 1984) and those found in the present chickens are in general agreement. They are also very similar to the Penner serogroups from sporadic cases of enteritis over a wide area of the UK (Jones, Sutcliffe & Abbott, 1985) and the present isolates from the Southampton area (Table 6). There are two notable exceptions: serogroup 21 was found to be high in incidence in Southampton chickens (10·2%) and humans (6%), but low (2%) in chickens and humans (2%) previously reported in the UK (Jones *et al.* 1984; Jones, Sutcliffe & Abbott, 1985); the frequency of serogroup 31 was high (12%) in UK chickens (Jones *et al.* 1984) and Southampton chickens (8·2%), but low in general in UK human strains (1·8%) and in Southampton (2%) strains. The former suggests a direct link between human infections and local chickens and the latter indicates the possibility of a chicken strain of low virulence for humans.

Comparisons of frequency distribution of Lior serogroups show a close agreement between isolates from sporadic cases in the UK (Jones, Sutcliffe & Abbott, 1985) and Southampton and from chicken in Southampton (Table 6). Lior serogroup 11 was the only obvious difference in being low in Southampton cases (2%) but of a similar high incidence in UK humans (8·1%) and Southampton chickens (14·2%). It is important to note, however, that less than half of the Lior serogroups were of similar Penner serogroups indicating that only about half of the human isolates were the same as the chicken isolates.

It is also of interest to note that a calculation of the percentage frequencies of
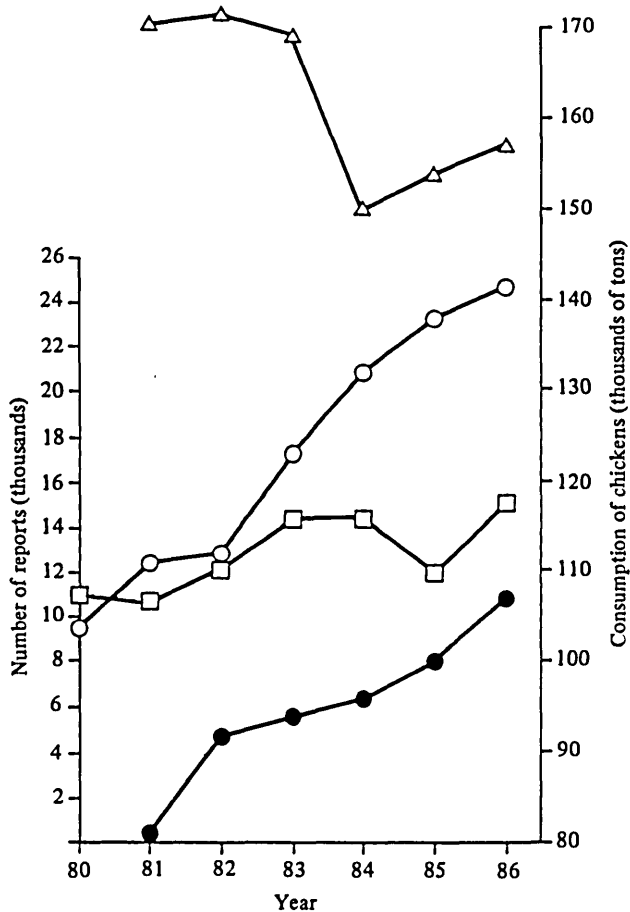
Fig. 1. Gastrointestinal infections 1980–6. Consumption of fresh and frozen chickens, 1981–6. △, consumption of frozen chickens; ○, human cases of campylobacter enteritis; □, human cases of salmonella; ●, consumption of fresh chickens.

isolation of Lior serogroups isolated from human cases from eight USA states (Patton *et al.* 1985) show a clear high incidence of serogroup 4 (17%) which is comparatively rare in the UK. It would be useful to determine whether this was common in chickens or other animal foods in those areas. In the UK there are now strong indications that retailed contaminated fresh chickens present a high potential source of infection and that this is often realized. Further evidence of this could now be more readily obtained by applying measures to reduce or eliminate this source by intervention at the farm (Pearson *et al.* in preparation) and looking for a consequent reduction in the number of sporadic cases of enteritis.

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