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Author Contributions:

Genevieve Greene: methodology, validation, formal analysis, investigation, data curation, writing – original draft and preparation, writing – review and editing, and visualization.

Leonard Koolman: validation, investigation, writing – review and editing, and visualization.

Paul Whyte: resources, data curation, writing – review and editing, visualization, supervision, and funding acquisition,

Helen Lynch: writing – review and editing.

Aidan Coffey: writing – review and editing, funding acquisition.

Brigid Lucey: writing – review and editing.

Olwen Golden: writing – review and editing.

Lisa O'Connor: writing – review and editing.

Declan Bolton: conceptualization, methodology, validation, formal analysis, resources, data curation, writing – original draft preparation, writing – review and editing, visualization, supervision, project administration, and funding acquisition.

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Testing barrier materials in the development of a biosecurity pen to protect broilers against *Campylobacter*

Genevieve Greene [1, 2], Leonard Koolman [1], Paul Whyte [2], Helen Lynch[2, 3], Aidan Coffey [4], Brigid Lucey [4], Lisa O'Connor [5] and Declan Bolton [1,*]

[1] Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland
[2] School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
[3] Department of Agriculture, Food and the Marine, Backweston, Celbridge, Kildare, Ireland
[4] Department of Biological Sciences, Munster Technological University, Cork, Ireland
[5] Food Safety Authority of Ireland, George's Dock, Dublin, Ireland

***** Correspondening author:

Dr. Declan J Bolton,

Teagasc Food Research Centre,

Ashtown, Dublin 15, Ireland

Tel: 353 1 805 9539

Email: declan.bolton@teagasc.ie

Abstract: Previous studies demonstrated that commercial broiler flocks could be protected from *Campylobacter* colonisation using a bird pen, termed the "biosecurity cube", constructed from four polycarbonate sheets (1m high x 2.5m long x 6mm thick) supported at the corners by 4 x 1m high wooden columns. However, this design had issues with airflow and potential for upscaling. A biosecurity cube composed of four galvanised steel mesh panels (3.44m long x 1.25m high) was therefore developed onto which different barrier materials, preventing contact between the test birds and the main flock, were attached. The objective of this study was to test a range of barrier materials including cardboard, wire mesh, polyurethane film and later (at the suggestion of broiler industry personnel), flyscreen mesh. Initial studies suggested that while the cardboard and wire mesh were ineffective, the polyurethane film protected the birds. Further validation (over 2 separate trials, 7 cubes for each barrier material) demonstrated that polyurethane and flyscreen mesh were effective. It was concluded that a biosecurity pen infrastructure based on galvanished steel mesh panels surrounded by polyurethane film or flyscreen mesh was effective at protecting the birds from *Campylobacter* but upscaling studies will be undertaken before full implementation.

Keywords: biosecurity, *Campylobacter*, broiler, poultry, barrier

1. Introduction

Campylobacter spp. are microaerophilic Gram negative, spiral shaped bacteria (Facciolà et al., 2017). Every year these bacteria cause approximately 250,000 cases of gastroenteritis in the European Union (EU) costing an estimated €2.4bn in health care and lost working days (Bolton, 2015; EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2019; EFSA, 2014; Gracia et al., 2016). Although ubiquitous in warm blooded animals, the primary reservoir is in birds, and broilers are the main source of *Campylobacter* infections in humans (EFSA, 2011). The European Food Safety Authority (EFSA) recently reported that 26% of broilers and 38% of broiler carcasses were contaminated with *Campylobacter* in 2018 (EFSA, 2019).

It is generally agreed that the most effective place to control *Campylobacter* in the poultry chain is on the farm and effective biosecurity is the most appropriate method of achieving this objective (EFSA, 2011). However, with multiple sources of *Campylobacter* it is difficult to consistently implement all of the control measures required (Perez-Arnedo & Gonzalez-Fandos, 2019). Thus, our research group recently developed and validated an additional biosecurity measure. Referred to as the 'biosecurity cube', this broiler pen consisted of 4 polycarbonate sheets (1m high x 2.5m long x 6mm thick) supported at the corners by 4 x 1m high wooden columns. Four slits (50cm high x 8cm wide), lined with industrial 50mm thick bristle strips, allowed the feeder and drinker lines to run through the unit. This biosecurity cube housed a sub-flock at the same stocking density as the main flock and prevented *Campylobacter* infection of the test birds in 5 different flocks even when the main flock was infected as early as 14 days into the production cycle (Battersby, Whyte, & Bolton, 2016).

Protecting the entirety of the flock (approximately 30,000 birds) using this design would have adversely affected airflow and thus temperature control in the broiler house. Moreover, the extensive use of polycarbonate sheets would be prohibitively expensive. The biosecurity cube was therefore redesigned to facilitate upscaling. The polycarbonate sheets were replaced with a galvanised steel mesh, with the redesigned cube composed of 4 galvanised steel mesh panels (1m high x 3.43m long) bolted at the corners (**Figure 1**). Slits were cut in 2 ends to accommodate the rise and falls of the feeder and drinker lines. To prevent direct contact between the test and control birds (main flock) it was important to encircle the cube with a physical barrier material approximately 0.5m high. The objective of these studies was to test the ability of a range of barrier materials, including cardboard, wire mesh, and polyurethane film initially and later in more extensive trials polyurethane and flyscreen mesh, in preventing direct contact between test birds and the general flock and in doing so protect the test birds.

2. Materials and Methods

2.1 *Description of farm*

This study was conducted on a commercial broiler farm in county Monaghan (Ireland) which consisted of three broiler houses on site as well as a separate containment facility that housed dairy cattle in the winter months. Two of the three broiler houses and the containment facility were located adjacent to each other while the third broiler house was situated on a separate concrete apron. This third house was used for this study. Each flock had a population of approximately 30,000 birds. A fan based ventilation system was used in the broiler house.

Flocks were thinned twice, first thin usually occurred around Day 28 and final thin occurred between Day 35 and 36.

2.2 *Description of biosecurity cube*

The biosecurity cube consisted of a 4 galvanised steel mesh panels (3.44m long x 1.25m high) (Cill Dara animal compounds limited, Kildare, Ireland) bolted at the corners (**Figure 1**). This structure was surrounded by a barrier, approximately 0.5m, composed of cardboard, a wire mesh (B&Q, DIY Store, Liffey Valley, Dublin, Ireland), polycarbonate film (B&Q, DIY Store, Liffey Valley, Dublin, Ireland) (**Figure 2**) or flyscreen mesh (Midge Mesh Roll, Goss Fly Screens, Louth, Ireland). Four slits down two sides of the cube allowed for feeder and drinker lines to run directly through the cube without disruption. The cubes were constructed in the broiler house of a commercial flock enclosing an area of 11.8m^2 which was stocked at the same density as the main flock (approximately 21 birds per m^2).

2.3 Description of the study design

In the first study 3 materials (cardboard, wire mesh and polyurethane film) were tested on 3 separate occasions, with polyurethane selected for upscaling. In the second study the biosecurity cubes were upscaled and both polyurethane and flyscreen were implemented on 2 separate occasions testing each barrier material a total of 7 times.

2.4 *Sample collection*

On day 1 (chick arrival) 10 papers lining the chick crates were tested for *Campylobacter*. Thereafter composite fresh faecal samples (2 x 10 samples in the cube and 10 x 10 in the main flock (first study), and 2 x 10 samples in each cube and 5 x 10 in the main flock (second study)) were obtained on days 7, 14, 21, 28 and 35.

2.5 *Campylobacter testing*

All samples were tested using ISO methods 10272-1 and 10272-2 for the detection and enumeration of *Campylobacter* spp (ISO, 2017a, 2017b). Chick paper samples were processed by cutting a 10cm^2 square from each paper and stomaching for 60 seconds in 90mls of Bolton broth (CM983B, Oxoid, Cambridge, UK) supplemented with 5% lysed horse blood (HB037, Cruinn Diagnostics, Dublin, Ireland) and 1% Bolton broth selective supplement (SR183E, Oxoid, Cambridge, UK). 1ml of each sample was plated out in duplicate onto modified Charcoal Cefoperazone Deoxycholate agar (mCCDA) (CM0739, Oxoid, Cambridge, UK) supplemented with CCDA selective supplement (SR0155, Oxoid, Cambridge, UK) and tazobactam sodium salt at a concentration of 1mg/L, to improve the selectivity of the agar (Fisher Scientific, Dublin, Ireland) (Smith et al., 2015). Both the sample inoculated broths and mCCDA plates were incubated microaerobically at 42°C for 48hours using Anaero Jars (AG0025A, Fannin, Dublin) with Campygen atmosphere generation kits (CN025A, Oxoid, Cambridge, UK). 10µl of enriched samples was then streaked out onto tazobactam supplemented mCCDA to test for the presence or absence of growth.

For faecal samples, 10g of the composite sample was weighed out and aseptically added to 90ml of Bolton broth. After stomaching for 60 seconds serial dilutions were prepared using 1ml of sample in 9ml of Maximum Recovery Diluent (MRD) (CM0733B, Oxoid, Cambridge, UK). 100µl of each sample dilution was plated out in duplicate these plates and the faecal sample inoculated broths were then incubated as before.

2.6 *Campylobacter confirmation*

Five suspect colonies were randomly selected from each sample and streaked onto Mueller Hinton agar (CM0337, Oxoid, Cambridge, UK) supplemented with 5% defibrinated sheeps blood (SB054, Cruinn Diagnostics, Dublin, Ireland) and incubated microaerobically at 42°C for 48 hours. Isolates were then subjected to biochemical tests including: aerobic growth, L-alanine test (Oxoid Biochemical Identification System (O.B.I.S.), Thermo scientific, Hampshire, UK), Oxidase test (Fisher Scientific, Dublin, Ireland), and growth on chromogenic agar (RAPID' *Campylobacter* Medium, BioRad, Dublin, Ireland). After biochemical testing a representative cohort of isolates were randomly selected for further confirmation via PCR. DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Manchester, UK) and speciated using conventional PCR (Wang et al., 2002).

2.7 *Analysis of results*

In this study there were 3 possible scenarios; [1] the control and test birds were infected with *Campylobacter* at the same time; [2] the control birds were infected with *Campylobacter* but infection of the test birds was delayed by the cube, and [3] the control birds were infected but the test birds remained *Campylobacter* negative. As it was not possible to continuously test the birds, the time of infection was estimated based on the data reported by Koolman et al (Koolman, Whyte, & Bolton, 2014). These authors monitored *Campylobacter* growth in broilers and consistently observed an increase in these bacteria of 1.5 $\log_{10}$ CFU/g per day once the birds were infected. Thus, the time of infection was estimated using the equation;

$C_t / 1.5 = T_{si}$

$T_m - T_{si} = T_i$

therefore;

$T_i = T_m - (C_t/1.5)$

C_t = *Campylobacter* count at the time of testing in $\log_{10}$ CFU/g

T_{si} = time since first infection in days

T_m = time when the birds first tested positive in days

T_i = estimated time of infection in days

3. Results and Discussion

This study focused on evaluating the effectiveness or otherwise of the different barrier materials, all of which prevented direct contact between the test birds and the litter (bedding material-faecal mixture) outside the cube, and then upscaling to investigate the potential for implementation within the poultry industry. The wire mesh and the cardboard were not effective barrier materials because they did not prevent bird-to-bird contact, which occurred directly through the holes in the mesh and at the slits cut in the cardboard to facilitate the rise and fall of the feeder and drinker lines. In contrast both the polyurethane film and flyscreen material protected the test birds by preventing direct contact between infected birds in the main flock and the test birds in the cubes.

With multiple sources of *Campylobacter* on broiler farms it is difficult to consistently implement full biosecurity (Battersby et al., 2016). Once one bird is infected the bacteria can reach high concentrations in the caeca within 3 to 4 days (Newell, 2002), which is continuously shed in the faeces (Evans & Sayers, 2000). Broilers are coprophagic and the faecal-oral route facilitates the rapid dissemination of *Campylobacter* within the flock so

every bird is infected by 5 to 7 days after entry of the pathogen into the flock (Evans & Sayers, 2000; van Gerwe et al., 2009). In our experiments, the wire mesh and cardboard were not effective in protecting the test birds. In both studies the control birds were *Campylobacter* positive after 21 days with faecal counts of approximately 5.0 $\log_{10}$ CFU/g faeces (**Table 1**). Throughout this study all *Campylobacter* isolates were *Campylobacter jejuni*. The test birds were also infected with *Campylobacter* after 21 days with similar counts, suggesting direct contact between the birds is sufficient to facilitate immediate transfer of the organism. This was not unexpected as experimental studies have shown that a dose as low as 40 CFU is sufficient to infect a chicken (Cawthraw, Wassenaar, Ayling, & Newell, 1996).

The polyurethane film protected the birds in the biosecurity cube for at least 2.8 days, during which time approximately 250 birds (in each pen) surrounded by 30,000 positive broilers remained *Campylobacter* negative until harvest (**Table 1**). Interestingly, it was estimated that the main flock remained *Campylobacter* negative until approximately 31 days, which corresponds well with first thinning (at day 29) plus a lag period of 1 to 2 days estimated by Koolman et al (2014) before *Campylobacter* start to multiply within the flock.

As the polyurethane cube was effective in the initial study, further validation was undertaken on 2 separate occasions (validation trials 1and 2). Furthermore, at the suggestion of the poultry industry, flyscreen material was included at this stage of the development process. In the first trial each material was tested on 3 cubes. During the second trial each material was tested on 4 cubes. In the first validation trial, the control birds were positive by day 35 (6.1 $\log_{10}$ cfu/g faeces) but the polyurethane barrier delayed infection in 1 cube by 1 day and in the other 2 cubes by at least 3.9 days so these birds were *Campylobacter* negative at harvest (**Table 2**). Similar results were obtained for the flyscreen except that 1 of the 3 cubes completely failed with the test birds being infected (5.8 $\log_{10}$ cfu/g faeces) at the same time as the control birds in the main flock. When this was repeated (validation trial 2) the control birds were once again infected by day 35 (5.9 $\log_{10}$ cfu/g faeces) but on this occasion all of the birds in the cubes, regardless of barrier material (polyurethane or flyscreen) remained negative and were *Campylobacter* free at harvest.

4. Conclusions

It was concluded that that a biosecurity pen infrastructure based on galvanised steel mesh panels surrounded by polyurethane film or flyscreen mesh was effective at protecting the birds from *Campylobacter* but further upscaling studies are required before full implementation can be considered.

Author Contributions:

Genevieve Greene: methodology, validation, formal analysis, investigation, data curation, writing – original draft and preparation, writing – review and editing, and visualization.

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All authors have read and agreed to the published version of the manuscript.

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Table 1. The ability of the different barrier materials to protect the test birds from *Campylobacter*

	Time to detection of *Campylobacter* positive birds (days)	*Campylobacter* count at the time of detection (t_d) (log_{10} CFU/g)	Estimate of time the birds were initially infected (days)	Estimated duration of protection time of the test birds in the cube (days)
		Cardboard		
Control	21	4.9	17.7	NA
Test	21	5.3	17.5	0
		Wire mesh		
Control	21	5.8	17.1	NA
Test	21	5.2	17.5	0
		Polyurethane film		
Control	35	4.2	32.2	NA
Test	birds remained negative	ND[1]	NA[2]	at least 2.8

[1]ND = not detected; [2]NA = not applicable

Table 2. Retesting the biosecurity cubes constructed using polyurethane and flyscreen mesh barrier materials

Validation trial number	Validation trial test number	Time to detection of *Campylobacter* positive birds	*Campylobacter* count at the time of detection (t_d) (log_{10} CFU/g)	Estimate of time the birds were initially infected (days)	Estimated duration of protection time of the test birds in the cube (days)
1	Control	35	6.1	30.9	NA
2	Control	35	5.9	31.1	NA
		Polyurethane film			
1	Test 1	birds remained negative	NA	NA	at least 3.9
1	Test 2	birds remained negative	NA	NA	at least 3.9
1	Test 3	35	4.3	32.1	1
2	Test 1	birds remained negative	NA	NA	at least 3.9
2	Test 2	birds remained negative	NA	NA	at least 3.9
2	Test 3	birds remained negative	NA	NA	at least 3.9
2	Test 4	birds remained negative	NA	NA	at least 3.9
		Fly screen			
1	Test 1	birds remained negative	ND	NA	at least 3.9
1	Test 2	birds remained negative	ND	NA	at least 3.9
1	Test 3	35	5.8	31.1	0
2	Test 1	birds remained negative	NA	NA	at least 3.9
2	Test 2	birds remained	NA	NA	at least 3.9

		negative			
2	Test 3	birds remained negative	NA	NA	at least 3.9
2	Test 4	birds remained negative	NA	NA	at least 3.9

[1]ND = not detected; [2]NA = not applicable

Figure 1. The biosecurity cube consisted of a 4 galvanised steel mesh panels (with slits to facilitate the rise and fall of the drinker and feeder lines) bolted at the corners, to which cardboard, wire mesh, polyurethane film or flyscreen mesh were added.

Figure 2. The biosecurity cube with a barrier of polycarbonate film. Test birds can be seen contained within, with the general flock surrounding the cube.

Highlights:

- This study provides further evidence of biosecurity cube protection of broilers
- A biosecurity cube framework could protect broilers from *Campylobacter*.
- Polyurethane and flyscreen barriers prevent *Campylobacter* colonisation.

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