

# Microbial Ecology of *Campylobacter jejuni* in a United Kingdom Chicken Supply Chain: Intermittent Common Source, Vertical Transmission, and Amplification by Flock Propagation

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**A study of *Campylobacter jejuni* on a broiler chicken farm between 1989 and 1994 gave an estimated isolation rate of 27% (3,304 of 12,233) from a 0.9% sample of 1.44 million broiler chickens from six to eight sheds over 32 consecutive rearing flocks comprising 251 broiler shed flocks. During the study, *C. jejuni* was found in 35.5% of the 251 shed flocks but only 9.2% (23 of 251) had *Campylobacter* isolates in successive flocks, with 9 of those 23 sheds having the same serotype between consecutive flocks, indicating a low level of transmission between flocks. Analysis of a systematic sample of 484 of 3,304 (14.6%) *C. jejuni* isolates showed that 85% were of 10 serotype complexes but 58% were of 3 serotype complexes, indicating a high degree of strain similarity throughout the entire study. The three commonest types were detected in 8 of 32 flocks during the 5-year study period, suggesting an intermittent common external *Campylobacter* source. This hypothesis was tested by a retrospective cohort analysis of *C. jejuni* rates and types by reference to hatchery supplier of the 1-day-old chicks. Isolation rates of *C. jejuni* and frequency distribution of types were determined in 6-week-old broiler chickens identified by the hatchery supplying the original chicks. The isolation rate of *C. jejuni* in broilers, supplied by hatchery A, was 17.6%, compared to 42.9% ( $P < 0.0001$ ) for broilers reared from chicks supplied by hatchery B. In two instances, when both hatcheries were used to stock the same farm flock, *Campylobacter* isolates were found only in those sheds with chicks supplied by hatchery B. Thus, the frequency distribution of *Campylobacter* types for chickens supplied by the two hatcheries over the 5-year period showed marked dissimilarity. These findings suggest that the isolation rate and type of *Campylobacter* isolates in broiler chickens was associated with the hatchery supplying chicks. The lack of diversity of types and the intermittent high positivity of sheds is evidence for a common source of *C. jejuni* introduced by vertical transmission rather than contamination at the hatchery or during transportation.**

Reports of *Campylobacter* infection in humans have continued to rise for 2 decades despite the identification of broiler chicken, milk, water, and contact with pets and farm animals as sources of infection (2). While several studies have indicated poultry meat to be an important source of *Campylobacter jejuni* infection of humans (8, 24, 28), the etiological fraction attributable to this source is poorly defined and recent studies have shown the presence of raw or cooked poultry in the home to be protective (1, 17). Live broiler chickens can be colonized by *C. jejuni* at levels of  $10^4$  to  $10^8$  CFU/g (27), and colonization rates vary from 0 to 100%, with point prevalence rates frequently above 30% (2). Cross contamination of poultry carcasses occurs during processing via contact with gut contents, resulting in final contamination levels of up to  $10^2$  to  $10^6$  CFU/g in 30 to 40% of birds sold to the consumer (4). Transmission of *C. jejuni* to humans from poultry is thought to occur predominantly by cross contamination of foods eaten raw and by the handling of raw carcasses. The gastroenteritis that ensues has the potential to cause life-threatening illness, includ-

ing Guillain Barré syndrome (20). Identification of the mechanism for *C. jejuni* transmission in the poultry industry would be an important step in the development of intervention protocols to reduce or eliminate *Campylobacter* colonization of broiler chickens.

*C. jejuni* can be pathogenic for young chicks (27) but is generally not so for adult birds. One study has shown that 75% of chickens can harbor *C. jejuni* in their intestinal tracts between the ages of 5 and 7 weeks (32). Suggested sources and routes of transmission of *C. jejuni* to broiler poultry vary with locality and the type of study undertaken (29); they include contaminated water supplies (23) and horizontal transmission within and between broiler-rearing sheds from the environment and also from insects, rodents, and free-living wild birds. It should be noted that the term "horizontal transmission" is used in addition to describe the sequential cross contamination that may occur between successive sheds and flocks through different time periods. Vertical transmission, to date, is believed to be unlikely (2, 10, 14, 30, 31, 33).

This paper reports the results of a 5-year longitudinal study to determine the incidence and types of *Campylobacter* isolates in broiler chickens on a small rearing farm in southern England and a retrospective cohort study of the effect of the hatchery on *Campylobacter* colonization.

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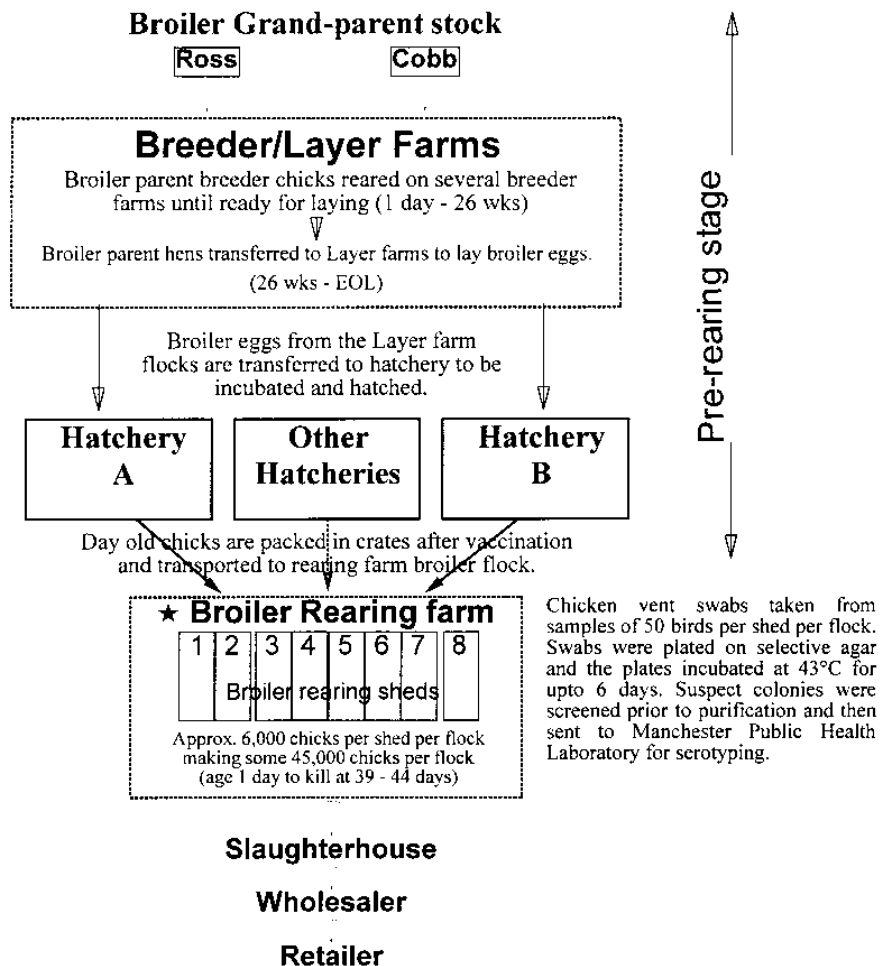


FIG. 1. Schematic diagram of broiler supply chain to a broiler-rearing farm. ★, chicken vents sampled (broiler-rearing farm sheds); EOL, end of lay (when broiler parents stop laying).

**MATERIALS AND METHODS**

**The hatcheries.** Figure 1 shows the broiler-rearing flowchart for chicks supplied to the rearing farm under study. Hatcheries are supplied with eggs from various layer farms, the output from a given layer farm being termed a layer farm flock, and incubate a sufficient number of eggs in electric incubators to ensure the required number of day-old chicks for delivery to the rearing farm on the day that they are required. After hatching, the chicks are manually sorted, counted (usually electronically), and manually sexed before being put into crates (or baskets) and spray vaccinated for infectious bronchitis virus. The day-old Cobb or Ross chicks in the transport crates are delivered by temperature-controlled lorry to the rearing farm and transferred manually to the rearing sheds (or houses). The total chick delivery for one date to the rearing farm is termed a placement or broiler flock. The hatchery and layer farm flock numbers for each broiler flock of chicks were recorded at the time of their delivery to the broiler-rearing farm.

**The broiler-rearing farm.** The broiler-rearing farm investigated during this study had eight broiler-rearing wooden sheds or small houses, varying in size from 288 to 325 m<sup>2</sup>. The sheds were built above concrete floors on low concrete block walls and were equipped with a bell drinker system which piped borehole water to the chicks. Each shed accommodated between 5,500 and 6,300 birds. Before deliveries of new chick flocks, the broiler litter (a mixture of woodshavings and poultry manure) was removed from the rearing sheds by bulldozers and the sheds were swept clean, pressure washed, disinfected, and fumigated before new shavings were distributed. Poultry feeders and drinkers were also thoroughly cleaned between flocks. The broiler flock-rearing sheds were warmed by propane gas brooder heaters controlled to provide a shed temperature of 35°C during the first 3 days, gradually reduced to 21°C at or after 28 to 30 days.

The chicks for each broiler flock were reared from 1 day of age to 39 to 44 days of age. When the broiler birds reached the desired weight, they were caught by hand, placed in crates, and transported to the slaughterhouse. Rearing sheds for which exact proportions of mixed hatchery flocks were not available or with birds

from two other hatcheries (10%) were excluded from the analysis listed in Table 1.

**Longitudinal study.** Between March 1989 and March 1994, 32 consecutive flocks of broiler poultry were reared, yielding 6 and 8 rearing sheds per flock (a total of 251 broiler shed flocks), and live chickens were sampled between 38 and 44 days of age by vent swabs being taken from a planned sample of 50 live birds per shed for each flock of birds. During the 5-year study period, the farm reared a total broiler population of about 1,440,000 birds, of which a total of 12,233 birds were sampled from the 251 shed flocks. About 45,000 chicks were reared per broiler flock, from which the study design was to sample 400 birds (0.9%) consisting of 50 birds per shed.

**Cohort study.** A retrospective study of the influence of the hatchery of supply on *Campylobacter* isolation rate and frequency distribution of type was undertaken in 1995.

**Detection, confirmation, and typing of *Campylobacter* isolates.** Swabs from cloacal vents of broiler chickens were used to inoculate selective agar media (5, 7) at the rearing farm at the time of swabbing. The isolation media were incubated at 42°C for 48 h to 6 days under microaerobic conditions. Suspect colonies were Gram stained and screened for cytochrome oxidase production and absence of aerobic growth. Presumptive *Campylobacter* isolates were purified on blood agar and sent to the Manchester Public Health Laboratory for confirmation of identification, biotyping, and serotyping (6, 15, 25).

**Data analysis.** Sampling regimen, results of microbiological testing for *Campylobacter* isolates, and serotyping results were collated, entered, and validated in a database using SMARTWARE II (Angoss Software Ltd., Guildford, Surrey, United Kingdom) software and analyzed by SAS 6.08 (SAS Institute Inc., Cary, N.C.) and EXCEL version 5.0 (Microsoft) on an Intel Pentium personal computer running at 90 MHz with 32 MB of RAM. Data entry and validation were from copies of the original laboratory records and followed a standard protocol.

TABLE 1. *Campylobacter* isolation rate (CIR) for 32 broiler flocks from 1989 to 1994

Yr	Flock Mo	Total no.			% CIR <sup>a</sup>
		Of sheds	Of swabs	Positive	
1989	March	8	400	400	100.0
	May	7	350	39	11.1
	July	7	338 <sup>b</sup>	333	98.5
	September	8	400	200	50.0
	November	8	400	0	NIL
1990	January	8	400	57	14.2
	March	7	350	126	36.0
	May	8	400	50	12.5
	July	8	400	83	20.8
	September	8	400	378	94.5
1991	November	8	400	0	NIL
	January	8	400	0	NIL
	March	8	400	0	NIL
	May	8	400	0	NIL
	July	8	400	387	96.8
1992	September	8	400	238	59.5
	November	8	400	0	NIL
	January	8	400	0	NIL
	March	8	400	168	42.0
	May	8	400	5	1.2
1993	July	8	400	158	39.5
	September	8	400	0	NIL
	November	8	400	131	32.8
	December	8	400	0	NIL
	February	8	400	0	NIL
1994	April	8	200 <sup>c</sup>	0	NIL
	June	8	400	289	72.2
	August	8	399	222	55.6
	October	6	300	40	13.3
	December	8	296 <sup>d</sup>	0	NIL
1994	January	8	400	0	NIL
	March	8	400	0	NIL
Total		251	12,233	3,304	27.0

<sup>a</sup> The CIR for the first 16 flocks in this table was 36.7%; that for the second 16 flocks was 16.9%. NIL, negative sample (i.e., no *Campylobacter* isolates found).

<sup>b</sup> No samples were taken from shed 1, and only 38 birds from shed 8 were sampled.

<sup>c</sup> Only 25 birds sampled from all eight sheds.

<sup>d</sup> Twenty-five birds sampled from sheds 7 and 8; 23 birds sampled from sheds 5 and 6.

## RESULTS

**Prospective longitudinal study.** Table 1 gives the *Campylobacter* isolation rates for each flock. The overall rate was 27% (3,304 of 12,233) during the period between March 1989 and 31 March 1994 (Table 1), falling from 51.5% in 1989 to 27.6% in 1993. Figure 2 shows the distribution of *C. jejuni* isolations from >40-day-old broiler chickens for successive flocks. Marked differences in isolation rates were found between successive broiler flocks, with a higher prevalence recorded during the first 16 flocks (36.7%) than during the second half of the study (16.9%). Flocks with *Campylobacter* isolates occurred more often in late summer (July and August). Adjacent flocks had markedly different isolation rates. *C. jejuni* was found in sheds for consecutive flocks on only 23 occasions (9.2%). Shed isolation rates were greater than or equal to 90% in only 14 flocks. There were a total of 165 negative broiler shed flocks (69%), 70 in the first half and 95 in the second half—an increase of 29%. No *Campylobacter* isolates were detected in 174 of the 251 (69%) shed flocks.

**Frequency and distribution of *C. jejuni* serotypes.** The frequency distribution of Penner serotype complexes found in the

484 *C. jejuni* isolates typed over the 5-year study is given in Table 2. These results indicated that over 58% of the strains were one of three serotype complexes, with eight serotype complexes accounting for >80% of the strains typed. The occurrence and frequency by flock of all the *C. jejuni* serotypes found during this study are shown in Table 3. Most serotype complexes occurred between four and six times in different flocks, excluding nontypeable strains (type X), with two serotype complexes appearing only once (complexes F and J).

**Retrospective cohort study.** Table 4 lists a comparison of the number of positive swabs for *C. jejuni* per flock in broilers reared from chicks supplied by the two main hatcheries. The *Campylobacter* isolation rate for broilers reared from chicks supplied by hatchery A was 17.6% (1,219 of 6,945), compared to 43.0% (1,756 of 4,088) for hatchery B ( $\chi^2 = 843$ ,  $df = 1$ ,  $P < 0.0001$ ). Table 5 gives a summary of the number of positive broiler shed flocks analyzed by the hatchery source of the chicks. Hatchery A supplied over 75% of flocks with *Campylobacter* isolation rates of less than 5%, whereas hatchery B's chicks were more likely to be positive.

Three broiler flocks had chicks from both hatcheries. In the March 1992 chart (Fig. 3), chicks in sheds 1 to 3 were supplied by hatchery A and gave a mean isolation rate of 0.7%, whereas the chicks in the remaining five sheds were supplied by hatchery B and gave a rate of 67%. In November 1992, chicks in sheds 1 to 4 were supplied by hatchery A, with no positive samples found (Fig. 4). Those in shed 5 were from both hatcheries (isolation rate of 44%), and in sheds 6, 7, and 8, all were supplied by hatchery B, giving a rate of 73%.

Sixty percent of the *Campylobacter* isolates in chicks from hatchery A were types B and E, whereas 59% of the *Campylobacter* isolates in chicks reared from eggs supplied by hatchery B were types A and C.

## DISCUSSION

*Campylobacter* transmission in broiler chicken-rearing farms has been considered to be propagated by horizontal transmission between and within the flocks and sequential flocks, with debate about the relevant contributions of different possible sources and routes for colonization (13, 14, 18, 23, 32, 33). Evidence presented in an earlier study showed that shed design and hygiene between flocks, water supply, and distribution system (18, 23), as well as the presence of rodents, birds,

TABLE 2. Penner serotype frequency distribution for 484 *C. jejuni* strains typed from 3,304 isolates and frequency of the 10 commonest serotype complexes from hatcheries A and B

Serotype complex	Frequency (%)	Cumulative frequencies (%)	Frequency (%) from:	
			Hatchery A	Hatchery B
A	22.4	22.4	9.6	28.2
B	21.5	43.9	49.6	3.4
C	14.5	58.4	0.1	30.5
D	6.2	64.7	6.2	3.8
E	6.0	70.7	11.0	1.7
F	4.7	75.3	0.1	8.9
G	3.3	78.6	0.1	5.1
H	3.1	81.7	0.1	6.0
J	1.7	83.4	4.1	0.1
K	1.4	84.8		
L	0.7	85.5		
M	0.0	85.5		
X <sup>a</sup>	14.0	99.5	18.5	10.0

<sup>a</sup> Nontypeable strains.

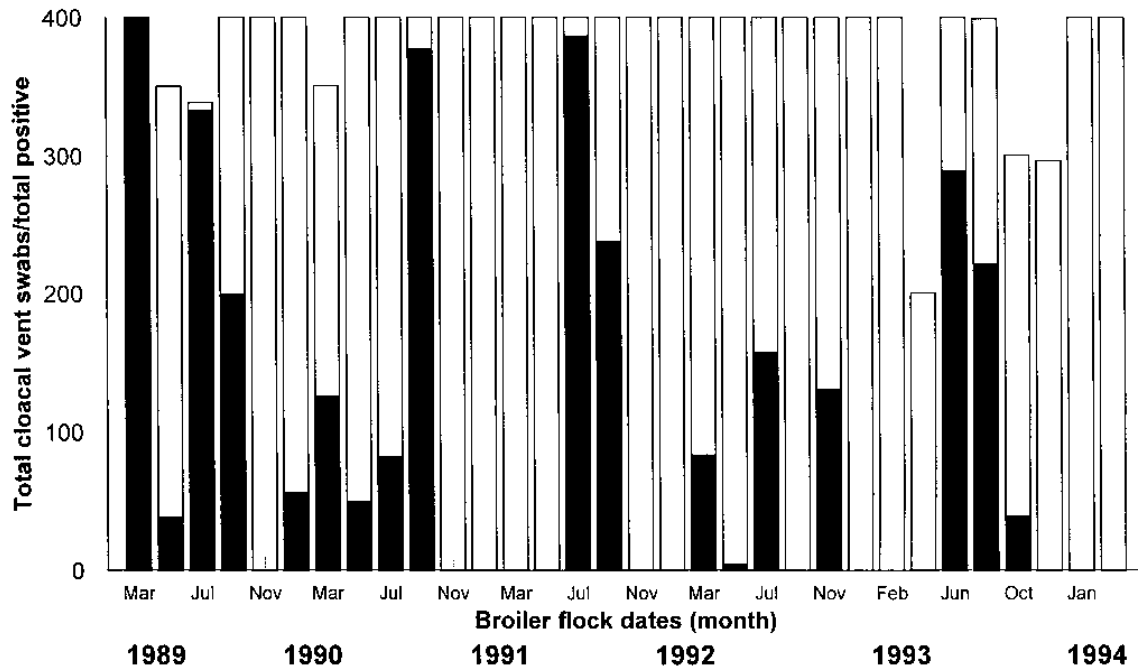


FIG. 2. *C. jejuni* isolation rates from >40-day-old broiler chickens by flock. □, number of birds tested per flock; ■, number of samples with *C. jejuni* per flock.

TABLE 3. Distribution of serotype complexes by broiler flock

Yr	Mo	% of strains												
		Of serotype complex										X <sup>a</sup>	Other	Negative
Flock		A	B	C	D	E	F	G	H	J				
1989	March	100												0
	May										5.6	5.6		88.8
	July	28.3	2.7			16.8		25.1			25.5			1.5
	September	27.5									20	2.5		50
	November													100
1990	January											14		86
	March				21.4					14.3		0.3		64
	May				12.5									87.5
	July				18.5				1.3		1.0			79.3
	September	19.8	33.6			33.6					7.5			5.5
	November													100
1991	January													100
	March													100
	May													100
	July			77.1				2.3	12.5		4.9			3.2
	September			44.7							3.6	11.3		40.4
	November													100
1992	January													100
	March						39.1		0.9			1.8		58.2
	May													100
	July			0.3							37.8	0.9		61
	September													100
	November		20.2						11.4		1.4			67
	December													100
1993	February													100
	April													50
	June		68.9											31.1
	August		45.0								10.5			44.5
	October	12.7												87.3
	December													100
1994	January													100
	March													100

<sup>a</sup> Nontypeable strains.

TABLE 4. *Campylobacter* isolation rate by flock and hatchery supplier of chicks

Flock		No. of samples from:					
		Hatchery A		Hatchery B		Other hatchery	
Yr	Mo	Total	Positive	Total	Positive	Total	Positive
1989	March			400	400		
	May			350	39		
	July			338 <sup>a</sup>	333		
	September					400 <sup>b</sup>	200
	November				400	0	
1990	January					400 <sup>c</sup>	57
	March	350	126				
	May	50	0	300	50	50 <sup>d</sup>	0
	July			300	33	100 <sup>e</sup>	50
	September	400	378				
1991	November	400	0				
	January	400	0				
	March	400	0				
	May	400	0				
	July			400	387		
1992	September			400	238		
	November			400	0		
	January			400	0		
	March	150	1	250	167		
	May	400	5				
1993	July	400	158				
	September	400	0				
	November	200	0	150	109	50 <sup>f</sup>	22
	December	200	0			200 <sup>g</sup>	0
	February	400	0				
1994	April	200 <sup>h</sup>	0				
	June	400	289				
	August	399	222				
	October	300	40				
	December	296 <sup>i</sup>	0				
1994	January	400	0				
	March	400	0				
Total		6,945	1,219	4,088	1,756	1,200	329

<sup>a</sup> No samples were from shed 1, and only 38 birds were sampled from shed 8.

<sup>b</sup> No hatchery records available at all for the eight sheds.

<sup>c</sup> Chicks from both hatcheries A and B, but records unavailable for individual rearing sheds.

<sup>d</sup> Chicks from both hatcheries A and B in shed 2; proportion records unavailable.

<sup>e</sup> Chicks from another hatchery (C) in shed 1 and also with those from hatchery B in shed 2.

<sup>f</sup> Chicks from both hatcheries A and B in shed 5; proportion records unavailable.

<sup>g</sup> Chicks from another hatchery (D) in sheds 1 to 4.

<sup>h</sup> Only 25 birds sampled from all eight sheds.

<sup>i</sup> Twenty-five birds sampled from sheds 7 and 8; 23 birds sampled from sheds 5 and 6.

insects, and other farm animals and cross contamination between sheds by human footwear (13), all may play a role. The broiler-rearing farm investigated in this study was operated by a single management and was supplied almost exclusively by two hatcheries. It was studied continuously by a consistent methodology (7) for detecting *C. jejuni* between 1985 and 1994, since it was found to be the origin of a continuous-source, poultry-associated outbreak of campylobacteriosis in southern England, starting in November 1984 (24). The isolation rates of *C. jejuni* were determined with the selective media (5, 7) shown to give the highest sensitivity for detecting *C. jejuni* in chickens. The overall rate in 44- to 49-day-old broiler chickens, reared at the farm during 1985 and 1986, was 85% (based on samples from 510 broilers collected over 19 weeks, each week

relating to a different shed of one particular flock of broilers). Throughout this time, the broilers being reared had only one *C. jejuni* serotype complex (Lior type 1, Penner type 4 complex [L1P4c]), which subsequent investigations revealed had been introduced via the water distribution system and had persisted by colonizing the water header tanks in each shed. Tank cleaning eliminated the outbreak strain serotype L1P4c, which was thereafter replaced by other serotypes. L1P4c was isolated very rarely from broilers on the farm over the next 8 years and was never detected during the period from March 1989 to March 1994. From July 1986 to March 1987, multiple farm-based interventions, including water and line disinfection, replacement and vigorous cleaning of the header water tanks, and cleaning the distribution system (23), were successful in reducing the *Campylobacter* isolation rates from 85 to 7%. After the interventions were stopped, the isolation rates rose to 80%. Between April 1987 and February 1989, simplified interventions were introduced, resulting in a reduction of the isolation rates to 20.5%. During the 1989 to 1994 study, simplified interventions, water tank cleaning and line disinfection, were adopted as standard farm practice and an overall *Campylobacter* isolation rate of 27% was achieved (Table 1). Not surprisingly, a decrease in isolation rates from 36.7 to 16.9% was observed between March 1989 and March 1994. Against this background of diminishing horizontal transmission there became apparent a pattern of intermittent shed positivity within the same broiler flock. During this study period, on only 23 occasions (9.2%) was *C. jejuni* isolated from successive flocks in a shed, and only 9 of the 23 yielded the same serotype, showing that there was little evidence of horizontal transmission between sheds once the overall isolation rate had been reduced to 37% on this farm. Thus, horizontal transmission was the likely explanation only when the same serotype predominated in consecutive flocks within the same shed. Conversely, when a new serotype predominated in the next flock, throughout the shed and in all or the majority of sheds, this was suggestive that there was a common source of introduction to the whole farm. Results of the study reported here demonstrate such a pattern of introduction of new serotypes. In six broiler flocks, a new serotype colonized broilers throughout all or most of the sheds within a single flock: serotype complex D was introduced in March 1990; serotypes A, B, E, and X were introduced in September 1990; serotype C was introduced in July 1991; serotype F was introduced in March 1992; serotype X was introduced in July 1992; and serotype B was introduced in June 1993. It is concluded that these represent common-source, intermittent introductions of serotypes that subsequently predominated across most or all of the sheds. The hypothesis proposed in this study was that these introductions could be traced either to the hatchery supplying the 1-day-old

TABLE 5. Number of broiler shed flocks from which *C. jejuni* was isolated, by hatchery

Hatchery	No. (%) of shed flocks			
	Total	With <i>Campylobacter</i> isolation rate of:		
		>95%	5-95%	<5%
A	149	14 (9.4)	23 (15.4)	112 (75.2)
B	79	25 (31.6)	16 (20.3)	38 (48.1)
C	4	0	0	4 (100)
D	1	0	0	1 (100)
Other	18	5 (27.8)	2 (11.1)	11 (61.1)
Total	251	44 (17.5)	41 (16.3)	166 (66.1)



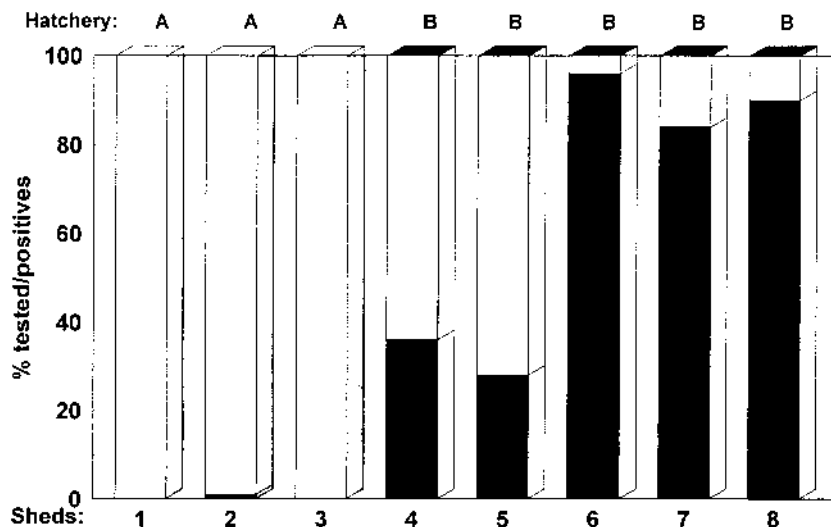


FIG. 3. *C. jejuni* prevalence in eight sheds during March 1992 flock indicating the hatchery supplier of the chicks for each shed and the *Campylobacter* isolation rate (50 birds sampled from each shed = 100%).

chicks or to vertical transmission of *C. jejuni* from broiler breeder grandparents or parents to their chicks and then through to the layer flocks, with *Campylobacter* amplification occurring within sheds. The range of serotype complexes found in this study was very restricted compared with that in previous reports (16, 22, 26), indicating that the source in this study was more likely to be from parent breeder farms than from delivery vehicles, chick transport crates, or cross contamination within the hatchery. It is noteworthy that the literature on *C. jejuni* infection of eggs occurred with a limited variety of strain types (30, 31) which unfortunately were not detailed as to serotype.

Hatchery A was associated with an isolation rate of 17.5%, compared to 49% in hatchery B, and quite different types of *C. jejuni* and a similar difference in occurrence of *Campylobacter* isolates occurred when both hatcheries supplied different sheds within the same broiler flock. This result, taken together with a restricted range of serotypes, the different serotypes for each hatchery, and a reduced likelihood of colonization in

broilers supplied by hatchery A, is evidence of an intermittent, common source of *Campylobacter* isolates originating from the parent breeder or layer farm flocks through vertical transmission.

What led previous investigators to discard the hypothesis of vertical transmission? A low rate of isolation from naturally or experimentally infected eggs has led investigators to suggest that vertical transmission of *C. jejuni* is not an important route of transmission for the broiler industry (6, 10, 14, 30, 31, 33), although one report by Shanker et al. in 1986 (31) found that two chicks were colonized with *C. jejuni* at the time of hatching after injection of 167 eggs with *C. jejuni*. Since natural infection was not demonstrated by their results, the authors concluded that their data did not support biological vertical transmission. Early work by Doyle had demonstrated the potential for vertical transmission when 67% of individually caged hens were shown to excrete *C. jejuni* over a 42-week study (9). One percent of eggs had external contamination (2 of 226), and the

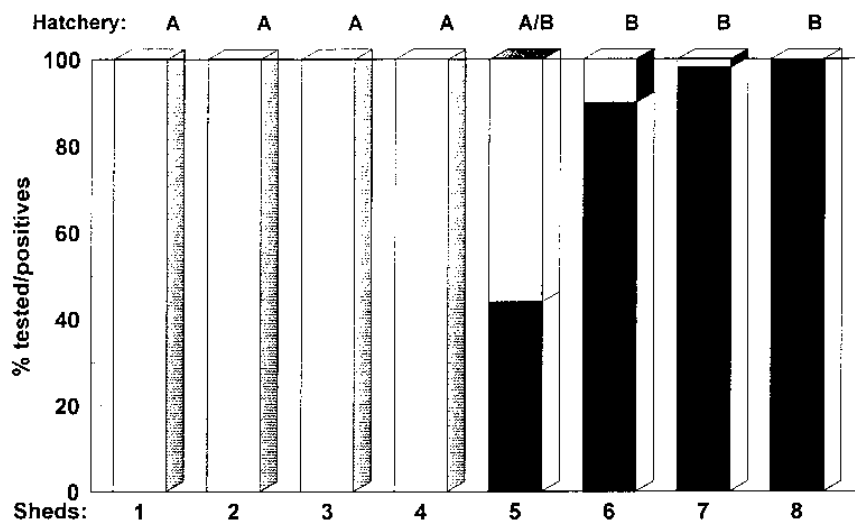


FIG. 4. *C. jejuni* prevalence in eight sheds during November 1992 indicating the hatchery supplier of the chicks for each shed and the *Campylobacter* isolation rate (50 birds sampled from each shed = 100%).

organism could be recovered from the inner shells and membranes of refrigerated eggs. There are three published reports of *C. jejuni* being isolated from egg contents, one reporting recovery of *C. jejuni* from egg shells and membranes (30) and the other two reporting recovery from the egg contents (12, 21). An epidemiological association between eating undercooked eggs and acquiring *Campylobacter* infections was shown during investigations of an outbreak that occurred in Minnesota in July 1982, when 26 of 81 people became ill (32% attack rate) (11). The role of vertical transmission of *C. jejuni* in parent or layer farm bird flocks needs to be reexamined in the light of the new evidence presented here and of inferences from the studies cited above (3, 11). We postulate that the most likely explanation of our finding is of low-level vertical transmission from hatchery supplier of 1-day-old chicks of a restricted range of *C. jejuni* types amplified by horizontal transmission within the shed environment of a single broiler flock. These results were elucidated by eliminating the bulk of extraneous-source horizontal transmission by the 4-year intervention studies carried out on this farm.

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