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MINIREVIEW

Sources of *Campylobacter* Colonization in Broiler Chickens

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Campylobacter jejuni and its close relative *Campylobacter coli* (hereafter jointly referred to as *C. jejuni* or campylobacters) are gram-negative, thermophilic, obligate microaerophilic bacteria that are ubiquitous in temperate environments. Both species colonize the intestinal mucosa of most warm-blooded hosts, including all food-producing animals and humans (87; reviewed in reference 67). However, the favored environment appears to be the intestines of all avians, including wild birds, chickens, turkeys, quails, ducks, and even ostriches (41, 62, 90, 102, 109, 111, 116). Campylobacters generally colonize avians as a commensal organism, with the possible exception of ostriches (107). In contrast, in humans, particularly in children and in adults in industrialized countries, infection is associated with acute enteritis (97). It is generally assumed that campylobacters contaminate poultry meat during processing, surviving throughout the food chain supply to constitute a risk to human health.

Human campylobacteriosis is the most common cause of food poisoning in much of the industrialized world (115), and the reduction and/or elimination of *C. jejuni* in the food chain, particularly from chicken products, is a major strategy in efforts to control this disease (2). One approach to this goal is to prevent *C. jejuni* colonization of broiler chickens. Such an approach has been used to control *Salmonella* contamination in poultry, but the measures put in place for *Salmonella* contamination have been generally unsuccessful for *C. jejuni*. This is considered a reflection of differences in the physiology, epidemiology, and ecology of these organisms.

This paper reviews the descriptive epidemiology and ecology of *C. jejuni* in broiler flocks and, where possible, assesses this information in terms of the potential effectiveness of targeted biosecurity measures to prevent flock colonization.

PHYSIOLOGY AND ECOLOGY OF *C. JEJUNI* COLONIZING BROILERS

In chickens, *C. jejuni* colonizes the mucus overlying the epithelial cells primarily in the ceca and the small intestine but may also be recovered from elsewhere in the gut and from the spleen and liver. Experimentally, the dose of viable *C. jejuni* required to colonize chicks and chickens can be as low as 40 CFU (16). However, this dose and the kinetics of colonization may be dependent on both the bacterial strain (78) and chicken strain (P. Barrow, unpublished data). Once colonization is established, campylobacters can rapidly reach extremely high numbers in the cecal contents, as high as 10^9 CFU in experimentally challenged birds (112) although this level may be lower in naturally colonized birds (81).

Campylobacters are readily detectable in the feces of colonized birds. Under experimental conditions, in vivo-passaged organisms can exhibit an enhancement of colonization potential of at least 1,000-fold in most strains (78) and up to 10,000-fold in some strains (16). This presumably reflects the upregulation of bacterial factors important for colonization. As chickens are coprophagic, fecal shedding is presumably an important factor in the dissemination of organisms around large broiler flocks once the first bird becomes colonized. Certainly, once flock colonization is detected, bird-to-bird transmission within flocks is extremely rapid, and the majority (up to 100%) of birds in a positive flock are colonized within only a few days (64, 85). These colonization kinetics indicate that measures to reduce the "within-flock prevalence" at slaughter are likely to be unsuccessful, which is relevant to quantitative risk assessment models currently under development (42).

Interestingly, epidemiological investigations of commercial flocks indicate that naturally acquired flock colonization is age dependent. Newly hatched chicks appear to be free of campylobacters. In Europe this negativity persists until at least 10 days of age (the so-called lag phase), and most flocks become infected only 2 to 3 weeks after the placement of chicks into a broiler house (26, 53). Colonized chickens usually show no observable clinical symptoms of infection even when young chicks are exposed to high doses under experimental conditions.

The duration of colonization and shedding in poultry has not been fully determined. It is generally accepted that colonization in chickens persists at least for the life span of a broiler. In conventionally reared birds, this is usually less than 47 days. However, in experimentally challenged birds, persistence of colonization may vary among campylobacter strains (78). Moreover, after 8 weeks, colonization may gradually reduce in terms of both the number of organisms recoverable from the cecal contents and the number of colonized birds (1). Self-limitation of infection has been reported in other naturally colonized birds; for example, gulls can become negative for *Campylobacter* spp. within a period of 4 weeks (36). The factors involved in the self-limitation of colonization are unclear but may involve acquired immunity. The experimental challenge of chickens induces circulating and mucosal antibody responses directed against a range of *C. jejuni* antigens (14, 80). The efficacy of such antibodies in preventing or limiting infection remains unknown. However, elderly hens can be antibody pos-

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TABLE 1. Prevalence of campylobacters in broiler flocks from selected countries

Country	No. of farms	No. of flocks	No. of samples	Sample type	Prevalence (%)	Reference
US	8	32		Ceca/feces	87.5	92
UK	23	49			76	50
		100		Cloaca	>90	26
		151		Cloaca	45	27
Denmark		8,911	89,110	Cloaca	42.5	113
		4,286	57,000		39.6 (*C. jejuni*) 5 (*C. coli*)	114
Norway	176	176			18	57
Sweden	18	287			27	6
Germany		12	509		41.1	4
Italy				Cloaca	80	Borvelli and Sola, conference presentation, 2000
France	75	75		Feces	42.7	76
Canada		45		Cloaca or ceca	44.4 (*C. jejuni*)	9
Chile			300	Feces	19.7 (*C. jejuni*) 6 (*C. coli*)	31
Taiwan				Cloaca	24.1	17
Malaysia		10	138		53.7 (*C. jejuni*) 28.3 (*C. coli*)	81
Japan			200		45	96

itive without colonization, suggesting that the antibody response may be associated with elimination of infection (33). Nevertheless, as yet, vaccination of chickens against *C. jejuni* colonization has had only partial success (59, 70, 77).

The reason for the lag phase is unknown. This phenomenon has also been detected in preliminary studies in organic broilers (68). As such birds are frequently exposed, even when young, to this environmentally ubiquitous organism, this lag phase is likely to be an inherent property of the chick. The avian intestinal niche undergoes many physiological changes during the first few weeks of life, including the maturation of mucosal immunity and shifts in the microflora. Moreover, several changes in stock management may occur during this period, including altered feed composition, even in organically produced birds. The impact of such changes on host susceptibility to infection is unknown. Interestingly, preliminary evidence suggests that young birds removed from commercial flocks during this lag phase are more resistant to experimental infection than specific-pathogen-free birds (68) and that this resistance reduces with time. Explanations for these observations include an inhibitory effect produced by commensal organisms in the gut of young chicks (82) and the presence of maternal antibodies which may be protective and which decline by about 14 days of age (14, 80). Recent experimental data suggest that such antibodies can effectively reduce the susceptibility of chicks to challenge (Sahin et al., 102nd Annu. Meet. Am. Soc. Microbiol., Salt Lake City, Utah, 2002, abstr. Z13). Another factor which might contribute to the lag phase is antimicrobial treatment, but this is unlikely in organic flocks. As an understanding of the nature and mechanism of the lag phase could provide insight into the susceptibility of birds to *C. jejuni* infection under commercial conditions, more research on this aspect of colonization is urgently required.

PREVALENCE OF CAMPYLOBACTERS IN BROILERS

A recent report of a joint Food and Agriculture Organization/World Health Organization Expert Consultation on the risk assessment of *Campylobacter* spp. in broiler chickens (www.who.int/fsf/Documents/Bangkok_Campy_02_En.pdf) indicated that there is a linear relationship between flock prevalence and the probability of human campylobacteriosis. Thus, reduction of the prevalence of positive flocks should contribute substantially to the reduction of human disease.

Limited data exist on the prevalence of campylobacter-positive poultry flocks in different countries. Such studies are expensive and time- and resource-consuming, and only certain countries, for example, Sweden and Denmark, appear to be doing this routinely. In most countries, the nature of the poultry industry makes random sampling and sample collection at the farm level difficult.

The proportion of broiler flocks colonized with campylobacter varies among countries (Table 1). However, this variation may reflect, at least in part, different sampling and isolation methods used. In the United States, a recent survey (92) indicated that nearly 90% of flocks are colonized. In Europe, this prevalence varies from 18 to >90%, with the northernmost countries having substantially lower figures than southern European countries. In Sweden, for example, recent national surveys indicate that flock positivity is less than 10% (L. Engvall, personal communication). The reason for this is unknown. Zootechnical parameters, including the number of animals per farm, climatic conditions, and distance between farms, may all influence flock prevalence. It is also possible that the poultry industries in these northern European countries are less mature (and therefore exploit newer facilities) and more closely regulated than elsewhere in Europe. It remains to be seen whether similar trends occur in other regions of the world.

Several surveys have indicated a seasonal variation in the prevalence of poultry flock colonization (41, 57, 76, 110; D. G. Newell, E. Hartnett, M. Madsen, J. Engberg, T. Hald, A. Wedderkopp, and A. Engvall, presented at the 10th International Workshop on *Campylobacter*, *Helicobacter*, and Related Organisms, Baltimore, Md., 1999). There is generally a higher rate of infection in summer than in winter, and the timing of this peak also appears to vary with latitude. Some studies have

failed to demonstrate seasonality in the prevalence of *C. jejuni* in poultry (27, 50), but this may be a consequence of the sample size and flock selection or geographical location. The number of cecal organisms per bird (110) and the strain types present (48) may also be seasonally related. The reason for these seasonal variations is unknown but may reflect levels of environmental contamination. Certainly, poultry houses have more ventilation in the summer, increasing the contact of the birds with the outside environment. Individually caged hens also have a seasonal variation in excretion rates (24), suggesting that, under circumstances limiting bird-to-bird contact, climatic changes influence colonization

Preliminary data analysis (Newell et al., presented at the 10th International Workshop on *Campylobacter*, *Helicobacter*, and Related Organisms, Baltimore, Md., 1999) suggests that the seasonal variation in humans coincides with or even precedes rather than follows that in poultry, which may indicate a seasonality in common environmental sources yet to be identified. Seasonally associated shedding has been reported in wild birds (23), cattle (89), and sheep (56), but the relationship between this and seasonal colonization in broiler flocks is unknown.

The prevalence of flock positivity is also dependent on flock size (6) and the type of production system. Flock positivity is generally higher (up to 100%) in organic and free-range flocks (43) compared to intensively reared flocks This presumably reflects the level of environmental exposure of such birds as well as the increased age of the birds at slaughter.

In all cases where identification to species level has been undertaken, the majority (80 to 90%) of colonized broiler flocks were determined to be colonized with *C. jejuni*. The remainder were primarily colonized by *C. coli* and occasionally by *C. lari* (104). In the United Kingdom (5), Sweden (7), and the Netherlands (53), studies suggest that conventionally reared broiler flocks are predominantly colonized with a limited number (generally one to two) of *C. jejuni* subtypes. In contrast, in other countries, such as the United States (44, 47, 66) and Australia (100), multiple campylobacter strains are commonly isolated from broiler flocks. This may indicate either that in some countries, conventionally reared flocks are exposed to several sources potentially containing different campylobacter strain types or that there is a single source which contains multiple strain types.

SOURCES OF POULTRY COLONIZATION

Current understanding of the sources of poultry colonization by human enteric pathogens is largely derived from work undertaken on salmonellae. With this organism, recognition of the importance of both vertical and horizontal transmission routes of infection has been pivotal in the development of appropriate control and prevention strategies. With campylobacters, most studies have focused on horizontal transmission, but most recently, vertical transmission has been more thoroughly investigated.

Vertical transmission. The vertical transmission of campylobacters to flocks via contaminated eggs remains controversial. Breeder hens are usually colonized by multiple strains of *C. jejuni* (51). These organisms can be recovered from various segments of the reproductive tract, including the oviduct (10, 12). Genotyping has demonstrated that diverse strains are recoverable and some may be identical to those seen in the feces, suggesting that at least some of these organisms ascend from the cloaca (12, 45). *C. jejuni* has also been recovered from rooster semen, suggesting that the genital tract of hens may also be infected venereally (21). Whether the presence of campylobacters in the oviduct results in the colonization of chicken embryos is unknown. Few studies have been undertaken to determine the proportion of egg contents contaminated. In early studies, *C. jejuni* was recovered from about 1% of eggs from colonized hens (24, 84). Potentially, this prevalence of infected eggs could be higher as bacterial survival in experimentally infected eggs may be short-lived (20, 65).

Intact egg shells appear to be permeable to *C. jejuni*. Over 4% of eggs can be experimentally infected with *C. jejuni* by immersion in a suspension of organisms (3), indicating that contact with fecal material could result in egg contamination. However, in eggs experimentally infected in this way, the bacteria are restricted to the inner shell or membranes rather than the egg contents (24, 65). These results suggest that the natural infection of egg contents, if it occurs, is primarily due to fecal contamination of the external surface and penetration via shell cracks (24). Moreover, chicks could become orally infected during hatching from such egg shell contamination.

Experimental studies therefore indicate that *C. jejuni* can penetrate egg contents, either via oviduct colonization or, more likely, by fecal contamination of egg shells. However, whether this generates colonized chicks and, consequently, campylobacter-positive flocks remains questionable. The inoculation of chorioallantoic membranes with *C. jejuni* is lethal to chicken embryos (60), although embryo susceptibility is chicken lineage and bacterial strain dependent. Only about 10% of experimentally infected eggs produce viable chicks (20, 83). Nevertheless, the chicks that hatch contain *C. jejuni* in their intestinal tract (20) and could therefore act as a source of flock colonization. *C. jejuni is* not recoverable from hatched chicks or tray liners (51, 61, 74). However, based on a direct correlation between hatchery source and broiler flock positivity, Pearson et al. (74) concluded that vertical transmission was an external source of *C. jejuni broiler* flock colonization.

In contrast, Jacobs-Reitsma et al. (52) sampled two broiler farms in the Netherlands, each of which possessed its own hatchery and breeding flocks. On one of the farms, the laying hens were colonized by *C. jejuni* throughout the survey period (eight consecutive broiler cycles). Broilers from these parent flocks were found to be colonized with *C. jejuni* in one production cycle and *Campylobacter* free in another, suggesting that there was no direct relationship between flock positivity and hatchery source. Additional support for this comes from experimental colonization studies. In particular, over the last 10 years, approximately 180 experiments have been undertaken in our laboratory, each with an uninfected control group of at least 10 1-day-old chicks. These control chicks (n = at least 1,800) were hatched from the eggs of four flocks of non-campylobacter-free commercial laying flocks from England, the United States, and Germany and raised in closed incubators. The cecal contents of each chick was cultured at 6 days of age for *C. jejuni* by a technique which had a detection limit of 200 CFU per g of cecal contents. Under these conditions, no evidence of naturally acquired colonization has ever been ob-

served (S. Cawthraw, unpublished data), suggesting that vertical transmission had not occurred in these chicks.

More recently, the discriminatory power of molecular typing has allowed more detailed investigations into the potential role of vertical transmission as a source of broiler house contamination. Some comparative studies of strains from broiler flocks and from the parent flocks supplying them suggest that vertical transmission is rare, if it occurs at all (18, 75). In a further extensive study in the Netherlands, strains from 21 breeder flocks were compared by riboprinting with strains recovered from progeny broiler flocks. Only on one occasion did such strains match by ribotype, but by amplified fragment length polymorphism, these strains were not identical (52), shedding doubt on the relatedness of these strains. However, in a more recent comparison of strains from commercial broiler breeder flocks and their respective progeny using DNA sequence analysis of the short variable region of the *flaA* gene (22), genotypic identity between strains indicated that breeder hens are a potential sources of poultry flock contamination with campylobacters.

In the event of vertical transmission, it would be expected that organisms would be detected in an affected flock immediately after hatching. This is certainly observed with the vertical transmission of salmonellae in chickens. However, as mentioned previously, there is a consistent lag in the detection of *C. jejuni* colonization in chickens, which suggests that vertical transmission of this organism is uncommon. It is, of course, possible that small numbers of organisms may be present in the hatching chick, but the growth of these organisms is constrained by environmental factors such as maternal antibodies. Indeed, more sensitive molecular detection techniques such as Southern blot hybridization and PCR have detected *Campylobacter* DNA in fluff and egg shells of hatchery waste (46) and in the cecal contents of some chicks less than 3 weeks of age (19). Nevertheless, viable organisms were not recovered, even with enrichment techniques, suggesting that this DNA may not be associated with live cells.

In conclusion, the debate on the role of vertical transmission as a source of flock colonization continues. However, overall the most pragmatic approach is to control the horizontal sources that appear more obvious risks and then determine the role of vertical transmission in any residual problem.

Horizontal transmission. The houses used for intensively reared broilers can largely be considered closed environments. However, campylobacters are ubiquitous in the environment in and around broiler houses. These organisms constitute a substantial risk of flock contamination. Nevertheless, no direct relationship between the detection of such environmental campylobacters and flock positivity has been observed (92).

Internal contamination of the broiler house may be due to the residual presence of these organisms either from previous campylobacter-positive flocks or associated with resident populations of vermin or insects. Organisms from the external environment would need to be transported into the house either in utilities (such as feed, litter, and water), by human activities (associated with the entrance of farmers, maintenance staff, veterinarians, and catching crews and their equipment), or by the unofficial entrance of domestic or wild animals, birds, and insects.

Many epidemiological studies have been undertaken to identify the risk factors associated with *C. jejuni* flock infection. Such studies have generally involved a questionnaire-based approach coupled with detection of *C. jejuni* flock positivity (7, 26, 40, 50, 76). With these data, univariate analyses of farm management data are undertaken, followed by logistic regression analysis to identify risk-increasing and risk-reducing factors. There are substantial differences in the risks reported from these surveys. Such differences are presumably a reflection of variation in poultry management practices. Some common risk factors can, however, be identified. These include poor house maintenance (as indicated by large rodent populations and poor building repair), poor hygiene barriers and inadequate staff compliance (including insufficient use of boot dips and changing outer clothing), insufficient cleansing and disinfection between flocks (including the reuse of old litter), short empty periods, close location to other poultry sites or farm animals, flock thinning, and contaminated water supplies (especially poorly cleaned water pipes or polluted stored water). With such a variety of risk factors, the accurate identification of sources is essential to ensure that the most effective interventions are put in place. In the following sections, each potential source is reviewed and assessed for relative importance.

(i) Feed and litter. The dry conditions of feed and fresh litter are considered lethal to *C. jejuni*. Since the organisms are not isolated from clean dry litter, it is widely accepted that feed, feed additives, and fresh litter are not potential sources of infection (7).

(ii) Water. The ability of *C. jejuni* to survive in water under experimental conditions is well recognized. Campylobacters can be isolated from the water lines and reservoirs of broiler houses, and these strains may be phenotypically (74) and genotypically (I. Ogden, unpublished data) identical to those recovered from the feces of chickens in the same houses. However, water contamination usually follows rather than precedes colonization of a flock (25, 58, 61), suggesting that this is a consequence of the tracking up through the water lines of organisms excreted from the birds. Certainly, water cups become campylobacter positive at about the same time that flocks become positive (6). Detection of *C. jejuni* in broiler house water sources prior to stocking and flock positivity has proved difficult (106). However, in natural water supplies, shearing forces, high oxygen concentration, UV, nutrient deprivation, and antimicrobials all cause the bacteria to become environmentally stressed. Thus, the viability of *C. jejuni* in water under these conditions may decline rapidly. In addition, the methods necessary for recovery from water contribute to the physical damage and reduced recoverability (49).

The effect of water-related environmental stresses on campylobacters has been investigated extensively. Experimental evidence suggests that under such conditions *C. jejuni* generates forms that are viable but nonculturable, especially in the presence of biofilms derived from poultry houses (101). The survival of such forms for periods of up to 4 months has been recorded (79), but survival times appear to vary with water temperature, strain, the aquatic system used, and previous growth conditions (11, 98, 99). There has been considerable debate as to whether such water-stressed bacteria retain infectivity. Although water-borne human outbreaks have been recorded (108), experimental animal studies have had variable

success in demonstrating the infectivity of environmentally stressed *C. jejuni* (8, 13, 38, 55, 63, 105), especially in chicks. One study (30) even suggested that the colonization potential of culturable *C. jejuni* for chicks is severely compromised by long-term exposure to water. However, the infectivity of water-stressed organisms in chickens, like recoverability, may be strain dependent (95).

Several epidemiological studies have investigated the relationships between water source (well or mains water) and broiler flock *Campylobacter* positivity. Most studies found that the water source was a low-risk factor (6, 50, 53), but this is disputed in other studies (73). Intervention studies have provided a clearer indication that the addition of sanitizers can significantly reduce either the probability that a flock will become positive (26, 54, 57) or the proportion of positive birds (73). Although the levels of chlorination normally found in potable waters would normally be considered lethal to *C. jejuni*, there is preliminary evidence that water-borne protozoa, such as *Tetrahymena pyriformis*, can act as reservoirs for *C. jejuni* in broiler house water systems. Experimental cocultivation of *C. jejuni* with such protozoa appears to reduce the susceptibility of the bacteria to chlorine as well as to the disinfectant Virudene and to extend the viability of the organisms (W. Snelling, personal communication). Such factors may contribute to the recent observations by Stern et al. (94) that chlorination of flock drinking water had no effect on *C. jejuni* colonization. There is as yet no explanation for the reported observations that acidification (76) and chlorination (91) can sometimes increase the risk of colonization.

Overall, the evidence suggests that *C. jejuni*-contaminated water constitutes a risk, albeit relatively low, of colonization for broiler flocks. Nevertheless, further research is required to establish the role of water line and reservoir contamination in flock colonization and the value of water sanitizers as a control measure.

(iii) Broiler house cleansing and disinfection. The carryover of infection from a positive flock to a new flock in the same house is an obvious potential source. This may be particularly pertinent in countries where used litter is routinely left in the houses between crops. In the United Kingdom and other European countries, where used litter is removed and houses are cleaned between flocks, longitudinal studies indicate that infection is not predictable from the *Campylobacter* status of the last flock in the house (26). Negative flocks often follow positive ones (6), and positive flocks can occur even in newly constructed broiler houses (37). There are no published reports of *C. jejuni* isolation from the floors and walls of emptied, cleansed, and disinfected houses (and many groups have searched). Even chicks raised on litter removed from a broiler house positive for *C. jejuni* did not become colonized over a 7-week period (72), indicating that under such conditions, the organisms poorly retain colonization potential.

In one recent United Kingdom study, only 15% of sequential flocks in 100 houses showed evidence of genotypically identical strains (85), consistent with the carryover of strains from one flock to the next. Other studies have similarly shown that the genotypes of strains can vary from one crop to another (W. Jacobs-Reitsma, unpublished observations). This suggests that routine house cleansing and disinfection are largely adequate for *C. jejuni* decontamination and confirms evidence from earlier studies with serotyping methods (53). However, this observation may also be true in houses where emptying, cleansing, and disinfection are not routinely carried out between flocks (47), indicating the poor survival of organisms under these environmental conditions. Nevertheless, flock positivity has been linked to the "turnaround" time in a house (40, 114), suggesting that longer periods (over 14 days) between sequential flocks reduce residual bacterial contamination in or around a previously positive house. Clearly, the hygiene-related practices during the time between the removal of one flock and the placement of new birds, with respect to *Campylobacter*-positive flock status, requires further investigation.

(iv) Aerosols. Although campylobacters can be isolated from air (6, 58), there is an assumption that *C. jejuni* cannot survive for long periods within the dehydrating conditions of aerosols. However, when the atmosphere is heavily laden and humid, *C. jejuni* can apparently travel considerable distances and retain infectivity, at least to humans (69). Within closed broiler houses, the location of ventilation fans (34) affected the risk of flock positivity, and the use of air conditioning increased this risk (76). In a multipen study (85), the pens first infected were located closest to vents juxtaposed to the incinerator used for the disposal of dead birds from the whole site. This suggested that aerosols might have been involved.

The role of aerosols is unclear, and air is a broiler house commodity that would be impossible to control. Nevertheless, the use of vents that take in air from potentially contaminated areas should be avoided in house design.

(v) Human traffic and activities. The main human traffic in and out of a broiler house is the farm staff for the purpose of routine animal husbandry (71). In industrialized countries, asymptomatic carriage of *C. jejuni* by humans is rare (97). However, as persons working in endemically infected environments appear to be immune (15), poultry-house workers may fecally carry *C. jejuni* more frequently. This is still speculative, but staff should be encouraged, and provided with the facilities, to wash their hands routinely before and after entering a house (35).

Campylobacters can potentially be carried into the house from the external environment via boots, external clothes, and equipment. The level of biosecurity required of farm staff and other broiler house visitors to ensure flock negativity has yet to be established, but the presence of a hygiene barrier, including an anteroom with a walk-over bench and the use of house-dedicated footwear, was considered the single most important risk factor in one recent Danish study (40). In longitudinal sampling surveys within a broiler house where bird movement was restricted (85), the first birds colonized were close to unofficial doors (without hygiene barriers) used by the staff. This would be consistent with the trafficking of campylobacters into the house by farm staff. However, positive samples from the boots of farm staff are usually only reported once the flock has become *Campylobacter* positive (47). Nevertheless, the risk of flock positivity increased with two or more people involved in farm management (76). This risk is greater when staff have been tending other poultry or pigs prior to entering the house (7, 57) but can be reduced by the use of effective boot dips (50) or of house-dedicated boots (35, 40).

The extent of *Campylobacter* contamination in the environment around broiler houses will obviously contribute to this

risk. *C. jejuni* can be recovered from both standing water and soils. Recently, genotyping techniques have shown that at least some of these environmental isolates, particularly those from standing water, recovered before flock positivity, had the same genotypes as isolates subsequently recovered from the broiler flock (47, 68). Thus, clean and intact concrete aprons can reduce the risk of carrying in contaminated material. Equipment routinely taken into the broiler house should also be considered and disinfected, for example, buckets and bags to remove dead birds and weighing machines.

Biosecurity measures should also apply to visitors. Maintenance personnel, especially those traveling between farms, and their equipment may be instrumental in the transmission of *C. jejuni* between flocks, but this has not been fully investigated.

Thinning procedures, reducing bird density within the broiler house, are in common practice in many European countries, including the United Kingdom, but occur infrequently in some other countries, such as the United States. This practice apparently enables higher productivity and is required for an optimal retail supply of birds of different weights. Thinning occurs at about 35 days, depending upon the size and weight of the birds, and is undertaken by catching crews routinely traveling between farms and the poultry abattoir with their catching equipment and crates. The crates in particular are contaminated with *C. jejuni* from previous flocks and from inadequate washing procedures (47, 69, 88), and as shown by genotyping, these strains may contaminate end products following processing.

Thinning appears to be a major risk factor in the introduction of *C. jejuni* into the broiler house (50). Hald et al. (39) demonstrated that all flocks which were *C. jejuni* negative at the time of batch depletion became positive by the following week when the remaining birds were sent for slaughter. Such contamination routes may also be important during the final depopulation, which can take place over several days. Flocks which are only partially positive at slaughter may well represent such late contamination events (V. Allen, unpublished data).

(vi) Wild and domestic animals and insects. The environment around broiler houses varies considerably but is usually rural. Wildlife, including rodents and birds, will readily occupy such environments and be naturally attracted to spilled feedstuffs and wastes. *C. jejuni* can be isolated from the feces of wild rodents (32), in particular the intestines of mice trapped in broiler house environments (47), albeit probably as a transient colonizer. Genotypic studies suggest that such mice become positive with the same strain shed by chickens in the broiler house (47) but are unlikely to be the source of this colonization. In epidemiological studies, the presence of rats and mice on a farm can lead to an increased risk of flock colonization, but this is not usually considered a significant risk factor (7, 57), especially when farms operate an effective vermin control program.

Moreover, campylobacters can also be recovered from other wild animals, such as deer, foxes, rabbits, and badgers (N. French, personal communication). An even greater risk is free-ranging livestock (pigs, cattle, and sheep) and domestic pets (cats and dogs) around the broiler houses. Although such animals are unlikely to enter houses, they may excrete campylobacters in substantial numbers, and this can result in contamination of boots, other external clothing, and equipment taken into the houses. Certainly, genotypically identical strains can be found in cattle kept next to the broiler house and the broilers within the house (37, 68), and the presence of other farm animals on the farms is a risk factor for flock colonization (35). Concrete aprons around houses that are regularly cleaned and disinfected, the enclosure of grazed areas, and restrictions on the freedom of movement of domestic pets should be effective measures to reduce these risks.

Insects, including flies (37), darkling beetles (*Alphitobius diaperinus*) (53), and cockroaches (*Periplaneta americana* and *Blatta orientalis*) (103), in and around broiler houses, have been reported to be carriers of campylobacters. The bacteria may survive on or within these insects for only a few days (28). Nevertheless, resident insects in a house are associated with an increased risk of flock colonization (76), but as carryover from one house to another is relatively infrequent (86), this seems to be a relatively low risk.

The final potential wildlife source to be considered is wild birds. *C. jejuni* has evolved to most effectively colonize the avian gut, and not surprisingly, many wild birds, including waterfowl, pigeons, and passerines, are colonized (23, 29, 116). The prevalence of colonization may be dependent on age, species, habitat, season, and migratory behavior (109, 116). Campylobacters are frequently isolated from wild-bird feces around broiler houses, and studies with molecular epidemiological tools have reported that the strains isolated from such samples can, on occasion, subsequently be recovered from the cecae of broilers in those houses (47, 68, 93). The frequency of observation of wild birds in closed broiler houses seems to be debatable. Clearly this is dependent on the house integrity and management practices. In particular, the practice of opening houses for cooling purposes may provide considerable opportunity for wild-bird access. Given the potential importance of such a source, this issue should be addressed and data obtained at the earliest opportunity.

CONCLUSIONS

The lessons learned from *Salmonella* control in poultry have been of little help in the control of *C. jejuni* in the same environment. This is a reflection of significant differences in the ecology and physiology of these organisms.

Horizontal transmission is generally considered the most significant cause of *C. jejuni* infection in broiler flocks. Campylobacters are ubiquitous in the environment and could be readily carried into the house by a number of vehicles, including human activity associated with routine flock management. However, this has yet to be proven by reproducible observation, confirmed by genotyping, of strains in the environment which subsequently result in flock colonization.

The literature suggests that standard biosecurity procedures are inadequate for the maintenance of flock negativity. This is a consequence of high exposure, low dose, and rapid bird-to-bird transmission rates. Nevertheless, stringent biosecurity may either delay positivity or reduce the number of flocks that become positive. However, it is generally considered that adequate biosecurity procedures are difficult to sustain in the farm environment (71). For example, routine procedures such as the effective use of hygiene barriers, hand washing, and boot

disinfection may be readily undertaken under normal conditions, but during emergencies, such as fan failure, such procedures may be ignored. Well-designed and well-located farms, the development of appropriate standard operating procedures to minimize risk factors, staff education, and incentives to maintain biosecurity at the highest level would all contribute to the reduction of flock positivity. Nevertheless, at least in some countries, such as the United Kingdom, the development of supplementary on-farm control strategies may be required to achieve predominantly *C. jejuni*-negative flocks.

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