Epidemiol. Infect. (1993), **110**, 601–607

The colonization of broiler chickens with *Campylobacter jejuni*: some epidemiological investigations

T. J. HUMPHREY[1], A. HENLEY[1] AND D. G. LANNING[2]

[1] *Food unit, Public Health Laboratory, Church Lane, Exeter EX2 5AD, UK*
[2] *L M Agriculture, Willand, Cullompton, Devon EX15 2PJ*

SUMMARY

Between June 1990 and July 1991, broiler chickens from 49 flocks from 23 farms were examined for the carriage of *Campylobacter jejuni* at slaughter. Thirty-seven flocks (76%) were campylobacter-positive. Prevalence of campylobacter-colonization was not associated with any of a variety of factors such as water source and broiler house floor structure. There was also no apparent seasonal variation in carriage. Investigations on one farm indicated that dipping boots in disinfectant before workers entered broiler houses either delayed or prevented colonization with *C. jejuni*.

INTRODUCTION

Campylobacter jejuni is an internationally important food-borne human pathogen. While a variety of foods of animal origin have been implicated as vehicles of infection [1] contaminated chicken meat is probably the most important [2,3]. Live chickens are frequently colonized with *C. jejuni* and the intensive nature of poultry production can facilitate cross-contamination with the result that a high proportion of chicken carcases may be campylobacter-positive [4]. A reduction in the prevalence of carcase contamination would have clear benefits for the public health.

The route by which growing broiler chickens acquire *C. jejuni* is unclear. Suggestions have included contaminated drinking water [5], the poultry house environment [6] and other sources, including farm workers, cats, dogs, flies and rodents [1].

Humphrey and colleagues [7] demonstrated that it was possible to identify campylobacter-negative broiler flocks and that this appeared to be associated with the production of inhibitory metabolites by caecal microflora. A variety of environmental factors and management practices might contribute to controlling the campylobacter-status of chickens and it was decided to investigate this.

In a longitudinal study lasting approximately 12 months, the campylobacter status of broiler flocks was assessed by the examination of caeca collected from up to 100 birds per flock at slaughter. Investigations were also undertaken with growing broiler flocks. The campylobacter-status of the flocks in the study was investigated with respect to a variety of environmental and production factors.

MATERIALS AND METHODS

Production system

The collaborating broiler operation is of medium size, killing approximately 300000 birds per week. It has one hatchery, 15 broiler-breeder flocks and 41 broiler farms. The company is fully integrated and purchases breeding stock as day-old chicks. All feed is produced by the company at a separate mill.

Factors considered

The company keeps detailed records on all aspects of chicken production and it was possible to use these to examine the potential influences of a variety of factors (Table 1).

The impact of improved on-farm hygiene

In a separate study on one farm, the impact of improved hygiene on campylobacter colonization of growing broiler flocks was investigated. The farm comprises three broiler houses, each containing 35000 birds, and operates an 'all-in', 'all-out' policy. Each house is divided into two sections of equal size and these are separated by a central area in which water tanks, feed hoppers, etc. are situated. The houses are entered from the outside through doors situated at each end of this central area and there are also two internal doors to each section of the house. As part of normal company policy, farm staff were asked to dip their boots routinely in 1% phenolic disinfectant (Sterilite White Farm Disinfectant, Coventry Chemicals Limited). The disinfectant, 15–20 litres, was placed in containers in the central area by each of the four internal doors. During three separate flock cycles, the disinfectant concentration was increased to *c.* 3% and additional containers were placed outside the external doors of some houses. The disinfectant was changed at least once per week and often more frequently depending upon weather conditions and frequency of usage. The houses with additional and more concentrated disinfectant were identified and it became mandatory for staff to dip their boots in both the 'external' and 'internal' containers of disinfectant before entering these broiler houses. In the first and third trials, the middle house (no. 2) was used as the experimental house while, with the other two houses, normal company policy was observed. In the second trial, additional boot disinfection was made mandatory before entering houses nos. 1 and 3. Farm staff were regularly questioned and reminded about the need for 'boot-dipping' by research staff and company management. The houses on the farm were surrounded by concrete and were *c.* 6 m apart.

Sample collection and microbiological examination

The campylobacter-status of either broiler or breeder flocks at slaughter was established by culturing caecal contents collected at evisceration in the processing plant by company staff. Caeca were placed immediately into sterile plastic bags and usually stored at +4 °C for 2–24 h until microbiological examination. On a few occasions, pressure of work meant that there was a delay between collection and examination, and caeca were stored at −20 °C for up to 7 days.

When growing broiler flocks were investigated, samples were collected at house

Table 1. *Factors considered in a study of the epidemiology of* Campylobacter jejuni *in broiler chicken production*

Factors considered	No. of farms	No. of flocks	No. (%) campylobacter-positive flocks
Individual to farms			
Water source			
Mains	17	31	22 (71)
Borehole	6	18	13 (72)
Drinker type			
Bell	9	16	12 (75)
Cup	1	3	3 (100)
Both	13	30	20 (67)
Floor type			
Earth	9	15	10 (67)
Concrete	10	24	18 (75)
Both	5	10	7 (70)
Flock size			
< 20000	4	5	4 (80)
21–100000	12	23	16 (70)
> 100000	7	21	17 (81)
Other farming activities			
Yes	7	16	13 (81)
No	16	33	22 (67)
*Standards of farm hygiene**			
Good	13	26	16 (62)
Average	7	11	9 (82)
Poor	3	12	10 (83)
House surroundings			
Concrete	6	17	11 (65)
Earth	4	8	5 (63)
Mixed	13	24	19 (79)
Common to all farms			
Date of sampling		–	–
Identity/age of parent flock		–	–
Location		–	–

* Assessed by experienced company staff who considered aspects such as cleanliness of the farm environment and inside the broiler houses; frequency of grass cutting, particularly in the areas immediately around the broiler houses; adherence to pest control policies; attitudes, experience and interest of farm staff; cleanliness and scope of toilet and hand-washing facilities.

filling and at 2–3 day intervals thereafter until the birds became campylobacter-positive. On each visit to each farm up to 50 chicks were culled and 10 litter samples were collected from each broiler house.

With chicks up to 2 weeks of age, the entire intestine was removed, using aseptic techniques and examined for campylobacters. Samples were stored in the same way as caeca.

At intervals during the investigation, 25 g samples of either broiler feed, feed components or hatchery waste (egg shells, dead chicks and box liners) were collected and examined for campylobacters [8].

Caeca were placed on clean, disposable paper towels on removal from either frozen or refrigerated storage. Part of the surface of each caecum was sterilized using a red hot scalpel blade which was then used to incise the wall of the organ. A sterile cotton-wool swab of the contents was then cultured for campylobacters [8].

The outer surfaces of the intestines from young chicks up to 2 weeks of age were sterilized by flaming in industrial methylated spirits. The tissue was macerated with 9–10 volumes of campylobacter-selective broth and then cultured. Feed samples (25 g) were added to 225 ml broth and examined for campylobacter using similar techniques.

Campylobacter isolates were confirmed as *C. jejuni* by either biochemical tests or using a latex agglutination test (Mercia).

Survival of campylobacter in poultry feed

An overnight culture (43 °C in Robertson's cooked meat medium) was diluted to 10^{-4} in BPW. Volumes, 0·1 ml, were added to 10 g samples of unmedicated broiler feed in 25 ml screw-capped sterile bottles. The initial inoculum was approximately 10^3 cells of *C. jejuni* per gram of feed. The inoculated feed was mixed by shaking and stored at 20 °C for 48 h. At intervals, 10 bottles were removed and the feed from each added to 90 ml campylobacter-selective broth. The number of viable *C. jejuni* was estimated using an MPN technique capable of detecting one cell in 10 g of feed [8].

RESULTS

The prevalence of campylobacter-positive flocks

Forty-nine broiler flocks from 23 farms were examined for campylobacter-colonization. Nine separate farms contributed three or more flocks. Thirty-seven flocks (76%) were campylobacter-positive. Mean prevalence of colonization in positive flocks was 50%. In 13, the proportion of campylobacter-positive birds exceeded 75% and in 5 it was less than 10%. All isolates were *C. jejuni*.

None of the factors listed in Table 1 influenced either the presence of *C. jejuni* in a broiler flock or the number of birds carrying the bacterium. For instance, birds in houses with concrete floors receiving chlorinated mains water were as likely to be colonized with campylobacter as birds in houses with earth floors supplied with water from a bore hole. There was also no significant seasonal variation in campylobacter carriage either in the prevalence of positive flocks or the proportion of birds in these flocks colonized with *C. jejuni*.

While the majority of broiler flocks became campylobacter-positive by the time they reached slaughter weight, 12 flocks remained uncolonized. These were not associated with any particular farms and this meant that successive flocks on some farms were not necessarily colonized. Thus on one farm, flocks tested on 19 August and 19 June carried *C. jejuni* whereas the bacterium was not isolated from caeca taken from birds killed on either 19 February or 19 April. A similar pattern was observed on other farms.

In addition to the investigation with broiler flocks, 10 broiler-breeder flocks were each sampled once at slaughter. All were positive for *C. jejuni*. Mean prevalence of colonized birds was 72% with a range of 62–100% of those sampled.

Development of campylobacter colonization in young broiler flocks

With six separate broiler flocks, from three different farms, attempts were made to try and determine the time at which birds became campylobacter-positive. *C. jejuni* was not isolated from any chicks either at house filling or during the first 5 days of life. One hundred and twenty samples of hatchery waste were also campylobacter-negative. Birds in all six flocks became colonized with *C. jejuni*, however, by the time they were 3-weeks old. Mean time for the prevalence to reach 5% was 9 days with a range of 6–18 days. No litter samples were campylobacter-positive until *C. jejuni* had been detected in the chicks.

Survival of Campylobacter jejuni *in broiler feed*

One hundred and forty-six 25 g samples of either finished broiler feed or feed ingredients were examined for the presence of campylobacters. All samples were campylobacter-negative.

Death rates of *C. jejuni* added to feed and stored at *c.* 20 °C appeared to be rapid. Thus, although the bacterium could still be detected in the majority of samples within 3 h of inoculation, by 24 h the population of culturable cells had fallen from 1×10^3/g to undetectable levels ($< 1/10$ g).

Impact of improved on-farm hygiene

Farm workers on one broiler farm, with houses with concrete floors and a bore hole water supply, were asked to dip their boots in strong phenolic disinfectant before entering either 1 or 2 of the 3 houses on the farm. This was the only potential intervention measure in operation on the farm at that time.

'Boot dipping' influenced the time at which the chickens acquired *C. jejuni*. In all three trials, birds in the control houses, where boots may not always have been dipped, became campylobacter-positive 7–10 days after hatching. In the first trial, birds in the houses where boot-dipping was mandatory remained uncolonized until slaughter. In trials two and three, colonization was delayed by 2 and 3 weeks respectively.

DISCUSSION

The economics of the United Kingdom poultry industry are finely balanced and modern poultry slaughter lines are designed to function at speeds which largely preclude prevention or reduction of contamination. For this reason, control of *C. jejuni*, and other poultry-associated human pathogens, may be more successful on the farm. An important prerequisite for this is to identify factors which may influence colonization. Results presented in this paper suggest that no one factor is important on its own (Table 1) and this may present difficulties in the formulation of intervention measures. It is clear, however, that although *C. jejuni* has been isolated from egg shells [9], vertical transmission from colonized breeder flocks is unlikely. Hatchery waste samples were campylobacter-negative and there was a delay of up to 3 weeks before chicks became campylobacter-positive. No birds were found to be carrying *C. jejuni* until they were at least 6 days old.

For reasons as yet unexplained some flocks of broiler chickens remain negative for *C. jejuni*, and other campylobacters, during the rearing period. This was not associated with any of the factors investigated (Table 1) and such flocks may

either precede or be followed by flocks infected with campylobacters. This phenomenon may be associated with anti-campylobacter metabolites produced by certain caecal micro-organisms [7, 10] and this is to be investigated further.

The rapid death rate of *C. jejuni* in broiler feed suggests that contaminated feedstuffs are unlikely to be important in introducing campylobacters into broiler flocks. Thus the treatment of feed with organic acids, which can be a successful measure against salmonellas [11, 12], may have no impact on campylobacters.

Boot-dipping in phenolic disinfectant appeared to be effective in either preventing or delaying the colonization of chickens with *C. jejuni*. This may indicate that the environment is an important source of *C. jejuni*. Once it appears in flocks, horizontal spread can be rapid. Results presented here, and in other studies [13], suggest that improvements in on-farm hygiene may either limit or prevent this. Research in the Netherlands [13] has demonstrated that effective cleaning and disinfection following flock clearance, coupled with such measures as boot-dipping, a hygiene barrier and separate shoes to be worn in the broiler house prevented campylobacter-colonization on a farm where previous flocks had been positive for *C. jejuni*. Similar work in Sweden (Engstrom, personal communication) has also shown that the above measures can prevent, in the long term, the colonization of broiler flocks with campylobacters.

Such measures are relatively inexpensive when compared to modification of slaughter techniques and should be investigated further.

ACKNOWLEDGEMENTS

We are grateful to the staff, particularly Julian Anderson and Helen Collier, and directors of Lloyd Maunders Ltd for their unfailing help and courtesy and to Mrs P. Goodman for her typing of the manuscript. The financial assistance from MAFF and the PHLS is gratefully acknowledged.

REFERENCES

1. Blaser MJ, Taylor DN, Feldman RA. Epidemiology of *Campylobacter jejuni* infections. Epidemiol Rev 1983; **5**: 157–75.
2. El-Shenawy MA, March EH. *Campylobacter jejuni* and foodborne campylobacterosis: a review. Egypt J Dairy Sci 1989; **17**: 181–200.
3. Oosterom J, den Uyl CH, Bänffer JRJ, Huisman J. Epidemiological investigations on *Campylobacter jejuni* in households with primary infection. J Hyg 1984; **92**: 325–32.
4. Aho M, Hirn J. Prevalence of campylobacteria in the Finnish broiler chicken chain from the product to the consumer. Acta Vet Scand 1988; **29**: 451–62.
5. Pearson AD, Colwell RR, Rollins DM, et al. Transmission of campylobacter on a poultry farm. In: Kaijser B, Falsen E, eds. Proceedings of the 4th International Conference on Campylobacter. Göteborg, Sweden, 1987.
6. Kazwala RR, Collins JD, Hannan J, et al. Factors responsible for the introduction and spread of *Campylobacter jejuni* infection in commercial poultry production. Vet Rec 1990; **126**: 385–91.
7. Humphrey TJ, Lanning DG, Mead GC, Inhibition of *Campylobacter jejuni* in vitro by broiler chicken caecal contents. Vet Rec 1989; **125**: 272–3.
8. Humphrey TJ. Techniques for the optimum recovery of cold injured *Campylobacter jejuni* from milk or water. J Appl Bacteriol 1986; **61**: 125–32.
9. Doyle MP. Association of *Campylobacter jejuni* with laying hens and eggs. Appl Environ Microbiol 1984; **47**: 533–6.

10. Schonei JL, Doyle MP. Reduction of *Campylobacter jejuni* colonisation of chicks by caecum-colonising bacteria producing anti-*C. jejuni* metabolites. Appl Environ Microbiol 1992: **58**: 664–70.
11. Humphrey TJ, Lanning DG. The vertical transmission of salmonellas and formic acid treatment of chicken feed: a possible strategy for control. Epidemiol Infect 1988: **58**: 43–9.
12. Hinton M, Linton AH, Perry FG. Control of salmonella infections in broiler chickens by the acid treatment of their feed. Vet Rec 1988: **123**: 416–21.
13. Van de Giessen A, Mazurier SI, Jacobs-Reitsma W, et al. Study on the epidemiology and control of *Campylobacter jejuni* in poultry broiler flocks. Appl Environ Microbiol 1992: **58**: 1913–17.