Food Microbiology 64 (2017) 1–6

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Evaluating and improving terminal hygiene practices on broiler farms to prevent *Campylobacter* cross-contamination between flocks

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ARTICLE INFO

Article history:
Received 23 June 2016
Received in revised form
26 September 2016
Accepted 26 November 2016
Available online 26 November 2016

Keywords:
Disinfection
Broiler units
Campylobacter
Hygiene

ABSTRACT

The objectives of this study were to evaluate current cleaning practices in broiler houses by testing a range of sites after cleaning and disinfection and to test the efficacy of the most commonly used methods in a commercial broiler house after flock harvesting. Cleaning procedures on 20 broiler houses (10 separate farms) were examined by testing a range of sampling points (feeders, drinkers, walls, etc.) for total viable count (TVC), total *Enterobacteriaceae* count (TEC) and *Campylobacter* spp. after cleaning and disinfection, using culture based methods. In a second experiment, the six most commonly used commercially available disinfectants and/or detergent products were evaluated. The results of the first study demonstrated that critical areas in 12 of the 20 broiler houses were not effectively cleaned and disinfected between flocks as the tarmac apron, ante-room, house door, feeders, drinkers, walls, columns, barriers and/or bird weighs were *Campylobacter* positive. Thermal fogging with the combination of potassium peroxymonosulfate, sulfamic acid and sodium chloride (5%, v/v) or the glutaraldehyde and quaternary ammonium complex (0.3%, v/v) were the most effective treatments while other disinfectant treatments were considerably less effective. It was therefore concluded that farmers should review their broiler house cleaning and disinfection procedures if *Campylobacter* cross-contamination between successive flocks is to be prevented.

1. Introduction

Campylobacteriosis is the most common bacterial gastroenteritis in the developed world. The 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). Despite over thirty years of research and numerous studies, protecting broilers from infection during primary production is still a major problem. Although a range of sources, including other farm animals (Acke et al., 2009; Ogden et al., 2014), personnel (Ridley et al., 2011), flies (Hald et al., 2007), rodents (Meerburg et al., 2006), equipment (Agunos et al., 2014; Battersby et al., 2016) and the environment around the broiler house have been identified (Ogden et al., 2014) and addressed using biosecurity measures, *Campylobacter* infections have persisted on the majority of farms.

The Codex Alimentarius Commission define disinfection as 'the reduction of the number of microorganisms in the environment to a level that does not compromise food safety or suitability' (CAC, 2003). Recent research by our group suggested that inadequate cleaning and disinfection of key sites such as the tarmac apron, ante-room, house door, feeders, drinkers, walls, columns, barriers and bird weighs compromise food safety by contributing to cross-contamination between successive flocks on the broiler farm (Battersby et al., 2016). The persistence of *Campylobacter* on equipment and in the environment has previously been reported in poultry slaughterhouses (Peyrat et al., 2008). These findings contradict the commonly held belief that, as *Campylobacter* are susceptible to commonly used disinfectants (Avrain et al., 2003), inadequate cleaning is not a source of *Campylobacter* for new flocks on broiler farms (Evans and Sayers, 2000; Stern et al., 2001; Herman et al., 2003).

Disinfectants typically have multiple target sites in the bacterial cell and affect a wide range of bacterial species (Poole, 2002). Solid

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http://dx.doi.org/10.1016/j.fm.2016.11.018
0740-0020/

surfaces need to be cleaned prior to disinfection to remove organic matter and the disinfectant applied at the required concentration and for sufficient contact time (Langsrud et al., 2003). Moreover, equipment must be hygienically designed to allow for effective cleaning and disinfection (Cerf et al., 2010). The cleaning agents used in the food industry are typically halogens (iodophors and chlorines), alcohols, oxidizing agents, phenols, aldehydes and/or quaternary ammonium compounds or combinations thereof. Commercial formulations for cleaning and disinfecting broiler farms typically use oxidizing agents such as potassium peroxymonosulfate ($KHSO_5$) and hydrogen peroxide(H_2O_2), aldehydes (glutaraldehyde, $C_5H_8O_2$) and quaternary ammonium compounds (C_nH_{2n+1} where n = 8 to 18). Other compounds used in these formulations include sodium hydroxide (NaOH), limonene ($C_{10}H_{16}$) and sulfamic acid (H_3NSO_3). In addition to disinfection, sodium hydroxide and the d-enantiomer of limonene also have detergent properties (dissolve grease, oils, fats and/or protein based deposits) while sulfamic acid is an acidic cleaning agent used for descaling activities such as removing limescale.

Once introduced into the flock, *Campylobacter* is quickly spread to all birds, grows rapidly within each bird and large numbers are shed, heavily contaminating the broiler house environment and equipment (Battersby et al., 2016). Although essential for pathogen control and prevention (Payne et al., 2005; Chima et al., 2012), cleaning and disinfection may therefore be extremely difficult (Bøhm, 1998). In addition to the high concentration of *Campylobacter*, high levels of organic matter, incorrect dilution and ineffective application methods also inhibit effective disinfection (Bøhm, 1998; Payne et al., 2005; Huneau-Salaün et al., 2007). As evaluation studies have not been previously undertaken and information on the effectiveness of terminal hygiene practices in broiler houses is limited, advice for farmers is lacking. The objectives of this study were therefore to evaluate current cleaning practices in broiler houses by testing a range of broiler house sites after cleaning and disinfection and to evaluate the most commonly used methods in a commercial broiler house after flock harvesting.

2. Method and materials

2.1. Farm sampling

Various detergent and disinfection products are available for use on Irish farms, with choice and application solely up to the farmer. The standard mode of application is the use of mechanical sprayers, due to ease of application, cost efficiency and rapid application. However, with increased hygiene auditing and the decreasing turnaround times between flocks the need for effective, drier methods of disinfection has resulted in an interest in thermal fogging applications.

Ten farms (20 broiler houses) indicative of the differing cleaning and disinfection practices in use were chosen and sampled after terminal disinfection and prior to house restocking. All farms were contracted to a major broiler processor in the Republic of Ireland and were located in counties Monaghan and Cavan.

2.1.1. Sample collection

A range of environmental swabs within and outside each house were taken using sterile sponge swabs pre-moistened with 10 ml maximum recovery diluent (TSC Swabs UK), including; feeders (3 m^2), drinkers (3 m^2), bird weigh points (1.5 m^2), the middle section of wooden barriers separating male and female birds (1.5 m^2), wooden support columns (1 m^2), walls (the front, middle and back of each house on both sides (1.7 m^2), the concrete apron directly in front of the large vehicular access doors (3 m^2) and directly in front of the personnel access door (1.5 m^2), the step-over hygiene barrier and the floor of the ante-room of each house (1.5 m^2) as described in Battersby et al. (2016). Swabs were transported to the laboratory at 4 °C using cooler boxes and analysed within 12 h.

2.1.2. Isolation of Campylobacter spp. from environmental swabs

Swabs were added to 100mls 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), and stomached for 30 s. Serial dilutions were prepared in maximum recovery diluent (MRD, CM0733B Oxoid, Cambridge, UK), and 100 μl aliquots were plated in duplicate onto modified *Campylobacter* blood free selective agar (mCCDA, CM0739b; Oxoid, Cambridge, UK) supplemented with cefoperazone and amphotericin (SR0155E; CCDA selective supplement, Oxoid, Cambridge, UK). These plates were incubated at 42 °C for 48 h under microaerobic conditions, generated in Anaero Jars (AG0025A; Fannin, Dublin) using Campygen atmosphere generation kits (CN025A; Oxoid, Cambridge, UK). Swabs were also incubated for 48 h under microaerobic conditions and re-plated on mCCDA using the procedure described above.

2.1.3. Campylobacter confirmatory tests

All *Campylobacter* isolates were confirmed using Gram reaction (3% [w/v] KOH; Sigma Aldrich, Ireland) and standard biochemical tests including: the oxidase test (Oxoid, Cambridge, UK) and L-ala test (Sigma Aldrich, Ireland) followed by streaking on Brilliance CampyCount Agar(Oxoid, Cambridge, UK).

2.2. Investigating the effectiveness of commonly used disinfectants and detergents

2.2.1. Treatment selection

The most commonly used broiler house cleaning and disinfection procedures were selected for inclusion in this study including the chemical formulations and application methods used.

2.2.2. Selection of farm for treatment

One farm was selected for the chemical treatment evaluation study. This farm consisted of four broiler houses on one tarmac apron. The houses had the capacity to rear between 25,000–30,000 broilers per rearing cycle which was typically 40 days with 7–14 day turnover. The farmer was fully trained and had experience in the disinfectants and application methods used. Three replications of each chemical treatment were carried out.

2.2.3. Treatment with detergent

The test farm was sampled on the day of slaughter, immediately after flock and litter removal. Following sampling, detergent was applied at the recommended concentration and following the manufacturer's instructions (Table 1) followed by rinsing with a power hose. Houses were swabbed again 12 h later using the sampling method described previously.

2.2.4. Treatment with disinfectant

Swabs were taken immediately before disinfectant was applied. Disinfectant was applied at the recommended concentration and following manufacturer's instructions (Table 1) and the houses washed down. Houses were swabbed 12 h later following the sample collection method described previously. After thermal fogging the houses were left idle for 24 h, as a safety precaution, before the researcher was permitted to swab.