Biosecurity-Based Interventions and Strategies To Reduce Campylobacter spp. on Poultry Farms

D. G. Newell,1,2* K. T. Elvers,3 D. Dopfer,4 I. Hansson,5 P. Jones,6 S. James,3† J. Gittins,7 N. J. Stern,8 R. Davies,9 I. Connerton,9 D. Pearson,10 G. Salvat,11 and V. M. Allen3

Veterinary Laboratories Agency, Addlestone, United Kingdom1; Foodborne Zoonoses Consultancy, Andover, United Kingdom2; University of Bristol, Langford, Bristol, United Kingdom3; Central Veterinary Institute of Wageningen University and Research Centre, Lelystad, The Netherlands4; CRL-Campylobacter, National Veterinary Institute, Uppsala, Sweden5; University of Reading, Reading, United Kingdom6; ADAS Pwllpeiran, Cwmystwyth, Aberystwyth SY23 4AB, United Kingdom7; Russell Research Center, ARS, USDA, Athens, GA8; University of Nottingham, Nottingham, United Kingdom9; VION Agriculture Ltd., Livingston, United Kingdom10, and Agence Française de Sécurité Sanitaire des Aliments (AFSSA), Laboratoire d’Étude et de Recherche Avicoles Porcines et Piscicoles, Ploufragan, France11

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The prevention and control of Campylobacter colonization of poultry flocks are important public health strategies for the control of human campylobacteriosis. A critical review of the literature on interventions to control Campylobacter in poultry on farms was undertaken using a systematic approach. Although the focus of the review was on aspects appropriate to the United Kingdom poultry industry, the research reviewed was gathered from worldwide literature. Multiple electronic databases were employed to search the literature, in any language, from 1980 to September 2008. A primary set of 4,316 references was identified and scanned, using specific agreed-upon criteria, to select relevant references related to biosecurity-based interventions. The final library comprised 173 references. Identification of the sources of Campylobacter in poultry flocks was required to inform the development of targeted interventions to disrupt transmission routes. The approach used generally involved risk factor-based surveys related to culture-positive or -negative flocks, usually combined with a structured questionnaire. In addition, some studies, either in combination or independently, undertook intervention trials. Many of these studies were compromised by poor design, sampling, and statistical analysis. The evidence for each potential source and route of transmission on the poultry farm was reviewed critically, and the options for intervention were considered. The review concluded that, in most instances, biosecurity on conventional broiler farms can be enhanced and this should contribute to the reduction of flock colonization. However, complementary, non-biosecurity-based approaches will also be required in the future to maximize the reduction of Campylobacter-positive flocks at the farm level.

Campylobacteriosis is a common cause of acute bacterial enteritis worldwide. In England and Wales, the prevalence of human campylobacteriosis peaked in 2000, with over 57,000 cases reported to the Health Protection Agency (HPA), and it remains the most commonly identified cause of intestinal infectious disease in those countries (37). Similarly, campylobacteriosis is the most common zoonosis throughout the European Union, with nearly 200,000 reported cases in 2009 (23). In the United States, the relative importance of this disease in terms of causes of food-borne illnesses may not be so high (79), but even so, it is estimated that 9% of cases are caused by campylobacters.

Recent source attribution studies indicated that the handling and consumption of poultry meat account for approximately 30% of cases of campylobacteriosis (24). However, molecular typing techniques suggest that Campylobacter strains associated with the poultry host overall cause up to 80% of human infections. It is therefore speculated that non-food-borne routes of transmission from poultry to humans are also important (24). Such routes could, for example, occur through environmental contamination directly from poultry farms. Thus, it seems likely that interventions to control and prevent poultry flock colonization at the farm level, as well as interventions targeting poultry meat processing, will be needed to substantially reduce the prevalence of human campylobacteriosis. Current control strategies for reducing Campylobacter in poultry production on farms propose the use of biosecurity measures to exclude the organisms from the flock and/or complementary, non-biosecurity-based approaches, such as antibacterial treatments, probiotics, or vaccination, to prevent establishment or reduce levels of colonization.

Although the sources of Campylobacter on poultry farms have been reviewed previously (59), to date there has been no comprehensive review of the effectiveness and feasibility of on-farm interventions against Campylobacter. The overall objective of this study was to critically review and evaluate appropriate interventions and strategies through the systematic review of the relevant peer-reviewed published literature. Systematic reviewing has been developed and used extensively in recent years in the medical and pharmaceutical sectors (19). Such reviews provide a structured scientific method to give a documented, reproducible, and comprehensive profile.

* Corresponding author. Mailing address: Foodborne Zoonoses Consultancy, Silver Birches, Werherw, Andover SP11 7AW, United Kingdom. Phone: 44 1264720464. E-mail: diane.newell@btinternet.com.
† Present address: Food Refrigeration & Process Engineering Research Centre, The Grimsby Institute, Grimsby, North East Lincolnshire, United Kingdom.
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of the evidence, while minimizing bias and errors (19). These approaches may or may not include meta-analysis. Such reviews are now widely accepted by policy makers as a basis for providing evidence-based recommendations. Systematic reviews commonly comprise the following components: a clearly formulated and focused research question, systematic and explicit methods to identify, select, and critically appraise relevant research, and the collection and synthesis of data from the selected studies (19). This approach has enabled a critical review to be completed in this study and has informed the provision of unbiased advice for policy makers.

MATERIALS AND METHODS

Literature review. The primary literature search was undertaken using the following electronic databases and conference proceedings: MEDLINE, Current Contents, Cab Abstracts, Food Science and Technology Abstracts, Agricola, BIOSIS Previes, Web of Science, Agris International, and Foodline Science. The search was conducted on all literature from 1 January 1980 to 1 September 2008. There was no restriction on language. The Boolean search string used was as follows: Campylobacter AND (chickens OR chicken OR turkeys OR turkey OR fowl OR broiler OR broilers OR poultry).

All identified citations (n = 4,316) with respective abstracts or full records, including keywords, were uploaded into the commercial reference management software package Endnote X1. Duplicate references were removed using a function within the software.

Relevance screening, quality assessment, and data extraction, collation, and synthesis. An international research team of 13 individuals was formed; all members were experts in various aspects of the research topic. This team defined and monitored the review approach, criteria, and outputs. The titles and abstracts of citations were screened for relevance by using the following criteria: (i) any reference not pertaining to poultry was removed, and (ii) any reference not pertaining to Campylobacter jejuni and/or Campylobacter coli or to thermophilic campylobacters or unspecified campylobacters was also removed. Two reviewers, chosen for their expertise and availability, independently screened all the references. Where necessary, disagreements between the two primary reviewers, largely resulting from a lack of clarity in the interpretation of publication results, were resolved by majority decision, based on the expertise within the research team. The resulting subset of references (n = 2,621) formed a second library in Endnote X1.

A second phase of relevance screening was undertaken, and at this stage, all abstracts were obtained where feasible. The two reviewers read and screened all of the abstracts, and references were selected on the basis of the following: they (i) described an effect on Campylobacter, (ii) described an effect applicable to live chickens or turkeys, and (iii) described an experimentally tested or as yet to be tested on-farm Campylobacter control intervention. References were excluded if (i) they described only Campylobacter prevalence in chickens or turkeys or (ii) they described only molecular typing or antimicrobial resistance of Campylobacter. At this stage, 384 references were selected, and these formed the final library in Endnote X1. These references were also exported to an Excel spreadsheet (Microsoft) to enable additional searching and sorting. In the final phase of relevance screening, references were further excluded if they did not relate to on-farm biosecurity. This subset of the final library comprised 173 references.

The full articles were obtained for all of these references. Some articles were in languages other than English. Where feasible, these were translated by members of the review team, or translations were purchased. Each report was scanned into a PDF file for ease of distribution and then sent to at least two members of the review team, on the basis of their expertise, for critical appraisal. The reviewers worked independently to assess the validity of and extract the data from each study, using a standardized questionnaire-based approach. Each completed questionnaire provided a record of the data collected and the information necessary for summarizing and synthesizing the results. The relevant information collected included authorship; date of publication; an indication of whether the paper was an abstract, review, or primary research (on-farm or laboratory based); the research question; the methods used, including the sampling frame, controls, and statistical analysis; and any effect on Campylobacter reported.

The data from the questionnaires were then entered into a spreadsheet to allow the information to be summarized in tabular format. Preliminary analysis of this meta-data set indicated that due to the diversity in reported methodologies and the inadequate number of reported experiments with similar objectives and designs, a formal meta-analysis was not possible.

RESULTS AND DISCUSSION

Identification and assessment of the selected literature. After the removal of duplicates, 4,316 references were screened for relevance. A total of 384 references passed the initial two phases of relevance screening. Full papers for six of the reports could not be obtained, and these were excluded. Two duplicate citations were identified and eliminated.

Of the studies reviewed, 173 were related to biosecurity on farms, including transport. The remainder were related to probiotics, including feeding regimens and competitive exclusion, and to other factors that influenced colonization or reduced numbers of Campylobacter, and they were not considered in this report. For the production of the manuscript, additional references were included, where relevant, to support interpretation and discussion.

Only 78 of the 173 publications described primary research. The majority of these primary studies related to broiler chickens, but seven were on turkeys and one was on multiple species. Similarly, the vast majority of the papers referred to conventional intensive production, with a minority (n = 7) investigating, at least in part, free-range, organic, or backyard systems.

The major research strategy of the papers reviewed was to identify the sources of Campylobacter in poultry flocks in order to introduce targeted interventions to disrupt transmission routes. The approach used generally involved risk factor-based surveys related to culture-positive or -negative flocks (or birds), usually combined with a structured questionnaire. In addition, some studies, either in combination or independently, undertook intervention trials. Most studies employed cross-sectional surveys, collecting data from a variety of points along the food chain, such as “at slaughter” or “just before slaughter,” or on farms at various stages through the bird/flock life cycle. Some studies undertook sequential sampling throughout the broiler production period. These approaches were generally supplemented with attempts to identify potential on-farm sources of infection by the culture of environmental samples, i.e., samples taken from water, hatchery waste, poultry houses, etc. Such studies indicate where viable organisms may survive but do not accurately identify sources of flock infection. In more recent years, strain typing has been applied to compare isolates from the environment with those from chickens.

The review of the short-listed studies revealed several confounding factors that affected the usefulness of some of the data collected. The literature survey was from 1980 on, so the earlier articles were relatively old, and many industry practices have changed in the interim. The studies were performed in different countries, with the potential for country- or region-specific risk factors reflecting environmental and climatic differences. Furthermore, the poultry industry structures, practices, facilities, legislation, etc., could vary fundamentally between countries, introducing major differences in baseline information which may not be evident from the publications. Despite the broadly similar approaches adopted by the studies, i.e., identifying risk factors, the methodologies used were extremely variable. There were differences, for example, in sampling design, types of samples collected, detection methods,
sampling points, and sampling periods, as well as in the design, delivery, and analysis of the surveys.

The approaches to study design and analysis of the on-farm risk factors raised a number of epidemiological and statistical issues and questions regarding the validity of the results or conclusions reported. For example, many studies applied convenience sampling strategies, but few indicated the expected order of magnitude of the effects of given risk factors upon the transmission of Campylobacter into poultry flocks. The methodologies for the detection of positive flocks varied, and sample size calculations were not always applied during planning. In some studies, the proportion of shedding or colonized chickens in a flock was used as a measure of positivity, even though this is unreliable given that within-flock transmission can be very rapid. In addition, confounding factors such as the age of the birds and the season at testing, which might mask the true relationship between a predictor and outcome variables, were often not considered.

The inclusion of molecular typing of flock and environmental isolates substantially enhances the power of epidemiological studies. However, widespread contamination of the farm environment occurs rapidly, i.e., within 3 to 5 days of the time when a flock becomes positive (72). Thus, without longitudinal sampling of both the flock and the external environment, there is no way of knowing the direction of transmission (chicken to environment or vice versa) (72). Recovery of campylobacters from the environment is generally poor because the stresses on the organism are high and their viability is compromised (71). Improved culture methods, such as enrichment or the use of appropriate transport media, are essential to obtain optimal recovery from such sites (61). Even then, isolates recovered are fragile and frequently lose viability during storage or subsequent growth (V. Allen, unpublished data). The methods of sampling and isolation used may influence both the species and subtype of the isolates recovered, and the use of different methods for different samples may generate skewed populations (61). Strategies such as those to identify strain-specific DNA from environmental samples (71) may partly overcome this bias. In addition, the representativeness of the population sampled is a major issue. The typing of sufficient isolates to represent the whole population is resource consuming (60). It is clear that even from the same apparent source, there are many types recoverable, but that only a proportion of these may be associated with flock infection (83).

The characteristics of methods used for Campylobacter strain typing are debatable (60, 94). A wide range of typing methods are available, which vary in discriminatory power, cost, rapidity, and practicality (94). An appropriate method needs to be selected on the basis of the question asked, not just because of convenience or consistency with other studies. For accurate identification of sources of poultry infection, discriminatory power is very important, but such methods are resource intensive. Therefore, they tend to restrict the numbers of isolates that can be investigated. In order to address such issues, layered typing strategies have been recommended, leading to the confirmation of identical strains (60). There is growing evidence that the genetic instability observed in Campylobacter strains (93) occurs during chicken colonization and that the variants generated can have different colonization properties (73). The rate of instability is unknown, but given the relatively small number of clearly identified strain types isolated from intensively reared flocks at slaughter (for example, see reference 4), this incidence appears to have little effect on the molecular epidemiology of infection within poultry flocks. Nevertheless, genetic instability should be actively considered in the interpretation and attribution of sources of chicken colonization on poultry farms.

**Preventing vertical transmission as a potential intervention against flock colonization.** Vertical transmission is usually taken to include the internal contamination of the egg within the reproductive tract before completion of shell deposition. However, it can be interpreted more loosely to also include the pseudovertical transmission of organisms from parent flocks to progeny via routes such as fecal contamination of shells.

In this systematic review, there were 10 primary on-farm research studies investigating the potential for vertical transmission of Campylobacter (14, 15, 43, 45, 50, 63, 64, 81, 85, 90). These studies showed that viable campylobacters are recovered infrequently from internal egg contents (63, 81), although these organisms can easily be recovered from the oviduct (78). Moreover, infection of fertile egg contents significantly reduces the viability of resultant chicks (81). Additional evidence for the role of vertical transmission via Campylobacter-contaminated egg contents is reviewed elsewhere (59).

Attempts have been made to isolate campylobacters from broiler or turkey hatchery fluff, debris, and/or liners (14, 15, 45, 50). Most studies were unable to recover organisms, and even PCR failed to detect organisms (15). However, in one report (14), 0.75% of samples were culture positive. Thus, hatchery debris may be fecally contaminated with hen-derived viable organisms, which might be consumed by associated chicks. However, the contribution of this to later flock infection would appear to be small for several reasons. The first reason is the presence of a lag phase. Under experimental conditions, as few as 40 organisms, recently passed through a chicken and administered intragastrically, will maximally colonize a 1-day-old bird within 3 days (20), even in the presence of maternal antibodies (17), whereas commercial broiler and turkey flocks consistently demonstrate a lag phase of Campylobacter colonization of at least 2 weeks (50, 59, 85). Second, cloacal swabs from 230 24-h-old chicks (63) and whole homogenized gastrointestinal tracts of 144 turkey pouls of up to 7 days of age (50), all derived from Campylobacter-positive parents, were culture negative. Furthermore, progeny flocks derived from Campylobacter-positive parent flocks can remain uncolonized (15, 43, 45, 81), especially when placed under experimental high-biosecurity containment (85). Finally, comparative molecular typing indicates that there are few (43, 63), if any (64), identical isolates recovered from parent and progeny flocks.

**Sources of horizontally transmitted Campylobacter in the poultry farm environment.** Horizontal transmission after chicks are placed on a farm appears to be the normal route of infection for intensively reared flocks. The sources of such contamination are multiple and various. As a consequence of their fastidious growth requirements, campylobacters can naturally multiply only within a warm-blooded host, usually within the gastrointestinal tract. Since all mammals and birds (wild and domestic) are considered potential hosts, the environment (soil, water, pasture, etc.) must be contaminated frequently with these organisms. Therefore, the fecal material from all
livestock on or in the locale of a poultry farm is considered a high risk to poultry flocks.

Because of the large numbers of campylobacters (up to \(10^9\) organisms g\(^{-1}\)) (18) in the feces of colonized poultry, the greatest infection risk is from the other poultry on the same site. Moreover, longitudinal studies, regardless of country of origin, management system, or poultry species, have shown that the risk of Campylobacter colonization in a flock is directly related to the age of the birds (59). Therefore, the risk of Campylobacter colonization in any target poultry house increases with the presence of other flocks of older ages. Such a management practice is common on organic and free-range farms. The risk also increases with the number of houses on-site, even when an all-in-all-out system of rearing is used. In Northern Ireland (54) and France (68, 69), the infection risk increased when there were three or more houses on the same site (odds ratio [OR], 2.4 and 13.2, respectively), while in the Netherlands, farms with five or more houses had an elevated risk (OR, 3.02) (11), and in Great Britain, flocks on farms with up to eight houses were at high risk (26).

Although many risk factor surveys point to the presence of nonpoultry livestock on-farm as a potential source of flock infection, few have demonstrated that this risk is real. In those that have reported such positive results (16, 91), the ORs can be high (7.5 and 6.3, respectively). However, though these results may be significant in univariate analysis, they may have only low, or even no, significance in a multivariate model (54). Despite the limited evidence, most publications conclude that the presence of livestock on a poultry farm should be minimized to reduce the risk of infection (for example, see reference 58), and when their presence cannot be avoided, effective hygiene barriers should be employed (33, 48).

Molecular epidemiological investigations related to other livestock on farms are more enlightening. Using phenotypic or genotypic methods, those strains colonizing target poultry flocks can sometimes be found in adjacent livestock, including cattle and pigs (45, 46, 71), and in longitudinal studies this occurrence can be detected prior to poultry flock colonization, indicating that the direction of transmission is from the livestock to the broilers. However, this seems to be a relatively rare event (46), and surprisingly, the majority of the strains in adjacent livestock are not recovered subsequently from broilers. Moreover, models from the Netherlands (49) indicate that removal of other livestock from a poultry farm would reduce infection only from 44% to 41%.

Campylobacter has also been recovered from the feces of most wild mammals (such as mice, rats, badgers, foxes, and rabbits), pets (dogs and cats), and wild birds. Thus, these animals are also potential sources of contamination for the poultry flock environment. They may either enter the house and provide a direct source of contamination or fecally contaminate the surrounding environment. However, once again, the evidence for such animals as sources of transmission is sparse and contradictory. Some Campylobacter strains isolated from wild bird feces found on a farm were later recovered from broiler flocks (39), but other studies showed no significant overlaps in the Campylobacter populations from wildlife and broilers (21, 64), indicating that the role of wildlife as a risk to flocks is unclear.

The evidence for rodents as infection sources is also circumstantial. The presence of rodents on farms can have a strong association with flock positivity (27, 54), and the efficacy of vermin control is a risk factor (3, 41). Although rodents are detected within the poultry houses of some modern farms (25), the importance of this risk may be low, as Campylobacter carriage is detected infrequently in captured rodents (47).

**Routes of horizontal transmission into a poultry house.** A conventional poultry house that is modern and well maintained and with limited access points should be considered a potentially biosecure premises. Inactive or passive transgressions of the biosecurity perimeter of such a house may be through the import of essential commodities such as water, feed, and air. Active transgressions require the carriage of campylobacters from the external environment, which may occur by vectors, such as vermin, flying/crawling insects, or humans, that are excreting campylobacters or have been exposed to fecal material containing these organisms (62).

House age is often considered to be a proxy for the integrity of the structure, and therefore the completeness of the biosecurity barrier, particularly against farm pests. Older houses may be less well maintained and unsuitable for adequate cleaning. However, in risk factor studies from Sweden (7) and France (56), no statistically significant difference was reported between the prevalence of colonized flocks and the age of the houses.

Active carriage into a poultry house. Human traffic is a very important vehicle for campylobacters entering the poultry house from the external environment (7, 16, 25, 33, 40, 48). Farm staff handling of other neighboring livestock, especially poultry, increases the risk of Campylobacter-positive flocks, and both the number of staff members looking after the house and the number of visits they undertake per day are directly related to that risk (41, 68). Campylobacters have been isolated from the clothes, hands, and boots of farm staff, managers, catchers, and lorry drivers (38, 66), and molecular epidemiology provides evidence that these strains are often subsequently associated with flock colonization (38, 46, 71, 72). It seems likely, therefore, that people entering the poultry house can track in campylobacters from the external environment. Campylobacters are widespread in the environment around poultry houses (36, 70) even before bird placement, and Campylobacter isolation rates outside broiler houses are independent of whether a farm has a high, medium, or low risk of flock infection (33), suggesting that most farms have similar levels of environmental contamination.

Standing water or puddles are particular on-farm sites from which campylobacters can be recovered (12, 38, 56). Campylobacters survive well in water (10): there is a close association between such environmental contamination and the weather (36), and recovery is highest just after rain. The genotypes of isolates from puddles, soil, and close surroundings are commonly identical to those later isolated from the flock when it becomes positive (36, 39, 46, 75). Although there are no published intervention trials supporting the following, the hygiene scores of farms in Norway correlate directly with flock positivity and environmental contamination (46), while in Senegal, farms with a poor record of cleaning and disinfection of house surroundings had an increased risk of Campylobacter infection (16).

Disposal of broiler farm waste, which might constitute a
reservoir of organisms from previous positive flocks (38), is a further obvious risk. *Campylobacter* can survive in poultry litter (76). If the distance between the stacked used litter and the poultry house is less than 200 meters, then the risk of flock infection may increase 5-fold or more (3, 16). The removal of dead birds from the farm also reduces the risk of a positive flock (25). Such poultry wastes may be a source of persistent *Campylobacter* clones on poultry farms (64).

The poultry farmer may not be the only human tracking campylobacters into a poultry house. It is widely stated that thinning or partial depopulation is a significant risk factor for flock positivity, and this was confirmed by the recent European baseline survey of *Campylobacter* in poultry flocks (22). Many catching crews are based within poultry company plants and, like maintenance personnel, travel from farm to farm with their own vehicles, equipment, boots, and clothing, frequently without due regard to personal hygiene or biosecurity. Certainly the boots, clothes, and vehicles of personnel involved with the depopulation process become contaminated with campylobacters at the abattoir (42) or other farm sites.

During transportation to processing plants, birds frequently defecate onto both the crate surfaces and other birds (86, 95), potentially leading to increased carcass contamination with campylobacters (87). Transport crates and the modules in which they are transported can remain microbiologically contaminated following washing (9, 89) and when returned to the farm for loading during final or partial depopulation (2, 12, 34, 55), even though they are apparently visually clean (1). Subsequently, these strains from transport crates can externally contaminate birds during transport and holding at the processing plant (34, 39, 67, 84).

During partial depopulation, crate contamination can result in positivity of the remaining birds on the farm. For example, in one study, the flock-colonizing strain on a farm following thinning was indistinguishable from a strain recovered from the catcher's hands during thinning and very similar to a strain isolated from an empty crate (2). However, the transmission of campylobacters during thinning is not inevitable (5, 7). Several risk factor surveys found a statistically significant risk associated with thinning (5, 11, 28, 65, 68) and found that this risk was greatest when the thinning crews were large (65), not farm dedicated, or poorly educated (7). However, a study in the Netherlands (77), based on 1,737 flocks, questioned the importance of thinning in flock *Campylobacter* prevalence when data were adjusted for the confounders of bird age and season. Furthermore, when thinning occurs only a few days before final depopulation, it is likely that the within-flock prevalence will still be low at slaughter, thus minimizing the importance of this potential transmission route. However, in some countries, often where thinning is considered a financial necessity, the time between thinning and final depopulation can be long enough for maximal flock colonization to occur (2).

Clearly, farm worker hygiene is an important factor in reducing the risk of *Campylobacter* colonization. Levels of such hygiene vary significantly between farms, companies, and countries. Industry recommendations are relatively standard and include the provision of appropriate hygiene barriers, hand washing facilities, boot dips/changes, etc. However, the implementation of such procedures is variable. It is not surprising, therefore, that the use of house-specific boots (12, 25, 91) and clothes (11, 33), the use of overshoes (65), and the effective use of boot dips (11, 25, 26, 54, 91) are all associated with a reduced risk of flock infection. The relative efficacy of house-specific boots compared with boot dips remains unclear from available intervention studies (90, 92), and a combination of both methods might even be beneficial.

Hygiene barriers at the entrance to poultry flocks vary in location, structure, and, most importantly, use. The purpose of a hygiene barrier should be to physically separate the “dirty” outside environment from the “clean and protected” inside environment. In the most basic situations, a hygiene barrier for a poultry house is a physical point (possibly as simple as a line drawn on the floor) at which there is a boot dip and/or a change of footwear. Hygiene barriers may also enable/ensure a change of outer clothes (overalls) and provide hand washing facilities. Most risk factor surveys have considered the presence and/or use of hygiene barriers, but variations in barrier type and effective use may confuse interpretation of the data (58). Nevertheless, the strict use of a hygiene barrier can reduce the risk of flock infection by about 50% (7, 25, 92) and seems especially important when there are other livestock on the farm (33, 90, 92). All of the intervention studies investigated included hygiene barrier use (26, 92).

These human traffic-related observations are interpreted as indicating failures of basic hygiene procedures. The rapidity and extent of dissemination of campylobacters throughout the poultry farm once one house becomes positive (72) suggest that hygiene procedures should be undertaken both going into a house and on leaving a house. Although it is possible to ensure that flocks remain negative under experimental containment, only trained and committed technical staff can prevent campylobacters from spreading out of positive flocks and prevent their entrance into adjacent flocks (51, 85).

There are vehicles for transmission other than humans entering and leaving poultry houses. Insects, including flies and beetles and their larvae, could also be active carriers of campylobacters from the external house environment. Although the ambient body temperature of insects is unlikely to support *Campylobacter* growth or colonization, flying insects such as houseflies are attracted to and feed off feces, potentially containing campylobacters from livestock and other animals. These insects could then deposit this fecal material at the next location, which might be a poultry house (29, 30). Early experimental studies demonstrated that campylobacters could be recovered from up to 20% of the external surfaces and 70% of the viscera of houseflies exposed to contaminated fecal material (80).

Some insects, such as litter beetles, may also be permanent residents within poultry houses, living in protected environments deep within the walls and potentially carrying fecal contaminants from previous flocks. However, if such insects were frequent carriers of campylobacters, it could be anticipated that identical genotypes would regularly be passed to subsequent flocks, which is an infrequent event (82).

The evidence for the role of insects in poultry house contamination is contradictory. The presence of insects or the use of insecticide was not a statistically significant factor in risk surveys in Sweden (7) and France (68), and in on-farm studies, campylobacters either were not cultured from insects by use of the best recovery techniques or were recovered only after the
flock became positive (6, 45, 58) and then were not cultured from the insects' internal organs (47). In contrast, in a Danish study undertaken in 2004 (29), 8.2% of 49 flies captured from around poultry houses were culture positive for campylobacters, while 70.2% of 47 flies were PCR positive. Moreover, intervention studies undertaken in the same country (31, 32) demonstrated that the use of fly nets to prevent flies from entering a house reduced the incidence of Campylobacter flock positivity from 51.4 to 15.4% during the period from June to November.

Enhanced biosecurity measures may be particularly important at specific times of the year. Seasonality was an observed risk factor or a confounder of risk in many of the publications reviewed (5, 7, 11, 25, 35, 40, 41, 44, 46, 48, 53, 54, 65, 68, 69, 77). The seasonal risk peak generally occurs in late summer/early autumn, but the timing, extent, and sharpness of this peak can vary between countries and may be related to the latitude of the country (22). Such seasonality may become clearly visible only when the background prevalence levels are low. The reason for the seasonal trend is unknown, but one recent hypothesis is that it is related to the breeding period of flies (32).

Inactive and passive transmission into the poultry house. Once a flock becomes positive, the poultry house and surrounding farm environment become widely contaminated (38, 39), and this contamination can persist for several weeks (46). Thus, previous flocks in the same house could constitute a risk of subsequent flock infection, and this risk might increase with shorter downtimes between flocks, which can be as short as 3 to 10 days. While studies in Sweden (7) found that there was no relationship between risk of infection and downtime, studies in France indicated that longer downtimes, i.e., 25 days instead of 15 to 20 days, have a decreased risk (68, 69).

Campylobacter recovery from inside the poultry house after cleaning and disinfection and before or during placement of chicks has not been reported (16, 25, 38, 90), and flock infection does not correlate with the thoroughness of cleaning and disinfection (3, 11), implying that poultry house cleaning regimes are, in general, efficient at inactivating Campylobacter. This conclusion is consistent with multiple longitudinal studies undertaken with sequential flocks which report that carryover of Campylobacter infection from one flock to a subsequent flock in the same house is limited or negligible (21, 25, 45, 54, 83) and that colonization can occur just as readily in new houses as in previously used houses (27). However, French studies indicate that the operator commitment to disinfection is an important factor (41, 65).

Molecular epidemiological investigations of carryover from one flock to a subsequent flock in the same house have been particularly useful. In a study (83) of strains from 100 sequential flocks in Great Britain, typed by flaA typing, possible strain carryover from the previous flock in the same house occurred in only 16% of cases. Notably, when carryover did occur, not all of the strains were transmitted to the subsequent flock, suggesting that survival to cause infection may be strain specific. In similar studies using multiple flock cycles in selected houses in the Netherlands (45, 90), the lack of consistent carryover suggested that the contamination source was due to persistence in the external environment rather than within the house.

Feed, litter, water, and air are all potential passive vehicles of campylobacters entering the poultry house. The presence of campylobacters in feed (57) and litter (45) has been investigated, but both materials are normally Campylobacter-free. These materials might become contaminated by the feces of wild birds during transport and storage, but the lack of moisture provides a hostile environment for campylobacters. However, when litter becomes wet, the risk of infection can increase by up to 2-fold (7). Although the type of litter used appears to have no effect on the risk of flock infection (58), reused litter (which is a regular occurrence in some countries, but not in Europe) may provide some protection, at least in turkeys, possibly as a result of enhancing the diversity of the gut flora (50).

Campylobacters can survive for weeks in water (10) and therefore persist in runoff from the pastures of grazing animals and in wastewater (42), potentially reaching the water table, surface waters, or water reservoirs. Routine chlorination for potable water is fully effective against Campylobacter. However, water treatment failure or the use of unchlorinated water sources, such as wells or bore holes, may facilitate the delivery of viable Campylobacter to poultry houses (59).

The evidence for drinking water as a source of flock colonization is largely circumstantial. Many studies have attempted to isolate campylobacters from water supplies in poultry houses. In most cases, no isolates were recovered (27, 35, 39, 48, 51, 63, 74), but campylobacters are notoriously difficult to recover from water, even when their presence is detectable by immunofluorescence (63). The reasons for this remain unclear but may be related to their physiological state as a result of environmental stress responses. In several studies, campylobacters were isolated from water supplies, but only after the flock became positive (27, 38, 50), suggesting tracking up of the water lines from contaminated drinkers.

There has been no evidence from typing studies that isolates from poultry house water supplies have gone on to cause colonization in flocks (96), even in sequential flocks. However, the water source or frequency of disinfection of water lines is a statistically significant risk factor in some studies in Europe (3, 7, 25, 26, 38), but not in others (33, 54, 58, 68), risk factor studies. High risk due to lack of sanitation and adequate cleaning might be confounded by poor general hygiene (7).

One intervention investigation targeting water sanitation by using laboratory- and field-based trials (88) showed no statistically significant reduction in flock prevalence as a result of chlorination, but another field trial (63), albeit a poorly controlled one, indicated that daily treatment to give 0.2 to 0.4 ppm free chlorine reduced within-flock prevalence from 81% to 7%. Two other studies (35, 48) have suggested that water treatment to remove viable campylobacters could reduce infections by about 50%, but no evidence was provided.

Several studies have sampled air in and around broiler houses for campylobacters. The results indicate that air becomes contaminated only once the flock in the house is colonized (63). Nevertheless, different types of ventilation systems, in particular horizontal (5) and static (68) systems, were risk factors in Icelandic and French studies, respectively.

Poultry management practices could affect not only the risk of flock exposure and colonization but also the susceptibility of birds to colonization. Chickens appear to be highly susceptible to colonization with Campylobacter, and the minimum infec-
tious dose of fresh strains for a bird is lower than 40 organisms (20). The reasons for and factors which affect successful colonization are unknown. Campylobacter is considered to be commensal in chickens, and there is no recorded effect on bird weight gain or feed conversion, but the effects on bird health and welfare remain debatable. In most studies (7, 33, 48), no effect on bird health was observed, but an association with some causes of bird mortality has been suggested (13). The effect of the use of antimicrobials in a flock is also questionable, with one study (38) in Belgium reporting no statistically significant difference, while a similar study in France (68) showed a protective effect. Such effects may, of course, be dependent on the antimicrobial susceptibility of the Campylobacter strains present. Studies on stocking density or flock size (5, 25, 48, 65) have also produced inconclusive outcomes.

Biosecurity-based interventions. In many countries, interventions are clearly required to reduce the levels of Campylobacter reaching the public, either through poultry meat or via the environment (24). At the farm level, preventing flock colonization would clearly be most effective, but delaying the onset of infection until close to slaughter could also reduce the extent of environmental contamination, as well as possibly the proportion of birds colonized and the levels of that colonization. In combination with delaying the onset of infection, reducing the age of slaughter may be a very effective, albeit noneconomic, strategy to reduce human exposure to campylobacters from poultry.

A conventional poultry house, used for the production of intensively reared poultry, should have limited and protected entrances. The delivery of proven effective interventions should be relatively easy. However, experience over the last 20 years has shown that this is not always the case in practice. The poultry industry tends to be well established and highly integrated. In order to meet consumer demand, there needs to be a constant flow of flocks, which tends to involve short crop turnaround times, increasing intensification and scale, and low profit margins. Existing biosecurity protocols are generally perceived to be adequate by the industry, but the consistency with which they are applied can be variable. Proposed changes to industry practices, in order to be acceptable, would need to be underpinned by strong research evidence, especially if they involve extra costs.

To date, few intervention studies have been reported, and the quality of their design, implementation, and analysis has been questioned. The provision of adequate controls and sampling frames can be very problematic in such a varied environment. Controlling farmer behavior is rarely considered, even though this is clearly a major factor in transmission. Even in well-designed studies, the benefits of improved biosecurity may be underestimated, because interventions that delay the onset of infection could produce flocks that may not be 100% colonized or colonized to maximalecal levels at slaughter. Such flocks would still be classified as positive at slaughter, and the intervention might be interpreted as unsuccessful even though the number of flock-associated campylobacters entering the processing plant would be reduced.

At this time, it seems that vertical transmission can be discounted as a source and that focus should be placed on horizontal transmission. The majority of Campylobacter infections in conventionally reared flocks appear to be transmitted horizontally into the poultry house from some external reservoir, probably via a variety of routes and vehicles. It should be possible to exclude these organisms by using biosecurity-based approaches. Nevertheless, under practical conditions, it has proved extremely difficult, though not impossible, to provide the stringent biosecurity measures required to effectively and consistently exclude infection from such poultry houses.

The seasonality of Campylobacter infections in poultry indicates that the relative importance of potential reservoirs and transmission routes can change over the course of the year. This may be a key observation in some regions, such as Scandinavia (22), but the absence of a clear seasonal peak in other countries with high Campylobacter-positive flock prevalences (22) indicates that the background, nonseasonal reservoirs can predominate, and these therefore need to be addressed first. As these background infections are reduced, then the seasonal peak will become clearer, sharper, and more consistent with that seen in Scandinavian countries.

The critical point in the transmission chain is likely to be the first bird that becomes colonized in the house. This bird then acts as an extremely efficient amplifier of the organism, which is then rapidly disseminated throughout the house via "coprophagic" activity and contamination of litter, water, feed, and dust particles. Moreover, any positive flock then becomes a significant source and risk of contamination for other flocks on the farm and for the general environment. Therefore, preventing the colonization of that first bird is essential for the flock, farm, and environment to remain campylobacter negative.

Campylobacter contamination of the farm environment is widespread even before the birds are infected (70–72). However, identifying the environmental origins of those strains which later colonize the flock has proved difficult even with modern tracking techniques. It is necessary to distinguish between true sources, where colonization of a host results in bacterial amplification, and those sources which are environmental reservoirs or sites of survival. The bacterial properties required for these disparate environments are quite different, and environmental survival is increasingly recognized as important in host-to-host transmission of campylobacters. However, strain-specific variations in responses to environmental stress could mean that not all viable campylobacters found in and around the farm have equal capacities to colonize chickens (73).

It seems likely that there are multiple originating hosts for those Campylobacter species later found in poultry flocks. From the environmental surveys described above, humans and certain wildlife species, such as geese, starlings, and rodents, are unlikely to be originating host sources, but livestock such as cattle and pigs, and particularly other poultry, may be. However, more investigation is required to establish the extent of wildlife colonization.

Because other livestock on the farm can contribute to the risk of flock infection (16, 91), establishing single-species farms should be an obvious intervention. However, in order to maintain farm profits during the change to single-species farming, additional poultry houses would have to be established. Unfortunately, the biosecurity benefits accruing from removal of mixed farming would almost certainly be outweighed by the added risk from increasing the number of poultry houses (11,
54, 68). This is presumably a reflection of the high risk of dissemination of campylobacters between adjacent houses.

Given that many poultry farms are considerable distances from other livestock or other poultry farms, it is logical to assume that a variety of vectors and vehicles are involved in transmission. Molecular epidemiology certainly confirms that poultry transport and other vehicles and associated human traffic on the farm are contaminated with campylobacters that can later cause infection in flocks. However, recent investigations suggest that flies can also be vectors for introduction of Campylobacter into poultry houses. The importance of flies in various countries is difficult to assess at the moment. Both the distances that various fly species, including the house fly (Musca domestica), can travel and the duration of Campylobacter carriage by such flies require research. Moreover, the types of flying insect and their potential involvement in Campylobacter transmission are most likely determined by temperature and geography and therefore are potentially country specific. In temperate zones, such flies are unlikely to account for infections year-round. Nevertheless, in Denmark, it appears that over 2/3 of the positive flocks during the seasonal peak may be due to flies, and there was a statistically significant reduction in flock positivity of over 60% in houses protected by fly nets (31, 32). Obviously, such intervention studies need to be repeated in other countries and climates.

The risk of passive carriage of campylobacters into the farm by commodities such as feed, litter, and air are minimal, so it seems that no interventions are required here. However, the role of water remains debatable. Since most poultry farms use municipal water supplies which are chlorinated, water seems to play a minor role in the overall contribution to the background levels of infection in poultry farms, and basic biosecurity (chlorinated mains water, covered header tanks, cleaning and disinfection of tanks, and lines between flocks) seems to maintain these low risk levels.

The relative risks of the various routes into the house and then to the flock remain unclear, but traffic into the house by farm personnel and visitors is a high risk that should be reduced by best hygiene practices and effective use of hygiene barriers. From the literature reviewed, it is apparent that the consistent application of simple biosecurity measures, such as the use of boot dip disinfection as specified and changing into house-specific boots and overalls, can reduce flock colonization by 50% (26). Therefore, even though the strict hygiene practices of primary breeding flock facilities can fail to keep a flock Campylobacter-free, and despite evidence that the maintenance of such high hygiene standards is dependent on staff compliance and difficult to sustain, the effective use of hygiene barriers for conventionally reared flocks is feasible and practical and will contribute to the exclusion of Campylobacter. The approaches required to deliver and sustain best hygiene practice are currently unknown, and a concerted effort is needed by the poultry industry to define and establish the required standards. This should include the development of strategies to motivate staff and focus attention on the needs for biosecurity while acknowledging the difficult environment in which they are working. The continuous education of farm staff (7), the use of modern communication tools and forms of inducement, such as financial incentives, should all be considered. The sustainability of enhanced biosecurity measures is difficult to assess, but in at least one intervention study (92), initial reductions in prevalence of infection were unsustainable in the absence of monitoring.

There is sound evidence that equipment and vehicles entering the farm environment can be contaminated with campylobacters and that these can potentially colonize a previously negative flock. Crates and modules used for partial depopulation are good examples of this kind of breach in biosecurity. Several interventions have been suggested to reduce this risk. Storing soiled transport cages for 48 h between uses resulted in smaller numbers of campylobacters but is unlikely to be practical due to costs and space requirements (9). Spray washing of transport coops reduces Campylobacter numbers, but immersion in a chemical sanitizer does not (8). Detergent effectively reduces the numbers of campylobacters suspended in the crate wash water but does not eradicate organisms attached to crate surfaces (84). Factory-based trials have been undertaken using model rigs to allow the evaluation of existing, enhanced, and alternative decontamination protocols, such as ultrasound (1, 89). Clearly, greater efforts should be made to ensure that crates and modules, and the vehicles carrying them, are effectively decontaminated before entering the poultry farm environment.

Generating and maintaining a clean farm environment is one way of reducing the risk of tracking campylobacters into the poultry house. The role of contaminated surface water, such as puddles and ponds, as sources of infection, especially during rainy periods, has been established. Site drainage should therefore be an important factor in farm design and maintenance, and surface puddles should be minimized, as these provide ideal conditions for Campylobacter survival.

Whether enhanced biosecurity can provide similar benefits to free-range poultry flocks remains unknown. Surveys of free-range flocks (organic or nonorganic) generally indicate a higher prevalence of colonization than in conventionally reared flocks (52, 53, 74). Free-range birds are also slaughtered at an older age than conventionally reared birds. In longitudinal studies of free-range flocks (21, 41), many become positive before being allowed onto pasture. Most of the free-range flocks which are negative at that stage subsequently become positive, presumably as a result of environmental contamination (41). This assumption was recently questioned (21) because of a lack of similarity by molecular typing between Campylobacter populations isolated from the free-range chickens and those isolated from the wild birds in the environment of the farm. Nevertheless, alternative or complementary strategies may be particularly important to protect free-range birds from Campylobacter colonization.

In conclusion, this systematic review of the literature on on-farm biosecurity-associated interventions against Campylobacter in poultry flocks has highlighted a paucity of detailed understanding of both the sources of flock infection and those measures which might be effective for the prevention of flock positivity. Moreover, many of the studies reviewed were compromised by poor design, sampling, and statistical analysis. No one route, vehicle, or vector has clearly been identified as a target for intervention. Although the rigorous application of general biosecurity measures is likely to reduce flock infection, sustaining such measures on the farm appears to be extremely difficult and needs to be supported by farm worker education.
and incentives. Even so, it appears that additional and complementary interventions, such as probiotics, vaccination, etc., will be required to reliably achieve Campylobacter-negative flocks at slaughter.

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