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Colonization of Broiler Chickens by Waterborne *Campylobacter jejuni*

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Chickens on a broiler farm in southern England were found to be colonized with *Campylobacter jejuni* of a single serotype, Lior 1 Penner 4. The farm was the sole supplier of a local slaughterhouse associated with a campylobacter outbreak in 1984 caused by this serotype. The serotype persisted on the farm for at least 18 months after the outbreak; its prevalence in the human population served by the farm remained high until it disappeared from the farm in 1986. The possible sources and routes of transmission of *C. jejuni* to the broilers on the farm were investigated. The results showed that vertical transmission, feed, litter, small mammals, and environmental or airborne cross-contamination between sheds or successive crops could be excluded as persistent sources of *C. jejuni*. The predominant source of *C. jejuni* on the farm was shown to be the water supply. Direct microscopy and fluorescent antibody methods revealed presumptive campylobacters throughout the farm's water system. Campylobacter-free chickens raised in an animal house and given water from the farm supply became colonized with the serotype of *C. jejuni* endemic on the farm (Lior 1 Penner 4). An intervention program based on water chlorination, shed drinking system cleaning and disinfection, and withdrawal of furazolidone from feed reduced the proportion of birds colonized with campylobacter from 81 to 7% and was associated with a 1,000- to 10,000-fold reduction in campylobacters recoverable from the carcasses. Two months after the end of the intervention program colonization of the birds returned to high levels (84%), indicating that there was a temporal association between intervention and reduced colonization with *C. jejuni*. Investigations continue to establish the general applicability of these findings.

The consumption of fresh chicken has been associated epidemiologically with outbreaks of gastroenteritis due to *Campylobacter jejuni* both in the United Kingdom and in the United States (2, 5, 10). Chicken is the second most common food item associated with outbreaks of campylobacter infections in England and Wales according to Communicable Disease Surveillance Centre data. Most reported cases of enteritis due to campylobacter appear to be sporadic. There is a considerable body of evidence which suggests a link between such cases and the handling of, or cross-contamination from, poultry. First, chicken is much more frequently contaminated with campylobacters than red meats are (3, 10, 31); second, the level of contamination is often high (13); third, several studies in which discriminatory typing methods were used have shown a close correspondence between the strains of campylobacters found in human infections and those found in chickens (16, 20, 23); and finally, an association between poultry and sporadic campylobacter infections has been shown by a number of studies (7, 10, 14).

The present study was undertaken as a result of an outbreak of *C. jejuni* infections in Bournemouth, United Kingdom, that began in November 1984 and was shown to have been caused by *C. jejuni* serotype Lior 1 Penner 4 (complex 4, 13, 16, 50) Lior biotype II (designated L1 P4). The outbreak was associated with a catering college which was supplied with fresh chicken by a single wholesaler (wholesaler A), who obtained all of the chicken that it distributed from a single farm. This farm and its immediate surroundings were investigated to determine the source(s) of the organisms colonizing the chickens. Evidence was obtained that poultry from the farm caused sporadic human campylobacteriosis in the population served by the farm for at least 18 months after the recognition of the catering college outbreak. A report on this investigation is being prepared for publication elsewhere.

MATERIALS AND METHODS

The farm. There were 18 poultry sheds on the farm on two adjacent sites (sites 1 and 19). The sheds were built of wood on concrete floors. Each shed had a crude climate control system and separate water and feed supply systems (Fig. 1). Only birds grown on site 1 and in two of the nine sheds on site 19 were supplied to wholesaler A. Birds grown in the other sheds on site 19 were sent to a different slaughterhouse.

Each of the sheds supplying wholesaler A contained about 5,000 birds. Approximately 1,250 birds were sent daily to the slaughterhouse, a different shed being emptied each week. The birds in a single crop usually came from one hatchery (not always the same one for each crop) but were sometimes drawn from more than one hatchery. Each hatchery was

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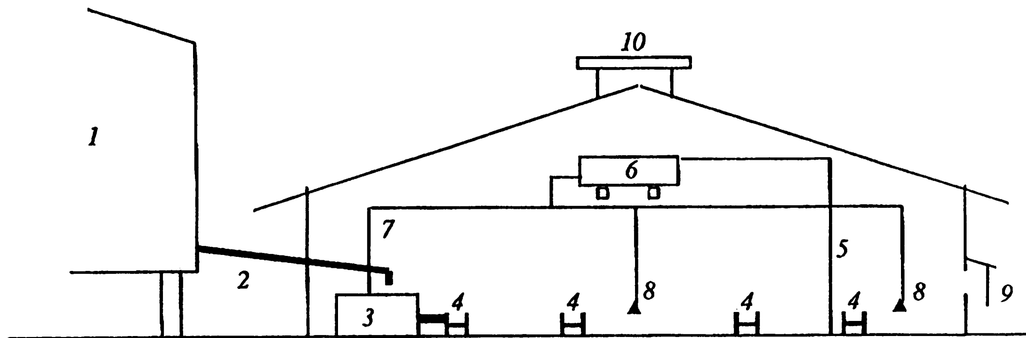

FIG. 1. Diagram of chicken shed, showing climate control and feed and water supply systems (not to scale). 1, Feed silo; 2, feed auger; 3, feed bin; 4, feed tracks; 5, rising main (in shed lobby); 6, header tank (12 gallons [ca. 55 liters]); 7, drinker line; 8, drinkers; 9, air inlet; 10, air extractor fan.

supplied by several laying flocks, and therefore the chicks supplied to the farm came from several sources.

After each shed was emptied, the drinkers and feed distribution tracks were dismantled and removed from the shed. The spent litter was removed mechanically, and the shed and fitments were washed with a high-pressure washer; Microsol disinfectant (cresylic acid [Micro-Biologicals Ltd., Hampshire, United Kingdom]) was added to the water at a rate of 30 ml/liter. Fresh litter (wood shavings) was then spread, the fitments were replaced, and the shed was treated with formaldehyde-glutaraldehyde vapor pumped into the shed via a Swingfog model SN11 vaporizer (Motan GMBH, Isry, Germany).

The birds were fed dried mixes (Nitrovit Ltd., Yorkshire, United Kingdom) as follows: B510 starter crumbs for the first 10 days of life, B511 grower pellets for the next 18 days, and B512 finisher pellets until the end of the crop (day 49)). Virginiamycin was added to the feed mixes at a concentration of 20 mg/kg in the starter crumbs and grower pellets and at a concentration of 5 mg/kg in the finisher pellets as a growth promoter, and salinomycin was added at a concentration of 60 mg/kg to all of the mixes as a coccidiostat. The nitrofuran antibiotic furazolidone was added to the starter crumbs at a concentration of 0.02% and to the finisher pellets between days 28 and 35 at a concentration of 0.03% as a prophylactic to prevent infections with *Escherichia coli*. The feed was delivered to the farm in trucks, from which it was blown into silos outside the sheds. From the silos it was moved on demand into bins in the sheds by auger systems and distributed around the sheds by a metal belt system running in tracks (Fig. 1). The feed bin in each shed was open topped.

The source of the water used on the farm was a small-bore well (borehole) sunk to a depth of 30 m. A pump at the top of the borehole was operated twice daily to lift water from a depth of 17 m in the borehole and pump it through a pipe (diameter, 2 in. [ca. 5 cm]) to a 12,500-gallon (ca. 56,500-liter) field reservoir located approximately 1 mile (1.609 km) up a hill. During pumping the water was supplied directly to the sites, but when the pump was not running, the water flowed by gravity through the same 2-in. pipe from the field reservoir to both farm sites, numerous cattle troughs, and eight residences.

The automatic chlorination system for the water supply had broken down, and it was the responsibility of a local resident to pour 0.75 pint (ca. 400 ml) of a concentrated sodium hypochlorite solution into the borehole once a week. Monitoring of chlorine levels in water taken directly from the borehole pump bleed valve revealed free chlorine levels exceeding 4 ppm 5 min after such chlorination, but after 90 min of pumping, chlorine was no longer detectable. Tests in the field reservoir showed levels of 0.4 ppm of free chlorine (0.6 ppm of bound chlorine) 3 h after the addition of the hypochlorite to the borehole, but after 48 h neither free nor bound chlorine could be detected.

Each shed on the farm had a 12-gallon (ca. 55-liter) header tank mounted near the roof (Fig. 1). On site 1 these tanks were fed directly from the common water distribution system, but on site 19 they were fed from an intermediate 500-gallon (ca. 2,275-liter) tank on the site. The water entered each shed via a rising main which fed into the header tank via a float valve which prevented backflow. The water was then distributed around each shed in 19-mm galvanized iron drinker lines (usually three per shed). Bell type poultry drinkers with demand valves were connected to the drinker lines via plastic tubes.

The water system could be divided broadly into two parts, which were subjected to rather different physical conditions. The first part, called the source in this paper, comprised those parts of the water system which were mainly outside the chicken sheds and were therefore not influenced by the shed environment. This included the borehole, the field reservoir, and the rising mains where the water entered the shed (these rising mains were in the shed entrance lobbies). The second part, called the supply, included those parts of the system which were within the sheds and were subject to the physical conditions in the sheds (dust, raised temperature, ammonia in the air). These included the header tanks, drinker lines, and drinkers (Fig. 1).

Detection of campylobacter. (i) Culture. The campylobacter isolations were performed on Preston medium (3) or on VPT medium (28). The enrichment procedure and broth used were based on the procedure and formulation of Humphrey (15) (without adjustment of pH and without cephalosporin). Inoculated broth cultures were incubated at 43°C for 20 to 24 h and then subcultured onto Preston medium. After inoculation, plates were incubated microaerobically at 43°C for up to 6 days. The plates were examined daily, and suspect colonies were subcultured, Gram stained, and tested for cytochrome oxidase production and the absence of aerobic growth. Strains identified as campylobacter isolates were sent to the Manchester Public Health Laboratory for serotyping by the Lior and Penner methods (18, 24).

(ii) IFA testing. Five strains of *C. jejuni* were used for the

TABLE 1. Campylobacter strains used in the fluorescent antibody tests

Strain	Source	Isolation source	Type[a]	Comment
BS	R. E. Black, Center for Vaccine Development, Baltimore, Md.	Laboratory	27	Swarmer variant
E8	A. L. Bourgeois, Naval and Medical Research Unit III, Cairo, Egypt	Human child	07	Diarrheic stool
HC		Human male	07	Blood
F^-	D. G. Newell, Central Veterinary Laboratory, Weybridge, England	Laboratory	NT[b]	Flagellated variant
M^+	D. G. Newell, Central Veterinary Laboratory, Weybridge, England	Laboratory	06	Aflagellate variant

[a] Penner serotype.
[b] NT, nontypeable.

indirect fluorescent-antibody (IFA) tests (Table 1). The strains were biotyped by using a battery of tests (26, 29) and were serotyped by the Penner method (24). The strains were grown on agar plates, harvested, and washed twice in 0.85% NaCl. The cultures were adjusted turbidimetrically to an optical density at 540 nm of 1.0 in either 0.5% formaldehyde or saline. The saline-suspended cells were placed in a boiling water bath for 20 min. Intravenous, intramuscular, and subcutaneous injections of both the formalinized and heat-killed whole-cell antigen preparations were used to raise anti-campylobacter sera in adult New Zealand White rabbits (Hazleton Dutchland, Inc., Dever, Pa.). Serum was collected and titrated by an immunoglobulin G, antibody capture, enzyme-linked immunosorbent assay. The sera were pooled and tested against a variety of strains of *C. jejuni* and *Campylobacter coli* available at the University of Maryland.

For the detection of campylobacter cells in water, samples were filtered through 0.2-μm-pore-size polycarbonate membrane filters (Nuclepore, Pleasanton, Calif.) previously stained in a 0.02% irgalan black solution (6, 12). These preparations were moist heat fixed at 56°C for 30 min. For solid, semisolid, or highly turbid samples, when filtration was not possible, a suspension of sample prepared in phosphate-buffered saline (PBS) was smeared on a glass slide, air dried, and fixed with 95% ethanol prior to IFA staining.

The samples were then incubated successively in a moist chamber at 37°C for 30 min with fluorescent antibody rhodamine (Sigma Chemical Co., St. Louis, Mo.), an appropriate dilution of the pooled anti-campylobacter hyperimmune serum, and fluorescein-labelled goat anti-rabbit immunoglobulin G (Difco Laboratories). The filters or smears were examined for cellular fluorescence by epifluorescent microscopy. At least 50 fields per sample were examined. Samples containing large numbers of definite campylobacter cells, either clumped or singly, were graded 3+; samples containing definite campylobacter cells in moderate numbers, either clumped or singly, were graded 2+; and samples containing presumptive campylobacter cells with vibrioid or helical morphology in low to moderate numbers were graded 1+. Samples not containing fluorescent campylobacter cells were graded 0. Replicate samples were examined by two independent observers who were blind to the nature and potential significance of the sampling program.

Examination of water samples. Samples of water were taken from different points in the water system, including the borehole, reservoir, rising mains, header tanks, drinker lines, and drinkers. These water samples were collected in sterile 10-liter plastic containers and stored at 4°C. The effluent from the slaughterhouse was discharged into a river which, several miles (1 mile = 1.609 km) downstream, flowed close to the borehole. Water samples (300 ml) were collected from five points on this river (above and below the slaughterhouse discharge point, just below the borehole, and at two points between the slaughterhouse and borehole).

All of the water samples were treated in the same way. Volumes of 100 to 500 ml were filtered through 0.45- or 0.22-μm pore-size, 47-mm-diameter, cellulose nitrate membrane filters (Sartorius 47ACN). Each filter was placed face down on a fresh Preston or VPT agar plate, incubated microaerobically at 43°C for 24 h, and then removed from the plate and placed face down on another Preston or VPT plate. Both plates were then incubated microaerobically at 43°C for up to 5 days. In addition, 10 ml of water was added to 10 ml of double-strength campylobacter enrichment broth; this preparation was incubated at 43°C and then subcultured onto Preston medium. Water samples were investigated further by the fluorescent antibody test, using either polyvalent or absorbed typing sera (see above) (26a). The water samples for fluorescent-antibody testing were packed in insulated crates after chilling to 4°C and were sent by air freight to the University of Maryland with a maximum transit time of 14 h. Serotype-specific, absorbed sera were used to examine identical water samples that had been collected chronologically from a single point in the system in order to study any changes that were occurring in the serotypes in the supply over time.

A decreased substrate concentration had been shown to enhance metabolic activity in nonculturable campylobacter cells from streamwater microcosms (25a). Additional tests were performed on the water samples to maximize the recovery of campylobacters. These tests were done in media which were prepared at various dilutions to provide decreased nutrient concentrations in an attempt to approximate more closely the oligotrophic conditions of the drinking water. In addition, to avoid temperature shock to the cells, cultures were first incubated at ambient temperature, and then there was a gradual increase to the conventional culture temperatures of 37 and 43°C. For these tests 10-ml volumes of water were added to 10-ml volumes of each of the different dilutions of broth.

At the slaughterhouse the chicken carcasses were cooled by immersion in large water tanks (100 to 300 gallons [ca. 455 to 1,364 liters]); samples of water from these tanks were tested for campylobacters by direct plating onto selective media and by IFA microscopy. When levels of campylobacters were expected to be very low, a multiple-tube culture method in enrichment broth was employed, and levels were derived from most-probable-number tables.

Isolation of campylobacters from chickens. (i) Colonization of birds from the farm. Cloacal swab samples were taken

TABLE 2. Colonization of broilers in the feeding experiment[a]

Group	Part 1							Part 2							
	Furazolidone present in starter crumbs	No. of birds	No. of birds positive on:					Furazolidone present in feed	No. of birds	No. of birds positive on:					
			Day 1	Day 12	Day 18	Day 31			Day 31	Day 36	Day 37	Day 50	Day 57	Day 64
A	No	15	0	0	0	0	Yes	4	0	0	0	0	3	4
							No	4	0	0	0	2	4	
B	No	15	0	2	0	0	Yes	4	0	0	1			
							No	4	0	4				
C	No	15	0	0	0	0								
D	Yes	20	0	1	20	20								

[a] Broilers were kept in an animal house, fed a diet with or without furazolidone, and supplied with farm water that was either not treated (group A), autoclaved (group C), or autoclaved and seeded with *C. jejuni* (groups B and D). On day 31 the birds in groups C and D were slaughtered, and furazolidone was added to the diet of one-half of the remaining birds in groups A and B.

from 0.5 to 2% of the birds from a single shed (25 to 100 birds) each week from May 1986 to March 1987 when they entered the slaughterhouse. The swab samples were taken after the birds had been stunned and killed, but before they were immersed in the scald tank. The samples were usually taken at the slaughterhouse to minimize disturbance to the birds in the sheds, but on some occasions, chickens were caught at random in the appropriate shed, and a cloacal swab sample was obtained. The swab samples were plated directly onto selective medium at the time of collection.

(ii) Vertical transmission. A total of 650 fertile (candle-clear) eggs from the hatchery were opened aseptically, and samples of the contents were plated onto Preston medium and incubated as described above. In addition, cloacal swab samples were taken from 230 24-h-old chicks and cultured.

(iii) Processed chickens. The numbers of organisms on processed broilers (both eviscerated and non-eviscerated) were determined by immersion in PBS, using the method described by Hood et al. (13). If numbers were expected to be low, 10-, 1-, and 0.1-ml aliquots of the immersion fluid were inoculated into double- and single-strength enrichment broths. The number of broths from which campylobacters were grown was used in conjunction with most-probable-number tables to obtain the numbers of campylobacters recovered from each bird.

Isolation of campylobacters from feed and the environment. (i) Feed. Samples of fresh feed were taken from the feed bins in the sheds. Feed that had been exposed to the birds was sampled from the feed distribution track. Subsamples of 10 g of material were inoculated into 90 ml of enrichment broth, antibiotic supplement was added either immediately or after 3 h, and the samples were incubated at 43°C. The broth media were subcultured onto selective media after incubation for 24 h.

(ii) Litter. Samples of litter were taken from unopened (i.e., unexposed) bales of litter and from the floors of the housing units at intervals during the 49-day periods when the sheds were occupied by chickens. The litter samples were treated in the same manner as the feed samples.

(iii) Air. Air samples were taken in the sheds with a Pool Bioanalysis Italiana Surface Air System sampler (Cherwell Laboratories Ltd., Bicester, United Kingdom). Volumes of 60 and 900 liters were sampled from different sheds, and the samples were incubated on VPT plates.

(iv) Wildlife. Small mammals (rodents and insectivores) were trapped in the vicinity of the poultry sheds or in the shed lobbies in Longworth live traps (Longworth Scientific Instrument Co., Abingdon, United Kingdom) or in break-back traps. They were transported to the laboratory, killed by cervical dislocation (if necessary), and dissected aseptically. Samples taken from the ileum and colon were macerated in 0.25 ml of sterile PBS until a homogenate was obtained. This homogenate was plated directly onto Preston medium, and the preparations were incubated microaerobically at 43°C for up to 6 days (11). In addition, the spleens of some of the animals were bisected aseptically, impressions of the cut surfaces were made on blood agar plates, and the plates were incubated as described above (11).

Wild birds were caught in mist nets around the farm, and vent swab samples were taken. These samples were plated onto Preston agar and incubated as described above.

(v) Other environmental samples. Swabs moistened with sterile PBS were used to take samples from the walls and floors of the poultry sheds, and these samples were plated directly onto Preston or VPT selective media or incubated in enrichment broth and then plated. The plates were incubated microaerobically at 43°C for up to 6 days.

Feeding experiment. Water from the borehole, from the field reservoir, and from drinker lines (but not from the actual drinkers, which tended to be contaminated with fecal material) was collected in sterile 10-liter containers and stored at 4°C.

A total of 65 1-day-old birds that were negative for campylobacters were taken from the birds supplied to the farm and were reared under laboratory conditions. In part 1 of the experiment (Table 2) the birds were divided into three groups of 15 (groups A, B, and C) and one group of 20 (group D). The birds in groups A, B, and C were fed feed from the farm (without furazolidone) by using the same feeding regime as the birds on the farm (starter crumbs for 10 days, grower pellets for 20 days, and finisher pellets thereafter). Group A was given untreated farm water, and group B was given autoclaved farm water which had been seeded with 10^2 to 10^3 *C. jejuni* cells per ml; the strain of *C. jejuni* used (strain 38175) was a serotype L1P4 strain, came from poultry from the farm, and had been passaged only twice since initial isolation. Group C received unseeded autoclaved farm water. Group D was fed starter crumbs containing 0.02% furazolidone for the first 14 days of life and given autoclaved farm water seeded with 2.0×10^3 *C. jejuni* 38175 cells per ml.

Vent swab samples were taken from each bird immediately after it arrived in the laboratory and once a week thereafter. Two birds from each group were killed each week, and postmortem examinations were performed by the Ministry of Agriculture, Fisheries and Food Veterinary

TABLE 3. Summary of results of the sampling program to determine the source of campylobacter colonization of farm poultry

Sample type	Culture[a]		IFA test[b]		
	No. of samples	% Positive[c]	No. of samples	No. of replicates[d]	% Positive[e]
Poultry					
Eggs	650	0	0	NS[f]	NS
49-Day-old broilers	2,925	37	0	NS	NS
Single flock	300	0	0	NS	NS
Farm water					
Source	62	0	93	175	62
Supply	77	0	54	80	58
Feed					
Fresh	2	0	2	2	0
Exposed	16	0	3	3	67
Litter					
Fresh	0	0	3	5	0
Used	38	0	14	28	93
Environment					
Shed walls			0	NS	NS
Shed floors	19	0	0	NS	NS
Fan			0	NS	NS
Other sources			0	NS	NS
Air[g]					
Poultry farm	7	0	0	NS	NS
Abattoir	4	75	0	NS	NS
Rodents (mammals)	141	2	0	NS	NS
Abattoir immersion water	28	96	2	3	100
River water[h]	130	67	13	20	50

[a] Vent swab samples were plated directly onto Preston or VPT medium.
[b] See text.
[c] Percentage of campylobacter culture-positive samples.
[d] Total number of replicates examined by the IFA test (50 to 100 microscopic fields were observed per replicate).
[e] Percentage of IFA-positive replicates.
[f] NS, not sampled.
[g] The volume of each replicate was 60 to 900 liters.
[h] River samples were obtained from the point nearest the farm and three other locations upstream.

Investigation Service Laboratory at Itchen Abbas, Hampshire, United Kingdom.

After 31 days, in part 2 of the experiment (Table 2), one-half of the remaining birds in groups A and B were switched to a diet of finisher pellets containing 0.03% furazolidone. The water treatments for each group were not altered. The birds in groups C and D had to be destroyed on day 31 because of demands for space in the animal house.

Intervention procedures. All water system hygiene, tank-cleaning, water disinfection, and chlorination procedures were standardized as far as was possible on a working farm. A sodium hypochlorite solution was added daily to the borehole and reservoir to give a concentration of 0.2 to 0.4 ppm of free chlorine in all of the shed rising mains. The levels of free and combined chlorine were determined with diethyl-*p*-phenylenediamine sulfate 1 (DPD1) and DPD3 tablets by using a Lovibond comparator. The original galvanized iron header tanks in the sheds were replaced with more easily cleaned tanks made of glass-reinforced plastic (GRP) or plastic material, and each tank was fitted with a draincock and lid. Disinfection of the shed plumbing systems was standardized by using known concentrations of a quaternary ammonium compound (Aquasan; Micro-Biologicals Ltd.). Prophylactic furazolidone was withdrawn from both starter feed and finisher feed for part of the program. To summarize, the following intervention techniques were used to reduce waterborne transmission of *C. jejuni* to broiler chickens: (i) hot water pressure washing of chicken drinkers; (ii) replacing galvanized water header tanks with header tanks made of GRP or plastic material with fitted lids; (iii) filling header tanks with the quaternary ammonium compound at the recommended working strength (equivalent to 1 ml/6 liters of water); (iv) flushing water lines with treated header tank water and holding for at least 24 h; (v) treating the input water supply and reservoir with chlorine to attain not less than 0.2 ppm of free chlorine in the rising mains in the sheds; and (vi) stopping the use of prophylactic furazolidone in the feed (at 0 to 10 and 28 to 35 days). These techniques were introduced over a period of several weeks starting in mid-September 1986 and were continued until 16 December 1986. The period during which the full intervention schedule was in place was mid-October to mid-December.

RESULTS

The results of attempts to detect campylobacters in broiler chickens and the different potential sources examined are summarized in Table 3.

Poultry. The investigative team arrived at the farm in April 1986. On 17 June 1986, the first flock to be placed since the team's arrival was sent to slaughter. The isolation rate for campylobacters from poultry on the farm between 13 May and 17 June 1986 inclusive was 112 of 175 birds (64%). A total of 68 of these isolates (60%) were typed, and 58 (85%) were serotype L1 P4 isolates. No isolations of campylobacters were made from 100 birds examined during the following week. Between 1 July and 9 September *C. jejuni* was detected in 364 of 450 (81%) of the birds but serotype L1 P4 isolates were rarely found (3 of 96 birds).

(i) Vertical transmission (infection of eggs or chicks from

TABLE 4. IFA test detection of *Campylobacter* sp. in the source and supply parts of the water system on the farm

Site	No. of samples tested	No. of replicates[a]	% Positive[b]	Grade[c]
Source				
Borehole	27	61	62	2+
Field reservoir[d]	16	36	67	2+
Rising mains	50	78	59	1+ or 2+
Supply				
Header tanks	14	23	6	2+
Drinker lines	36	48	48	1+ or 2+
Drinkers	5	11	88	2+ or 3+

[a] Number of replicates examined by the IFA test (50 to 100 microscopic fields were examined per replicate).
[b] Percentage of IFA test replicates positive for campylobacters.
[c] IFA grade (see text).
[d] Samples were taken from the inlet and from the reservoir.

parent flocks). Campylobacters were not isolated from any of the 650 fertile eggs from the hatchery or from any of the 230 newly hatched chicks examined.

(ii) Campylobacter counts for processed birds. The mean count obtained by PBS immersion of six uneviscerated (New York-dressed) birds examined in April 1986 was 4.3×10^5 campylobacter organisms per bird (range, 1.4×10^5 to 1.1×10^6 organisms per bird). The mean count obtained for 16 oven-ready broilers examined by immersion in April, May, and June 1986 was 2.3×10^6 campylobacters per bird (range, 2.5×10^4 to 2.0×10^7 campylobacters per bird). In August 1986 the mean count for six New York-dressed birds was 2.1×10^7 campylobacters per bird (range, 2.4×10^6 to 8.1×10^7 campylobacters per bird), and the mean count for six oven-ready birds was 3.1×10^6 campylobacters per bird (range, 1.7×10^6 to 4.8×10^6 campylobacters per bird).

Water. A total of 185 samples of water were taken between May and December 1986 from the borehole, field reservoir, rising mains to the sheds, and header tanks in the sheds. All attempts to isolate campylobacters directly from the water samples by the variety of direct and enrichment methods described above were unsuccessful.

Examination of the water samples (more than 1,000 replicate filtrations) by IFA microscopy led to the conclusion that the vibrioid bacteria observed frequently in clumps by this method were *C. jejuni*. These organisms were found in water from both the source and supply parts of the system (Tables 3 through 5). All of the sites examined were colonized intermittently. Campylobacters were detected at all levels in

TABLE 5. IFA test detection of campylobacters in samples of water taken directly from the borehole

Site	No. of samples tested	No. of replicates[a]	Grade[b]
Pump bleed valve	5	18	2+ or 3+
Water column (5–17 m)	8	17	0 or 2+
Water column (18–30 m)	10	16	0 or 1+
Bottom water	4	4	0 or 1+
Water column-sediment interface	3	8	1+ or 3+
Bottom sediment	1	2	3+

[a] Number of replicates examined by the IFA test (50 to 100 microscopic fields were examined per replicate).
[b] IFA grade (see text).

the borehole, as well as in the sediment at the bottom. The predominant serotype that was initially present in the poultry was L1 P4, but after several months, this serotype disappeared and the Penner 6 serotype predominated in the chicken flocks. Testing of identical water samples collected chronologically from a single point in the water distribution system (a rising main tap [Fig. 1]) revealed that both serotypes were found in the system from April to early September.

Campylobacters were isolated from 67 of the 130 water samples obtained from the river (Table 3). Positive samples were obtained from all five sampling points.

Feed and the environment. (i) Feed and litter. Campylobacters were not isolated from 38 litter samples and 18 feed samples examined between May 1986 and March 1987 (or from an additional 27 unexposed and 12 exposed feed samples [the latter taken from sheds containing known positive birds] sampled during 1989). IFA detection methods were successful, however, in identifying campylobacters in some of the samples of exposed feed from the feed track and from exposed litter. No vibrioid campylobacters were observed in fresh (i.e., unexposed) feed or litter.

(ii) Air samples. No isolations of campylobacters were made from any of the air samples taken from three broiler sheds containing known positive birds and one shed containing negative birds between 28 April and 1 July, although other microbial contamination was high. Counts of 1 to 3 campylobacters per liter were obtained from air samples taken from inside the slaughterhouse on two occasions when positive cloacal swab samples were obtained from birds being processed but not on a third occasion, when cloacal swab samples from the birds were negative.

(iii) Wildlife. Typeable campylobacters were isolated from 3 of 141 (2%) small mammals trapped on and around the farm. The endemic strain, serotype L1 P4, was isolated from one water shrew (*Neomys fodiens*). The results of this part of the study have been described in detail by Healing and Greenwood (11).

Nineteen wild birds were caught around the farm. These were members of seven species (blue tit [*Parus caeruleus*], great tit [*Parus major*], house sparrow [*Passer domesticus*], blackbird [*Turdus merula*], chaffinch [*Fringilla coelebs*], robin [*Erithacus rubecula*], and pied wagtail [*Motacilla alba*]). No campylobacters were isolated from any of these birds.

(iv) Other equipment and environmental sources. No campylobacters were isolated by culturing from any of the environmental sites, including the drinkers, drinking lines, water header tanks, walls, ceilings, floors, fans, and climate control units. A distinctive, thick biofilm and mineral accumulation that was not effectively removed by cleaning before the introduction of a new flock was found widely distributed throughout the inside of the supply part of the water system (i.e., within the sheds). Scrapings of this material failed to yield any growth of campylobacters, but examination by the slide fluorescent-antibody method revealed clumps of vibrioid campylobacter cells. Examination of the biofilm by electron microscopy revealed cells morphologically indistinguishable from vibrioid campylobacters.

Feeding experiment. The results of the feeding experiment are summarized in Table 2. During part 1 of the experiment (days 1 to 31) no campylobacters were isolated from birds in groups A and C (birds given untreated and autoclaved farm water, respectively). In group B (birds given seeded water) transient colonization was detected by using swab samples in two birds at day 12, but these birds were negative by day

TABLE 6. Proposed sources of *C. jejuni* in chickens

Proposed source	Results and comments
Vertical transmission (parent to egg or chick)	650 candle-clear eggs culture negative, 250 1-day-old chicks culture negative
Feed	
Unexposed	Culture negative, IFA negative, physical state unlikely to support survival of campylobacters
Exposed	Culture negative, IFA positive, physical state unlikely to support survival of campylobacters
Litter	
Unexposed	Culture negative, IFA negative, physical state unlikely to support survival of campylobacters
Exposed	Culture negative, IFA positive, possibly inhibitory to campylobacters
Air	Culture negative on the farm but culture positive in the slaughterhouse, aerosols constantly produced in the slaughterhouse
Wildlife	
Mammals	<2% positive, little evidence of ingress into sheds
Birds	Culture negative, rare in sheds
Human transfer	Viable campylobacters not recovered from environment, different serotypes in adjoining sheds on some occasions
Water	Culture negative, IFA positive, feeding experiments positive, proportions of birds colonized decreased during intervention and increased after intervention stopped

14 and remained negative thereafter. *C. jejuni* was isolated from two other group B birds at a postmortem examination. The other birds in this group remained negative throughout this part of the experiment. One bird given furazolidone in the starter feed and seeded water (group D) was positive after 11 days, and all of the birds in this group were positive by day 18.

In part 2 of the experiment, three of the four birds in group A fed finisher pellets containing furazolidone from day 31 were campylobacter positive 26 days later (when they were 57 days old), and all four birds were positive after an additional 1 week. Two of the four birds in group A given feed without furazolidone were positive when they were 50 days old, and all four were positive by the time that they were 57 days old. In group B one of the four birds given feed containing furazolidone after day 31 was positive 7 days later, and the four birds given feed without furazolidone were positive after 5 days (when they were 36 days old).

All of the isolates obtained from birds during this experiment were serotyped. Both the isolates recovered from birds given seeded water and the isolates recovered from birds given untreated water from the farm were serotype L1 P4 isolates.

Intervention program. The proportion of birds colonized was high (80.9% [364 of 450 birds]) before the intervention program, fell to 7% (63 of 900 birds) during the time of the full program, and returned to the previous high level (84.2% [379 of 450 birds]) 6 weeks after the end of the intervention procedures. No birds were slaughtered over the 2-week Christmas period, and for the following 4 weeks, the age of birds at slaughter was a mixture of 49 and 56 days. In the period immediately after Christmas more than 95% of the 56-day-old birds were positive, although the 49-day-old birds were negative.

PBS immersions of processed broilers from the slaughterhouse were carried out during the intervention period. Before intervention the numbers of campylobacters on oven-ready birds had exceeded 3.0×10^6 campylobacters per bird and on New York-dressed birds had exceeded 8.0×10^7 campylobacters per bird (see above). Thirty New York-dressed chickens were tested in November and December 1986, and the numbers of recoverable campylobacters did not exceed 1,500 campylobacters per bird; campylobacters could not be detected on seven of these birds (<45 campylobacters per bird). During the same period the numbers of campylobacters from the cooling tanks in the slaughterhouse also fell to very low levels (<1 campylobacter per ml). The numbers on carcasses fluctuated early in 1987 when a mixture of 49- and 56-day-old birds was being slaughtered, but the mean for three uneviscerated birds examined on 17 February was 2.4×10^5 campylobacters per bird (range, 8.7×10^4 to 4.5×10^5 campylobacters per bird), and the mean for six oven-ready birds examined in March was 8.3×10^4 campylobacters per bird (range, 8.0×10^3 to 2.2×10^5 campylobacters per bird.) The numbers obtained from cooling tank waters during this period rose to ca. 10^3 campylobacters per ml and sometimes exceeded 10^7 campylobacters per ml.

DISCUSSION

The proportions of broilers sampled either at slaughter or at the point of sale that were found to be colonized with campylobacters in a number of different studies have ranged from 22 to 87% (4, 9, 22, 32), and the prevalence of *C. jejuni* in live broiler chickens can be highly variable (31). Chicken farms are not necessarily colonized all of the time, and even when some sheds are colonized, others may be free. Within colonized flocks, the proportion of birds colonized is often high, and fecal samples may contain sizeable concentrations of *C. jejuni* cells (e.g., 10^7 CFU/g of feces [9a]).

There are a number of routes by which broiler chickens could theoretically become colonized with campylobacters. These include vertical transmission (infection passing from parent via egg to the chick); contaminated feed, water, or litter; wildlife; carryover within the shed from previous crops; and cross-contamination from adjacent sheds via the air, litter, wildlife, insects, or human transfer. Once the organism has entered a shed, either the birds may acquire it only via the primary source or bird-to-bird spread may occur. The proportion of birds colonized at the end of the crop, the date when the colonization is first detectable, the frequency of different serotypes, and the pattern of serotypes within and between sheds are all factors which can be

investigated, as can each potential colonization route. The results of our investigations into these different sources of campylobacters on the farm are summarized in Table 6.

The persistence of a single campylobacter serotype (L1 P4) on the farm despite the fact that the birds on the farm came from several different laying flocks, together with the failure to isolate campylobacters from a large number of candle-clear eggs or from newly hatched chicks, suggested that vertical transmission (i.e., infection from parent via the egg) of the organisms did not occur during this study. These results agree with those of other workers (8, 17, 21, 27) and suggest that campylobacters are rarely transmitted to broiler chicks by this route.

Neither feed nor fresh litter seems to be a likely source of campylobacters. The former is dried and pelleted, often contains antibiotics, and is air blown into the silos. *C. jejuni* is very sensitive to dehydration and dies rapidly in aerosols. The litter used on the farm was wood shavings. These are dry and resinous (being mainly softwood) and come directly from sawmills. *C. jejuni* could not be isolated from fresh feed or litter or from exposed material, but it was shown by IFA tests to be present on exposed feed and litter. Interestingly, it could not be isolated from the exposed litter, but this may have been due to interference by other bacteria or by breakdown products produced by the composting of the litter. Spent wood shaving litter is extremely inhibitory to the growth of salmonellas (19a). Its effect on campylobacters is less well understood, but experimental work has shown that litter artificially contaminated with campylobacters can infect chickens under laboratory conditions (19). However, the litter used in that study was rice husks and not wood shavings. The breakdown products formed by the composting of these two different substances may well have totally different effects on bacterial survival and growth. Additional evidence that neither feed nor litter was likely to have been responsible for the introduction of the organisms onto the farm is the persistence of the L1 P4 serotype in birds from the farm for at least 18 months. Since the wood shavings used as litter came from several sawmills and there were up to five different feed deliveries per crop, it is unlikely that these materials could result in the introduction of only one serotype into many different crops of chickens over an extended period.

Cross-contamination between sheds by contaminated air, dust, litter, or human transfer remains a possibility. Viable campylobacters were not recovered from litter, environmental, or air samples taken on the farm, but the extensive movement between sheds by farm staff (boot dips were not in use) could have resulted in the transfer of organisms from shed to shed. However, after the disappearance of the L1 P4 serotype there were occasions when there were numbers of different serotypes on the farm, and adjacent sheds differed in the types present, suggesting that transfer between the sheds was not occurring as the predominant mode of transmission.

It is unlikely that a single serotype of campylobacter would have persisted in the broilers on the farm for weeks or months if wildlife was the source. The frequency of detection of *C. jejuni* in the small mammal species caught (ca. 2%) was so low as to exclude any possibility of these animals being a key factor in the transmission of *C. jejuni* to poultry. In addition, none of the rodent species caught which are known to enter buildings was colonized with the organisms (11). Wild birds are often colonized with campylobacters (29), but none of the wild birds sampled during this study was positive. Starlings (*Sternus vulgaris*) were nesting in the roofs of two of the sheds on site 1 (one pair in each shed), but none were nesting in the other sheds. No starlings were caught during the wild bird sampling program, and so it is not known whether they were carrying campylobacters. Wild birds were unable to enter the main part of the sheds during crop production, and few were seen in the sheds during turnaround. Wild birds are not, therefore, likely to have been an important direct source of campylobacters in the sheds on this farm during the study.

The remaining theoretical possibility of a transmission vehicle was water. There was a high colonization rate of the birds, with the predominance of a single serotype across at least six crops of 50,000 birds, which indicates that there was an intermittent or continuous common source. Water transmission, with or without pipework colonization, was biologically the most plausible remaining source and route of transmission to explain our findings. Initially however, this seemed unlikely, since although campylobacters were recovered with ease from river waters (Table 3), they could not be isolated from any sample of water from the farm water system. Extensive efforts were made to overcome this problem. Large volumes of water, collected repeatedly from all parts of the source and supply systems, were sampled and processed in ways designed to allow recovery of damaged cells, the detection of very low numbers of bacteria, and the gradual adaptation of organisms from oligotrophic environments to the comparatively rich nutritional conditions of artificial culture media. None of these methods was successful in recovering campylobacters.

Electron microscopy revealed vibrioid bacteria resembling campylobacters in shape and general morphology in samples of water taken from the drinkers and their supply lines. An extensive sampling program was undertaken between April and July 1986, and the samples were examined by fluorescent antibody tests. A total of 60% of all source and supply samples of water consistently showed evidence of *C. jejuni*, and the organisms were found throughout the water system from the soil-water interface at the bottom of the 30-m borehole to the biofilm of the pipework within the chicken sheds. The fact that vibrioid IFA-positive *C. jejuni* was found in large numbers at the soil-water interface in the absence of lactose-fermenting coliforms or *Escherichia coli* suggests that *C. jejuni* may be a normal inhabitant of the aquatic ecosystem.

It is unclear why it was not possible to grow these vibrioid campylobacters despite the use of special methods designed for the recovery of environmental bacteria. One possible explanation is the laboratory-proven existence of viable but nonculturable *C. jejuni* (26). *C. jejuni* was detected in large numbers by IFA tests in the first of a series of samples of water taken at 1-min intervals from a shed rising main. The results of our examination of scrapings of material from the lining of the water system and the observations from sequential sampling described above suggest that the organisms may have been accumulating in the biofilm of the pipework. This process has been described for *Legionella* spp. (34). A latex agglutination method was used subsequently to study water samples from farms from which campylobacters could not be grown (33). Small numbers of samples (6 of 57 samples) were found to be positive by this means, a finding consistent with the possibility that viable but nonculturable campylobacters were present (33).

Feeding of water from the farm to campylobacter-free birds resulted in colonization of a small number of birds with the serotype L1 P4 isolate endemic on the farm. This recovery was after a period of time greater than the normal

grow-out period, but the experiment was carried out in a laboratory environment very different from the conditions prevailing in a chicken shed. This result, together with the results of the IFA tests of the supply water, suggested that intervention procedures designed to disinfect the water might be successful in reducing campylobacter colonization of the birds. It was not possible to identify a single suitable intervention procedure because of the widespread distribution of campylobacters in the water system and because of current hygiene practices on the farm. Accordingly, a series of intervention procedures was formulated on the basis of the findings of the study.

Furazolidone was added to the diet of one group of birds in the feeding experiment for the first 10 days of life. The rapid colonization of all of the birds in this group following withdrawal of the antibiotic suggested that furazolidone might have increased the likelihood that the birds on the farm would become colonized with campylobacters. As a result, the withdrawal of furazolidone from the poultry feed was included as one of the intervention procedures. Other intervention procedures were designed to improve the general hygiene of the water supply system and to maintain a reasonably constant detectable level of free chlorine in the water itself.

While the intervention program was operating on the farm, both the proportion of birds from the farm entering the slaughterhouse that were colonized with *C. jejuni* and the number of organisms recoverable from processed carcasses fell markedly from the levels measured before the intervention program. After the end of the intervention program, both the proportion of birds colonized and the number of organisms on the carcasses (and the numbers of organisms recoverable from the slaughterhouse cooling tanks) returned to their previously high levels. The results of these studies provide strong evidence which suggests that the route via which campylobacters were colonizing the birds was predominantly the water supply.

The design of this study had certain limitations imposed partly by the fact that it was undertaken on a working farm and partly because of possible commercial implications to the farm if it were identified publicly as the source of poultry which caused a campylobacter outbreak. Although the birds coming out of the slaughterhouse apparently remained contaminated with campylobacters during the intervention period, the numbers recoverable fell to low levels. The infective dose of campylobacters can be less than 1,000 organisms (1, 25); however, since campylobacters do not multiply on foods, the complete elimination of these organisms from broilers, while desirable, may not be necessary in order to achieve a reduction in human cases associated with this source.

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REFERENCES

1. **Black, R. E., M. M. Levine, M. L. Clements, T. P. Hughes, and M. J. Blaser.** 1988. Experimental *Campylobacter jejuni* infection in humans. J. Infect. Dis. **157**:472–479.
2. **Blaser, M. J., D. N. Taylor, and R. A. Feldman.** 1983. Epidemiology of *Campylobacter jejuni* infections. Epidemiol. Rev. **5**:157–176.
3. **Bolton, F. J., C. Dawkins, and D. N. Hutchinson.** 1985. Biotypes and serotypes of thermophilic campylobacters isolated from cattle, sheep and pig offal and other red meats. J. Hyg. **95**:1–6.
4. **Bolton, F. J., and L. Robertson.** 1982. A selective medium for isolating *Campylobacter jejuni/coli*. J. Clin. Pathol. **35**:462–467.
5. **Brouwer, R., M. J. A. Mertens, T. H. Siem, and J. Katchaki.** 1979. An explosive outbreak of *Campylobacter* enteritis in soldiers. Antonie van Leeuwenhoek J. Microbiol. **45**:517.
6. **Daley, R. J., and J. E. Hobbie.** 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. Limnol. Oceanogr. **20**:875–882.
7. **Deming, M. S., R. V. Tauxe, P. A. Blake, S. E. Dixon, B. S. Fowler, T. S. Jones, E. A. Lockamy, C. M. Patton, and R. O. Sikes.** 1987. *Campylobacter* enteritis at a university: transmission from eating chicken and from cats. Am. J. Epidemiol. **126**:526–534.
8. **Doyle, M. P.** 1984. Association of *Campylobacter jejuni* with laying hens and eggs. Appl. Environ. Microbiol. **47**:533–536.
9. **Grant, I. H., N. J. Richardson, and V. D. Bokkenheuser.** 1980. Broiler chickens as potential source of *Campylobacter* infections in humans. J. Clin. Microbiol. **11**:508–510.

9a. **Greenwood, M.** Unpublished data.

10. **Harris, N. V., N. S. Weiss, and C. M. Nolan.** 1986. The role of poultry and meats in the etiology of *Campylobacter jejuni/coli* enteritis. Am. J. Public Health **76**:407–411.
11. **Healing, T. D., and M. H. Greenwood.** 1991. Frequency of isolation of *Campylobacter* spp., *Yersinia* spp. and *Salmonella* spp. from small mammals from two sites in southern Britain. Int. J. Environ. Health Res. **1**:54–62.
12. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33**:1225–1228.
13. **Hood, A. M., A. D. Pearson, and M. Shahamat.** 1989. The extent of surface contamination of retailed chickens with *Campylobacter jejuni* serogroups. Epidemiol. Infect. **100**:17–25.
14. **Hopkins, R. S., and A. S. Scott.** 1983. Handling raw chicken as a source for sporadic *Campylobacter jejuni* infections. J. Infect. Dis. **148**:770.
15. **Humphrey, T. J.** 1986. Technique for the optimum recovery of cold injured *Campylobacter jejuni* from milk and water. J. Appl. Bacteriol. **61**:125–132.
16. **Kakoyiannis, C. K., P. J. Winter, and R. B. Marshall.** 1988. The relationship between intestinal *Campylobacter* species isolated from animals and humans as determined by BRENDA. Epidemiol. Infect. **100**:379–387.
17. **Lindblom, G.-B., E. Sjogren, and B. Kaijser.** 1986. Natural campylobacter colonisation in chickens raised under different environmental conditions. J. Hyg. **96**:385–391.
18. **Lior, H., D. L. Woodward, J. A. Edgar, L. J. Laroche, and P. Gill.** 1982. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat labile antigenic factors. J. Clin. Microbiol. **15**:761–768.
19. **Montrose, M. S., S. M. Shane, and K. S. Harrington.** 1985. Role of litter in the transmission of *Campylobacter jejuni*. Avian Dis. **29**:392–399.

19a. **Morgan-Jones, S. C.** 1984. Proc. Int. Symp. Salmonella, p. 377.

20. **Munroe, D. L., J. F. Prescott, and J. L. Penner.** 1983. *Campylobacter jejuni* and *Campylobacter coli* serotypes isolated from chickens, cattle and pigs. J. Clin. Microbiol. **18**:877–881.
21. **Neill, S. D., J. N. Campbell, and J. A. Green.** 1984. *Campylobacter* species in broiler chickens. Avian Pathol. **13**:777–785.
22. **Park, C. E., Z. K. Stankiewicz, J. Lovett, and J. Hunt.** 1981.

Incidence of *Campylobacter jejuni* in fresh eviscerated whole market chickens. Can. J. Microbiol. **27**:841–842.

23. **Pearson, A. D., T. D. Healing, P. Sockett, and D. M. Jones.** 1989. Campylobacter: the third agent in the food poisoning outbreak, p. 245–281. *In* O. Goldring (ed.), *Salmonella* and *Listeria*. EAG Scientific, London.
24. **Penner, J. L., and J. N. Henessey.** 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp *jejuni* on the basis of soluble heat-stable antigens. J. Clin. Microbiol. **12**:732–737.
25. **Robinson, D. A.** 1981. Infective dose of *Campylobacter* in milk. Br. Med. J. **282**:1584.

25a. **Rollins, D. M.** 1987. Ph.D. dissertation. University of Maryland, College Park.

26. **Rollins, D. M., and R. R. Colwell.** 1986. Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. Appl. Environ. Microbiol. **52**:531–538.

26a. **Rollins, D. M., R. R. Colwell, and A. D. Pearson.** 1988. Campylobacter IV. Proc. 4th Int. Workshop Campylobacter Infect., abstr. 202, p. 285.

27. **Shanker, S., A. Lee, and T. C. Sorrell.** 1986. *Campylobacter jejuni* in broilers: the role of vertical transmission. J. Hyg. **96**:153–159.
28. **Skirrow, M. B.** 1977. Campylobacter enteritis: a 'new' disease. Br. Med. J. **2**:9–11.
29. **Skirrow, M. B.** 1980. Differentiation of enteropathogenic campylobacter. J. Clin. Pathol. **33**:1112. (Letter.)
30. **Skirrow, M. B., and J. Benjamin.** 1980. '1001' Campylobacters: cultural characteristics of intestinal campylobacters from man and animals. J. Hyg. **85**:427–441.
31. **Smith, H. W.** 1971. The epizootiology of salmonella infection in poultry, p. 37–46. *In* R. F. Gordon and B. M. Freeman (ed.), Poultry disease and world economy. British Poultry Science, Edinburgh.
32. **Stern, N. J., M. P. Hernandez, L. Blankenship, K. E. Deibel, S. Doores, M. P. Doyle, H. Ng, M. D. Pierson, J. N. Sofos, W. H. Sveum, and D. C. Westhoff.** 1985. Prevalence and distribution of *Campylobacter jejuni* and *Campylobacter coli* in retail meats. J. Food. Prot. **48**:595–599.
33. **Sutcliffe, E. M., D. M. Jones, and A. D. Pearson.** 1991. Latex agglutination for the detection of *Campylobacter* species in water. Lett. Appl. Microbiol. **12**:72–74.
34. **Wright, J. B., I. Ruseska, M. A. Athar, S. Corbett, and J. W. Costerton.** 1989. *Legionella pneumophila* grows adherent to sufaces in vitro and in situ. Infect. Control Hosp. Epidemiol. **10**:408–415.