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Protecting broilers against *Campylobacter* infection by preventing direct contact between farm staff and broilers

Tara Battersby [a, b], P. Whyte [b], D. Bolton [a, *]

[a] Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland
[b] UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

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ABSTRACT

The objective of this study was to test the hypothesis that farm staff are the primary vector of *Campylobacter* transmission into broiler flocks. On 3 different farms and 5 different flocks (3 flocks on farm 1 and 1 flock on each of farms 2 and 3) a small section of the broiler house (3 × 2 m (farm 1) and 1 m × 1 m (farms 2 and 3)) was sectioned off using Perspex or plastic sheeting. This 'biosecure cube' (BC) was populated with 25–125 chicks (test birds), a small subset of the general population of up to 30,000 (control) birds in the broiler house. The BC area incorporated the water and feed-lines thus the test and control birds had access to the same feed, water and air. However, unlike in the general broiler house, the farm staff had no direct access to this sub-population. Dead birds were aseptically removed by the researchers. The birds were tested for *Campylobacter* (faecal and/or caecal samples), on the day of chick arrival and every 7 days thereafter. In farm 1-flock 1 the general broiler population was *Campylobacter*-positive after 21 days but the test birds remained negative until day 35. The general broiler population in the other 4 flocks were *Campylobacter* positive as early as day 14, but in all cases the test birds remained negative. Moreover BC broilers were significantly ($P < 0.05$) heavier than the control birds (400 g on average), at first thinning. It was therefore concluded that preventing direct contact between the farm staff and the broilers prevents *Campylobacter* infection in broilers.

1. Introduction

Campylobacter spp. are microaerophilic, fastidious, zoonotic pathogenic organisms (Silva et al., 2011), which, although ubiquitous in the environment, preferentially colonise farmed poultry (Newell & Fearnley, 2003). Campylobacteriosis is the most common gastroenteritis in the developed world and its incidence in the EU is conservatively estimated at 9 million cases per annum costing €2.4 billion (EFSA, 2011). Poultry are the primary source accounting for 50–80% of cases (EFSA, 2011). Approximately 83% of the 70 million broilers produced in Ireland each year are infected with *Campylobacter* (EFSA, 2010a).

Multiple sources of *Campylobacter* have been identified on broiler farms, including flies (Hald et al., 2004; Hald, Sommer, & Skovgård, 2007), rodents (Meerburg, Jacobs-Reitsma, Wagenaar, & Kijlstra, 2006), water (Pérez-Boto et al., 2010), adjacent livestock (Doyle & Erickson, 2006), pets (Whiley, van den Akker, Giglio, & Bentham, 2013), and dirty equipment (Agunos, Waddell, Léger, & Taboada, 2014). Thus preventing *Campylobacter* ingress into a poultry house is reliant on good biosecurity, including the application of foot dips, an ante-room with clean and dirty zones, effective terminal hygiene, house specific footwear, hand washing facilities, effective litter management, equipment hygiene and rodent control activities (Bord Bia, 2008). However, even when these are in place, flocks are still regularly infected, primarily due to a failure of farm staff to consistently apply biosecurity procedures (Newell et al., 2011), resulting in farm staff (and other personnel) serving as a major vehicle of *Campylobacter* carriage into the broiler house (Allen et al., 2008).

The objective of this study was therefore to test the hypothesis that farm staff are the primary source of *Campylobacter* transmission into broiler flocks and preventing direct contact between them and the birds would protect the flock against *Campylobacter* infection.

* Corresponding author.
E-mail address: declan.bolton@teagasc.ie (D. Bolton).

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2. Method and materials

2.1. Description of the farms used in the study

This study was initially undertaken on one farm (farm 1) using 3 different flocks (flocks 1, 2 and 3) at different times. It was then extended to include 2 additional farms (farms 2 and 3) using one flock per farm (flocks 4 and 5). There were approximately 33,000 birds in flocks 1 to 3, 22,000 in flock 4 and 35,000 in flock 5. The broiler farms all used fan based controlled ventilation and each had between 2 and 5 broiler houses in close proximity on a single site with a tarmac apron. Thinning or partial depopulation of flocks was carried out once in each flock, typically between day 32 and 37, at which point the experiment was terminated.

2.2. Description of biosecure cube used in the study (flocks 1–3)

The 'biosecure cube' (BC) used on farm 1 (flocks 1 to 3), consisted of 6 mm thick clear polycarbonate sheets (Goldstar Plastics, Dublin) on all 4 sides supported by four 1 m high wooden columns (Wood Workers, Dublin) on each corner (Picture 1). The total internal floor area was 6 m^2. Four slits in the polycarbonate sheets (50 cm high×8 cm wide), lined with industrial 50 mm thick bristle strips (Ibex Industrial Brushes, UK), allowed the feeder and drinker lines to run through the unit. The top of the unit was initially covered with a fly screen mesh (flock 1 only) (PetScreenMesh®, Modern Flyscreens, Tullamore, Offaly, Ireland) with a pore size of 0.914 mm, but this had to be removed after approximately 10 days as it became clogged with dust. Exactly 125 'test' birds were reared within this BC to ensure the stocking density was the same as that in the rest of the broiler house. Farm staff were instructed not to enter or interfere with the unit under any circumstances. If equipment failed or a fatality occurred the researchers were informed and carried out the necessary actions.

2.3. Description of 'biosecure cube' used for flocks 4 &5

The 'biosecure cube' (BC) unit used on farms 2 and 3 (flocks 4 and 5), consisted of clear polyethylene sheets (B&Q, Dublin) tacked onto a wooden frame consisting of 4 sides, each made from 4 × 1 m wooden slats (40 cm × 20 cm) (Picture 2). The total internal area was 1 m^2. The four slits in the polyethylene sheets which accommodated the feeder and drinker lines were secured with Universal tape. No fly screen was applied to these units. Stocking density placed inside the BC on each occasion was equivalent to that in the rest of the house, with 25 birds placed inside each time. As per above, farm staff were instructed not to enter or interfere with the unit under any circumstances.

2.4. Sample collection

Samples were collected from each flock on the day of chick arrival and every 7 days during the broiler rearing period. These included; [1] 40 air Samples (tested for *Campylobacter* and Total Viable Count's (TVC)); [2] 100 faecal samples (10 pooled samples each containing 10 fresh faecal samples, collected directly from the broiler house floor; [3] 10 faecal samples collected from the floor of the BC; [4] 3 × 50 g of feed from the feed auger supplying the feed line that included the BC; [5] 3 L of the broiler house water supply and [6] 10 caecal samples, each collected once per week from 10 randomly selected 'control' birds. Once the flock tested positive for *Campylobacter* (or the flock reached 21 days), caecal testing was extended to include the birds within the BC (10 per week from flocks 1 to 3 and 5 per week from flocks 4 and 5).

Air samples were taken using a AI3P Air Ideal 3P unit (Biomérieux, France). Ten litres were sampled for each air plate. Plate count agar (PCA, CM0325B Oxoid, Cambridge, UK) was used to collect total viable counts and modified *Campylobacter* blood free selective agar (mCCDA, CM0739b, Oxoid, Cambridge, UK) supplemented with cefoperazone and amphotericin (SR0155E, CCDA selective supplement, Oxoid, Cambridge, UK) plates were used to detect *Campylobacter*.

Water samples were collected using 3 × 1 L water sampling bottles (VWR International Ltd, Dublin). The tap was sprayed with 70% ethanol, flamed and the water allowed to run for 5 min before water samples were taken.

Samples were taken up to first thin which normally occurred between 32 and 37 days. Samples were transported to the laboratory at 4 °C in a cool box and processed within 24 h.

Bird weights were obtained by the poultry veterinarian *post mortem* on days when the birds were removed for caecal testing and/or from day 21 onwards. Post day 21 is considered to be the 'developmental phase' for broilers and a minimum of 25% of the birds were sampled for weights on days 21, 28 and 35.

2.5. Campylobacter isolation

To detect *Campylobacter*, samples were both direct plated and enriched according to the Horizontal Method for Detection and Enumeration of *Campylobacter* spp. (ISO 10,272, 2006). Composite faecal samples were prepared by adding 25 g to 225 mL of Bolton broth (CM983B, Oxoid, Cambridge, UK) supplemented with 5% lysed horse blood (SR048C, Lennox, Dublin) and a selective supplement containing cefoperazone, vancomycin, trimethoprim and cycloheximide (SR183E, Bolton broth supplement, Oxoid, Cambridge, UK), to give a 1:10 dilution and stomached for 30s. After mixing, serial dilutions were prepared using maximum recovery diluent (MRD) (CM0733B Oxoid, Cambridge, UK) and 100 μL aliquots were plated out on modified mCCDA for each composite sample. Sample inoculated broths were also enriched at 37 °C for 5 h followed by 42 °C for 48 h under microaerobic conditions using Anaero Jars (AG0025A, Fannin, Dublin) with Campygen atmosphere generation kits (CN025A, Oxoid, Cambridge, UK). Samples were plated out on mCCDA following incubation.

Caecal samples were both direct plated and enriched as per ISO 10,272: 2006. Briefly, 1 g of caecal material was added to 9 ml of Bolton Broth and vortexed. Serial dilutions were prepared in MRD, and 100 μL volumes plated out on mCCDA. The remaining broths containing caecal contents were enriched by incubating under microaerobic conditions as above at 37 °C for 5 h followed by 42 °C for 48 h. After incubation, samples were plated out on mCCDA.

Air plates were incubated as follows; PCA air plates were inverted and incubated at 30 °C for 48 h, mCCDA were inverted and incubated under microaerobic conditions at 37 °C for 5 h followed by 42 °C for 48 h. After incubation plates were inspected for colonies.

Water samples (3 L) were initially filtered through 0.45 μm (Millipore, MA, USA) membranes. Filters were then aseptically transferred to 100 mL Bolton enrichment broth and incubated at 37 °C for 5 h followed by 42 °C for 48 h. After enrichment the samples were plated out on mCCDA.

Feed Samples were analysed by adding 10 g to 90 mL Bolton Broth followed by vortexing for 30s. The samples were then incubated as previously described and plated out on mCCDA after enrichment.

2.6. Campylobacter identification

All presumptive *Campylobacter* isolates were confirmed initially using standard biochemical tests; Gram reaction (3% [w/v] KOH,