ON-FARM FACTORS FOR *CAMPYLOBACTER* CONTAMINATION OF BROILERS: LITERATURE REVIEW AND OVERVIEW OF BROILER FARMING IN NEW ZEALAND

Prepared as part of a New Zealand Food Safety Authority contract for scientific services

by

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May 2008

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Client Report
FW0679

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ACKNOWLEDGEMENTS

The authors would like to thank the Poultry Industry Association of New Zealand (PIANZ) and its member companies for cooperating with this project and supplying data and information

We would also like to thank Drs Ruff Lowman (Health Canada) and Laura Powell (VLA, UK) for supplying information on research in progress.

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1 INTRODUCTION

This report is part of a project investigating on-farm risk factors for *Campylobacter* contamination of poultry flocks in New Zealand. It is intended to contribute to the identification of such factors that offer opportunities for risk management. The preparation of this report will be followed by farm visits during early 2007.

This report covers two topics:

1. Review of the scientific literature regarding on-farm risk factors for *Campylobacter* infection in broilers.
2. Overview of broiler farming in New Zealand, a national perspective collated from information supplied by major poultry producers.

The first topic (Sections 1-3) is intended to provide an overview of currently available scientific information on factors which cause *Campylobacter* infection in poultry broiler flocks during the grow-out period on farms. The focus is on chickens rather than other types of poultry. We have reviewed information from both New Zealand and overseas.

The scientific information was obtained by the following methods:

- On-line PubMed searches;
- Review of references included in literature reviews by (Boxall, 2005) and (Ramabu, 2002);
- Scientific reports assembled for previous ESR projects concerning *Campylobacter* in poultry; and,
- Personal contacts with scientific groups overseas who provided information on work in progress.

The second topic, the overview of broiler farming (Section 4), has been assembled from information supplied by member companies of the Poultry Industry Association of New Zealand. This information was provided in response to a letter sent in November 2006.

2 INVESTIGATIONS INTO INDIVIDUAL RISK FACTORS

2.1 New Zealand Studies

2.1.1 Environmental sources

An investigation into potential sources of contamination during thinning has been conducted for a Masters thesis (Ramabu, 2002;Ramabu *et al.*, 2004). The sampling was done at a primary processing plant in the North Island, and involved a number of fomites relevant to the process of catching/thinning. These were sampled at the plant after cleaning but prior to visiting farms:

Pallets (75% positive for *Campylobacter jejuni*)
Crates (58%)
Truck bed (47%)
Truck wheels (50)
Drivers boots (54%)
Catcher's boots (67%)
Forklift wheels (31%)
Tractor wheels (0%)

The high rate of contamination was taken as evidence for the potential introduction of the bacterium from these sources during thinning. It was also evident that the cleaning procedures did not effectively eliminate *Campylobacter*. Another experiment demonstrated that boots used while visiting a contaminated flock could carry bacteria and infect a flock in a separate area.

New Zealand data are available concerning the introduction into broiler flocks of *Campylobacter* by darkling beetles (*Alphitobius diaperinus*) (Bates *et al.*, 2004). Four broiler flocks were monitored in two different sheds. Beetles were recovered from all four flocks and *Campylobacter* isolated from beetles in three of the flocks. Typing was performed on some isolates, and on one occasion the same type was found in both broilers and beetles at the same time. However, the number of samples tested was small and the direction of transmission could not be deduced.

Darkling beetles appear to be more common in broiler farms in the North Island; they are rarely seen on farms in the South Island, which are principally located around Christchurch (Brendon Hasson, Tegel, personal communication, September 2006).

2.2 International

2.1.1 Vertical transmission

The role of vertical transmission, i.e. via eggs from breeders to broilers, is one that would seem a potential source of *Campylobacter* introduction to broilers if the breeder flock is infected with the organism (Cox *et al.*, 2002b). However, the data suggest that eggs from breeder flocks are rarely, if ever, contaminated by *Campylobacter*, irrespective of the status of the breeder flock. For example, examination of hundreds of eggs from breeders found only

two contained *Campylobacter* (Shanker *et al.*, 1986). In addition, eggs challenged with *Campylobacter* on the surface did not show penetration of the organism although a few injected with the organism did result in colonization of the hatched chicks. Shell penetration in 3 of 70 artificially contaminated table eggs was shown in another survey (Shane *et al.*, 1986), and recovery of the organism from egg contents occurred in only one case. However, in a field survey none of 35 eggs surfaces was contaminated with *Campylobacter*, and the organism reduces in viability rapidly when inoculated onto egg shell and allowed to dry (Cox *et al.*, 2001). Similar results were reported in American studies (Baker *et al.*, 1987;Doyle, 1984).

Campylobacter was not isolated from newly hatched chicks in a Swedish study (Engvall *et al.*, 1986), or reported in data from Israel for 360 one-day-old chicks (Pokamunski *et al.*, 1986). None of 106 American hatchery samples were positive for the organism (Jones *et al.*, 1991).

One group in the USA has been a strong proponent of vertical transmission (Cox *et al.*, 2002a;Cox *et al.*, 2002b). Data were produced using genotyping that showed the same clones being isolated in breeder and derived broiler flocks. However, the number of isolates was small and no context was provided as to whether the particular clones which appeared to be transmitted were commonly occurring, i.e. there was no statistical analysis to say that the distribution of types observed was significantly different from that which might occur randomly in the same manner as performed for other data sets where the risk from vertical transmission was described as "very low" (Jacobs-Reitsma *et al.*, 2001;Jacobs-Reitsma, 1995). Similar conclusions have been drawn in a Danish study (Petersen *et al.*, 2001). More recent work by the American group and others has reported a lack of evidence for vertical transmission (Callicott *et al.*, 2006). This work was based in Iceland where eggs are imported from grandfather flocks in Sweden, so negating any possibility of horizontal transmission. Sequencing of the *flaA* SVR PCR product was unable to demonstrate *Campylobacter* with the same allele passing from grandparent to parent flocks.

Another study indicating the occurrence of vertical transmission, in this case from the hatchery has been published (Pearson *et al.*, 1996). Here the isolation rate of *C. jejuni* from broilers reared from chicks from one hatchery was 17.6% compared to 42.9% for another in an operation supplied almost exclusively by the two hatcheries. Because of this difference in intra-flock prevalence, and the low diversity of serotypes among isolated from the two hatcheries, it was concluded that the contamination was unlikely to have resulted from sources such as contaminated delivery vehicles, crates etc. This was taken to indicate that the contamination was occurring because of intermittent low-level vertical transmission from the parent breeder or layer farm flocks.

2.1.2 Biosecurity

A study of *Campylobacter* isolates from broiler farms and their environs indicated that introduction of the pathogen came from the environment, in one case from cattle to broilers via the farmer's boots (Van de Giessen *et al.*, 1998). Hygiene measures were introduced and included, *inter alia*, thorough cleaning and disinfection of broiler houses, including the entrance room, between broiler flocks, repair of cracks in the walls and floor, and introduction of a hygiene barrier at each shed. At the hygiene barrier separate boots and

overalls had to be worn before entering the shed. Flock prevalence reduced from 66% to 22% at one farm, and from 100% to 42% at another. Despite the improvement it was noted that *Campylobacter* was not completely eliminated and that the hygiene barriers are difficult to maintain.

A trial was conducted to assess whether flock contamination could be reduced through the implementation of biosecurity measures (Gibbens *et al.*, 2001). The controls included the use of a standard protocol for 1) cleaning and disinfecting the sheds and 2) hygiene measures for people entering the shed. It was found that *Campylobacter* infection at 42 days of age was reduced by more than 50% in the intervention flocks compared to the controls.

2.1.3 Cleaning and disinfection between flocks

The carry over of the same genotype of *Campylobacter* has been demonstrated between sequential flocks on 16% of occasions where sequential flocks in the same broiler house were positive (Shreeve *et al.*, 2002). Confidence in the data is high as three approaches were used in the genotyping (*fla*A and *fla*B typing and PFGE typing). A similar study identified that at least 63% of flocks were infected by a persistent clone (Petersen and Wedderkopp, 2001).

The formation of biofilms may create conditions favourable to the survival of *C. jejuni*. This has been studied in biofilms produced by three Gram positive bacteria (not further characterised) and a pseudomonad isolated from chicken sheds. The biofilms from these organisms were formed on PVC coupons. Enhanced survival of the pathogen was demonstrated at both 12 and 23°C over seven days (Trachoo *et al.*, 2002). Consistent with other data on the survival of this organism, survival at 12°C was superior to that at 23°C. A possible reason for the enhanced survival was attributed to protection from oxidative stress. If biofilms form in drinking water distribution systems, and the cleaning and sanitizing occurring between flocks is inadequate to remove the biofilm, then survival from an infected flock to a subsequent one could occur. Persistent flock infections with one serotype of *Campylobacter* have been shown, and this was attributed to the survival of *Campylobacter* in the broiler house water system (Pearson *et al.*, 1993).

The inclusion of *Campylobacter* in biofilms formed by other bacteria isolated from broiler sheds has been shown to increase the resistance of the organism to the action of chemical sanitisers (Trachoo and Frank, 2002). A concentration of 50 ppm sodium hypochlorite completely inactivated *Campylobacter* in biofilms within 45 seconds, while other sanitizers quaternary ammonia, peracetic acid, peracetic acid/peroctanoic acid mixture) were less effective at the same concentration of active ingredient.

The potential for contaminated rice hull and wood shaving litter to infect *Campylobacter*-free birds has been demonstrated (Line, 2002;Line, 2006;Montrose *et al.*, 1985) as it has elsewhere with moss peat litter (Kazwala *et al.*, 1992). The data indicated that the presence of a normal litter flora did not influence the colonisation of the birds. *Campylobacter* does not survive well when inoculated onto pine shavings litter and allowed to dry (Cox *et al.*, 2001).

2.1.4 Feed and water

A British study showed the link between the presence of *C. jejuni* in drinking water and colonisation of broilers (Pearson *et al.*, 1993). A single serotype persisted on a farm for 18 months (Byrd *et al.*, 2001), *Campylobacter*-free chickens supplied with water from the farm became colonised, and the proportion of positive birds reduced from 81% to 7% when the water was chlorinated and the shed drinking system cleaned and disinfected. Chlorination of drinking water to 2-5 ppm chlorine made no difference to the colonisation of chickens under experimental conditions or raised commercially in the USA (Stern *et al.*, 2002), although differences in husbandry in the US industry and that in the UK where the Pearson *et al*. (1993) study was carried out were noted. In UK husbandry it is reported that litter is mechanically removed between flocks, and houses are washed, disinfected and fumigated prior to the placement of the next flock, whereas these practices do not occur in America (Stern *et al*., 2002). Data from 11 farms in New Zealand found that none of the grow out houses were provided with water with 2 ppm free available chlorine (FAC), and only three consistently provided water exceeding 0.2 ppm FAC (Boxall *et al.*, 2003). One house did not chlorinate broiler drinking water.

When feed was withheld, with or without drinking water being provided, there was an increase in cloacal, and more noticeably caecal, carriage of *Campylobacter* (Willis *et al.*, 1996). In contrast feed withdrawal was shown not to result in increased caecal carriage, but did increase carriage in the crop, resulting in an increase from 25% to 62.4% carriage (Byrd *et al.*, 1998;Byrd *et al.*, 2001), the same result for crop carriage being reported elsewhere (Byrd *et al.*, 2001;Willis *et al.*, 2000). The addition of 0.44% lactic acid to drinking water during the period of feed withdrawal was found to reduce the prevalence of *Campylobacter* in the crop, and it was recommended that this intervention could be adopted as a CCP (Byrd *et al.*, 2001).

The addition of yeast at 1 or 100 g/kg feed was not found to influence the colonisation of chickens by *Campylobacter* (Line *et al.*, 1998).

No feed or water samples were positive for *Campylobacter* in an Israeli study (Pokamunski *et al.*, 1986), and none of 78 feed mill samples or 10 feed and water samples from the broiler shed was positive in an American report (Jones *et al.*, 1991). Similarly none of 64 Swiss feed samples was positive for the organism (Ring *et al.*, 2005).

2.1.5 Flies and other insects

The carriage of *Campylobacter* by flies has been well established. Prevalences of 50.7% and 43.2% were determined in a Norwegian study (Rosef and Kapperud, 1983) in flies sampled in the Autumn from the environs of poultry and pig rearing units, respectively. No positive flies were detected in samples from cattle barns and turkey farms. Most isolates were *C. coli* (90.1%) with 6.2% being identified as *C. jejuni*. In contrast no surface sterilised insect sample, was positive for the organism in another study (Jones *et al.*, 1991). *Campylobacter* isolated from flies caught in broiler shed ante-rooms was of the same sero and biotype as the associated *Campylobacter* infected flocks (Berndtson *et al.*, 1996a).

Flies exposed to infected chickens become contaminated within five days (Shane *et al.*, 1985), and when these flies were transferred to a unit containing *Campylobacter*-free birds all birds became intestinally contaminated within eight days, and 70% had contaminated bile. In addition, water and litter became contaminated. This paper also reported on the degree and site of contamination with different feeding periods. When flies were allowed to feed on a solution containing *Campylobacter* 6/32 feet and ventral surfaces became contaminated and 22/32 abdominal contents were contaminated. With a shorter feeding time of 2 hours, 3/32 feet and ventral surfaces and 30/32 abdominal contents were contaminated.

Some evidence for insect transmission of *Campylobacter* between flocks has been presented based on typing data (Van de Giessen *et al.*, 1998).

More recent papers have discussed the postulated correlation between fly population dynamics and human case distributions, and the suggested link between flies and broiler infections. In an attempt to explain the strong seasonality of *Campylobacter* infections in the UK, it was claimed that the only convincing potential seasonal driver was fly transmission (Nichols, 2005). A correlation was shown between the peak in human cases and the period of the year when the larval development time for *Musca domestica* was at its shortest. In Denmark 8.2% of flies trapped in the environs of broiler sheds were contaminated by *C. jejuni* as determined by culture, 56.4% positive by PCR, and a mean of 917 +/-73.5 flies passed through the ventilation system into the broiler house per day (Hald *et al.*, 2004). 27 of 28 isolates from flies, dogs and broilers were indistinguishable by PFGE using two restriction enzymes.

Danish work reported the isolation of *Campylobacter* from four species of litter beetle (Skov *et al.*, 2004). Contaminated beetles were always associated with an infected flock, and beetles tested between flocks were negative. It was therefore concluded that the beetles were not responsible for carrying over *Campylobacter* from one flock to the next. *C. jejuni* has been shown to survive in darkling beetles for a few days (Hazeleger *et al.*, 2001) and isolated from arthropods caught in outdoor flock rearing areas (Ring *et al.*, 2005).

If the data above can be transferred to New Zealand then there is potential for broiler flocks to become contaminated by flies contaminated with *Campylobacter*; carriage has been shown in flies, flies can enter sheds, chickens can acquire infection from contaminated flies and the subtypes isolated from flies can also infect broilers.

Currently the industry in New Zealand does not implement control programmes designed to prevent the entry of flies into broiler sheds. Such fly control measures are not practised internationally, and the practicality of fly control procedures has not been demonstrated in the commercial industry.

2.1.6 Vertebrate pests

Small mammals trapped in the Cascade mountains of Washington, USA, or their faecal pellets were only infrequently (<1%) found to carry *Campylobacter* (Pacha *et al.*, 1987), and in another study no isolations were made (Jones *et al.*, 1991). An undisclosed number of rabbit faeces samples were negative for the presence of *Campylobacter*. A Dutch study

detected *Campylobacter* in 8/83 house mice and 1/8 brown rats, but not in other mammals (shrews and voles) trapped on organic farms (Meerburg *et al.*, 2006).

In New Zealand *Campylobacter* was not isolated from any of 72 possum faecal samples tested, and only one of 197 samples of possum faeces contained *C. coli* (Devane *et al.*, 2005).

In Denmark campylobacters were isolated from 24.8% of cloacal swabs taken from 540 wild birds (Kapperud and Rosef, 1983). Isolations were common in gulls from rural areas, and around 67% of the isolates from gulls were identified as *C. jejuni*. In the USA 35% of 445 ducks tested for the presence of *C. jejuni* contained the organism as did 10% of intestinal and faecal samples from wild birds (Craven *et al.*, 2000), in Japan 13 from 507 sparrow faecal samples yielded *C. jejuni* (Chuma *et al.*, 2000) and 39.3% of wild bird faeces contained *Campylobacter* in a study conducted in Barbados (Workman *et al.*, 2005).

In New Zealand 65.2% of faecal samples from ducks were positive (Devane *et al.*, 2005). An overall carriage rate of 55.9% was determined for *Campylobacter* spp. (approximately 2:1 *C. jejuni* to *C. lari*) in the faeces of the red billed gull from Canterbury and Rotorua (Rodgers, 2000). Faecal samples from individual gulls contained between 2.2×10^3 and 9.4×10^3 *Campylobacter* g^{-1}. Genotyping showed that some of the types isolated from human cases were also isolated from gull's faeces and, while this does not show that the human case isolates were derived from gulls, it does show that types present in gulls can cause disease in humans.

Studies in the Manawatu determined that <10% of wild bird faecal samples from children's play areas were contaminated with *C. jejuni*, and MLST typing identified clonal complex ST-45 among the faecal isolates. This complex includes strain ST-45 which is a common human pathogen (French *et al.*, 2005). Within the ST-45 group of isolates, four different *Sma*I PFGE macrorestriction profile subtypes could be distinguished, all of which have been isolated from human cases and poultry in New Zealand (Nigel French, Pers. Comm.).

2.1.7 Partial depopulation

A Dutch study was unable to find an association between partial depopulation of flocks and the *Campylobacter* status at slaughter in a study of 808 flocks depopulated in one event compared to 84 where partial depopulation was practiced (Bouma *et al.*, 2003). A further contribution by these authors using data for 1737 flocks also concluded that partial depopulation was not a significant risk factor for *Campylobacter* prevalence at slaughter (Russa *et al.*, 2005).

In direct contradiction workers from Denmark concluded that "batch depletion of broiler houses increased the prevalence of *Campylobacter* spp.-infected broilers in the flocks" (Hald *et al.*, 2001). The much larger number of flocks considered in the first two studies would indicate that more weight should be given to them than the Danish study, although the possibility of *Campylobacter* introduction during depopulation is logical, and the presence of *Campylobacter* on crates used for depopulation has been reported (Van de Giessen *et al.*, 1998).

2.1.8 Transport and crate contamination

Transport and holding prior to slaughter has been shown to increase both the prevalence and numbers of *Campylobacter* on chickens (Stern *et al.*, 1995). A first set of experiments compared counts prior to and following transport in disinfected plastic crates. In this case transport did not result in an increase of *Campylobacter* numbers in the caeca, but the number of *Campylobacter* on the birds increased significantly (around 2,800 fold). In a field trial transport occurred as in normal commercial practice. In this case the *Campylobacter* prevalence increased from 12.1% to 56.0%, and the exterior contamination increased around 270 fold.

Only one of five flocks of turkeys showed an increase in the prevalence of *Campylobacter* spp. after transport and holding (Wesley *et al.*, 2005). Similarly the prevalence of flock contamination with *Campylobacter* for broilers was not shown to increase significantly, although the prevalence was already very high prior to transport (57.1%) (Whyte *et al.*, 2001). However, counts of *Campylobacter* in faecal samples increased significantly after transportation by between 0.86 and 0.71 $\log_{10}$/g. Further holding was not shown to result in decreased shedding of the pathogen.

Campylobacter could be isolated in numbers in excess of 3,600 MPN per crate on several occasions in a British study and there was a significant increase in contamination by *Campylobacter* when the birds were caught and placed in crates (Slader *et al.*, 2002). Experiments involving alteration of the concentration of detergent and exposure times found that it was possible to reduce *Campylobacter* in the washwater, but not to eliminate it from crates. This would indicate that *Campylobacter* washed from the crates could effectively be killed while in suspension, while those cells remaining on the crates were more recalcitrant to the effect of the washing and disinfection, presumably due to the protective effect of the soil in which they would occur. Five of seven crates sampled at the farm prior to loading with birds were positive for *Campylobacter*. Colonisation of the caeca did not occur within the 2 h that the birds were held in crates, i.e. the contamination was external.

Crates were found to be frequently contaminated (57%) by *Campylobacter* prior to being used to transport birds (Hansson *et al.*, 2005). At one slaughterhouse where 85% of the crates were contaminated, the crates were used more than once a day, and did not dry between uses. Twenty-six slaughter groups which were negative at the farm level were transported in contaminated crates and 42% of these slaughter groups had been contaminated at slaughter, while 15% of slaughter groups negative at the farm level became positive when transported in uncontaminated crates. Typing data were able to support a hypothesized crate to bird contamination route in some instances. A somewhat lower level of crate contamination (10%) was found in an Icelandic study (Stern *et al.*, 2003), but crates arriving at US farms were contaminated at rates between 6.2 and 30.0% in another paper (Stern *et al.*, 2001).

Cleaning and disinfection, as practiced in Northern Ireland, appeared to have little effect on the prevalence of contamination, with 69% of crates, modules and vehicles testing positive prior to cleaning and 57% positive after cleaning (McKenna *et al.*, 2001). Similar results have been shown in a Dutch study (Jacobs-Reitsma and Bolder, 1997) which also presented data indicating the potential for contaminated crates to lead to bird colonisation. A Belgian

survey reported an increase in the prevalence of contaminated crates after cleaning, with the proportion positive increasing from 54 to 60% (De Zutter, 2000).

The type of flooring of the crates (wire mesh or solid) had no effect on the presence of the pathogen on carcasses assessed after defeathering (Buhr *et al.*, 2000).

Berrang and Northcutt demonstrated that drying of crates for 24 or 48 hours, with or without a spray wash, was effective in reducing the level of *Campylobacter* contamination to an undetectable level (Berrang and Northcutt, 2005). However, the practicality of these extended drying times in a commercial operation was not commented on.

3 EPIDEMIOLOGICAL INVESTIGATIONS

3.1 New Zealand

A poultry industry supported PhD project (Boxall, 2005) carried out a series of studies on broiler grow-out farms in the northern part of the North Island of New Zealand to:

- Determine the prevalence (farm, flock, bird) of *Campylobacter* infection in broiler production,
- Determine the time at which *Campylobacter* colonisation arises in birds within a grow-out house and in the associated environment,
- Determine risk and protective factors for flock *Campylobacter* infection by multivariate logistic regression, and
- Examine one risk factor (chlorination of water) in detail (discussed in Section 2.1.4).

3.1.1 Prevalence of *Campylobacter* infection on commercial broiler farms in New Zealand

Cloacal swabs were collected from 1200 birds from 80 distinct flocks over an eight week period. A total of 71 samples from eight flocks (flock prevalence = 10%) tested positive for *Campylobacter.* The flocks were situated on seven farms (farm prevalence = 14.5%). The bird prevalence on different farms ranged from 6.6 to 100%.

3.1.2 Timing of colonization

Twelve sheds, representing two companies, were monitored from three weeks after addition of day-old chicks until final depopulation (Boxall, 2005). Birds, food, water, litter, boot-dip, boots and the environment immediately surrounding the shed were tested for the presence of *Campylobacter jejuni.*

Birds from seven sheds were colonized by the final depopulation, while birds from three sheds had been colonized by the first partial depopulation. In all cases, detection of colonisation in birds occurred prior to or at the same time as detection of the orgnaism in the environment. Birds in one shed colonized with one *C. jejuni* type, were colonized by another type ten days later. Birds in another shed were colonized with a type different from that isolated from their environment.

3.1.3 Risk and protective factors for *Campylobacter* infection

Over a period of 13 months, 810 flocks of commercial broilers from two different companies in 219 grow-out houses on 77 farms were examined for *C. jejuni* by analysis of pooled caecal contents, taken at slaughter. The impact of a range of farm management, biosecurity and flock demographic factors were assessed by multivariable logistic regression to determine their influence on flock infection status.

Protective factors for *C. jejuni* infection included:

- Hard pathways (gravel, asphalt, concrete) (OR = 0.28)
- Being near another broiler farm (OR = 0.20)
- Using reticulated town water supply (OR = 0.09)
- Using tunnel or cross-flow grow-out houses (OR = 0.15 and 0.24 respectively)

- Using a Chore-Time™ feed delivery system (OR = 0.18)
- Chlorinating the bird water supply (OR = 0.11, winter only)

Risk factors for *C. jejuni* infection were:
- Rodents seen on farm (OR = 2.29)
- Nib-wall construction on grow-out house (OR = 4.70)
- Gas heaters used during brooding (OR = 5.33)
- Cattle farmed on the same property (OR = 2.66)
- Extra workers employed on farm (OR = 2.61)

Sanitisation of the annex (the service area adjoining the grow-out shed) was protective in summer (OR = 0.13), but a risk factor in winter (OR = 2.19).

Although partial depopulation (thinning) had an elevated odds ratio compared to all-out flocks, this was not statistically significant.

The author discussed the identified risk and protective factors and concluded that they were biologically plausible as each could contribute to:
- Potential sources of *Campylobacter* introduction or persistence,
- Water activity in the shed environment and potential for *Campylobacter* growth, or
- Awareness of hygiene or biosecurity requirements.

3.2 International

Two main types of epidemiological studies have been carried out to determine factors contributing to colonization of broiler flocks by *Campylobacter* spp.

Cross-sectional studies have been carried out in a number of countries. These studies determine the *Campylobacter* status of a flock by various techniques, such as testing fresh droppings, caecal swabs or intestinal contents (taken at evisceration). Information on farm management practices, broiler shed construction, biosecurity, etc. are collected in the form of a standardized questionnaire. Univariate analysis is used to determine factors contributing significantly to the *Campylobacter* status of flocks, while multivariate logistic regression is used to build a fuller model.

Environmental studies were carried out by collecting successive samples from flocks and the surrounding environment. Samples were tested for *Campylobacter* and sub-typed.

In addition to these studies several previous reviews of the literature have been carried out.

3.2.1 Cross-sectional studies

Table 1 summarises results from cross-sectional studies carried out in eight countries. While risk factors varied to some extent between different studies, a number of factors are common to the majority of studies. These are:

- Flock size. Larger flocks are associated with increased risk of *Campylobacter* colonization (Barrios *et al.*, 2006;Berndtson *et al.*, 1996b) and the effect was independent of bird density.
- Age of birds at slaughter. Broilers are usually not colonized with *Campylobacter* before three weeks of age. However, prevalence of colonization tends to increase with increasing age (Barrios *et al.*, 2006;Berndtson *et al.*, 1996a;Bouwknegt *et al.*, 2004;Neubauer *et al.*, 2005).
- Number of broiler houses on farm. More broiler houses on farms has consistently been associated with increased risk of *Campylobacter* colonization (Berndtson *et al.*, 1996a;Bouwknegt *et al.*, 2004;Refregier-Petton *et al.*, 2001).
- The presence of non-broiler farm animals on a broiler farm. Animals such as pigs, sheep, cattle and non-broiler fowl may carry *Campylobacter* and excrete it in their faeces, contributing to contamination of the environment surrounding the broiler house. Various studies considered the importance of non-broiler animals in the immediate vicinity of the shed (Hald *et al.*, 2000), on the same farm (Bouwknegt *et al.*, 2004;Cardinale *et al.*, 2004;Hald *et al.*, 2000;Kapperud *et al.*, 1993;Neubauer *et al.*, 2005;Van de Giessen *et al.*, 1996) or on near or adjoining farms (Bouwknegt *et al.*, 2004). While some researchers (Hald *et al.*, 2000) were able to determine separate significant relationships for the presence of pigs and cattle on the same farm, other studies were only able to determine a relationship for 'other animals' in general (Van de Giessen *et al.*, 1996).
- Hygiene measures. Hygiene measures or the lack of hygiene measures has been associated with *Campylobacter* colonization of broilers (Berndtson *et al.*, 1996a). Specific measures that have been mentioned include; separate boots for different broiler houses, clothing specific to broiler contact (Cardinale *et al.*, 2004), and hand washing before handling birds. Use of a disinfection boot dip was assessed to be important by some (Herman *et al.*, 2003;Van de Giessen *et al.*, 1996), while using shed-specific boots was determined to be important by others (Hald *et al.*, 2000;Van de Giessen *et al.*, 1996). The less people who have access to broiler house the lower the apparent risk of *Campylobacter* colonization (Refregier-Petton *et al.*, 2001). There has also been exhibited in an increased risk associated with loadout to slaughter when the staff work at several farms, as opposed to just the farm where the loadout is occurring (Berndtson *et al.*, 1996a). Cleaning of the area around the broiler shed was also assessed to be important in some studies (Cardinale *et al.*, 2004).
- Water source and chlorination status. (Herman *et al.*, 2003) suggested that water was important as a means of spreading *Campylobacter* within the broiler house, rather than as a source for introduction of *Campylobacter* into the broiler house. (Kapperud *et al.*, 1993) suggested that water may have been the source of *Campylobacter* colonisation, in some cases, with *Campylobacter* being isolated from the water supply outside the broiler house. The same biotype was subsequently isolated from broilers on the same farm. Lack of water disinfection was also found to be a risk factor (Kapperud *et al.*, 1993). Lack of cleaning of water cups has also been shown to be a risk factor for *Campylobacter* colonization (Berndtson *et al.*, 1996b). The use of groundwater for cleaning of the broiler houses was found to be a risk factor in one study (Van de Giessen *et al.*, 1996).
- Pest control. Presence of rodents (Berndtson *et al.*, 1996b;Kapperud *et al.*, 1993) or litter beetles (Refregier-Petton *et al.*, 2001) has been correlated with increased colonization of broilers in some studies.

- Broiler house construction or design. The type of ventilation system used in the broiler house has been shown to be important in several studies, with static systems leading to higher risk of colonization than dynamic systems (Refregier-Petton *et al.*, 2001). Vertical ventilation has been associated with increased risk of colonization (Barrios *et al.*, 2006), with the authors suggesting that these may provide a point of entry for wild bird faeces. Concrete, rather than dirt floors, reduce the risk of *Campylobacter* colonization (Cardinale *et al.*, 2004). This was ascribed to superior drying potential of concrete floors. Berndtson *et al*. (Berndtson *et al.*, 1996b) found a greater risk associated with sheet metal ceilings, than with wooden or concrete ceilings.
- Multiple or single depletion (thinning). Where the effect of multiple depletions was examined, the *Campylobacter* prevalence in later depletions was higher than in earlier depletions (Berndtson *et al.*, 1996b); (Hald *et al.*, 2000); (Barrios *et al.*, 2006), although this may be just a function of flock age.
- Feed. One study found a significant relationship between *Campylobacter* status of flocks and feed (Hald *et al.*, 2000), with feed brought in constituting a greater risk than feed grown on the farm. Another study suggested that 'vegetarian' feed (not including animal products) was associated with increased risk (Berndtson *et al.*, 1996b).
- Down period. A longer period between final depopulation and placement of a new flock appears to be protective for *Campylobacter* colonization (Berndtson *et al.*, 1996b;Hald *et al.*, 2000). This is probably due to the increased opportunity for complete drying.
- Season. Some studies have demonstrated a greater risk of *Campylobacter* colonization in Summer or Autumn (Barrios *et al.*, 2006;Bouwknegt *et al.*, 2004;Kapperud *et al.*, 1993;Refregier-Petton *et al.*, 2001)

Table 1: Summary of cross-sectional studies of risk factors for *Campylobacter* colonization of broiler poultry

Country	Year	Study details	Flock Prevalence (%)	Risk factors	Protective factors	Reference
Austria	1998-2001	445 broiler flocks, on 100 farms. Faecal samples collected from floor litter	24.5	• Age of birds at slaughter • Presence of pigs on the farm • Use of undisinfected equipment		(Neubauer *et al.*, 2005)
Belgium	1998-2000	18 flocks, followed from hatchery to slaughter. Fifty sample sources, describing the flock, their housing facilities and the environment tested for *Campylobacter*. Flock status assessed by analysis of 4-7 pools of 10 caecal droppings	39	• Movable material on farm (i.e. 'vectors' easily moved into and out of the broiler house) • Water in broiler house		(Herman *et al.*, 2003)
Denmark	1995	100 broiler batches (88 broiler flocks, 64 farms). Flock status assessed by 15 cloacal swabs per batch taken on arrival at the abattoirs.	52	• Lack of hygiene barrier (OR=3.1) • Animals (e.g. dogs, cats) allowed within immediate environment of broiler house (OR=1.7) • Livestock other than chickens on farm and lack of a hygiene barrier (OR=10.5 for cattle, OR=5.0 for swine) • Depletion in more than one batches (OR=6.8) • Broiler house less than 8 years old (OR=2.2) • Period between flocks less than 14		(Hald *et al.*, 2000)

Country	Year	Study details	Flock Prevalence (%)	Risk factors	Protective factors	Reference
				days (OR=5.0) • Feed purchased from dealer, rather than grown on site (OR=3.1)		
France	1999-2000	75 flocks, 15 pooled samples of five fresh droppings taken once between 30-48 days age.	42.7	• Season (Summer/Autumn) (OR=6.4) • Static ventilation system (OR=20.8) • More than two broiler houses per farm (OR=13.2) • Acidification of drinking water (OR=4.2) • Two or more people taking care of the house (OR=3.1) • Presence of litter beetles in change room (OR=5.0)	• Season (Spring) (OR=0.2) • Antibiotics used (OR=0.1)	(Refregier-Petton *et al.*, 2001)
Iceland	2001-2003	1091 broiler flocks on 36 farms, four pooled samples of 10 ceca tested from each catch lot at slaughter	15.4	• Age at slaughter • Flock size • Vertical ventilation		(Barrios *et al.*, 2006)
Netherlands	1991-1993	112 broiler flocks on 20 farms, status assessed by taking ten pooled samples of ten fresh droppings.	57	• Presence of other farm animals (OR=6.33) • Use of groundwater for cleaning broiler house (OR=5.00)	• Use of detergent for cleaning broiler house (OR=0.16) • Cleaning and disinfection of farm yard between successive broiler cycles (OR=0.23) • Use of separate boots for each broiler house (OR=0.19) • Washing hands before tending broiler flocks (OR=0.19) • Use of footbath	(Van de Giessen *et al.*, 1996)

Country	Year	Study details	Flock Prevalence (%)	Risk factors	Protective factors	Reference
					disinfection when entering broiler house (OR=0.32)	
Netherlands	1997-2000	495 broiler flocks assessed at 4-6 weeks by testing of 60 faecal samples, taken from the ground, and aggregated into five pooled samples.	26.3	• More than two broiler houses on the farm (3 or 4; OR=1.77, 5 or more; OR=3.02) • At least one other farm animal on the premises (OR=1.88) • Animals on nearby farms (OR=9.56) • Age of broilers (29-35 days; OR=2.34, 36-42 days; OR=3.96, >42 days; OR=3.02) • Season (summer; OR=3.48, Autumn; OR=2.59) • No broiler specific clothes and children allowed in broiler house (OR=28.0) • Hatchery (ORs in range 1.00 – 20.2, depending on the hatchery)	• Age of broilers (<21 days; OR=0.36) • Broiler specific clothes and no children allowed into broiler house (OR=0.38)	(Bouwknegt *et al.*, 2004)
Norway	1990-1991	176 broiler farms (one flock per farm, selected at random), 26-28 chickens per flock examined at slaughter by cloacal swab.	18	• Undisinfected water (OR=3.24) • Undisinfected surface water (OR=5.24) • Tending other poultry (OR=2.79) • Tending pigs (OR=4.26) • Rats seen on farm (OR=4.12) • Geographic area (OR=2.51) • Season (OR=2.95)		(Kapperud *et al.*, 1993)
Senegal	2000-2001	70 farms, status assessed by 12 pooled samples of five fresh droppings.		• Poultry house without cement floor (OR=4.15) • Other animals on farm (OR=7.52) • Use of chick transporting carton as feed plate (OR=5.28)	•	(Cardinale *et al.*, 2004)

Country	Year	Study details	Flock Prevalence (%)	Risk factors	Protective factors	Reference
				• Manure disposed of on farm (OR=15.57) • No specific clothes for farm workers (OR=4.52) • No cleaning and disinfection of house surroundings (OR=6.86)		
Sweden	NS	287 flocks from 18 farms. Caecal samples taken from 40 birds per flock at evisceration and pooled into ten samples of four.	27	• Flock size (OR=22.5 for flocks of greater than 25,000 birds) • Age at slaughter (ORs in range 2.9 to 27.4 for birds in age intervals over 32 days) • Small/weak birds (OR=2.3) • Two (OR=3.1) or four (OR=2.7) broiler houses on farm) • Asphalt floor (OR=1.4) • Sheet metal ceiling (OR=4.7 • Feeding trough from ante-room (OR=2.4) • Empty period less than 21 days (OR=2.4) • Wet litter (OR=2.5) • No or diffuse hygiene barrier (OR=2.3) • Divided slaughter (OR=14.6 for second batch) • Staff loading to slaughter work at several farms (OR=7.8) • Cleaning of water cups, seldom (OR=2.1) or never (OR=3.1) • Preceding flock *Campylobacter* positive (OR=2.1) • Traces found of rodents (OR=2.1) • 'Vegetarian' feed (OR=3.4)	• Wooden floor (OR=0.0) • Water from bore or dug well (OR=0.5) • Horses within 500 m (OR=0.2)	(Berndtson *et al.*, 1996b)

Country	Year	Study details	Flock Prevalence (%)	Risk factors	Protective factors	Reference
				• Poultry within 500 m (OR=1.7)		

A longitudinal study of *Campylobacter* in 100 flocks in England and Wales (Evans and Sayers, 2000) assessed flocks in terms of the time from placement when they first became - *Campylobacter*-positive. Risk factors for colonization were:

- Broiler house due for repair; and
- Boot dips used only after litter/chick arrival.

Significant protective factors were:

- Boot dips used only after house disinfection;
- Boot dip changed at least weekly;
- Water header tank disinfection adequate; and
- Dead birds removed from site.

A longitudinal study in the United Kingdom of 45 flocks from 23 broiler farms between June 1990 and July 1991 (Humphrey *et al.*, 1993) considered the impact of water source, drinker type, floor type, flock size, other farming activities, standard of farm hygiene, and house surroundings on the prevalence of *Campylobacter*-positive flocks. Overall 76% of flocks were *Campylobacter*-positive at slaughter, with a mean within-flock prevalence of 50%. None of the factors examined were found to influence either the presence of *Campylobacter* in a broiler flock or the proportion of birds within a flock that were *Campylobacter*-positive.

Colonisation of flocks was shown to occur by the time the birds were three weeks old and litter did not become *Campylobacter*-positive until after birds were colonized. A trial involving boot dipping in strong phenolic disinfectant demonstrated that this practice could delay or prevent flock colonization.

3.2.2 Environmental studies based on sub-typing

Table 2 summarises studies where molecular typing has been used to match *Campylobacter* types found to colonise broilers with *Campylobacter* types found in potential sources of broiler infection.

Table 2: Environmental studies to identify sources and transmission routes for *Campylobacter* colonization of broiler poultry

Country	Year	Samples from	Testing	Conclusions	Reference
NS	NS	Breeder farms, hatchery, broiler shed (droppings, litter, drinkers, feeders, air), surface water adjacent to sheds, footbath	Thermophilic *Campylobacters* identified and enumerated	Infection acquired after entry to broiler shed. Feed, water, litter and air unlikely to be initial source *Campylobacter* in surface water and boot wash suggests that *Campylobacter* was introduced from outside environment	(Kazwala *et al.*, 1990)
NS	NS	Faecal samples from domestic animals, farmers and their families. Rats, mice, swallows, sparrows, pigeons and houseflies. Damp litter, boot and equipment swabs. Drinking water, broiler feed.	Identification, biotyping and Penner serotyping of *Campylobacters*. Antibiotic sensitivity was also assessed.	*Campylobacter* was not isolated from the progeny of infected layers. Cattle, pigs, dogs, free-living birds, rodents and houseflies were often infected. Uninfected flocks had higher body weights and production indices.	(Annan-Prah and Janc, 1988)
Denmark	1997	Hatchery machinery and floor, broilers (cloacal swabs), water, mouse and mouse faeces, beetles, broilers from adjacent shed (cloacal swabs), mink, puddle water, anteroom floor swab	Culture, Penner typing, RFLP (*fla)*, PFGE	For one shed the dominant type was also isolated from a bird in an adjoining shed. In another shed the dominant type was found in a swab from the floor of the anteroom.	(Petersen *et al.*, 2001)
Netherlands	1990-1991	Flocks at 4-5 weeks and at slaughter, pigs on farms, vermin and companion animals, litter, feed, drinking water, environmental water, straw	Culture, Penner typing, RAPD typing	No evidence for transmission from other animals or environmental sources to broiler flocks. Some evidence for transmission of *Campylobacter* between successive flocks in one house.	(Van de Giessen *et al.*, 1992)
Netherlands	1992-1993	Breeder flocks (caecal droppings), broiler flocks (faeces), farm and companion animals, broiler house swabs, beetles and flies, drinking water and feed, delivery boxes and lorry, lorry and crates at depopulation	Culture, RAPD typing	Evidence for transmission from cattle on same farm. There was evidence that this was via farmer's footwear in one case. Insects and pigs were other potential sources of colonisation.	(Van de Giessen *et al.*, 1998)
Norway	2004	4 farms without other production animals, broiler caecal droppings and caecal samples at the abattoir, swab samples form several sites in the broiler house, anteroom, faeces	Culture, AFLP typing	AFLP clusters were used to identify sources of infection from the outdoor environment, including water puddles, farmers boots, and broilers at adjacent farms. Biosecurity risk factor scores for each farm showed that	(Johnsen *et al.*, 2006)

Country	Year	Samples from	Testing	Conclusions	Reference
		of other animals on-farm, courtyard, outdoor boots, nearby water sources, and vehicle wheels.		flocks on higher scoring farms became infected at the earliest age, and these flocks also had the largest genetic diversity suggesting multiple introductions.	
Sweden	NS	24 flocks in six houses on one farm; drinking water and faecal droppings weekly, house swabs, boot sole swabs, water-cup, feed-cup, insects, rodents, air	Culture and identification, Penner typing	No chickens colonised before two weeks of age. *Campylobacter*-positive flies found in anterooms of houses with positive flocks only.	(Berndtson *et al.*, 1996a)
United Kingdom	NS	Breeder flocks, broiler flocks at placement and at least weekly intervals, litter, feed, drinking water, air (in shed and downwind), various environmental samples from inside the shed and the environs.	Culture, MLST typing, serotyping, phage typing, *fla*A RFLP typing.	In most cases, *Campylobacter* types that developed in broilers were different from those in breeder flocks that the chick originated from. In two cases, *Campylobacter* isolated from puddles adjacent to the house before placement were identical in type to *Campylobacter* that subsequently colonised the flock in the house. *Campylobacter* were detected in some litter, feed, water and shed air samples, but only after the flock had become colonised. *Campylobacter* were also detected up to 30 metres downwind of the shed. *Campylobacter* were found on 58% of crates to be used for transporting birds to slaughter. In one case, a flock that was uncolonised a few days before slaughter was found to be positive at slaughter, with a type identical to that found on the transport crate.	(Bull *et al.*, 2006)
USA (Georgia)	1993-1994	Biotic and abiotic sources in grow-out houses and surrounding environment, sampling from one day prior to placement to 10 days post final depopulation	Samples cultured and isolates identified as *Campylobacter* by biochemical testing	Flies and beetle did not become *Campylobacter*-positive until after birds. Rats and mice in the vicinity of the houses were *Campylobacter*-negative. In all positive houses, owner/manager were concurrently in contact with *Campylobacter*-positive cattle. A transient colonisation in one house was associated with the only operation that also raised pigs, which were *Campylobacter*-positive. Drinking water and worker's boots were found to be positive, but not prior to flock infection.	(Gregory *et al.*, 1997)

NS = Not stated

3.2.3 Reviews

A review of available information on colonization of broiler poultry by *Campylobacter* (Sahin *et al.*, 2002) concluded that:

- It is likely that there is not a single dominating source for *Campylobacter* transmission and both vertical and horizontal transmission may be involved into introduction of *Campylobacter* into broiler flocks.

A review of the descriptive epidemiology and ecology of *Campylobacter jejuni* in broiler flocks (Newell and Fearnley, 2003) concluded that:

- While debate on the role of vertical transmission of *Campylobacter* is continuing, control of horizontal sources of colonization appear to be a more productive approach.
- Feed and litter are not potential sources of infection, but *C. jejuni*-contaminated water may represent a low risk of colonization. Further research on the value of water sanitizers is required.
- Broiler house cleansing and disinfection appears to be largely effective in preventing flock-to-flock carryover, although shorter turnaround times may carry more risk.
- Although *Campylobacter* can be isolated from the air in broiler sheds, the role of aerosols is unclear. However, the use of vents that take in air from potentially contaminated areas should be avoided when designing broiler houses.
- *Campylobacter* can potentially be carried into broiler houses via boots, external clothes and equipment and biosecurity measures such as an ante-room and shed-specific boots and clothing or a disinfectant boot dip appear to be important.
- *Campylobacter* may be present in the environment surrounding the broiler shed and a clean, intact concrete apron around the shed and routine disinfection of equipment taken into the shed can reduce the risk of colonization.
- Thinning (partial depopulation) appears to be a major risk factor for introduction of *C. jejuni* into broiler sheds, possibly due to carriage of *Campylobacter* from farm to farm on equipment or traffic of *Campylobacter* from the shed surroundings to the shed interior. Contaminated crates may also contribute to colonization during thinning and transport.
- While excretion of *Campylobacter* by domestic or wild animals was seen as contributing to the contamination of the shed surroundings and potential carriage into the shed on boots, clothing or equipment, *Campylobacter*-carrying animals and insects were viewed as low risk routes for broiler colonization.

A more recent report for the UK Food Standards Agency (Allen and Newell, 2005) provided further evidence of the benefits of biosecurity in the control of *Campylobacter* in poultry. The review was based on both published and unpublished scientific data, as well as discussions with poultry companies. Vertical transmission was considered sufficiently unlikely that the focus should remain on preventing horizontal transmission.

Although evidence for the effectiveness of biosecurity measures is sparse, the available evidence indicated that the following measures were important in the control of *Campylobacter*:

- Wearing protective clothing, house dedicated footwear, and/or dipping boots;
- Washing or sanitising hands;
- Cleaning and disinfecting the house and any equipment entering that house;
- Controlling visitors and their equipment and vehicles both to the house and the farm;
- Controlling pests and other animals on the farm.

Although numerous on-farm sources of *Campylobacter* have been identified, the relative importance of these cannot yet be established and so a programme including all feasible and practical biosecurity measure was recommended. Evidence for the value of biosecurity derived from the almost 100% colonisation of free-range flocks, and unpublished UK company data indicated that levels of around 30% of flocks colonised were achievable, prior to any depopulation. Evidence from several countries suggested that although best practice biosecurity can delay the onset of colonisation, prevention cannot be guaranteed. In addition, biosecurity may only be effective during the months outside the summer peak.

A systematic review of literature on sources and contributing factors for *Campylobacter* colonization on broiler farms has been published (Adkin *et al.*, 2006). A systematic review is a scientific method that locates, appraises and groups evidence from primary studies (CRD, 2001). Each source (variable identified as most probable cause of *Campylobacter* infection in a study) or contributing factor (factors associated with occurrence of *Campylobacter*, but not thought to be associated with the initial cause) was assigned a 'relevancy' score based on country of origin, sample size, strength of association and document type (journal or internet). Relevancy score could be positive or negative depending on whether an association was found or not. The extent of conflict between the findings of different studies was also assessed and expressed as a 'disagreement' source. Although the method used to calculate the disagreement score was not described, disagreement scores range from zero (all relevant studies either show an association or no association) to ten (equal numbers of relevant studies show an association and no association). Results are summarised in Table 3.

Table 3: Sources of *Campylobacter* infection and contributing factors ranked by relevancy (Adkin *et al.*, 2006)

Contributing Factor	Relevancy Score*	Disagreement Score#	Source	Relevancy Score*	Disagreement Score#
Depopulation schedule	14.17	0.0	Depopulation event	12.70	0.0
Hygiene barrier	10.13	1.14	Cross-house transfer	11.67	0.0
Multiple houses	9.80	2.50	On-farm staff	9.14	0.0
Parent company/abattoir	7.60	1.25	Other livestock	8.00	1.2
Season of rearing	7.44	3.69	Wild birds	-0.71	5.3
Disinfectant footbath	6.71	2.80	Small mammals	-4.10	4.3
Outside access	6.40	1.25	Insect carriage	-5.00	2.8
Number of staff	6.00	1.50	Dust/air	-5.25	1.3
Water disinfection	4.50	6.67	Carry over	-5.43	5.6
Presence of other animals	2.38	8.13	Vertical transmission	-5.84	7.9
Age at sampling	2.13	4.80	Water supply	-8.41	3.6
Flock stress	1.50	1.33	Litter	-9.00	0.0
Down-time and routine cleaning	0.30	10.00	Feed	-11.44	0.0
Insect presence	-1.00	4.00			

Contributing Factor	Relevancy Score*	Disagreement Score#	Source	Relevancy Score*	Disagreement Score#
Litter characteristics	-1.64	9.17			
Age of housing/state of repair	-2.67	4.50			
Ventilation/heating	-3.86	2.80			
Clothing routine	-4.00	1.50			
Performance of farm	-5.33	1.50			
Locality	-6.50	1.33			
Staff hygiene: hands	-6.50	3.00			
Medication usage	-7.33	2.57			
Broiler line/sex	-8.40	1.25			
Disease occurrence	-10.00	1.13			
Flock size	-10.38	1.14			
Floor/yard material	-10.71	1.17			
Water equipment	-11.13	1.14			
Feed equipment	-11.50	1.14			
Rodent control	-14.67	0.0			
Stocking density	-14.67	0.0			
Manure routine	-15.25	0.0			
Removal of dead birds	-16.00	0.0			

* A high positive relevancy score indicates a consistent positive association between the factor and *Campylobacter* colonization, considered to be relevant to the UK. A high negative score indicates a consistent lack of association.

A low disagreement score indicates that most or all studies were in agreement with respect to the association of the source or factor with *Campylobacter* colonization. A high disagreement score indicates inconsistency in findings between different studies.

The UK Advisory Committee on the Microbiological Safety of Food (ACMSF, 2005) reviewed information on *Campylobacter* and concluded that chicken meat was a significant source of human *Campylobacter* infection. In reviewing the literature they identified a number of potential sources of infection in the broiler process, including:

- Contaminated water;
- Vertical transmission from parent flocks;
- Contaminated feed;
- Carry-over from a previous flock;
- Domestic and/or wild animals and birds;
- Contaminated transport crates, vehicles and personnel at flock thinning and when birds are weighed or maintenance is carried out;
- Equipment at times other than thinning;
- Feed withdrawal;
- The external environment around the broiler house;
- Contaminated footwear and clothing of farm personnel and visitors; and
- Transfer of contaminated equipment between houses.

The review commented on the fact that flocks appear to only become susceptible to *Campylobacter* infection at 3-4 weeks of age and concluded that the overall strategy was to extend this *Campylobacter*-free status to slaughter age, either by prevention entry of the bacteria into the flock or by improving resistance of the flock to colonization (ACMSF, 2005).

This report also commented on a comparison of two broiler farms in the South-West of England, that differed markedly in prevalence of *Campylobacter* infection over a period of time. The Committee speculated that these difference could be influenced by the condition of the birds. The farm with higher levels of *Campylobacter* in the broilers also had consistently higher flock mortality, rejects at slaughter and hock and pad burns .

3.2.4 Intervention Studies

An intervention study in which infection in broiler houses was compared between control houses and houses employing a range of specified biosecurity measures involving cleaning and disinfection at previous depopulation and standard hygiene protocols to be followed by personnel entering the poultry house during broiler raising (Gibbens *et al.*, 2001).

Intervention flocks remained longer without infection and control flocks were nine times more likely to be infected at 42 days than intervention flocks, although the company to which the flock belonged was a significant confounding factor. The shed ventilation system and water sanitation were additional risk factors over and above the effect of intervention. Wall fans contributed a lower risk than ceiling fans, possibly due to greater ease of cleaning, while more frequent disinfection of water reduced the risk of infection.

Intervention measures included:

Procedures at previous depopulation

- Dust removal by blowing
- All internal surfaces washed with defined sanitizer
- Drying period between washing and disinfection ≥6 hours
- House dry before disinfection
- All internal surfaces disinfected using specified product at defined dilution rate
- Chick brooding equipment disposable or washed/disinfected in main house at the same time
- Adjoining rooms to poultry house hand washed and disinfected
- Water system cleaned; disinfected for ≥1 hour; iodine-based disinfectant
- Concrete areas on the site disinfected before litter is placed

Procedures implemented during the study period

- Two boot dips: on entry to the anteroom and on entry to main house
- Boot dip disinfectant as specified
- Use only dedicated boots and overall in study house
- Separate clean area of anteroom next to main entrance
- Hand sanitizer provided

The authors commented that these hygiene and biosecurity measure had the potential to lower *Campylobacter* prevalence in broiler from the current level of 80% to less than 40%.

3.2.5 Research in progress

3.2.5.1 Iceland

The extensive study of campylobacteriosis in Iceland is still in the process of being analysed, although some information has been published (Stern *et al.*, 2003). The research includes a multi-disciplinary longitudinal study integrating the Icelandic surveillance programs (human and veterinary) in an epidemiological study design, sampling the entire broiler production of three major integrators (90% of total Icelandic production during the study period 2001-2004), including all production levels from grandparent flocks in Sweden, to retail products; domestic livestock, wild birds, environmental samples, and expanded epi data on human cases (Ruff Lowman, Health Canada, personal communication, July 2006). The following (as yet unpublished) information has been supplied by Dr Lowman.

Spatial analysis of risk factors for broiler farm flock prevalence found the presence of cattle farms within a 5 km radius of broiler farms a significant risk factor during the April/May/June period from 2001 to 2004, with those broiler farms having UV treatment of the water supply to broiler flocks protected.

An epidemiological analysis of risk factors for colonization of broiler flocks from 2001 to 2003 found increasing flock age at slaughter, large increases in flock size and vertical ventilation ducts (OR of 5.3, versus horizontal style systems) as significant risk factors for flocks. Interventions on some farms to bird proof the vertical ventilation ducts provided the opportunity for analysis of the effect, but bird proofing did not emerge as a protective factor and vertical ventilation ducts remained a risk factor. A further analysis examined the potential for fly activity being important, with degree-days criteria used as a proxy for fly activity. This model was predictive for the peak July/August/ September months for *Campylobacter* in broiler flocks, but not for the April/May/ June period, when it is likely that waterborne sources are the predominant exposure pathway to flocks.

3.2.5.2 United Kingdom

The study in the Bristol area of the UK (Bull *et al.*, 2006) is now being followed by a three year UK wide research programme which has four objectives (L. Powell, Veterinary Laboratory Authority, Weybridge, personal communication, July 2006):

- Development and field validation of a EU draft protocol for a culture method to determine the prevalence of *Campylobacter* in broilers (similar to the existing protocol for *Salmonella)*
- Using survey samples to do latent class modelling for sensitivity and specificity to compare a culture and a non-culture method.
- Closer examination of thinning as a risk factor for introduction of *Campylobacter* into a flock
- Refinement of the existing *Campylobacter* in poultry quantitative risk model using data from the rest of the project.

The third objective promises to provide useful information relevant to the New Zealand project.

4 OVERVIEW OF BROILER FARMING IN NEW ZEALAND

To assemble an overview of broiler farming in New Zealand, the four major poultry production companies were approached in late November 2006 for summary information on farms. The companies were: Tegel Foods Ltd., Inghams Enterprises (NZ) Pty Ltd., PH Van den Brink Ltd., and Turks Poultry Farm Ltd. By the second week of January 2007 information had been received from four regions. The information provided has been summarised in the following sections. The data relates to approximately 500 sheds on approximately 130 farms.

4.1 Broiler Farms Size and Facilities

Information on the number of farms and numbers of birds per growing cycle for each farm are summarised in Table 4.

Table 4: Broiler farms and numbers of birds

	Region					% of total
Farm size (birds per cycle)	**A**	**B**	**C**	**D**		
25001-50000	5	1	4	11	**21**	**16.2**
50001-75000	5	5	9	12	**31**	**23.85**
75001-100000	5	13	10	3	**31**	**23.85**
1000001-200000	19	10	7	6	**42**	**32.3**
>2000001	4	0	0	1	**5**	**3.8**
	38	**29**	**30**	**33**		
					130	

The ventilation systems reported for the farms are summarised in Table 5. The most common form of ventilation is cross flow, which involves air intake by fans at one end of the building. More modern tunnel sheds have vents and/or panels at the sides of sheds which can be opened to enhance air intake by fans at the ends as well as sides of the shed. These vents or panels are screened which will prevent access by birds and other animals, but not insects.

Table 5: Shed ventilation type

	Region					% of total
	A	**B**	**C**	**D**		
Ridge	9	0	0	0	**9**	**1.8**
Cross flow	70	90	41	98	**299**	**59.8**
Tunnel	55	33	14	16	**118**	**23.6**
Mixed	0	0	73	0	**73**	**14.6**
Other	0	0	0	1	**1**	**0.2**
	134	**123**	**128**	**115**		
					500	

The shed design or construction information is summarised in Table 6. Older sheds are basically a wooden construction with timber framing. New sheds are free standing sandwich

panel type. All sheds in New Zealand will be of a controlled environment type i.e. totally enclosed sheds on a concrete or bitumen base where the ventilation for the shed is totally reliant on fans. Most sheds will have a large door at each end (equipment and bird movement) with a separate enclosed annex and doorway for people only movement.

Table 6: Shed design and construction

	Region					% of total
	A	**B**	**C**	**D**		
Wooden	21	0	86	N/S	**107**	**28**
Panel/steel	113	19	40	N/S	**172**	**45**
Timber/fibre linings	0	89	0	N/S	**89**	**23.2**
Fibre glass linings	0	6	0	N/S	**6**	**1.5**
Colour/steel linings	0	9	0		**9**	**2.3**
	134	**123**	**126**			
					383	

* N/S = not specified

Information on the number of sheds per farm is summarised in Table 7. Most farms had 3-4 sheds.

Table 7: Shed numbers per farm

	Region					% of total
Sheds	**A**	**B**	**C**	**D**		
1	1	0	0	1	**2**	**1.5**
2	7	0	4	10	**21**	**16.2**
3	9	11	4	9	**33**	**25.4**
4	16	9	11	7	**43**	**33.1**
5	2	2	5	1	**10**	**7.8**
6	3	6	4	2	**15**	**11.5**
7	0	0	1	1	**2**	**1.5**
8	0	1	1	2	**4**	**3.0**
	38	**29**	**30**	**33**		
					130	

Information about bird drinking water sources for farms is summarised in Table 8. The majority of farms have their own bore or well.

Table 8: Water source for broiler farms

	Region					% of total
Water source	**A**	**B**	**C**	**D**		
Bore/Well	35	5	28	28	**96**	**73.2**
Town/mains/ city	2	17	2	3	**24**	**18.3**
Spring water	1	0	0	1	**2**	**1.6**
River/creek	0	7	0	1	**8**	**6.1**
Dam	0		0	1	**1**	**0.8**
	38	**29**	**30**	**34**		
					131	

Information on drinker design is summarised in Table 9.

Table 9: Drinker design

	Processor					% of total
	A	**B**	**C**	**D**		
Nipple	101	29	51	46	**227**	**45.6**
Cup	33	94	75	69	**271**	**54.4**
	134	**123**	**126**	**115**		
					498	

Where the information was provided, the majority of nipple drinkers also had splash cups or guards.

Information regarding other animals on the farms is summarised in Table 10. From the information supplied, it was unclear whether adjacent livestock were on the broiler farm itself, or other surrounding farms.

Table 10: Other animals on or surrounding broiler farms

	Region					% of total
	A	**B**	**C**	**D**		
Farm/pet dog	N/S	8		N/S	**8**	**8.6**
Adjacent sheep	N/S	13		10	**23**	**24.7**
Adjacent cattle	N/S	27		26	**53**	**56.9**
Other (horses/pigs)	N/S	5		4	**9**	**9.6**
Excellent biosecurity (no stock)	N/S	8	13	4	**25**	**26.8**
Unspecified stock	N/S		17		**17**	**18.2**
		29	**30**	**34**		
					93	

N/S = not specified NB: Many farms had several different adjacent animal sources; therefore percentages don't total 100.

5 CONCLUSIONS

This review indicates that *Campylobacter* infection in broilers may have multiple sources, and identifying ways of preventing such infection may be difficult. Vertical transmission appears unlikely to be important, while thinning practices appear to be worthy of further investigation, despite not being identified as a risk factor in a New Zealand case control study (Boxall, 2005). All New Zealand broiler farms will practice thinning.

The information on boiler farms supplied by the industry suggest areas for further examination during farm visits. Many farms have their own water bore or well, which may be affected by ground water quality, particularly as chlorination does not always seem to be effective (Boxall *et al.*, 2003). A majority of farms have other livestock either on the broiler farm or adjacent farms. Almost all farms have multiple sheds.

The information in this report is a first step towards identifying risk factors for *Campylobacter* contamination in broiler on farms in New Zealand. This project will now augment this information with further data from industry sources and farm visits.

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