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On farm interventions to minimise Campylobacter spp. contamination in chicken

T. Lu D^{a,b}, M. Marmion^c, M. Ferone^c, P. Wall^{a,b,d} and A. G. M. Scannell^{b,c,d}

^aUCD School of Public Health, Physiotherapy and Sports Science, University College Dublin, National University of Ireland, Dublin, Ireland; ^bUCD Centre for Food Safety, University College Dublin, National University of Ireland, Dublin, Ireland; ^cUCD School of Agriculture and Food Science, Agricultural & Food Science Centre, University College Dublin, National University of Ireland, Dublin, Ireland; ^dUCD Institute of Food and Health O'Brien Science Centre South, University College Dublin, National University of Ireland, Dublin, Ireland;

ABSTRACT

1. This review explores current and proposed on-farm interventions and assess the potential of these interventions against *Campylobacter* spp.

2. Interventions such as vaccination, feed/water-additives and, most importantly, consistent biosecurity, exhibit potential for the effective control of this pathogen and its dissemination within the food chain.

3. Due to the extensive diversity in the *Campylobacter* spp. genome and surface-expressed proteins, vaccination of poultry is not yet regarded as a completely effective strategy.

4. The acidification of drinking water through the addition of organic acids has been reported to decrease the risk of *Campylobacter* spp. colonisation in broiler flocks. Whilst this treatment alone will not completely protect birds, use of water acidification in combination with in-feed measures to further reduce the level of *Campylobacter* spp. colonisation in poultry may be an option meriting further exploration.

5. The use of varied types of feed supplements to reduce the intestinal population and shedding rate of *Campylobacter* spp. in poultry is an area of growing interest in the poultry industry. Such supplements include pro – and pre-biotics, organic acids, bacteriocins and bacteriophage, which may be added to feed and water.

6. From the literature, it is clear that a distinct, albeit not unexpected, difference between the performance of in-feed interventions exists when examined *in vitro* compared to those determined in *in vivo* studies. It is much more likely that pooling some of the discussed approaches in the in-feed tool kit will provide an answer.

7. Whilst on-farm biosecurity is essential to maintain a healthy flock and reduce disease transmission, even the most stringent biosecurity measures may not have sufficient, consistent and predictable effects in controlling *Campylobacter* spp. Furthermore, the combination of varied dietary approaches and improved biosecurity measures may synergistically improve control.

Introduction

Campylobacter spp. are Gram-negative, thermophilic, nonspore-forming bacteria that are visible under the microscope as slender, helical or curved rods. It generally colonises the caeca of avian species as a commensal microorganism, with broiler chickens being a particular vector of concern (Hermans et al. 2011a; Natsos et al. 2019). While most *Campylobacter* spp. carriage is asymptomatic in the broiler host, infection in some individuals can lead to damage of the gastrointestinal lining, causing diarrhoea (Humphrey et al. 2014; Sahin et al. 2015). In humans, *Campylobacter* spp. infection causes an acute form of enteritis known as campylobacteriosis, while more serious sequalae include Guillain-Barré syndrome, reactive arthritis and Miller-Fisher syndrome (Hermans et al. 2011a; Pedersen et al. 2018).

The incidence and prevalence of human campylobacteriosis has increased in both developed and developing countries over the last 10 years. Compared to other bacteria, *Campylobacter* spp. is less known for outbreaks, and more for large numbers of sporadic individual cases. However, outbreaks caused by *Campylobacter* spp. do exist, with 4,936 outbreaks recorded by the Centres for Disease Control and Prevention in the United States between 1999 and 2008 (Batz et al. 2012).

The overall economic burden of campylobacteriosis is quite substantial (EFSA 2011). In the UK, the median estimated cost to patients and the health service during the 2008–2009 period was £50 million (95% CI: £33-£75 m). The additional cost of related Guillain-Barré syndrome hospitalisations were estimated at £1.26 million (Tam and O'Brien 2016). The annual costs of campylobacteriosis in the United States (Hoffmann et al. 2012) and in the EU (EFSA 2014; Bolton 2015) are quite high, estimated to be in the region of 2.9 USD billion and €2.4 billion, respectively.

Campylobacter spp. enter the food chain through poultry colonisation at the farm level. The dose of *Campylobacter* spp. required to colonise chicks and chickens can be very low, but, once established, populations within the caeca can rapidly reach high levels (between 105 and 109 CFU/g; Woodall et al. 2005; Stern 2008; Newell et al. 2011; Taha-Abdelaziz et al. 2018a). Because chickens remain colonised until slaughter, this almost inevitably results in carcase contamination during processing, which can, in turn, allow pathogen transmission to humans (Allen et al. 2008; Stern 2008; Jorgensen et al. 2011; Ridley et al. 2011; Hermans et al. 2012; Sahin et al. 2015).

There is little *Campylobacter* spp. genotype homogeneity, with genetic transfer playing an important role in the

CONTACT Ting Lu 🔊 ting.lu@ucdconnect.ie 🗊 UCD School of Public Health, Physiotherapy and Sports Science, University College Dublin, National University of Ireland, Dublin 4, Ireland

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ARTICLE HISTORY

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KEYWORDS

Campylobacter; contamination; on farm; interventions; chicken development of novel genotypes within a short space of time. High competency species, including C. jejuni, are capable of utilising external DNA, such as the virulence plasmid pVir, DNA acquired from the environment or obtained through transformation, to modify the cell genotype. As such, it is difficult to characterise Campylobacter spp. by genotype (Burnham and Hendrixson 2018). Further complicating this is phase variation in Campylobacter spp. gene expression, with over 30 genes differentially regulated in response to the external environment. This is vital as part of the adaptive process when Campylobacter spp. isolates from an avian reservoir, for example, the broiler caecum, and enter a new environment, such as the meat processor plant. Phase variation is particularly effective with regard to modulating externally expressed structures, such as lipooligosaccharide, capsular polysaccharide and flagellin, as well as altering cell motility (Burnham and Hendrixson 2018). Regulation of the stator protein gene motA in response to environmentallyinduced c-di-GMP reduces flagellar stability and alters a cell's ability to move (Wirebrand et al. 2018; Burnham and Hendrixson 2018). This altered genotype may become more adept at surviving external stresses, such as those within human consumers or the external environment.

There have been attempts to circumvent this genotypic variation and classify C. jejuni according to alleles of highly conserved genes through Multilocus Sequence Typing (MLST). The MLST has allowed researchers to trace the occurrence of live-bird associated C. jejuni sequence types (STs) from the farm level through the slaughter and manufacturing process to the end-product. These studies have revealed that the majority of C. jejuni STs found in endproduct meat are indistinguishable from those associated with live birds (Colles et al. 2010; Hastings et al. 2011). MLST studies allow for further analysis of flock characteristics and handling. Certain STs, for example, ST418 and ST 4227, are easily removed through washing and decontamination within the transport process. Some STs, such as ST-45, are associated with the caecal microbiome, and show resilience when exposed to decontamination stresses, such as disinfectant (Hastings et al. 2011). These resilient STs represent the most difficult to eradicate and are the most environmentally tolerant within the production cycle, and represent the worst-case scenario at a farm level, which may necessitate extra precautions or decontamination steps.

Many risk factors contribute to Campylobacter spp. colonisation of broiler flocks, indicating the difficulties in maintaining effective countermeasures against its entry into the broiler environment (Hermans et al. 2012; Natsos et al. 2018). In general, studies have indicated that horizontal transmission from environmental sources is the most significant cause of dissemination in flocks. Vertical transmission remains a controversial source of the microbe. (Bull et al. 2006; Callicott et al. 2006; Ridley et al. 2008; Workman et al. 2008; Zweifel et al. 2008; Ellis-Iversen et al. 2009; Allen et al. 2011; Patriarchi et al. 2011; Cox et al. 2012; Sahin et al. 2015). Bull et al. (2006) and Callicott et al. (2006) stated lack of evidence for vertical transmission of Campylobacter spp. in chickens while Cox et al. (2012) believed vertical transmission has been overlooked because of two reasons. One reason is that there is no ideal culturing procedure for recovering and isolating Campylobacter spp. It is difficult to routinely culture from certain types of samples. Another reason is that researchers have not fully accepted the role of the fertile egg in transmission. Most factors commonly associated with

Campylobacter spp. colonisation in broiler flocks are farmbased, including: (1) A lack of overall biosecurity on farms; (2) Presence of other animals in close proximity to poultry houses (including other poultry species, livestock, pets, and wildlife); (3) Increasing numbers of houses on a farm; (4) Slaughter age; (5) Size of flocks; (6) Partial depopulation (thinning); (7) Seasonal and climatic changes; (8) Use of ventilators; (9) Fly and insect population; (10) Use of old litter; (11) Farm equipment and (12) Transport vehicles and farm workers (Hald et al. 2004; Nichols 2005; Hald et al. 2008; Hazeleger et al. 2008; Stern 2008; Horrocks et al. 2009; Meerburg 2010; Newell et al. 2011; Patriarchi et al. 2011; Ridley et al. 2011; Wagenaar et al. 2013; Carron et al. 2018; Murphy et al. 2018).

Animal stress is an unavoidable aspect of broiler husbandry, and can have damaging effects upon the growth and well-being of the live bird. Common practices within the broiler house and in the lead up to slaughter cause increases in the production of stress-related hormones, such as corticosterone, leading to changes in animal behaviour, including increased pecking at self and others, agitation and defaecation (Scanes 2016; Rasschaert et al. 2020). Stress-inducing processes include shackling, cooping, transport, fasting/feed restriction and worker interactions, while excess heat, cold, light and movement restriction increase individual and group stress (Scanes 2016). Furthermore, corticosterone production, due to stress, is linked to an increase in gastrointestinal permeability. This causes increased defecation due to higher digesta water content and can cause the systemic infection of birds by gastrointestinal bacteria (Scanes 2016). This may cause illness in the bird if early in life, as chicks are particularly vulnerable to systemic Campylobacter spp. infection, or may spread to the end product (Humphrey et al. 2014; Sahin et al. 2015). These effects can have subsequent ramifications for the end-product, with faeces acting as a prominent vector for Campylobacter spp. between adjacent birds, especially in crated environments prior to slaughter, with increased pecking exacerbating this spread (Rasschaert et al. 2020).

The prevalence of Campylobacter spp.-positive poultry broiler flocks varies by region, season and production system (Bahrndorff et al. 2013; Murphy et al. 2018). In the United States, a 2001 survey indicated that nearly 90% of flocks are colonised on-farm (Stern et al. 2001). In Europe, the prevalence on-farm broiler flocks varies from 18 to > 90%, with Northern European countries having significantly lower figures than their Southern European counterparts (Newell and Fearnley 2003; Hermans et al. 2012; Sibanda et al. 2018). As contaminated poultry is the primary reservoir for human infections, the Food Safety Authority of Ireland (FSAI), European Food Safety Authority (EFSA), Health Protection Surveillance Centre (HPSC) and World Health Organisation (WHO) are actively seeking effective interventions within the poultry production chain. This is particularly important as antimicrobialresistant strains of *Campylobacter* spp. (Murphy et al. 2018) add to the economic burden of foodborne disease globally (EFSA and ECDC 2017).

Since *Campylobacter* is frequently found as a commensal organism in the avian caecum, it makes sense to introduce interventions that prevent this colonisation in the first instance. The application of strict biosecurity measures is the most effective method to prevent this colonisation (see Figure 1) (Rivoal et al. 2005; Nauta et al. 2007; Hermans et al. 2012; WHO 2013). However, biosecurity measures alone will not be sufficient as broiler chickens are at constant risk of contamination (Pattison

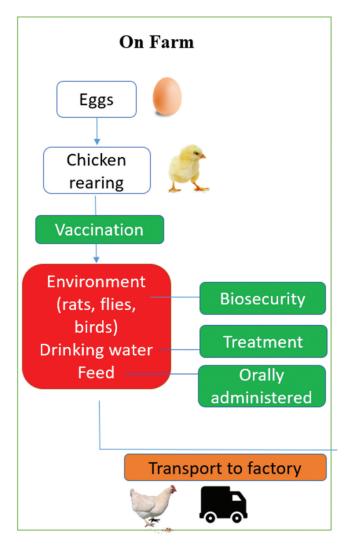


Figure 1. The Flow chart illustrates steps in chicken production on farms. Red steps indicate points at which *Campylobacter* spp. can pose a risk. Green steps indicate points at which *Campylobacter* spp. can be controlled. Orange steps indicate the possibility of risk/control, which depends on behaviour/actions.

2001; Sahin et al. 2003; Van Gerwe et al. 2005; Hermans et al. 2011b). Therefore, other promising specific pre-slaughter interventions (e.g. no-thin practices and improved transport hygiene) also must be considered. This literature review explores on-farm primary production practices to reduce the contamination of *Campylobacter* on poultry flocks.

Vaccination

Vaccination of poultry may be considered a logical starting point in the struggle to prevent initial colonisation. Unlike Salmonella spp., for which there exists a broiler vaccine, there is no such facility currently available for Campylobacter spp. (Wagenaar et al. 2013; Olofsson 2015). Given the extensive diversity in Campylobacter spp. genome and surface-expressed proteins, it is not easy to develop vaccines that can prevent associated illness (Ringoir and Korolik 2003; Walker 2005). Several Campylobacter spp. target structures have been the focus of in ovo vaccination studies, including DNA, flagellar subunits and liposomes, as well as a whole-cell bacterin suspension. However, these vaccination candidates did not elicit a protective humoral response when used in vivo (Liu et al. 2019; Vandeputte et al. 2019). Recent promising research (see Table 1) has shown that vaccination of chickens with recombinant Campylobacter spp. peptides can result in reduced C. jejuni caecal carriage compared to a nonvaccinated Campylobacter-challenged group. This indicated broiler protection from C. jejuni colonisation (Rice et al. 1997; Widders et al. 1998; Wyszyńska et al. 2004; Buckley et al. 2010; Neal-McKinney et al. 2014; Kobierecka et al. 2016; Taha-Abdelaziz et al. 2018a). Other promising vaccines include an oral candidate which utilises a combination of soluble PLGAencapsulated oligodeoxynucleotides containing unmethylated CpG motifs and Campylobacter spp. lysate to achieve significant reduction in caecal carriage in both layer and broiler chickens through the induction of an IgG response (Taha-Abdelaziz et al. 2018b). However, vaccination of poultry against Campylobacter is not yet regarded as a completely effective strategy (Hermans et al. 2011b). Broiler chickens are specially bred for fast growth and often slaughtered between five and six weeks of age. Vaccination of broilers is challenging due to the cost. A study conducted by Bull et al. (2006) found that Campylobacter spp. was rarely isolated from broiler flocks until the birds were at least three weeks old. Maternal antibodies in chicks are considered to have a role in preventing early colonisation of the chick's caeca. Further work is needed in this area to find a consistently successful vaccine, as well as in the development and establishment of a suitable and robust vaccination policy (Svetoch and Stern 2010). Approaches which need to be optimised include time of vaccination (live chick vs. in ovo vaccination), a potential mixture with other poultry vaccines, such as those currently used to prevent Table 1. Control measures to reduce Campylobacter prevalence and transmission in poultry flocks.

Intervention measures	Results	References
Vaccination	The median level of <i>C. jejuni</i> colonisation was reduced to 2.55*10 ⁴ to 1.1*10 ⁶ CFU/gram of caecal content compared with non-vaccinated control group (5.35*10 ⁷ CFU/gram).	(Neal-McKinney et al. 2014
	The median reduction of <i>C. jejuni</i> was 1 log ₁₀ to 2 log ₁₀ .in caecal contents. The reduction of <i>C. jejuni</i> is less than 2% in intestinal colonisation compared with unimmunised control birds.	(Kobierecka et al. 2016) (Widders et al. 1996)
	The overall reductions of <i>C. jejuni</i> colonisation in the vaccinated chickens ranged from 16 to 93% compared with non-vaccinated controls.	(Rice et al. 1997)
	2-log reduction of C. jejuni in caecal colonisation.	(Widders et al. 1998)
	>6 logs reduction upon homologous challenge.	(Wyszyńska et al. 2004)
	Reductions of 3.78 and 3.47 \log_{10} CFU/g were observed at days 21 and 28 post-challenge.	(Buckley et al. 2010)
	1-log to 4-log <i>C. jejuni</i> reduction in caecal colonisation. No effect upon homologous challenge.	(Layton et al. 2011) (Ziprin et al. 2002)
	Reduction of <i>C. jejuni</i> positive chickens (40/145 vs. 70/142 in control group).	(Khoury and Meinersmann 1995)
ygiene and biosecurity	ND	(Van de Giessen et al. 1998
farming practices	Reduced the risk of Campylobacter spp. infection by over 50% in intervention flocks.	(Gibbens et al. 2001)
	Reduced the percentage of Campylobacter spppositive flocks from 51.4 to 15.4%.	(Hald et al., 2007)
	Reduced the prevalence of <i>Campylobacter</i> spppositive flocks from 41.4 to 10.3%.	(Bahrndorff et al. 2013)
	The prevalence of <i>Campylobacter</i> -positive broiler flocks at slaughter decreased from 43% in 2002 to 27% in 2007.	(Rosenquist et al. 2009)
	Rodent control around the house reduced the risk of <i>Campylobacter</i> colonisation ($OR = 0.18$, 95% Cl 0.03–0.95).	(Allain et al. 2014)
Drinking water treatment	Crop contamination with <i>Campylobacter</i> was significantly reduced by lactic acid treatment (62.3%) as compared with the controls (85.1%).	(Byrd et al. 2001)
	Chlorination of flock drinking water was not effective in decreasing colonisation by <i>Campylobacter</i> spp.	(Stern et al. 2002)
	<i>Campylobacter</i> counts in the cloacal samples were about 2 log ₁₀ lower in the experimental group which receives monocaprin in drinking water.	(Hilmarsson et al. 2006)
	Capric acid (10 mM, 37°C, 10 min) reduced C. <i>jejuni</i> by \geq 6.8 log ₁₀ in 10 min.	(Thormar et al. 2006)
	A-1 (formic:acetic:propio- nic acids at 1:2:3) and A-2 (1:2:5) gave a high reduction rate at 3.03 and 3.22 log ₁₀ cfu/mL, respectively.	(Chaveerach et al. 2002)
	<i>Campylobacter</i> numbers were significantly lower in the treatment group (acidified drinking water) compared with group 2 (ordinary drinking water) on d 13, 15, and 19.	(Chaveerach et al. 2004)
	Chlorinated drinking water reduced the risk of <i>Campylobacter</i> colonisation ($OR = 0.5$, Cl 95% 0.2–0.9).	(Ellis-Iversen et al. 2009)
	The acidification of drinking water reduced the risk of <i>Campylobacter</i> colonisation (OR = 0.33, 95% Cl 0.13–0.86).	(Allain et al. 2014)
	Organic acids in drinking water of broilers can reduce the caecal <i>Campylobacter</i> spp. by 4.25log ₁₀ CFU load, but this did not reduce carcase contamination.	(Jansen et al. 2014)
Antibiotics (banned the use as growth promoters in animal feed since 2006 in EU)	The C. <i>jejuni</i> loads of the enrofloxacin-, neomycin-, and vancomycin-derived communities were decreased by 1 log, 2 logs, and 4 logs, respectively.	
	Antibiotic treatment at the beginning of the rearing period reduced the risk of <i>Campylobacter</i> colonisation ($OR = 0.20, 95\%$ Cl 0.07–0.55).	(Allain et al. 2014)
Transport to factory	The <i>Campylobacter</i> spp. in the caecal count significantly increases from $10^{5.44}$ CFU/g to $10^{6.15}$ CFU/g after transport.	(Stern <i>et al.</i> , 1995)
	Catching and placing birds in crates significantly increased the chance that the birds were contaminated with <i>Campylobacter</i> .	(Slader et al. 2002)
	Campylobacter on crates survived for at least 3 h after sanitisation	(Hastings et al. 2011)
	Chickens transported in <i>Campylobacter</i> -positive crates were more likely to test positive at the	(Hansson et al. 2005)
	slaughterhouse [relative risk (RR) = 2.9, 95% Cl 1.1–7.3]. The <i>Campylobacter</i> spp. prevalence in the crops of birds slaughtered after transport (24%) was significantly higher than that for turkeys slaughtered on-farm (3%).	(Wesley et al. 2009)

ND: Campylobacter spp. were not detectable (<1*10² CFU)

Newcastle Disease, Marek Disease, infectious bronchitis and infectious bursitis, as well as the suitability of the vaccination mixture for the *Campylobacter* spp. challenge in question.

Biosecurity

Farm biosecurity is a set of measures designed to protect a property from the entry and spread of pests and disease. It is considered to be the best strategy to prevent the colonisation and spread of *Campylobacter* spp. across poultry farms (Vandeplas et al. 2007; Hermans et al. 2011b). Biosecurity on a poultry farm must ultimately encompass appropriate decontamination methods from the point of entry to the poultry house, maintaining set hygiene standards within the house, through to bird removal at the end of the broiler rearing process. Foot dips, footwear changes, step-over-barriers, hand wash facilities, drinker/feeder hygiene, optimised in-house and bird management (i.e. litter, water, temperature, humidity), poultry house boundary maintenance and traffic management/hygiene all are highlighted as key factors in biosecurity protocols for blocking *Campylobacter* spp. contamination (Sahin et al. 2002; Newell and Fearnley 2003; Barrios et al. 2006; Workman et al. 2008). For terminal hygiene, a five-step guide with appropriate standards and recommendations at each phase has been previously described; (1) dry clean-out; (2) wet clean/wash; (3) disinfection; (4) drying out; and (5) de-infest (Burke 2018).

Farmers and personnel move in and around broiler houses, up to 150 times over the lifetime of a flock. This constitutes a significant risk for *Campylobacter* spp. introduction and spread (Wagenaar et al. 2006). The implementation of personnel hygiene and broiler house disinfection protocols have been found to decrease the prevalence of Campylobacter spp. by 50% (Gibbens et al. 2001), while effective rodent, wild bird and fly control have all been associated with reduced risks of colonisation (see Table 1) (Hald et al. 2001, 2007; Allain et al. 2014; Skarp et al. 2016). For example, in Nordic countries, the use of fly screens as a biosecurity measure is common practice, with 50 to 90% risk reduction achievable when used in combination with strict biosecurity measures (EFSA 2011). The introduction of fly-screens resulted in a reduction in Campylobacter spp.positive flocks in Denmark (Bahrndorff et al. 2013), from 41.4% between 2003 and 2005 (before use of fly screens), to 10.3% between 2006 and 2009 (after introduction of fly screens), whereas the prevalence reduction in the control houses (without fly screens) was not significant (from 41.8% to 36.0%, P > 0.05) during the same period. However, the use of fly screens can have a significant negative impact upon ventilation within poultry houses, which may account for the fact that their implementation is not universal.

Another measure which increases Campylobacter spp. contamination on farm is flock thinning. Thinning is the early removal of a proportion of birds, which have reached the correct market weight, to create space for the remaining flock's continued growth. The thinning process requires the entry of personnel and catching equipment into broiler houses, which can increase the risk of transmission within and between flocks. This leads to the contamination of up to 50% of flocks that were previously Campylobacter spp.-free (Allen et al. 2008; Hermans et al. 2011b, 2012; Skarp et al. 2016). In a recent study, Campylobacter spp. prevalence was found to increase to >85% in both high and low performance farms across all seasons at final depopulation, suggesting that infection was introduced during the thinning procedure (Smith et al. 2016). During thinning, and prior to slaughter, it is common practice to remove feed for up to 12 hours. This decreases the intestinal contents, and consequently reduces the risk of intestinal rupture during the in-factory evisceration process, thereby decreasing the probability of carcase contamination. Unfortunately, feed withdrawal introduces additional stress and is often associated with increased pecking of contaminated litter and coprophagy, resulting in an increased pathogen load in the intestine of the chickens (Corrier et al. 1999; Thompson and Applegate 2006).

A 'zero-thinning' policy in Iceland has had a positive effect on *Campylobacter* spp. reduction in broiler flocks, reducing the percentage of positive testing flocks from 40% to 15% per year (Strydom 2015). Whilst 'zero-thinning' is an option that is being considered by retailers, increasing pressure has been applied by industry partners to allocate additional payments to poultry farmers who 'zero-thin' to make up for lower performance due to reduced bird numbers.

Whilst on-farm biosecurity is essential to maintain a healthy flock and reduce disease transmission, even the most stringent biosecurity measures may not have sufficient, consistent, and predictable effects in controlling infection. This is not helped by the fact that assessment of the effectiveness of biosecurity measures in controlling flock prevalence is quite difficult under commercial settings in different production systems, and technique implementation can be cost prohibitive and hard to maintain (Newell et al. 2011; Dale et al. 2015; English et al. 2015). For example, a study conducted on Finnish poultry farms concluded that biosecurity costs approximately $\in 3.55$ per bird and added 8% to the total work time on broiler farms (Siekkinen et al. 2012). From a farmer's perspective, biosecurity interventions should be considered an investment, rather than an additional cost to production, that results in reduced numbers of *Campylobacter* spp.-positive flocks, and, potentially, an overall improvement in bird performance and quality.

Drinking water treatment

While Campylobacter spp.-typing studies showed no evidence that isolates from poultry house water supplies have caused colonisation in individual or sequential flocks (Zimmer et al. 2003; Hermans et al. 2011b; Jansen et al. 2014), the level of infection in poultry may be alleviated by treating drinking water. Various methods have been investigated, including chlorination, acidification, addition of monocarpic acid and probiotic treatments. However, the efficacy and consistency of such treatments varies across studies. Water chlorination (using 0.2-0.4 ppm free chlorine), combined with effective cleaning of the drinking system, was found to reduce the proportion of Campylobacter spp.-positive birds in a flock from 81% to 7% in early studies (Pearson et al. 1993). In addition, the treatment gave a 103 to 104-fold reduction in contamination levels in the carcase post slaughter. However, chlorination of flock drinking water (with 2-5 ppm chlorine) in line with commercial production practices in the United States did not result in reduction of Campylobacter spp. in poultry (Stern et al. 2002). This may be because, although this organism is sensitive to chlorine treatment when in free suspension, it appears to be more resistant to this treatment when in mixed populations with other organisms, such as protozoa (King et al. 1988; Snelling et al. 2005; Vieira et al. 2015). However, there are different forms of chlorine available, which should be taken into consideration.

The acidification of drinking water through the addition of organic acids has been reported to decrease the risk of colonisation in broiler flocks (see Table 1) (Allain et al. 2014; Jansen et al. 2014). The addition of lactic acid to drinking water during feed withdrawal has been shown to significantly reduce the incidence of Campylobacter spp. recovered from crop samples, with 62.3% of treated birds being colonised compared with 85.1% in the control groups (Byrd et al. 2001). In addition, it was found that permanent application of acidified drinking water did not negatively affect meat quality parameters or animal welfare. Whilst this treatment alone will not completely protect birds (Jansen et al. 2014), use of water acidification in combination with in-feed measures to further reduce the level of colonisation in poultry may be an option meriting further exploration.

Feed supplements

The use of various types of feed supplements to reduce the intestinal population and shedding rate of *Campylobacter* spp. in broilers is an area of growing interest in the poultry industry. Such supplements include pro- and pre-biotics, organic acids, bacteriocins and bacteriophage which may be added to bird feed and water (see Table 2). These will be further discussed below.

Table 2. Feed additives orally administered to reduce Campylobacter prevalence and transmission in poultry flocks.

Feed ingredients	Process conditions	Results	References
Probiotics	PoultryStar [®] sol	6-log reduction caecal colonisation by C. jejuni	(Gharib et al. 2012)
	Effective Microorganisms (EM)	No obvious effect of this product on the reduction of <i>Campylobacter</i> spp.	(Nuengjamnong and Luangtongkum 2014)
	Saccharomyces boulardii Lactobacillus acidophilus and	<i>Campylobacter</i> colonisation was not significantly affected by yeast treatment.	(Line et al. 1998)
	Streptococcus faecium	A 70% reduction in the frequency of <i>C. jejuni</i> shedding in colonised chicks.	(Morishita et al. 1997)
	A combination of <i>Citrobacter diversus</i> 22, <i>Klebsiela pneumoniae</i> 23, and <i>Escherichia coli</i> 25 (CE 3)	<i>C. jejuni</i> was not detected in the caeca of birds receiving the prevention treatment, CE 3 with mannose, representing a 62% reduction in the colonisation rate.	(Schoeni and Wong 1994)
Prebiotic		A significant one-log reduction of C. <i>jejuni</i> in the faecal samples.	(Santini et al. 2010)
	0.2% mannanoligosaccharide	Reduction in caecal <i>Campylobacter</i> load in broiler chickens fed mannanoligosaccharide.	(Baurhoo et al. 2009)
	0.1% xylanase	The growth scores of <i>C. jejuni</i> from caecal samples of broiler chicks fed the xylanase-supplemented diet were significantly lower.	(Fernandez et al. 2000)
Organic acids	5.7% lactic acid and 0.7% acetic acid caprylic acid	The number of <i>Campylobacter</i> decreased 2–3 ¹⁰ log CFU 3 to 4 logs reduction in caecal <i>Campylobacter</i> counts in. chicks fed with 7% caprylic acid.	(Heres et al. 2004) (De Los Santos et al. 2008b)
	0.05% butyrate coated beads	Butyrate-coated micro-beads are unable to reduce C. <i>jejuni</i> caecal colonisation in 2-week-old broiler chicks.	(Van Deun et al. 2008)
	1% caproic, caprylic, or capric acid sodium salt	Medium-chain fatty acids (MCFA) caproic, caprylic, and capric acid was not capable of reducing caecal <i>Campylobacter</i> colonisation <i>in vivo</i> .	(Hermans et al. 2010)
	1% Lodestar [™] C8–10	The number of C. <i>jejuni</i> bacteria required to colonise 50% of inoculated broilers was estimated 200 times higher in broilers fed with supplemented feed (log(10)4.8 CFU) than in control broilers (log(10)2.5 CFU).	(Van Gerwe et al. 2010)
	1.5% formic acid and 0.1% sorbate 2% formic acid and 0.1% sorbate	Reduced the colonisation of <i>C. jejuni</i> significantly. Prevented <i>C. jejuni</i> colonisation in chickens.	(Skånseng et al. 2010)
Plant extracts	Oil of marigold taegetes, ginger root, jasmine, patchouli, and gardenia	Bactericidal activity (BA50) values ranging from 0.003 to 0.007.	(Friedman et al. 2002)
	The ethanolic extract from Eleutherine Americana	The level of the tested isolates decreased by 2 to 5 log-fold within 8 h at 4 MIC.	(Sirirak and Voravuthikunchai 2011)
	0.25% thymol	Reduced from $7.38 \pm 0.20 \log_{10}$ to $6.74 \pm 0.14 \log_{10}$.	(Arsi et al. 2014)
	1% carvacrol 2% thymol treatments	Reduced from 7.38 \pm 0.20log ₁₀ to 6.85 \pm 0.17log ₁₀ . Reduced from 7.38 \pm 0.20log ₁₀ to 6.88 \pm 0.17log ₁₀ .	
	a combination of both thymol and carvacrol at 0.5%	Reduced from $7.38 \pm 0.2009_{10}$ to $4.10 \pm 0.55\log_{10}$. Reduced from $6.07 \pm 0.17\log_{10}$ to $4.10 \pm 0.55\log_{10}$.	
	1 mM thymol	Counts of <i>C. jejuni</i> were 6.43 and 6.87 log ₁₀ CFU/ml lower than those of controls after 24 and 48 h, respectively.	(Epps et al. 2015)
Seafood by- products	0.5% dose of the medium molecular weight chitosan	A significant reduction of <i>Campylobacter</i> .	(Arambel et al. 2015)
Bacteriocins	OR-7 derived from fermenting <i>L.</i> salivarius NRRL B-30 514	Significantly reduced the numbers of C. jejuni organisms from $10^{6.2}$ to $10^{>8.3}$ CFU per g of caecal material.	(Stern et al. 2006)
	125 mg E-760 kg ⁻¹ feed Bacteriocin E 50–52	Reduced the counts of <i>Campylobacter</i> by more than 8 \log_{10} CFU. The treatment eliminated detectable (<10 ² /g) levels of <i>C. jejuni</i> while control birds were colonised with 10 ^{8.40 ± 0.47} CFU/g of caecal content.	(Line et al. 2008) (Svetoch et al. 2008)
	1×10^4 or 1×10^8 CFU of the <i>E. faecalis</i> MB 5259	Not significantly lower caecal <i>Campylobacter</i> MB 4185 counts compared with the control groups.	(Robyn et al. 2013)
Bacteriophages	Phage 71 Phage CP8 and CP34	Reduce C. <i>jejuni</i> colonisation by 1 log over the time span of 30 days. Resulted in <i>Campylobacter</i> counts falling between 0.5 and 5 log_{10}	(Wagenaar et al. 2005) (Carrillo et al. 2005)
	Phage CP220	CFU/g of caecal contents compared to untreated controls. Resulted in a significant reduction in mean caecal <i>Campylobacter</i>	(El-shibiny et al. 2009)
	Phage cocktail (phiCcolBB35, phiCcolBB37, phiCcolBB12)	counts, by 2.1 log CFU/g. Reduced the titre of both <i>C. coli</i> and <i>C. jejuni</i> in faeces by approximately 2 log ₁₀ CFU/g.	(Carvalho et al. 2010)

MIC: minimal inhibitory concentration

Probiotics

Probiotics have been defined by the World Health Organisation as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (Food and Agricultural Organization of the United Nations and World Health Organization 2001). Probiotics work on the basis of setting up effective competitive exclusion (CE) circumstances between microbial species in the birds' gastrointestinal tract, the focus being to colonise the gut with 'good' bacteria while preventing 'bad' bacteria from finding a niche in which to grow (Bratz et al. 2015; Stef et al. 2016; Thomrongsuwannakij et al. 2016). A potential mechanism is the production of antagonism between pathogenic and probiotic bacteria, for example, through the secretion of antibacterial substances, adhesion site and receptor occupancy and competing for essential nutrients (Bermudez-Brito et al. 2012; Bratz et al. 2015). CE has been reported to reduce *Salmonella* spp. colonisation in chickens and turkeys (Doyle and Erickson 2008; Milbradt et al. 2014).

There are currently three microbial groups that are authorised as animal feed additives in the EU: lactic acid bacteria (LAB; mainly *Enterococcus*, *Lactobacillus* and *Bifidobacterium* spp.), *Bacillus* spp., and yeasts of the genus Saccharomyces. The reported effects of probiotics on intestinal Campylobacter spp. load reduction is not consistent and various depending on both the probiotic used and the strains targeted. For example, the administration of Bifidobacterium longum PCB 133 in feed was found to reduce C. jejuni by approximately 1 log in the faeces of experimentally infected chickens (Santini et al. 2010). An additional study screened 116 bacterial species, and reported six Bacillus spp. that reduced C. jejuni counts by at least 1 to 2 log in vivo (Arsi et al. 2015). Several commercially available dietary treatments, such as PoultryStar®, Lactobutyrin BRC, Biotronic® Top3, Excential Alliin Plus, Excential Butycoat, Adimix® Precision, Anta®Phyt product and Campylostat have been shown to significantly decrease colonisation in live broilers after 14 days of treatment when compared to the control group (Guyard-Nicodème et al. 2016). It is important to note that the four dietary treatments giving a mean reduction over 1 log CFU/g (Biotronic[®] Top3, Adimix[®] Precision, Anta[®]Phyt high and Campylostat) presented variability in *Campylobacter* spp. counts (>1.5 log CFU/g).

While further research is required to generate sufficient data to conclusively affirm probiotics as an effective tool in the control of Campylobacter spp. during poultry rearing, these studies indicated that the use of probiotics in CE trials is promising. Probiotic interventions require the development of probiotic species and strain mixtures that both survive the rigours of feed processing and the host environment, such as low pH, salt and enzymatic stress and temperature fluctuation, and provide a benefit to either the host or end-consumer (Shokryazdan et al. 2017). From this, LAB-based interventions may be considered as strong probiotic agents for use against colonisation in broiler chickens. These bacteria form a large part of the natural host microflora within the same caecal niche as Campylobacter spp. LAB are frequently characterised as probiotic candidates, with a number of them afforded 'generally recognised as safe' (GRAS) status as food additives. LAB are associated with the production of a broad range of antimicrobial compounds such as bacterocins, for example, lacticin 1317 and nisin, which have been documented as having inhibitory effects upon the growth of Campylobacter spp. in both in vitro and in vivo studies (Lohans et al. 2015; Saint-Cyr et al. 2016). The use of LAB species within probiotic mixtures can increase the efficacy of the treatment against Campylobacter and other putative pathogens, while reducing negative impacts upon the host.

Prebiotics

Encouraging the growth of beneficial microbes through the provision of specific nutrients (prebiotics) is thought to give additional and synergistic benefits on the control of Campylobacter spp. within the broiler (Hermans et al. 2011b; Arsi et al. 2015). Several studies have highlighted the benefits of adding prebiotics and probiotics to poultry diets (Guyard-Nicodème et al. 2016), although results depend upon the particular probiotic strains and prebiotic used and the target example, Campylobacter spp. selected. For when Bifidobacterium longum PCB 133 was combined with the prebiotic galactooligosaccharide, no noticeable increase in efficacy against Campylobacter spp. was observed (Baffoni et al. 2012).

More recent research has focussed on the use of prebiotics as natural antibiotic growth promoter (AGP) replacements. Various mannanoligosaccharide products either used alone or in combination with other ingredients, for example, the probiotic GalliPro and prebiotic TechnoMos have been shown to serve as alternatives to the AGP Neoxyval (Abudabos et al. 2015). This is due to the enhancement of broiler performance through improvements in the bird's intestinal morphology, in addition to the establishment of microbial balance associated with the modulation of intestinal microflora and the inhibition of pathogens. These types of additives could contribute to a reduction in *Campylobacter* spp. colonisation, with probiotics having been already reported to prevent pathogenic bacteria such as *Clostridium perfringens* and *Salmonella* spp. from colonising the gut (Abudabos 2013).

Based on the research discussed (Table 2), addition of prebiotics to poultry feed generally results in a detectable decrease in *Campylobacter* spp. populations in the poultry gastrointestinal tract. This indicated that adding prebiotics as feed supplements can potentially limit *Campylobacter* spp. in the poultry gastrointestinal tract. However, it remains unclear whether these reductions in colonisation will result in reduction of human campylobacteriosis cases. Prebiotics may be a promising strategy for controlling *Campylobacter* spp. in poultry, but it remains to be determined whether consistent overall reduction can be achieved in commercial operations.

Bacteriocins

Bacteriocins (BCN) are a group of antimicrobial peptides produced by bacteria when present in intensely competitive niches (Cleveland et al. 2001). Considerable research effort has been invested to investigate the use of bacteriocins as a food additive for protection against pathogens (Lewus et al. 1991; Scannell et al. 2000, 2001; Deegan et al. 2006; Coelho et al. 2007; Xie et al. 2009; Zacharof and Lovitt 2012; Goyal et al. 2018; Kimura and Yokoyama 2019). As a prophylactic treatment for poultry, significant progress has been made in the development of potent anti-Campylobacter bacteriocins from commensal microbes isolated from the chicken intestinal tract (Lin 2009; Zommiti et al. 2016). Although some bacteriocins, such as defensins and cathelicidins produced by the avian host, have been shown to dramatically reduce Campylobacter spp. colonisation in poultry, practical application for on-farm control has not been satisfactorily evaluated, most likely due to the high production cost of bacteriocins (Hoang et al. 2011).

Organic acids

Organic acids (including lactic acid, citric acid, malic acid, acetic acid, peroxyacetic acid, fumaric acid, gluconic acid, levulinic acid, pyruvic acid, caproic acid, caprylic acid and capric acid) are designated by the USFDA as GRAS feed additives. The antimicrobial efficacy of organic acids have been studied for decades (Scannell et al. 1997; Tamblyn and Conner 1997; Castillo et al. 1998, 1999; Ellebracht et al. 1999; González-Fandos et al. 2009; Mani-Lopez et al. 2012; Mohan and Pohlman 2016), with a particular emphasis on carcase decontamination. It is generally thought that the principal mechanism of action of organic acids occurs as a result of cytoplasmic acidification followed by the uncoupling of energy production and regulation, and through the toxic accumulation of dissociated acid anions within the cell. Organic acids have been tested as additives in drinking water and feed for Campylobacter and Salmonella spp. reduction in chickens, based on the assumption that ingested

organic acids lower the pH of the chicken gut, rendering this niche more hostile to colonisation (Byrd et al. 2001; Chaveerach et al. 2004; Hilmarsson et al. 2006; Van Immerseel et al. 2006; De Los Santos et al. 2008a, 2008b; Van Deun et al. 2008; Skånseng et al. 2010; Hermans et al. 2011a; Jansen et al. 2014).

Results from a comparative study on the effects of the commercial probiotic Primalac[®], organic acid Selko[®]-pH and plant extract Sangrovit[®] treatments found that, on day 49 of broiler life, all supplemented treatments showed a reduction in caecal content *Campylobacter* spp. colonisation. In addition, faecal samples showed reductions on days 35 and 42, which was in line with the age of slaughter for most mass-produced broilers (Gharib et al. 2012).

Although in vitro studies have demonstrated that organic acids, medium chain fatty acids (MCFA) or MCFA monoglycerides have strong bactericidal effects on Campylobacter spp., inconsistent results have been reported from in vivo trials. For example, caprylic acid in feed lowered the C. jejuni load in chicken caeca, and reduced carcase contamination during slaughter (De Los Santos et al. 2008a, 2008b). Conversely, the use of butyrate, acetate, propionate and l-lactate did not protect broilers from caecal colonisation, despite the marked bactericidal effect of butyrate in vitro (Van Deun et al. 2008). This was possibly due to the protective effect of mucus and the rapid absorption of butyrate by enterocytes. However, butyrate was able to protect Caco-2 cells from Campylobacter spp. invasion and translocation, two major virulence mechanisms, but not from a decline in transepithelial resistance (Van Deun et al. 2008). In addition, the use of a probiotic preparation containing Pediococcus acidilactici and Saccharomyces boulardii followed by the addition of acidifiers, such as formic and lactic acids, resulted in a significant reduction in Campylobacter spp. shedding and re-isolation, as well as reducing lesions (Abd EI-Ghany et al., Abd et al. 2015). These studies highlighted the potential of using the 'hurdle effect' to manage sequential supplementation of poultry flocks to prevent colonisation and transmission of Campylobacter spp. in poultry flocks.

Plant and marine extracts

A wide range of plant extracts and compounds have demonstrated strong bactericidal activity against *Campylobacter* spp. *in vitro* (Friedman et al. 2002; Sirirak and Voravuthikunchai 2011; Hermans et al. 2012; Robyn et al. 2013); however, the effectiveness of treatments varies considerably.

Natural plant extracts have been shown to have effects against other enteric pathogens. Thymol and carvacrol are phenolic plant extracts that act by altering cell membrane permeability, resulting in cellular content leakage and cell death (Lambert et al. 2001; Ultee et al. 2002). Chitosan is a natural byproduct derived from the deacetylation of chitin that can be obtained from crab and shrimp shell waste (Younes and Rinaudo 2015). Although the precise mode of action of chitosan is not completely understood, it is hypothesised that it may alter the permeability of the outer cell membrane of bacterial pathogens, disrupting cellular physiology and causing cell death. C. jejuni and C. coli have been reported as being sensitive to chitosan in vitro (Ganan et al. 2009; Mengíbar et al. 2011). In vivo, a 0.5% dose of chitosan was found to reduce caecal Campylobacter spp. counts in broiler chickens. In addition, RTqPCR analysis revealed that chitosan down-regulated the

expression of chicken colonisation genes when compared to a control (Arambel et al. 2015). This suggested that chitosan supplementation could be a potential strategy to reduce the enteric colonisation of *Campylobacter* spp. in pre-harvest chickens.

Bacteriophage

Bacteriophages can be applied as both pre- and post-harvest interventions to reduce the transmission of specific foodborne pathogens (Teng-hern et al. 2014). Bacteriophages invade bacterial cells and interfere with bacterial metabolism, causing bacterial lysis. Carrillo et al. (2005) were the first to perform bacteriophage treatment in chickens and discovered an effective reduction of Campylobacter spp. counts in the caecal contents of treated broiler chickens using pre-harvest interventions. The use of a combination of phages can provide a greater decrease in Campylobacter spp. levels in the caecal contents of infected broiler chickens than a singlephage approach (Wagenaar et al. 2005; Fischer et al. 2013). Phage type has a variable effect on Campylobacter spp. control as well. Wagenaar et al. (2005) found that phage 71 can reduce C. jejuni colonisation by 1 log over the period of 30 days while the addition of phage 69 resulted in a reduction of 1.5 log in CFU counts over the same period. These observations were in agreement with other studies that showed the colonisation of both C. jejuni and C. coli in chickens was successfully reduced upon the exposure to species-specific bacteriophages (see Table 2) (El-shibiny et al. 2009; Carvalho et al. 2010). Several studies on post-harvest interventions using bacteriophages against Campylobacter spp. were conducted to control pathogen contamination on food surfaces, such as chicken skin (Atterbury et al. 2003; Goode et al. 2003; Endersen et al. 2014). Although studies showed a small decrease in Campylobacter spp. levels on skin with postharvest phage treatment, the reduction was greater when freezing at -20°C was used in conjunction with the bacteriophage treatment (Atterbury et al. 2003).

Summary of in-feed treatments

It is always a difficult task to draw definitive conclusions from experimental research spanning different research eras, taking into account differences in experimental design and variations between trials within and between research groups. However, from the literature, it is clear that a distinct, albeit not unexpected, difference between the performance of in-feed interventions exists when examined in vitro compared to those determined from in vivo studies. This would explain why the global poultry industry is finding it difficult to establish consensus on a single acceptable feed additive that will obtain significant reductions in commercial settings. It is much more likely that pooling some of the discussed approaches within an in-feed tool kit will provide an answer. As it stands, there have been some positive results published, such as the combination of selected probiotics, plant extracts and organic acid products. In one study, Campylobacter spp. counts were not reduced by any fructooligosaccharide treatments (0.125%, 0.25%, or 0.5%) or mannanoligosaccharide (MOS) concentrations (0.04%, 0.08%, or 0.16%), but were reduced by the combination of Lactobacillus salivarius subsp. salicinius with 0.04% MOS in feed (Arsi et al. 2015). The results of this study indicated that selection and application of bacterial isolates in combination with selected prebiotics can reduce the carriage of caecal *Campylobacter* spp. in pre-harvest broilers (Arsi et al. 2015). Both bodyweight and feed intake in the probiotic-treated group were higher than in the control, while the villus height of the duodenum and jejunum in the probiotic and plant extract-treated groups were improved. It was concluded that the supplementation of organic acids in drinking water and the addition of probiotics and plant extract to broiler feed may reduce the incidence of *Campylobacter* spp. colonisation (Gharib et al. 2012).

Transportation to factory

At time of harvest, birds must be caught and transported to the poultry processing plant in crates. High stress during the catching process can cause defecation, increasing faecal contamination of adjacent birds and surfaces. Feed withdrawal is practised to minimise the amount of faeces produced and consequently prevent spread, but significant opportunity still exists for *Campylobacter* spp. spread within a flock. This may increase the microbial load of birds entering the slaughterhouse (Slader et al. 2002; Hastings et al. 2011; Newell et al. 2011). However, flocks that are contaminated immediately prior to slaughter tend to exhibit a lower level of carcase contamination than birds that had been colonised on farm, presumably because contamination is restricted to feathers (Hansson et al. 2007).

The design of the transport crate may facilitate the spread of faeces between birds, and the recycling of contaminated crates between the processing plant and the rearing farms poses a risk of Campylobacter spp. transmission, especially if depopulation takes place over an extended period. Over a twoyear period, a study by Hastings et al. (2011) indicated that 23.5% of empty crates used during thinning were contaminated (Allen et al. 2008; Hastings et al. 2011). Campylobacter can survive on transport cages in dried faeces at ambient temperatures (18-31°C) for up to 8 hours, with bacterial levels only being reduced by half after 24 hours (Berrang et al. 2004). Both transport vehicles and crates should be washed and disinfected after every journey in order to reduce crosscontamination between Campylobacter spp.-positive and negative flocks, and decrease the amount of contamination introduced into the slaughter facility (Allen et al. 2008). However, a number of studies have shown that many crates remain contaminated with Campylobacter spp. after cleaning, as they are very difficult to effectively sanitise (Corry et al. 2002; Hansson et al. 2005; Hastings et al. 2011).

Conclusions

The burden of *Campylobacter* spp. within the developed world is a constant pressure on health services, especially with emerging trends of frequent hospitalisations and the development of drug resistance in humans. The on-farm and in-transport interventions discussed could be vital interventions in the reduction of the incidence of contamination within the food-production chain. The role of the farm environment as the primary source of infection highlights its key role as a control point for the pathogen. Interventions such as feed and water-additives, vaccines and, most importantly, consistent biosecurity measures, exhibit potential for the effective control of this pathogen and its dissemination within the food chain. Furthermore, the combination of various dietary approaches and improved biosecurity measures may improve *Campylobacter* spp. transmission synergistically.

However, it can be hard to assess the impact of these interventions on a global scale. When analysing the literature, it is often difficult to obtain an accurate comparison of treatment effects between studies, due to differences that exist in experimental design, sampling protocols and target microbes. For example, some studies use naturally contaminated chicken while others artificially inoculate chickens with the pathogen of interest. In addition, the parts of the chicken (i.e. fillets, wings, whole carcases) used in the experiments vary from study to study. This can make it difficult to fully ascertain the effect of these proposed interventions on Campylobacter spp. transmission in retail poultry meat. The issue of cost of implementation must be taken into account, both in terms of loss of earnings and expenditure on the application of the discussed steps. This may be displeasing to producers, who may not want to take on the burden of the implementation of these processes. This may be remedied through provision of incentives, although the burden of cost may fall to the government, retailers or, ultimately, consumers.

It must be acknowledged that the multifaceted avenues of contamination that poultry meat is exposed to prior to consumption, such as the slaughter process and food handling, contribute to it being such a prevalent cause of disease beyond the on-farm causes discussed above (Lu et al. 2019). The evisceration and plucking steps of poultry carcase preparation are known critical control points, in which high levels of microbe transfer between contaminated and 'clean' carcases occur. Manual manipulation and poor food hygiene during preparation of chicken and adjacent foodstuffs (such as salad) contribute greatly to the spread of Campylobacter spp. among meals and preparation surfaces. These steps cannot be controlled by the aforementioned on-farm interventions. Thus, it is vital that in-factory interventions and high standards of food safety are implemented beyond the poultry husbandry steps discussed in this review. Campylobacter spp. contamination is a constant public safety issue, and a farm-to-fork approach is necessary to ensure that this prolific pathogen is managed.

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ORCID

T. Lu (b) http://orcid.org/0000-0003-1353-4960

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