Potential dissemination of *Campylobacter* by farmers' overalls in broiler farms

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SUMMARY

The aims of this study were to show whether overall garments worn by broiler farmers into sheds housing *Campylobacter* positive flocks could be contaminated, and if so, could loose debris shaken from these overalls transfer infection to other sheds. Ten overall garments from ten broiler sheds that housed *Campylobacter* infected broilers were tested for *Campylobacter* carriage. Loose debris shaken from two overalls tested positive for *Campylobacter* by enrichment. One of the two overalls also tested positive for *Campylobacter* when the overall garment was rinsed and the rinsate enriched for *Campylobacter*.

1 INTRODUCTION

In the poultry boiler industry, on-farm biosecurity measures are designed to minimize the risk of introducing infectious agents from outside the farm that could infect livestock, and to prevent cross contamination between sites within the farm. In 1991, in an effort to reduce *Salmonella* prevalence in retail chicken meats, a Code of Practice was introduced. This code was reviewed in 1995 (PIANZ 1995) and led to a lowering of *Salmonella* prevalence in retail chicken products (Wong 2004). However, while the code is effective in controlling *Salmonella* in the broiler industry, it does not appear to have had the same effect in controlling *Campylobacter* in the broiler sheds. It has been suggested that because *Campylobacter jejuni* and *C. coli* have an optimum growth temperature of 42ºC, which is very similar to the body temperature of chickens, these organisms are well adapted colonizers of the avian gut. Colonization of the gut by *Campylobacter* does not induce symptoms of disease so their presence in broilers are difficult to detect without laboratory testing.

The relative importance of various pathways on how *Campylobacter* is introduced into a broiler shed is not well understood and at present appears to be a random event occurring, at the earliest, when the chicks are around two weeks old (Stern and Robach 1995a). Once introduced the spread of the organism in the flock is rapid (Shanker *et al.* 1990) and a very high proportion, often 100% (Stern *et al.* 2001) of the birds become infected. Once infected, the flock tends to remain infected until slaughter (Jacobs-Reitsma 1995) with *Campylobacter* numbers in the caecal contents reaching as high as 10^{10} CFU g^{-1} (Cawthraw *et al*. 1996). On arrival at the processing plant the number of *Campylobacter* in the faeces is high, with a mean of $>10^6$ CFU g^{-1} reported by Stern and colleagues (1995b).

In view of the fact that a *Campylobacter*-infected flock within a broiler shed is difficult to recognize without microbiological testing, precautionary measures such as wearing dedicated overall garments to cover their farm clothings when going about their daily monitoring routine and moving from shed to shed in a poultry farm could prevent the spread of infection. While the growers in conjunction with the poultry industry association are continuously improving biosecurity on farms, the overalls or farm clothing that are worn by farmers, if not dedicated for each shed, could compromise any gains made by other biosecurity improvements. *Campylobacter* from a positive shed in a broiler farm could be transferred via non dedicated farm clothing by the farmer to a negative shed. A previous project called "On-farm risk factors for *Campylobacter* contamination of poultry" suggested this area for biosecurity improvement.

In section 2.6.3 of the Broiler Growing Biosecurity Manual (PIANZ 2007), which is promulgated by the Poultry Industry Association in New Zealand, visitors including contractors must wear dedicated clean clothing and hair nets when entering a broiler growing shed. Provision of shed specific clothing is specified only for operations that are "not all-in, all-out". Covering up of street shoes for visitors and dedicated footwear for farmers is also recommended.

The aims of this study were

- to determine if *Campylobacter* is present on the overalls of farmers on farms known to have *Campylobacter* present in the shed.
- to determine if *Campylobacter* can be transferred via loose particulate matter picked up by the overalls worn by poultry farmers when undertaking daily monitoring visits to multiple sheds.

2 MATERIAL AND METHODS

2.1 Collection of overalls from broiler farms

Poultry farms in the North and South Islands of New Zealand were recruited by broiler stock managers to participate in this project. Certain farms with a history of *Campylobacter c*ontamination were selected. The growers were issued with overalls by broiler stock managers to be worn over their normal farm clothing when stock was nearing maturation and close to harvesting. The overalls were dedicated to sheds. Only overalls from broiler sheds positive for *Campylobacter* were selected for testing. To do this, broilers were first thinned from sheds during the first cut where the *Campylobacter* status of each flock became known after laboratory testing of caecal contents as per National Microbiological Database programme. Once the laboratory notified the stock manager of the flock status in that particular farm, the stock manager visited the farm and collected the overall.

Five overalls were collected from poultry farms in the South Island and the other five from farms in the North Island. Each overall was placed in a large sterile plastic bag and sent to ESR Christchurch Science Centre in a chilly bin lined with frozen pads to keep samples cold (<8ºC).

2.2 Laboratory testing of overalls

A large sterile polyethylene sheet (120 x 80 cm) was placed on the floor of a room and the overall was shaken continuously for 5 minutes over the sheet. Particulate matter was collected from the surface of sheet with a micro sponge (130 x 70 x 25 mm, MDK-MS, BioMerieux, Brisbane, Australia) moistened with 9 ml of Peptone water. The sponge was immersed in 200 ml of Exeter broth in a whirlpak bag and incubated at 42ºC in microaerobic conditions for 48 h. *Campylobacter* was cultured by streaking a loopful onto an mCCDA plate and incubated for 48 h at 42ºC under microaerobic conditions. Presumptive *Campylobacter*-like colonies were confirmed by oxidase test and PCR.

To recover *Campylobacter* from the overalls after they had been shaken, the first three overalls were soaked in 2 L of buffered peptone water (BPW) in a large sterile stomacher bag (BA 6042, Stomacher™) and the remaining seven overalls in 2 L of Exeter broth instead of BPW.

Overalls treated in BPW were kneaded manually for 5 min by hand through the bag to loosen contaminated material that might have been trapped and dried on the overall fabric and the BPW drained into a smaller stomacher bag (BA6041, Stomacher™) and filtered through 22 µm membrane filters (4.7 cm diameter). The membrane filters were immersed in 50 ml of Exeter broth and incubated at 42ºC for 48 h. However, because of the amount of particulate matter and fibres that were washed from the overalls, the membranes became blocked quickly and had to be changed many times. Subsequently, for the remaining seven overalls, the method was changed to overcome the need for filtration. Each overall was soaked and kneaded in 2 L of Exeter broth, as described above. The Exeter broth (enrichment broth for *Campylobacter*) was drained into a stomacher bag and incubated at 42ºC for 48 h microaerobically. A loopful of Exeter broth culture from each sample was streaked on an mCCDA plate and incubated at 42ºC for 48 h under microaerobic conditions to culture for *Campylobacter*. Presumptive *Campylobacter*-like colonies were confirmed by oxidase test and PCR.

3 RESULTS

Ten shed-dedicated overalls that were worn by broiler farmers were submitted for testing. All came from sheds screened positive for *Campylobacter* following its confirmation from the pooled test of caecal contents from ten random birds after the first cut. *Campylobacter jejuni* was detected from the enrichment culture of sponges that contained debris fallen from the shaking of two overalls (Table 1). However, only the rinsate from one of these overalls showed presence of *Campylobacter*.

Table 1. In-used overalls taken from *Campylobacter* positive broiler sheds from poultry farms in the North and South Islands of New Zealand

Date Tested	Description	*Campylobacter* from sponge*	PCR identification	*Campylobacter* from overall rinsate†	PCR identification
2/07/2008	Farm A, Shed 1,	A		A	
2/07/2008	Farm A, Shed 2,	P	*C. jejuni*	P	*C. jejuni*
2/07/2008	Farm A, Shed 3,	A		A	
15/08/2008	Farm B, Shed 1,	A		A	
21/08/2008	Farm C, Shed 2,	P	*C. jejuni*	A	
22/11/2008	Farm D, Shed 1,	A		A	
22/11/2008	Farm D, Shed 2,	A		A	
22/11/2008	Farm D, Shed 3,	A		A	
22/11/2008	Farm E, Shed 1,	A		A	
22/11/2008	Farm E Shed 3,	A		A	

* "Sponge" represents the moist sponge that was used to swab loose debris that had fallen from an overall after shaking by hand over a plastic sheet for 5 minutes.
† "Rinsate" represents drained broth washings from an overall after it was soaked and kneaded in 200 ml of broth.
A – Absence; P - Presence

4 DISCUSSION

In New Zealand, much effort has gone into preventative biosecurity to secure the health and wellbeing of birds in a broiler farm. A broiler farm normally contains several growing sheds, each housing many thousands of broilers. Wearing dedicated outer uniform such as an overall garment for each shed in a broiler farm is not practiced widely although it is promulgated in the Broiler Growing Biosecurity Manual (PIANZ 2007). When a farmer wears non-dedicated farm clothing and moves from shed to shed while performing daily monitoring, there is a risk of spreading *Campylobacter* from a positive shed to another where broilers are not infected. A shed-dedicated clothing policy on a broiler farm could assist in keeping infectious material out of an uninfected broiler population within a shed or containment of infection if *Campylobacter* and/or other gastrointestinal infection are unknowingly contracted by the healthy birds.

Anecdotal evidence from one poultry company has suggested that a lower flock prevalence persisted following the introduction of dedicated shed clothing, but validation data are not available. However, this observation could also be caused by other effects including seasonal ones. If dedicated shed clothing is found to reduce the number of flocks with *Campylobacter*, this could reduce the number of birds with *Campylobacter* arriving at the processing plant and leading to a lower *Campylobacter* load at the start of processing and during evisceration. A *Campylobacter*-negative flock would help the processor to achieve the *Campylobacter* Processing Target.

The findings in this short study showed that *Campylobacter* could potentially be transmitted by farm clothing from shed to shed. In a positive shed, *Campylobacter* could be present in very large numbers in faecal material and be mixed onto the litter on the floor; a gram of chicken faeces could harbour $>10^6$ CFU of *Campylobacter* (Stern *et al*., 1995b). *Campylobacter* adhering onto loose debris and faecal material in a positive shed could be rubbed onto the clothing by the farmer's hands during routine work. When the farmer moves from a positive shed to a negative shed, *Campylobacter* could potentially be spread by sloughing off the garment through movement or rubbing by hand.

Although in this study, it was not known when contamination on the positive farm overalls occurred, it is possible that *Campylobacter* could have survived better and be recovered at a higher frequency from the overalls if contamination is fresh and newly introduced onto the garment. *Campylobacter* does not survive well in dry conditions (Humphrey 2001). The *Campylobacter* cells on freshly contaminated garments would remain viable for an hour and could be transmitted readily to another shed when sloughed off the garments, such as the case when the farmer is entering one shed after other.

In conclusion, having shed-dedicated overalls alongside other biosecurity control procedures such as routine uniform changes and sanitized laundering, dedicated footwear, wearing hair net and hand sanitation, could reduce the potential spread of *Campylobacter* from shed to shed. However, this practice is only partially adopted by some poultry growers because of the inconvenience factor thus diminishing the gains made by other on-farm interventions within the poultry industry.

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