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Thermotolerant *Campylobacter* during Broiler Rearing: Risk Factors and Intervention

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Abstract: Thermotolerant *Campylobacters* are one of the most important bacterial causative agents of human gastrointestinal illness worldwide. In most European Union (EU) member states human campylobacteriosis is mainly caused by infection with *Campylobacter jejuni* or *Campylobacter coli* following consumption or inadequate handling of *Campylobacter*-contaminated poultry meat. To date, no effective strategy to control *Campylobacter* colonization of broilers during rearing is available. In this review, we describe the public health problem posed by *Campylobacter* presence in broilers and list and critically review all currently known measures that have been researched to lower the numbers of *Campylobacter* bacteria in broilers during rearing. We also discuss the most promising measures and which measures should be investigated further. We end this review by elaborating on readily usable measures to lower *Campylobacter* introduction and *Campylobacter* numbers in a broiler flock.

Keywords: broiler, *Campylobacter*, intervention measures, risk factors

Introduction

Since 2005, thermotolerant *Campylobacter* infections, caused particularly by *Campylobacter jejuni* and *Campylobacter coli*, have become the most important cause of human bacterial gastroenteritis in many developed countries (EFSA 2010b, 2011, 2013). Worldwide, the broiler cecum has been demonstrated to be colonized to a high degree by *Campylobacter* species like *C. jejuni* (EFSA 2010c). Therefore, broiler chickens may serve as a potential reservoir for *Campylobacter* strains pathogenic to humans (Altekruse and others 1999; Fields and Swerdlow 1999; Friis and others 2010). In 2009, 90% of the human campylobacteriosis cases were caused by *C. jejuni*. *C. coli*, *Campylobacter lari*, and *Campylobacter upsaliensis* accounted for 2.5%, 0.2%, and 0.01% of the cases, respectively (EFSA and ECDC 2011). The remainder of the speciated isolates included other (unknown) species (EFSA and ECDC 2011). High intestinal colonization with *Campylobacter* during rearing has been shown to lead to carcass contamination during slaughtering (Herman and others 2003; Rasschaert and others 2006; Rosenquist and others 2006). Subsequently, handling and consumption of contaminated broiler meat constitutes a major risk factor for human *C. jejuni* infection (Friedman and others 2004). Lowering cecal colonization or carcass contamination has been shown in risk assessment studies to lead to a reduction of human campylobacteriosis cases (Messens and others 2007). Because the intestine of living poultry is the only niche where amplification of *C. jejuni* can occur throughout the food chain, control of *C. jejuni* colonization or shedding by broilers, and subsequently external *C. jejuni* contamination of broilers during rearing, would have a great impact on human campylobacteriosis incidence, as less *C. jejuni* will reach consumers.

In the European Union (EU), *Campylobacter* was the most commonly reported gastrointestinal bacterial pathogen from 2005 to 2009 (EFSA 2011). This results in 43.9, 45.6, and 50.3 cases per 100000 inhabitants or 190.566, 198.582, and 220.209 isolated cultures of *Campylobacter* from humans in the EU in 2008, 2009, and 2011, respectively (EFSA 2011, 2013). Because most cases of *Campylobacter*-caused enteritis are not reported, Havelaar and others (2012) made an estimate of the probable campylobacteriosis incidence for the 27 EU member countries and stated that only 1/47 cases were reported.

Gellynck and others (2008) calculated the costs of campylobacteriosis and its sequelae in Belgium and estimated them at 27 million euros per year. Extrapolation of these costs for the EU member states resulted in a total cost between 500 and 5000 million euros per year (EFSA 2011).

The Genus Campylobacter

In 1947, human infections were clearly associated with microaerophilic vibrios (*Vibrio fetus*) for the first time. These vibrios caused the death of a human fetus during pregnancy (reviewed by Vinzent and others 1947; Moore and Matsuda 2002). In 1957, King isolated 2 distinctly different types of vibrio organisms from blood cultures of patients. While the first type of the organisms was designated as *Vibrio fetus*, the second type was different. All organisms of this second type were isolated from patients with gastrointestinal disease and had a much higher optimal growth temperature (42 °C). These "related vibrios" were designated as the causative agents of the gastroenteritis (King 1957, 1962).

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The genus *Campylobacter* was proposed for the first time by Sebald and Véron in 1963 to distinguish certain bacteria from *Vibrio* spp. (Sebald and Véron 1963; Véron and Chatelain 1973; On 2001; Silva and others 2011). This distinction was based on their microaerobic growth requirements, their different G+C base content, and their nonfermentative metabolism. Finally, Dekeyser and others (1972) developed isolation procedures for thermophilic *Campylobacter* spp., followed by Skirrow (1977) who developed a more direct isolation technique. This technique was used for the isolation of campylobacters from human diarrheal stool samples. Using this technique, it became fully apparent that *Campylobacter* was one of the most important causative agents for acute human gastroenteritis (Skirrow 1991).

Currently, the family Campylobacteraceae consists of 3 genera: *Campylobacter, Sulfurospirillum,* and *Arcobacter* (Vandamme 2000, 2001; Vandamme and others 2005). The genus *Campylobacter* (Greek for curved rod) bacteria are small Gram-negative cells (0.2 to 0.8 μm × 0.5 to 5 μm) of curved, spiral, or S-shaped structure (Sebald and Véron 1963; Penner 1988). The genus *Campylobacter* has been in a state of flux, with new species defined at a rather rapid pace, and is reported to include currently 32 species with 6 species further divided into 13 subspecies (http://www.bacterio.net/ consulted on 06/23/2014). They typically have a low G+C content, ranging from 28 to 38 mol% (Véron and Chatelain 1973; Smibert 1984) and form an "S" or a "V" shape, when 2 or more bacterial cells are grouped together (Silva and others 2011). Generally, they have a single polar unsheathed flagellum (monotrichous) or a flagellum at each end (amphitrichous) of the cell, allowing a corkscrew-like movement (Penner 1988; Silva and others 2011).

C. jejuni, *C. coli*, *C. lari,* and some strains of *C. upsaliensis* have been referred to as thermophilic, meaning these species have an optimal growth temperature of 41.5 °C, which is the internal temperature of the chicken, and are able of growing at temperatures between 30 and 45 °C. Due to the absence of genes encoding for cold-shock proteins important in adaptation to low temperatures, in general they are incapable of growth below 30 °C (Silva and others 2011). There are *Campylobacter* species that have lower optimal growth temperatures (Penner 1988). Most species, albeit with some exceptions, grow best under atmospheric conditions with low oxygen tension (5% O_2, 10% CO_2, and 85% N_2), making them microaerophilic (Neill and others 1985; Penner 1988; Garénaux and others 2008). Due to these strict growth requirements *Campylobacter* spp. are unable of multiplying outside their animal host or in food products during processing or storage (Park 2002; Humphrey and others 2007).

However, *C. jejuni* can be present as a so-called viable-but-nonculturable (VBNC) form. The VBNC state has still not been fully elucidated; some authors consider this state a degenerating form (Medema and others 1992), whereas others authors claim that the VBNC state is a dormant state and that the organism is able to grow again under favorable conditions (Stern and others 1994).

Clinical Aspects of *Campylobacter*-Induced Disease in Humans

Human *Campylobacter* infection typically results in a form of gastroenteritis/enterocolitis, termed campylobacteriosis, characterized by watery, sometimes bloody diarrhea, fever, nausea, and abdominal cramps 1 to 5 d after initial infection (Ketley 1997; Allos 2001; Butzler 2004; Zilbauer and others 2008). Human infections are mainly caused by 2 species, *C. jejuni* and *C. coli* (Lastovica and Skirrow 2000; Galanis 2007; EFSA 2011), and can be caused by relatively low infective doses (<1000 cells). In developed countries, *C. jejuni* and *C. coli* cause, respectively, 89% to 96% and 4% to 11% of campylobacteriosis cases (Nachamkin and others 2000; Anonymous 2001a,b; Rosenquist and others 2003; Galanis 2007; EFSA 2010d, 2011). There are, however, regional or country differences as Uzunović-Kamberović and others (2007) demonstrated a higher prevalence of *C. coli* in sporadic human infections in Bosnia and Herzegovina (30%). Experimental infection studies with *C. jejuni* in humans demonstrated that oral inoculation doses as low as 500 to 800 cells were capable of causing illness (Robinson 1981; Black and others 1988). *Campylobacter*-mediated disease, in general, is self-limiting and normally depends on the virulence properties of the infecting strain and the immune status of the human host, and it lasts around 7 d (Zilbauer and others 2008).

Campylobacter infections can lead to severe chronic sequelae. Respectively, 1% and 0.1% of infections lead to reactive arthritis and Guillain-Barré syndrome (GBS) and the mortality rate is 5 in 10000 (Nachamkin and Blaser 2000; Nachamkin 2002; Butzler 2004; Galanis 2007; Zilbauer and others 2008). *C. jejuni* infection has also been linked with irritable bowel syndrome (Grover and others 2014). Finally, 1% of *Campylobacter* enteritis patients can develop bacteremia (Mead and others 1999).

C. *Jejuni* in Poultry and Poultry Meat: A Source for Human Campylobacteriosis

C. jejuni is considered to be a commensal organism establishing persistent, but benign, colonization in all types of poultry (broilers, layers, turkeys, ducks, fowl, quail, and ostriches) as well as wild birds (Stephens and others 1998; Newell and Wagenaar 2000; Sahin and others 2002; Waldenström and others 2002; Newell and Fearnley 2003; Dhillon and others 2006). *C. jejuni* is mainly found in the cecal and cloacal crypts of broiler chickens, in close association with the mucus layer (Beery and others 1988; Achen and others 1998; Meade and others 2009). Different reasons might be responsible for the intestinal colonization of broilers by *C. jejuni*. The growth requirements (optimal growth temperature of around 41.5 °C and a microaerophilic nature) of *C. jejuni* might be a reflection of the restricted ecological site the avian gut represents. This avian gut is characterized by limited oxygen levels and an internal temperature of 41 °C, which is higher than that of mammals (Park 2002) and constitutes an ideal environment for *C. jejuni* growth. Furthermore, *C. jejuni* is preferentially attracted to mucus-filled crypts in the ceca, due to chemotaxis toward cysteine and fucose components of mucins (Beery and others 1988; Hughdahl and others 1988; Baserisalehi and Bahador 2011). *C. jejuni* has also developed mechanisms to avoid mucus-clearing by undergoing several cycles of adherence, invasion, and escape from the epithelial cell layer in ceca, followed by fast replication in mucus and re-invasion of the cell layer (Van Deun and others 2008b). Additionally, the chick immune system is inefficiently activated upon *C. jejuni* colonization and expression of several antimicrobial peptide genes is reduced (Meade and others 2009; Hermans and others 2012b). All the aforementioned reasons contribute to the persistent colonization of *C. jejuni* in the avian gut.

Although *C. jejuni* in most cases cannot be isolated from broilers less than 2 to 3 weeks of age (Stern and others 1988; Newell and Wagenaar 2000; Sahin and others 2002), once 1 or a few broilers in a flock become colonized and act as "seeders," infection rapidly spreads throughout the entire flock. The rapid spread is due to (1) coprophagy by broilers combined with high fecal *C. jejuni* shedding by colonized broilers and (2) other transmission routes, like contaminated drinking water and litter

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(Berndtson and others 1996b; Herman and others 2003; Lee and Newell 2006). Once colonization is established, broilers can carry high cecal *C. jejuni* loads up to between 10^6 and 10^8 colony forming units (CFU)/g feces (Beery and others 1988; Meade and others 2009). Worldwide, 60% to 80% of all broiler flocks are colonized with *Campylobacter* spp. at slaughter age. The mean EU *Campylobacter* prevalence in broiler flocks was 71.2% in 2008. *C. jejuni* was responsible for an average of 41% of flocks colonized in the EU, while *C. coli* colonized an average of 31.9% of flocks (Herman and others 2003; Rasschaert and others 2006; Reich and others 2008; EFSA 2010a,b).

Especially chicken meat has been indicated as a primary route for human *C. jejuni* infection (Altekruse and others 1999; Corry and Attabay 2001; Park 2002; Mullner and others 2009). In the United States, it has been estimated that around 80% of *Campylobacter* spp. infections were foodborne in 1999 (Mead and others 1999). In Canada and Belgium, respectively, about 50% and ≥ 40% of human campylobacteriosis cases were associated with the handling and consumption of poultry or by cross-contamination during food preparation (Vellinga and Van Loock 2002; Galanis 2007). Research into the dioxin crisis in Belgium, which started in June 1999, demonstrated that, due to the withdrawal of poultry and eggs, a sudden change in food consumption had an immediate effect on the number of campylobacteriosis cases (Vellinga and Van Loock 2002). The number decreased by 40%, although only Belgian chicken meat was banned during this period and non-Belgian poultry was still on sale, indicating a poultry-related campylobacteriosis incidence above 40% (Vellinga and Van Loock 2002). Other indications implicating poultry meat in *C. jejuni* infections include a novel population genetics approach comparing *Campylobacter* DNA from patients to *Campylobacter* DNA found in livestock, wild animals, and the environment. This approach indicated chicken meat as a major source of *C. jejuni* pathogenic to humans (Wilson and others 2008). Havelaar and others (2012) found a significant correlation between the campylobacteriosis incidence rate per EU country and the prevalence of *Campylobacter* spp. on broiler chicken carcasses. Countries with high prevalence of *Campylobacter* spp. on broiler carcasses have a high risk of human campylobacteriosis.

For 26 EU member states and 2 non-EU member states, a *Campylobacter* baseline survey was conducted in 2008 on broiler carcasses, demonstrating the mean EU prevalence was 75.8% (EFSA 2011). The prevalence in the EU member states ranged from 4.9% in Estonia to 100% in Luxembourg (EFSA 2011). In the same baseline survey, enumeration of *Campylobacter* on carcasses (neck and breast skin) was also performed to estimate the distribution of *Campylobacter* counts. Respectively 46.6%, 12.5%, 19.3%, 15.8%, and 5.8% of broiler carcasses contained *Campylobacter* numbers (CFU/g) below 10, between 10 and 99, between 100 and 999, between 1000 and 10000, and above 10000. In 2009 it was demonstrated that the proportion of positive broiler meat samples at processing in the EU member states ranged from 9.0% in Belgium to 70.7% in Spain, whereas at retail, the proportion of positive broiler meat samples varied from 10.8% in The Netherlands to more than 75.0% to 79.8% in the Czech Republic, France, Slovenia, and Luxembourg. Harmonized sampling schemes were used in all the EU member states (EFSA 2011).

From 2008 to 2011, trained staff performed sampling as part of a monitoring program in Belgian slaughterhouses, meat-cutting plants, processing plants, and retail stores. This sampling indicated that 32% to 39% of carcasses at slaughter, 17% to 25% of carcasses at retail, and 7% to 14% of meat cuts were *Campylobacter*-contaminated (FASFC 2011, 2012). Habib and others (2008) reported that 48% of Belgian chicken meat preparation samples were *Campylobacter* contaminated with an average count of 1.7 ± 0.6 log CFU g^{-1}.

According to a quantitative risk assessment model (QRAM) performed by Messens and others (2007), lowering the *Campylobacter* shedding on the farm by 1, 2, or 3 log units could, respectively, result in a 55%, 84%, or 94% reduction in the number of campylobacteriosis cases and would be more efficient than trying to reduce the overall flock prevalence. The QRAM is based on the model developed by Hartnett (2001) and describes the chain from farm-to-fork in a modular fashion. At each stage the model estimates the probability that a bird/carcass/product is colonized/contaminated with *Campylobacter* and the associated microbial levels. Rosenquist and others (2003), in turn, stated that lowering the number of *C. jejuni* on the chicken carcasses by 2 log could reduce the incidence of campylobacteriosis associated with consumption of chicken meals 30 times. This demonstrates that an efficient *C. jejuni* risk management can also include methods to reduce the number of *C. jejuni* on carcasses between slaughter and retail, next to intervention at the farm level. In a risk assessment, Rosenquist and others (2003) obtained a similar reduction in campylobacteriosis incidence when flock prevalence was reduced 30 times. Establishing "strict hygienic barriers" or "biosecurity zones" at poultry houses seem to be the only preventive options shown to work in practice to reduce flock prevalence (Humphrey and others 1993; Kapperud and other 1993; Berndtson and others 1996a; Reiersen and others 2003). Rosenquist and others (2003) also obtained a similar reduction (30 times) in campylobacteriosis incidence when kitchen hygiene was improved, and thus cross-contamination during food handling. This magnitude of kitchen hygiene improvement will be difficult to attain, as it is not yet clear which specific measures will result in such an improvement. Some measures that can influence cross-contamination during food handling include hand-washing and cleaning of cutting boards (Rosenquist and others 2003). Reiersen and others (2003) described a decrease in human campylobacteriosis cases from 157/100000 inhabitants in 1999 to 75.4/100000 inhabitants in 2001 in Iceland due to the implementation of an intervention program that consisted of an educational program for farmers, an extensive surveillance program for *Campylobacter* in poultry, freezing all known *Campylobacter*-positive broiler flocks before they went to retail, and extensive consumer education. However, this decrease could not be linked to any specific intervention/mitigation implemented.

Risk Factors for *Campylobacter* Infection and External Contamination Of Broilers and Broiler Carcasses

Evaluation of the role of hatchery-mediated (vertical) transmission of *Campylobacter* in broiler flocks

In order to implement a control program for *C. jejuni* that is both successful and cost-effective, the relative contribution of risk factors in the entire poultry meat production chain must be known. Multiple strains of *C. jejuni* can be recovered from various segments of the reproductive tract of breeder hens, including the oviduct (Camarda and others 2000; Buhr and others 2002), up to an age of at least 42 weeks (Lindblom and others 1986). *C. jejuni* can also be isolated from the semen of roosters (Cox and others 2002b). This signifies that the first possible path for introduction of *C. jejuni* into a broiler flock is by a route going from the breeder hen through the egg to the chick. This transmission route was designated vertical transmission by Newel and others (2003). As

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will be made clear, different studies have refuted the importance of this transmission route for *C. jejuni* introduction into broilers.

If natural infection of egg contents occurs, it probably is primarily a consequence of fecal contamination of the egg shell surface and penetration via egg shell and egg shell cracks, as intact egg shells appear to be permeable to *C. jejuni* (Clark and Bueschkens 1985; Sahin and others 2003). Given that the eggs are infected, the question remains whether this generates colonized chicks and *Campylobacter*-positive flocks (King and others 1993). Experimental egg-penetration studies indicated *C. jejuni* transmission via the egg will most probably be very rare, as surface-challenged eggs gave *Campylobacter*-free progeny (Shanker and others 1986) and *Campylobacter* could be recovered only once in 70 homogenized egg contents (Doyle 1984; Shane and others 1986). *Campylobacter* is not detected in day-of-hatch chicks or in eggs using routine culture methodologies (Petersen and others 2001b; Herman and others 2003; Sahin and others 2003). Furthermore, in a field survey of laying farms, it was shown that hens fecally shedding *C. jejuni* did not produce infected eggs (Shane and others 1986). Different studies (Jacobs-Reitsma 1995; Callicott and others 2006; O'Mahony and others 2011; Patriarchi and others 2011) demonstrated that *Campylobacter* strains isolated from breeder flocks belonged to different genotypes than those isolated from their progeny.

In contrast, some studies identified vertical transmission as an important source for introduction of *Campylobacter* in broiler flocks (Pearson and others 1996; Cox and others 2002a). Cox and others (2002a) performed ribotyping and *flaA* SVR sequencing on isolates from broiler breeder flocks and from corresponding progeny broiler flocks and suggested the isolates were of clonal origin. Although breeders and progeny were housed only 60 miles apart, the potential for widely distributed *Campylobacter* clones as an alternative explanation was not considered in that study.

In conclusion, even if vertical transmission occurs, it is not a significant source for the contamination of chicken flocks with *Campylobacter*.

Evaluation of the risk factors for introduction of *Campylobacter* in broiler flocks by horizontal transmission during rearing

Different studies have been carried out to identify common risk factors for introduction of *Campylobacter* in broiler flocks during rearing by horizontal transmission. In the following sections, A to H, different risk factors that have been examined in these studies will be discussed. These risk factors are the most common ones identified or investigated and include litter, contaminated drinking water, insufficient cleaning and disinfection between flocks, short empty periods (< 14 d), poor house maintenance, poor hygiene barriers, inadequate staff compliance, flock thinning, infestation with insects and rodents, improper design and type of ventilation shafts, close location to other poultry sites or farm animals, and seasonal effects (Jacobs-Reitsma and others 1995; Herman and others 2003; Newell and Fearnley 2003; Bouwknegt and others 2004; Cardinale and others 2004; Barrios and others 2006; Johnsen and others 2006; Workman and others 2008; Rosenquist and others 2009).

Litter

Implication of used litter as a risk factor for *Campylobacter* introduction in broiler flocks has been investigated in on-farm studies as well as in controlled experimental studies. In a 1-y study by Berndtson and others (1996b) on 18 chicken farms, fresh litter was not identified as a risk factor for *Campylobacter* introduction in broilers. These authors also demonstrated that the *Campylobacter* status of broilers was not influenced by the use of straw or wood shavings as litter, litter bed thickness, litter storage time, or time passing between input of litter to input of day-old chickens. Payne and others (1999) established that broilers raised in a controlled environment on previously used litter, removed from a broiler house positive for *C. jejuni*, were negative for *C. jejuni*. Jacobs-Reitsma and others (1995) and Zweifel and others (2008), in their turn, could not isolate *Campylobacter* spp. from used litter samples taken from positive broiler houses.

Although fresh litter or dry used litter is probably not implicated in the introduction of *Campylobacter* in flocks, it plays a role in the spread of *Campylobacter* throughout the flock and in maintaining a persistent *Campylobacter* colonization. This might especially be true when litter is wet, as wet litter might function to keep the organism viable for colonization as *Campylobacter* is sensitive to dry environments (Smitherman and others 1984; Berndtson and others 1996b). Berndtson and others (1996b) demonstrated that 35% of wet litter beds and 18% of dry litter beds were found positive for *Campylobacter* during a 1-y epidemiological study in Sweden. Willis and others (2002) conducted a 1-y experiment with broiler chickens to assess the influence of cage and floor rearing environments on the isolation trends of *C. jejuni*. They found that the yearly average percentage isolation rates were significantly higher for the broilers held in the litter floor pen (65.4%) when compared to the broilers kept in wire cages (36.8%). Moreover, when the rearing period for broilers was increased, isolation rates for *C. jejuni* in caged birds decreased, but for birds housed on litter they stayed the same. This could be due to the presence of viable *Campylobacter* strains in litter capable of perpetuating colonization, which was supported by Shane (1991) and Montrose and others (1985). Results indicated that the survival of *C. jejuni* in litter prolonged periods of shedding by colonized birds up to 46 d. Berndtson and others (1996b) also established that flocks reared on wetter litter beds were found to be *Campylobacter*-positive more often than flocks raised on dry litter beds (odds ratio [OR] = 2.5). Thus, although fresh or used litter is not important in introducing *C. jejuni* into a broiler flock, it can be of importance in maintaining a constant *C. jejuni* colonization pressure and in spreading *C. jejuni* in a flock, once *C. jejuni* has been introduced, especially when litter is wet.

Drinking water

Although *C. jejuni* has been known to survive well in water, detection of *C. jejuni* in broiler house water sources prior to flock introduction and positive flock status has proven to be difficult (van der Giesen and others 1998). Experimental evidence suggests that in water *C. jejuni* is primarily present under a VBNC, form (Pearson and others 1993), especially when part of a biofilm (Trachoo and others 2002).

The relationship between water source (well or surface water) and broiler flock *Campylobacter* status has been investigated in several epidemiological studies. In most studies the water source was identified as a low-risk factor (Humphrey and others 1993; Jacobs-Reitsma and others 1995; Berndtson and others 1996a) and, due to excretion of live organisms by the birds, water contamination with *C. jejuni* follows rather than precedes colonization of a flock (Lindblom and others 1986). Pearson and others (1993), on the other hand, identified the water supply as the predominant source of *C. jejuni* infection. This seems to be an exceptional case, as *C. jejuni* was present as a biofilm in the entire water supply. *C. jejuni* was found throughout the farm's water

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system from the bottom of the bore-hole to the biofilm in the pipework in the poultry house. *Campylobacter*-free chickens raised in an animal house and given water from the farm supply became colonized with the serotype of *C. jejuni* endemic on the farm.

Additionally, Näther and others (2009) established that the use of nipple drinkers with trays (42% positive versus 13% positive when using nipple drinkers without trays) increased the *Campylobacter* prevalence in broiler flocks by acting as a vector for spreading *Campylobacter*. In an epidemiological study, Herman and others (2003) also identified (drinking) water in the broiler house as a significant risk factor for contamination of broilers, and concluded that contaminated (drinking) water is probably an important risk factor for spreading *Campylobacter* to the other animals of the flock.

Overall, the evidence suggests that *C. jejuni*-contaminated water constitutes a risk, albeit a relatively low one, for introduction of *C. jejuni* colonization in broiler flocks. Nevertheless, results with sanitizing measures, using chlorinated water and acidification of water, suggest drinking water plays a much more important role in the spread of *C. jejuni* throughout a flock than in the introduction of *C. jejuni* into a flock. The use of chlorinated drinking water reduced the risk of *C. jejuni* colonization of broiler batches significantly ($P = 0.004$; Ellis-Iversen and others 2009). Chlorinated drinking water was also able to lower the proportion of birds colonized with *Campylobacter* from 81% to 7%, and was associated with a 1000- to 10000-fold reduction in *Campylobacter* recoverable from the carcasses (Pearson and others 1993).

Cleaning, disinfecting, and empty periods in broiler houses

Carryover of *C. jejuni* infection from a positive flock to a new flock in the same house might be an obvious potential risk factor. The normal cleaning and disinfection processes carried out in houses between production cycles are likely to eliminate carryover of infection from one flock to the next (Anonymous 2002), but longitudinal studies indicate that infection is not predictable from the *C. jejuni* status of the last flock in the house, as negative flocks can follow positive flocks (Berndtson and others 1996a; Evans and Sayers 2000) and *vice versa*. However, there was a higher risk of positive infection status if the test result of the farm for the previous flock was *Campylobacter*-positive (OR = 1.60) (Chowdhury and others 2012). Different studies, sampling 3 to 388 broiler flocks (McDowell and others 2008; O'Mahony and others 2011), have given indications that adequate cleaning and disinfection of broiler houses and the broiler house ante-room contribute to the prevention of carry-over and cross-contamination of *C. jejuni* to successive flocks. These indications were based on extensive sampling and flaA-SVR sub-typing and multilocus sequence typing. Katsma and others (2007) reported that reducing transmission between different flocks on the same premises has less effect than reducing transmission between consecutive cycles in the same house. The authors demonstrated in a comprehensive risk assessment that 10% less transmission between flocks led to 41% infected flocks, but 10% less transmission between cycles in 1 house led to 31% infected flocks.

However, some studies indicate that cleaning and disinfecting of broiler houses have no influence on *Campylobacter* status. Wedderkopp and others (2001) did not demonstrate significant effects on *C. jejuni* status of 8911 broiler flocks when intensive cleaning and disinfection procedures were carried out. In this study, all broiler flocks slaughtered in Denmark in the years 1998 and 1999 were included. Hiett and others (2002) demonstrated little effect on *C. jejuni* contamination of 16 flocks when limited cleaning and disinfection was carried out in 4 different rearing facilities.

Flock positivity has also been linked to the empty-periods time in a house (Hald and others 2000; Wedderkopp and others 2001). Hald and others (2000) and Berndtson and others (1996b) identified a down-period of less than 14 d (OR = 5.0) or less than 21 d (OR = 2.4), respectively, as a significant risk factor. Longer periods (over 14 d) between successive flocks might reduce residual bacterial contamination in or around a previously positive house, resulting in less positive flocks. It has to be noted that an empty-period of 14 to 21 d is not commercially feasible. Wedderkopp and others (2001) later contradicted these findings and concluded that transmission of *C. jejuni* within the same broiler house from a positive flock to successive flocks is epidemiologically insignificant in an all-in all-out broiler production system.

Though important, and not to be neglected, cleaning/disinfection of broiler houses is not the main factor influencing *Campylobacter* status of a flock as is evidenced by the fact that positive flocks can occur in newly constructed broiler houses (Gregory and others 1997). Adequate cleaning and disinfecting should always be accompanied by adequate management and biosecurity practices, as well as pest control.

Human activity

As the main traffic in and out of a broiler house is that of farm staff for the purpose of routine animal husbandry and flock removal or possible flock thinning prior to slaughter, it can be expected that human activity plays a role in introducing *C. jejuni* into broiler flocks (Berndtson and others 1996b; Evans and Sayers 2000; Cardinale and others 2004). Human activity can introduce *C. jejuni* into a flock from environmental sources, from other livestock, livestock houses, and from equipment used by thinning crews also working at other farms or slaughterhouses.

Campylobacter present in the environment can potentially be carried into the house via boots, clothes, and equipment of the farmer or farm staff or of external staff responsible for flock thinning and transport of broilers to the slaughterhouse (Newell and others 2011). *Campylobacter* has been isolated from more than 50% of transport crates (up to 70.6%), catchers' and driver's boots and truck wheels (Herman and others 2003; Ramabu and others 2004). Samples of boots, wheels, and crates were taken before external staff departed from the processing plant, prior to each depopulation trip, and just after washing (Ramabu and others 2004). During transport, and after washing, transport crates were found to be *Campylobacter*-positive when loaded with *Campylobacter*-negative broilers (Herman and others 2003). *Campylobacter* strains found on farmer's boots, in water puddles, and on broilers in neighboring farms have been shown to be implicated in flock colonization by using molecular-typing methods (Johnsen and others 2006; Ridley and others 2008). However, *Campylobacter* on boots is mostly detected after positive flock status (Hiett and others 2002). In a longitudinal study with restricted bird movement carried out in a broiler house, the first birds to be colonized were closest to unofficial doors used by staff members not applying strict biosecurity measures (Shreeve and others 2000). Staff members were therefore presumed to be directly implicated in introducing *Campylobacter* into broiler houses. However, no research into the presence of other sources of contamination, for instance rodents, was done and securing the unofficial doors did not prevent infection.

Risk of positive flock status increased with the number of staff members (OR = 3.1 when the number of staff members exceeded 2) (Refrégier-Petton and others 2001) and also increased when staff had been tending pigs (OR = 4.86, $P = 0.037$) and, especially, poultry (OR = 6.43, $P = 0.007$) prior to working in

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the broiler house (Kapperud and others 1993; Berndtson and others 1996b). Genotypes of *Campylobacter* strains found in puddles and soil before positive flock status were commonly identical to isolates from the flock when it became positive (Hiett and others 2002; Johnsen and others 2006). *Campylobacter* spp. survive well in water (Blaser and others 1980) and are frequently isolated from on-farm puddles (Herman and others 2003; Messens and others 2009). The surroundings of a broiler house can therefore pose a risk for possible introduction into a broiler flock. Cardinale and others (2004) reported that Senegalese farm surroundings which were poorly cleaned and disinfected yielded an increased risk for *Campylobacter* introduction into the broiler flocks (OR = 6.86).

Apart from farm staff performing routine tasks, the highest risk for introducing *Campylobacter* into poultry flocks is posed by partial depopulation/thinning procedures approximately 1 week prior to slaughter (EFSA 2010a). Thinning allows farmers to raise a higher number of broilers per square meter (Katsma and others 2007). Catching crews performing these thinning procedures routinely travel between different farms and/or the abattoir with their catching equipment, outfit, and crates. Crates, boots, clothes, hands, transport vehicles, and other vehicles of thinning personnel have been investigated and were found to be contaminated with *C. jejuni* originating from previous positive flocks, other farms, and the abattoir. This *C. jejuni* contamination was present due to inadequate washing procedures (Newell and others 2001; Hiett and others 2002; Slader and others 2002; Ridley and others 2011b). Several studies (Hald and others 2001; Arsenault and others 2007; Allen and others 2008b; Ridley and others 2011b) demonstrated that flocks became *Campylobacter*-positive after thinning and that the colonizing strain could be isolated from personnel or equipment, especially transport crates (Arsenault and others 2007; Ridley and others 2011b). A statistically significant risk associated with partial depopulation was identified in several risk factor assessments (OR = 6.8) (Hald and others 2001; Jacobs-Reitsma and others 2001). In Sweden, Berndtson and others (1996b) even demonstrated that when the partial depopulation staff performed their duties at several farms, the *Campylobacter* isolation rate of flocks was 30%. On the other hand, on farms where staff never loaded at other farms, the *Campylobacter* isolation rate of flocks was only 5%. Additionally, O'Mahony and others (2011) concluded that the stressful thinning process might trigger the release of noradrenaline, which stimulates the growth and motility of *C. jejuni in vitro* (Cogan and others 2007). This event might contribute to rapid growth of the bacterium in the avian gastrointestinal tract, leading to increased shedding of *Campylobacter* and the subsequent rapid spread of the bacteria in the broiler flock. It is safe to conclude that the activity of both thinning crew and farm workers increases the risk of introducing a *C. jejuni* strain into a broiler flock and that this risk increases even more when biosecurity measures are not strictly applied.

Presence of wild birds and rodents on the farm

Wild birds and rodents are an important part of the environmental surroundings of broiler farms which are mostly situated in rural surroundings. It has repeatedly been demonstrated that *C. jejuni* could be isolated from wild birds (Siembieda and others 2011; Waldenström and others 2002), especially crows, gulls (Keller and others 2011), and passerine birds, and from birds captured on livestock farms (Sippy and others 2012). Although on rare occasions *C. jejuni*-colonized wild birds have been implicated in the direct or indirect infection of humans with *C. jejuni* (Gardner and others 2011), direct introduction of *C. jejuni* in broiler flocks by wild birds has been debatable as it is difficult for wild birds to enter closed broiler houses. However, *C. jejuni* can frequently be isolated from wild-bird feces around broiler houses; and, according to molecular studies, on some occasions these strains can subsequently be recovered from the ceca of broilers in the corresponding houses (Stern and others 1997; Hiett and others 2002).

C. jejuni can also be isolated from wild rodents (Fern and Park 1977; Annan-Prah and Janc 1988; Meerburg and others 2006), from their feces, as well as from their intestines (Hiett and others 2002). Results of epidemiological and risk assessment studies on the identification of rodents as risk factors for *Campylobacter* introduction into broiler flocks have been contradictory. Some studies have already put forward that the presence of rodents on a farm can lead to an increased risk of flock colonization (Kapperud and others 1993; Berndtson and others 1996b;McDowell and others 2008; Ellis-Iversen and others 2012). Hiett and others (2002) found the intestines of mice captured in broiler house environments to be *C. jejuni*-positive. Genotypic studies suggested, however, that these mice became colonized by a strain previously shed by broilers and were probably not the source of the *C. jejuni* strain. Overall, it can be suggested that rodents can play a role in *C. jejuni* colonization of broilers when the pressure of *C. jejuni* in the environment is high and pest control is not adequate, which according to Meerburg (2010) frequently happens. Because only a relatively low number of *C. jejuni* wildlife-derived strains had a clonal relationship to human and chicken strains, Petersen and others (2001a) suggested that wildlife (rodents as well as wild birds) had only limited importance as a reservoir of *Campylobacter* strains for infection of poultry and humans. Overall, if an effective rodent control program is installed and used properly the risk of *C. jejuni* introduction by rodents will probably be low (Evans and Sayers 2000).

Presence of insects and type of ventilation system

As poultry houses are large enterprises, a large number of insects, including flies, darkling beetles, and cockroaches, will be living in and around broiler houses (Umunnabuike and Irokanulo 1986; Salin and others 2000; Hald and others 2004). As most of these animals forage on animal feces, they have been reported to be carriers of *Campylobacter* (Umunnabuike and Irokanulo 1986; Jacobs-Reitsma and others 1995; Hald and others 2004), although bacteria may survive on or within these insects only for short periods (Evans 1992). Again, the evidence for the role of insects in poultry house contamination is contradictory. A survey carried out in Sweden (Berndtson and others 1996b) concluded that the presence of insects was not a statistically significant risk factor. In contrast, Hald and others (2004, 2008) found that in Denmark, during the month of July, hundreds of flies per day passed through the ventilation system into broiler houses and that 8.2% of flies captured in that environment had the potential to transmit *C. jejuni* from the outside to chickens in the broiler house. The authors concluded that an influx of large numbers of flies into broiler houses constitutes a considerable risk for *C. jejuni* colonization of broilers. Other Danish intervention studies (Hald and others 2007a,b) demonstrated that using fly nets significantly reduced the incidence of *C. jejuni*-positive flocks from 51.4% to 15.4% during the period from June to November.

Additionally, Jacobs-Reitsma and others (1995) could isolate *C. jejuni* from the internal contents of darkling beetles during an epidemiological survey at Dutch broiler farms. Though serotypes from strains isolated from insects and broilers on the farm were identical, the direction of the infection route was not clear, as *C. jejuni* could not be isolated from beetles prior to its isolation

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from broilers. Refrégier-Petton and others (2001) identified the presence of litter-beetles in the change room as a risk factor for *Campylobacter* contamination (OR = 5.0). As beetles will be present in the broiler house during consecutive production cycles, they can be important in the carryover of *C. jejuni* colonization to the next cycle. The risk of *C. jejuni* introduction posed by these beetles will be of lesser importance when empty periods are longer and broiler houses are adequately maintained. Hazeleger and others (2008) fed *C. jejuni* daily to darkling beetles or their larvae for 4 weeks but not 1 week prior to feeding the beetles or their larvae to the broilers. The authors subsequently demonstrated that, although *C. jejuni* could not be recovered from artificially inoculated darkling beetles or larvae after 1 week, broilers could become infected after eating these darkling beetles or their larvae. In conclusion, the need for a longer empty period can be recommended, coupled with the use of physical barriers (Hansson and others 2007b) (like fly nets) and insecticides (Salin and others 2003) for controlling insect populations in broiler houses in order to avoid or delay possible *C. jejuni* introduction.

Type of ventilation and ventilation systems used have also been identified as possible risk factors for introduction of *Campylobacter* into a broiler flock. Rushton and others (2009) demonstrated that natural ventilation enhanced the chance of *Campylobacter* contamination in broiler flocks (*t* value = 2.09). This could either be due to the influence of external weather conditions—since the temperature in a fan-ventilated environment is likely to be lower than that provided by natural ventilation—or due to the higher presence of *Campylobacter* vectors, such as flies. In addition, the authors hypothesized that forced ventilation might lead to fly mortality as flies hit fan blades. Barrios and others (2006) and Guerin and others (2007) identified the presence of vertical (respectively, OR = 5.3 and OR = 2.7) or the presence of vertical and horizontal (OR = 3.2) ventilation shafts, opposed to the presence of only horizontal ventilation shafts, to be significant ($P \leq 0.05$) risk factors for an increased *Campylobacter* colonization in the flock. The authors concluded that the increased risk might be related to the height of the vents, the potential for vectors such as flies to gain access to the house, and increased difficulties in properly cleaning and disinfecting the vents. The authors also recommended constructing horizontal ventilation systems in new broiler houses.

Presence and management of multiple broiler houses and other livestock on the farm and broiler age effect

The presence of multiple broiler houses on a broiler farm has been associated with increased risk of *C. jejuni* introduction into a broiler flock. This was described by Refrégier-Petton and others (2001) who identified an increased risk on farms with more than 2 houses (OR = 13.2 and $P < 0.05$), by Bouwknegt and others (2004) who identified a marked increase on farms with 5 or more houses (OR = 3.02), as well as by Ridley and others (2011a) and McDowell and others (2008). In a study performed by Alter and others (2011), to investigate the distribution and spread of *C. jejuni* genotypes between sequential and adjacent flocks, the data obtained suggested that a common environmental *C. jejuni* contamination was the source of *C. jejuni* infection in different adjacent broiler flocks rather than the survival of the *C. jejuni* serotype within the premise. Additionally, both the number of staff members (if > 1, OR = 2.03) (Chowdhury and others 2012) looking after different broiler houses and the number of visits they undertook daily were directly related to the risk of *C. jejuni* introduction into a flock (Berndtson and others 1996b; Newel and Fearnley 2003; Alter and others 2011; Chowdhury and others 2012). Overall, it can be concluded that due to the high environmental pressure of *C. jejuni*, the risk of *C. jejuni* introduction into a broiler house increases with the number of broiler houses present on the farm and spreading of *Campylobacter* to other houses can be facilitated by farm staff travelling between houses.

Next to the presence of multiple broiler houses on the farm, other neighboring livestock on the farm or on nearby farms has also been implicated in increasing the risk of *C. jejuni* -positive flocks (Kapperud and others 1993; Berndtson and others 1996b; van de Giessen and others 1998; Hald and others 2000; Bouwknegt and others 2004; Cardinale and others 2004; Guerin and others 2007), although McDowell and others (2008) could not find such an association. A possible explanation for McDowell's contradictory findings could have been the recent occurrence of foot and mouth disease in Northern Ireland and the subsequent increased farmers' awareness of biosecurity issues. An Icelandic analysis of environmental risk factors (Laberge and others 2006) showed that the presence of cattle within 5 km of the broiler farm increased the percentage of positive flocks on a broiler farm in the spring (OR = 6.7). Likewise, Bouwknegt and others (2004) stated that the presence of other farm animals on the farm (OR = 1.88), and the presence of animals on farms within 1 km (OR = 9.56), provoked significant risk increments. It can be hypothesized that this increase is linked to the presence of indirect vectors such as insects, rodents, personnel, and the absence of rodent control programs and hygiene barriers. Although, in general, the majority of strains recovered from adjacent livestock cannot be isolated from broilers, in some cases the same strains isolated from poultry flocks could be detected in other livestock present on the farm, such as cattle and pigs (Jacobs-Reitsma and others 1995; Gregory and others 1997; Johnsen and others 2006). According to Katsma and others (2007) this should not result in a ban on keeping other livestock on broiler farms, as this is less effective in reducing *C. jejuni* colonization of broilers than trying to reduce the transmission between successive production cycles. Again, effective hygiene barriers should be employed (Kapperud and others 1993; Hald and others 2000) when traveling between different broiler houses or from other livestock to broiler houses and *vice versa*, as the lack of an effectieve hygiene barrier results in an increased risk of *Campylobacter* contamination (OR = 3.1; Hald and others 2000). Hald and others (2000) stated that the presence of a hygiene barrier was the single-most important biosecurity measure for production of *Campylobacter*-free broilers.

The chance of *C. jejuni* introduction into broiler flocks has been shown to increase with the age at which broilers are being cleared for slaughter (Bouwknegt and others 2004; McDowell and others 2008; Chowdhury and others 2012), which is normally around 5 to 7 weeks of age. Bouwknegt and others (2004) demonstrated significant risk increments in broilers aged 29 to 35 d (OR = 2.34) and 36 to 42 d (OR = 3.96) compared to 22 to 28 d. The increasing risk of infection observed may simply result from the increased risk of introduction of a *C. jejuni* infection over time, originating from environmental and other sources, as exposure to a *C. jejuni* source becomes increasingly probable.

Seasonal effect

Several studies have reported that *Campylobacter* colonization was significantly elevated during the summer months (ORs ranging from 3.43 to 6.4; Kapperud and others 1993; Jacobs-Reitsma and others 1994; Refrégier-Petton 2001; Guerin and others 2008McDowell and others 2008). A possible explanation could be the elevated presence of flies during summer (Ekdahl

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and others 2005; Goulson and others 2005; Nichols 2005; Hald and others 2007a,b; Guerin and others 2008). Guerin and others (2008) hypothesized that, because houseflies can carry *C. jejuni* both internally and externally (Shane and others 1985), ingestion of *Campylobacter*-positive flies, contact with the bacteria on the external surface of the flies, or contact with fomites contaminated by *Campylobacter*-positive flies (Shane and others 1985) would possibly make broilers *Campylobacter*-positive. Because fly population size increases in summer months, the risk for these events to happen increases during this period. Apart from fly population size, temperature might also influence the prevalence of migratory birds (Luechtefeld and others 1980; Broman and others 2002) and other insects (Jacobs-Reitsma and others 1995) known to carry *Campylobacter*, and thus indirectly pose a low risk for *Campylobacter* introduction into broiler flocks.

Contamination of Broilers during Transport

During transportation to processing plants, birds frequently defecate onto both the transport crate surfaces and onto other birds, thereby contaminating their environment, as was demonstrated for turkeys by Wesley and others (2009). This increases the chances of externally contaminating both *C. jejuni*-positive as well as *C. jejuni*-negative broilers. Transport crates frequently remain microbiologically contaminated, even after washing (Slader and others 2002; Berrang and others 2004a; Ramabu and others 2004), although they appear visually clean (Allen and others 2008a). Three studies demonstrated that 60% and 71% of the transport crates were found to be contaminated with *Campylobacter* after cleaning and disinfecting (Slader and others 2002; Hansson and others 2005, Rasschaert and others 2007). As a result, *Campylobacter*-negative broilers can become externally contaminated, due to transport in these crates (Stern and others 1995; Hiett and others 2002; Slader and others 2002; Hansson and others 2005; Rasschaert and others 2007). Genotypes isolated from washed crates were also identified on broiler carcasses following transport and slaughter (Hansson and others 2007b; Lienau and others 2007). Additionally, although internal colonization of *Campylobacter*-negative birds during transport and holding at the processing plant is rare, Ridley and others (2011b) demonstrated that broilers could become detectably colonized by *Campylobacter* following brief exposure to commercially cleaned crates under experimental and nonstressed conditions. However, it has to be noted that broilers were examined at least 21 h after exposure, which is longer than normal transportation times. In contrast to experimental colonization results of Ridley and others (2011b), evidence for intestinal *C. jejuni* colonization due to transport in contaminated containers was not found by Rasschaert and others (2007). In conclusion, *Campylobacter*-negative as well as *Campylobacter*-positive broilers flocks transported in *Campylobacter*-positive transport crates are at risk of external *Campylobacter* contamination.

Contamination of Broiler Carcasses during the Slaughtering Process

Broilers contaminated with *Campylobacter* spp. during rearing and transport will likely produce contaminated carcasses following processing (Stern and others 1995; Herman and others 2003; Rasschaert and others 2006, 2008; Reich and others 2008). The levels of *Campylobacter* spp. on carcasses may increase and decrease during the entire slaughter process, but once contaminated, *Campylobacter* will be present on the carcass during the entire duration of the process. *Campylobacter* numbers can decrease during scalding, chilling, and freezing and can increase during defeathering and evisceration (Oosterom and others 1983; Izat and others 1988; Berrang and others 2004b; Rosenquist and others 2006). Because 71.2% of the broiler flocks in the EU are *Campylobacter*-positive (EFSA 2010a), it can be expected that many of the broiler flocks entering the processing plant are externally contaminated with *Campylobacter*. Several studies have recovered *Campylobacter* from broiler carcasses prior to entering and after leaving the scalding tank (Oosterom and others 1983; Izat and others 1988; Berrang and others 2000; Berrang and Dickens 2000).

During defeathering, contaminated feces leaking from the cloaca, due to mechanical pressure by rubber fingers, can lead to high broiler carcass contamination (predefeathering: 2.4 $\log_{10}$ CFU/g breast skin, postdefeathering: 4.2 $\log_{10}$ CFU/g breast skin) (Oosterom and others 1983; Berrang and others 2001). Especially on broiler carcasses with low external *Campylobacter* contamination but high *Campylobacter* counts in their ceca, carcasses of positive birds can become more externally contaminated due to visceral rupture during the evisceration procedure (an increase of 0.9 $\log_{10}$ CFU/ carcass, $P = 0.05$) (Berrang and others 2004b; Boysen and Rosenquist, 2009). At this point in the slaughter process, carcasses can carry the highest *C. jejuni* load, which, according to some studies, decreases slightly after chilling (Rosenquist and others 2006; Kuana and others 2008; Reich and others 2008; Boysen and Rosenquist 2009; Figueroa and others 2009).

Cross-contamination between birds within a flock and between successive flocks might occur during slaughtering and processing and cause external contamination of carcasses of both *Campylobacter*-positive and -negative broilers (Newell and others 2001; Herman and others 2003; Miwa and others 2003; Rosenquist and others 2003; Rasschaert and others 2006). Three potential routes of cross-contamination have been identified: (1) direct contact between carcasses, (2) indirect contamination via slaughter equipment and processing water, and (3) airborne spread via aerosols (Rasschaert and others 2008). Berndtson and others (1996a) isolated *Campylobacter* from all equipment sampled along the processing line, and Peyrat and others (2008) demonstrated overnight *C. jejuni* survival on food processing equipment surfaces, even after cleaning and disinfection. Surviving strains of previously processed *Campylobacter*-positive flocks might thus possibly be a source of poultry carcass contamination. Jones and others (1991) assessed that 20% of cloacal swabs of broilers entering the slaughterhouse was contaminated, but found 52% of the carcasses to be contaminated following immersion chilling and 31.6% of whole broiler carcasses sampled at retail outlets, indicating higher external contamination might arise at the end of the slaughter line.

Resistance to environmental stresses during processing, however, varies from strain to strain. Some subtypes, which survive carcass chilling, are able to contaminate the abattoir environment and cause cross-contamination of subsequent flocks (Newell and others 2001; Hunter and others 2009). It has indeed been proven that the genetic diversity of *Campylobacter* decreases as carcasses proceed through processing. An explanation could be that some subtypes persist on carcasses while others are unable to survive processing (Hunter and others 2009).

Possible Intervention Measures to Delay or Prevent *Campylobacter* Colonization of Broilers during Rearing

Biosecurity, cleaning, and disinfection measures

Biosecurity measures should be an integral part of every farms' program to combat the introduction of pathogens into livestock. As described previously, human activity is important in

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introducing *Campylobacter* into a broiler flock. This highlights the need for better hygiene control, not only for farm workers, but also for catching equipment and all personnel and the need for more effective cleaning and disinfection of vehicles, workers' equipment and bird-transport crates. Risk of *Campylobacter* introduction into a flock has been shown to be closely associated with not using house-specific boots and house-specific clothes, not using overshoes, and the ineffective use of boot dips (Humphrey and others 1993; Evans and Sayers 2000; Bouwknegt and others 2004; Puterflam and others 2005). The installation and strict use of an effective hygiene barrier with a boot dip, hand-washing facilities, and the possibility to change into clean clothes in the ante-room, before entering the broiler flock, can reduce the risk of flock infection (Berndtson and others 1996b; Evans and Sayers 2000; Hald and others 2000), and it seems particularly important when other livestock, especially poultry, is present on the farm (van de Giessen and others 1998; Hald and others 2000). The percentage of *Campylobacter*-positive flocks can be 30% to 50% higher when no hygiene barrier was present, depending on the presence of other livestock on the farm (Hald and others 2000). Guerin and others (2007) and Berndtson and others (1996b) noted that the use of a good boot-dipping procedure should be followed more closely on farms experiencing a high prevalence of *Campylobacter.* When farmers were frequently careless about boot-dipping (such as only dipping toes or heels, passing through the disinfectant very quickly, insufficient dipping of boots when organic material is present, and low frequency of changing the dip) the risk of *Campylobacter* colonization increased (Berndtson and others 1996b; Evans and Sayers 2000; Gibbens and others 2001; Guerin and others 2007; McDowell and others 2008). The hazard ratio for *Campylobacter* introduction when use of boot dips was inadequate amounted to 1.58 (Evans and Sayers 2000), meaning that under those circumstances *Campylobacter* introduction is 1.58 times more likely. Additionally, low frequency of changing the dip generated a strong chance of *Campylobacter* contamination (Fisher's exact test $P = 0.03$) (Gibbens and others 2001). Katsma and others (2007) demonstrated in a comprehensive risk assessment that, theoretically, increasing biosecurity on the farm could lower between-flock transmission from adjacent houses. Theoretically, if biosecurity is increased and this results in a 25% reduction of transmission, *Campylobacter* infection will almost extinct in broiler flocks.

Biosecurity measures may also help in reducing the influence of seasonality on the risk of *Campylobacter* introduction into a flock. A hypothesis stated that this seasonal influence is related to the breeding period of flies (Hald and others 2007b). The same study concluded therefore that installing effective fly screens in broiler houses in Denmark would most likely decrease the average yearly *Campylobacter* prevalence, especially by influencing *Campylobacter* introduction during summer months. In that study, preventing flies from entering broiler houses caused the number of *Campylobacter*-positive flocks at slaughter to drop from 51.4% to 15.4%.

The problem in applying additional biosecurity measures is that the precise effect on *Campylobacter* introduction is unknown and it is hard to set up specific control measures (Katsma and others 2007). Strengthening on-farm biosecurity measures, monitoring the prevalence of *Campylobacter*-positive flocks, and implementation of voluntary and regulatory poultry-focused control strategies resulted in a 50% decline in the rate of campylobacteriosis notifications and hospitalizations in New Zealand in 2007 (Sears and others 2011).

Ridley and others (2011b) and Rasschaert and others (2007) assessed that, despite enhanced biosecurity measures at farms, flocks negative at thinning were found to be positive at clearance, probably due to introduction of *Campylobacter* during partial depopulation. Studies demonstrated that standard crate-washing procedures could not effectively remove *Campylobacter* from surfaces of crates used during partial depopulation (Slader and others 2002; Ramabu and others 2004; Rasschaert and others 2007). As proven in a UK study on farmers' attitude toward adopting different on-farm biosecurity measures, most farmers would not be willing to stop partial depopulation, as this is estimated to have a high economic cost (Fraser and others 2010). Therefore, strict application of hygiene measures by thinning crews should be mandatory.

Evans and Sayers (2000) and Chowdhury and others (2012) also reported that flocks housed in buildings in need of repair, or old broiler houses (pre-1990), were colonized with *Campylobacter* more often than buildings in a good state, with the hazard ratio for broiler houses due for repair amounting to 2.45 (Evans and Sayers 2000). The difference in *Campylobacter* prevalence in different houses is probably due to the inability to adequately clean houses in a poor state, lack of physical barriers between a potentially contaminated external environment and broilers inside, the type of ventilation system, and the temperature regulation system. Old ventilation systems could, for instance, not be able to evacuate damp and moist air, in turn facilitating *Campylobacter* survival in the damp environmental materials (Chowdhury and others 2012). All these findings favor modern, new, and properly adjusted broiler houses, cleaning methods, ventilation systems, and temperature regulation systems. Reduction of the risk of carrying in *Campylobacter* via contaminated material, boots, and clothes might be attained by constructing clean and intact concrete aprons around broiler houses (Newell and Fearnley 2003).

Litter treatment

Various studies also investigated whether treating litter could delay or prevent *Campylobacter* spreading throughout the broiler flock. Two commercially available acidifying litter treatments tested by Line and Bailey (2006) indicated that, although *Campylobacter* prevalence was not influenced in broilers, both treatments caused a slight delay in the onset of *Campylobacter* colonization. In another survey, Poultry Litter Treatment® enhanced litter had no influence on *Campylobacter* counts (Pope and Cherry 2000). Poultry Litter Treatment® is a dry, granular acid, composed of sodium bisulfate, and is used extensively for poultry house ammonia control, litter acidification, and for on-farm pest management.

Drinking water treatment

Different intervention studies have provided proof that adding sanitizers to, chlorinating, or acidifying drinking water can either reduce the probability a flock becoming *Campylobacter*-positive or delay *Campylobacter* colonization (Kapperud and others 1993; Pearson and others 1993; Evans and Sayers 2000; Jeffrey and others 2001; Newell and Fearnley 2003; Hermans and others 2012a). Byrd and others (2001) assessed that by adding lactic acid to drinking water during the feed-withdrawal period prior to slaughter, crop and pre-chill carcass contamination was reduced by 20% and 15%, respectively. This is probably due to the reduction of bacterial numbers in both the drinking water and the broiler crop. Hermans and others (2012a) demonstrated *in vivo* that when a mixture of caproic, caprylic, capric, and lauric acids (medium chain fatty acids, MCFA) was added to drinking water, broilers were less susceptible to *C. jejuni* colonization, and *C. jejuni* survival in drinking water was prevented. The authors demonstrated that 60% of the birds receiving a *C. jejuni* dose of 2×10^3 CFU, and raised on

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control drinking water, were colonized, whereas none of the 10 birds receiving MCFA-supplemented drinking water were colonized in their ceca 24 h after inoculation ($P = 0.03$). In a 5-d *in vivo* experiment, however, *Campylobacter* colonization and transmission was not reduced, as MCFA supplementation to drinking water did not result in a significant ($P > 0.05$) reduction in the cecal *Campylobacter* load of these birds compared with birds receiving control water. Under commercial practices, 2 to 5 ppm chlorinated drinking water did not result in a reduced *Campylobacter* prevalence in broilers in the United States (Stern and others 2002). This may be explained by the fact that waterborne protozoa can potentially act as protecting reservoirs in drinking water systems of broiler houses (Snelling and others 2005). These protozoa appeared to reduce the susceptibility of bacteria to chlorine and other desinfectants in an experimental cocultivation study (Snelling and others 2005). Pearson and others (1993) obtained a reduction in the proportion of birds colonized with campylobacters from 81% to 7% and a 1000- to 10000-fold reduction in campylobacters recoverable from the carcasses. This reduction was the result of an intervention program based on 0.2 to 0.4 parts per million (ppm) drinking water chlorination, shed drinking system cleaning, and disinfection. Since the predominant source of *C. jejuni* on the broiler farm was the farm' water supply, these results are biased and use of chlorinated drinking water will probably not have comparable results on broiler farms with a different predominant source of *C. jejuni*.

An important point of interest is that addition of supplements to drinking water should not influence feed uptake and broiler growth. This also applies to supplements in feed as described below.

Use of plant-derived extracts in feed and drinking water

Plant-derived extracts harboring anti-*Campylobacter* activity can be added to feed or drinking water from day-of-hatch to prevent or delay *Campylobacter* colonization of broilers and to reduce *Campylobacter* transmission throughout the flock. The effect of these extracts will probably be highest in the crop of the animals thereby possibly reducing subsequent cecal colonization. Several *in vitro* studies have shown that plant-derived compounds and essential oils possess antimicrobial properties against *Campylobacter* (Cowan 1999; Friedman and others 2002; Lee and others 2004; Fisher and Phillips 2006; Yin and Chao 2008; Fujisawa and others 2009; Nannapaneni and others 2009; Hermans and others 2011a; Lu and others 2011; Klačnik and others 2012). Most of these studies have focused on the anti-*Campylobacter* activity of plant components under *in vitro* circumstances, although some have also indicated an anti-*Campylobacter* effect on retail products like chicken skin and ground beef (Yin and Chao 2008; Nannapaneni and others 2009). Most studies investigating anti-*Campylobacter* activity of plant-derived extracts have focused on the *Allium* family (Chinese leek, garlic, onion) or citrus oil fractions, although some other plant and seed extracts have also been tested (trans-cinnamaldehyde, *Alpinia katsumadai* seed extracts, roselle calyx extract, and protocatechuic acid) (Klančnik and others 2012).

Two studies assessed whether citrus essential oils were capable of inhibiting *Campylobacter* growth *in vitro*. The antimicrobial activity of these citrus essential oils is probably due to the chemical profile of the entire mixture and not due to a single compound (Caccioni and others 1998; Nannapaneni and others 2009). Nannapaneni and others (2009) concluded this because d-limonene, constituting the most important component of citrus oils, appeared to be up to 3 times less inhibitory toward *Campylobacter* than cold-pressed terpeneless Valencia orange oil. Fisher and Phillips (2006), in turn, determined that d-limonene had no antibacterial activity against *C. jejuni* and suggested that the inhibitory effect of the orange essential oils was due to linalool (MIC = 0.06% v/v). The inhibitory effect of these molecules is generally explained by their interaction with bacterial cell structural components (Belletti and others 2004).

Plant extracts of the *Allium* family have been shown to inhibit *C. jejuni* as well as *C. coli* growth *in vitro* (Lee and others 2004), presumably by the action of allicin, an organosulfur component, or allicin degradation products like allyl sulfides. All of these purified components display *in vitro* activity against *C. jejuni* strains (De Wet and others 1999; Lu and others 2012), but they have not been tested against *C. coli*. The activity of allicin is due to the S(O)S moiety which is able to interact with proteins and enzymes containing a -SH moiety, thereby altering their structure or influencing their activity (Cavallito and others 1944; Ankri and Mirelman 1999; Lu and others 2012). Although not all chemical reactions of allicin or its derived products in human or bacterial cells are known, it is clear that activity of allicin is lost due to interaction with sulfhydryl-containing compounds (-SH) like cysteine and glutathion (Smirnova and Oktyabrsky 2005; Fujisawa and others 2009). Fujisawa and others (2009) also demonstrated that Gram-positive bacteria are more sensitive to allicin than Gram-negative bacteria, probably due to the presence of proteins in the cell wall of the latter. Lee and others (2004) described that aqueous Chinese leek preparations have a higher anti-*Campylobacter* activity than aqueous garlic preparations. They also highlighted that the possible use of Chinese leek, garlic, or onion in feed to combat *Campylobacter* colonization in broilers should be investigated. Robyn and others (2013a) demonstrated that allicin at concentrations of 25 mg/kg was capable of completely and rapidly inhibiting *C. jejuni* growth *in vitro*, but no effect was seen *in vivo*, probably due to interaction of allicin with mucine, animal cells, and so on.

The problem with using citrus essential oils as additives for feed or drinking water is that these oils are tested *in vitro* only in view of later use as food preservatives, not for use during rearing. In only one study the effect of a plant extract with marked *in vitro* anti-*Campylobacter* activity was assessed in an *in vivo* broiler experiment. Hermans and others (2011a) demonstrated that trans-cinnamaldehyde, which originates from cinnamon oil, could not prevent or reduce cecal *C. jejuni* colonization, in a broiler seeder model, when used in feed. The marked *in vitro* activity could not even be validated in an *in vivo* model in which trans-cinnamaldehyde was directly injected into the broiler ceca, as this did not reduce cecal *Campylobacter* numbers.

Although garlic-derived chemical components give promising *in vitro* results, their use as antibacterial additives in feed and drinking water, as well as the chemical interactions in animal, human, and bacterial cells, should be researched more in depth. The organoleptic properties of citrus oils, *Allium* species and their active components can pose an important problem when these are used as anti-*Campylobacter* additives in feed or drinking water. These properties might have an influence on the taste of chicken meat when used as additives, but might also influence feed or water intake, which has not yet been checked. Additionally, it has not yet been investigated if passage through the broiler gastrointestinal tract influences anti-*Campylobacter* activity of plant-derived components. As has been clarified by Hermans and others (2011a), a clear *in vitro* anti-*Campylobacter* activity does not necessarily mean a similar *in vivo* activity. If these plant extracts will be used as feed additives to combat cecal *Campylobacter*

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colonization, it would be probably better to use them from day-of-hatch, rather than therapeutically in already colonized birds.

Use of organic acids in feed

Besides the application of organic acids in drinking water as a biosecurity measure, they might also be used as feed additives, coated or uncoated, to reduce *Campylobacter* prevalence in poultry.

Solis de los Santos and others (2008) postulated different mechanisms for the antibacterial activity of MCFAs in feed. They could lead to intracellular acidification (Sun and others 1998) due to dissociation within the protoplasm of caprylic acid, leading to inactivation of intracellular enzymes (Viegas and Sa-Correia 1991) and inhibition of amino acid transport (Freese and others 1973). They also suggested caprylic acid influences outer membrane proteins needed for bacterial adaptation to the host environment and for colonization (Solis de los Santos and others 2008).

Organic acids have been tested as feed supplements in therapeutic and prophylactic designs yielding conflicting results. Molatová and others (2011) provided chickens with feed supplemented with 0.25% of a coated or uncoated mixture of caprylic acid and capric acid (1:1) throughout their entire rearing period to test for a prophylactic effect. After oral *C. jejuni* challenge they assessed that both the coated and uncoated mixtures were able to reduce fecal *C. jejuni* numbers by 0.1 to 4.1 log CFU/g and by 1.0 to 1.9 log CFU/g, respectively. It was assessed that this reduction persisted up to 4 d after *C. jejuni* inoculation, but diminished or disappeared afterwards. Based on their results, Molatová and others (2011) recommended using MCFAs preferably 2 to 3 d before slaughter as a therapeutic agent. A result supporting this assumption was obtained by Solis de los Santos and others (2008, 2009, 2010) who demonstrated that supplementing caprylic acid to feed at different concentrations (0.7% and 1.4%) in the last 72 h prior to slaughter reduced *Campylobacter* numbers in broilers aged 10, 15, and 42 d by 3 to 7 logs CFU/g. When assessing the broiler performance scores, only the higher MCFA doses performed markedly poorer (Solis de los Santos and others 2008, 2009). Two studies obtained a prevention or reduction of broiler *C. jejuni* colonization when, respectively, 2% formic acid + 0.1% sorbate or a 1% MCFA mixture (1% C_8-C_{12}) was added to feed (Skånseng and others 2010; van Gerwe and others 2010). When broilers were fed only formic acid-supplemented feed (1% or 2%), Skånseng and others (2010), found only little effect. van Gerwe and others (2010) demonstrated that the *C. jejuni* dose necessary to colonize 50% of inoculated broilers was 200 times higher in broilers fed a MCFA mixture supplemented to their feed than in broilers given unsupplemented feed. Hilmarsson and others (2006) could also demonstrate a 1 to 2 log CFU/mL reduction of *C. jejuni* in cloacal swabs of 36-day-old naturally colonized birds when 0.24% monocaprin and 0.04% polysorbate 40 was supplemented to the feed and drinking water 3 d prior to slaughter. Monocaprin in water and feed was, however, not capable of preventing *Campylobacter* from spreading from artificially infected to noninfected 24-day-old chickens.

On the contrary, Hermans and others (2010) could not confirm any effect on cecal *C. jejuni* numbers when already colonized 28-day-old broilers were given feed containing 1% (w/w) encapsulated MCFA (caproic, caprylic, or capric acid) starting 3 d before euthanization. *C. jejuni* colonization was not even influenced when a 1% (w/w) sodium caprate solution was injected directly into the broiler cecum. The absence of reduction was presumed to be due to the protective effect of intestinal mucus, which was demonstrated *in vitro*. *C. jejuni* preferentially resides in mucus-filled crypts in the ceca (Beery and others 1988), indicating *C. jejuni* is located in a place where mucus will be exerting an influence on the activity of MCFAs. Van Deun and others (2008a) also did not demonstrate any reduction in *Campylobacter* colonization in 2-week-old broilers when adding butyrate to the feed in a seeder model. One of the reasons for the discrepancy between these studies might be the initial inoculation dose of *C. jejuni* used. The studies that did not demonstrate an effect when using MCFA supplemented in the feed (Van Deun and others 2008a; Hermans and others, 2010) all used an initial inoculation dose $\geq 10^5$ CFU/mL, while van Gerwe and others (2010) demonstrated that the *C. jejuni* dose necessary to colonize 50% of inoculated broilers was estimated to be 200 times higher in broilers fed with supplemented feed (4.8 $\log_{10}$ CFU) than in control broilers (2.5 $\log_{10}$ CFU). Some studies showing an *in vivo* effect used an initial inoculation dose $\leq 10^5$ CFU/mL (Skånseng and others 2010; van Gerwe and others 2010). Studies showing an effect using a higher inoculation dose $\geq 10^6$ CFU/mL (Hilmarsson and others 2006; Solis de los Santos and others 2008, 2009) used a different *C. jejuni* strain than Hermans and others (2010) and Van Deun and others (2008a), which could also be part of the explanation.

Providing broilers with acidified feed containing 5.7% lactic acid and 0.7% acetic acid resulted in reduced *in vivo Campylobacter* susceptibility (Heres and others 2004). However, the size of reduction was limited. The high level of organic acids and the low pH in the crop and gizzard may possibly constitute an improved bactericidal upper intestinal barrier (Heres and others 2004). Broilers provided with fermented liquid feed (FLF), a moistened feed with a high number of lactobacilli and a high concentration of lactic acid, showed reduced *in vivo Campylobacter* susceptibility (Heres and others 2003). The authors demonstrated that at any moment the probability to start shedding *Campylobacter* was 9 times higher for the control animals than for the animals that received fermented feed. FLF did, however, not consistently change the *Campylobacter* colonization level in the ceca.

Vaccination and passive immunization

De Zoete and others (2007) described that vaccines in broilers can only be considered effective if they meet 5 standards: (1) an immune response should be induced early on in young chickens and before contact with *Campylobacter* spp., (2) the vaccine should be cross-protective, providing broilers with a very high degree of protection from colonization with both *C. jejuni* and *C. coli* as they are the most frequent cause of human campylobacteriosis (Lastovica and Skirrow 2000; Galanis 2007; EFSA 2011), (3) it should be easy to deliver (orally, *in ovo*), (4) be cost-effective, and (5) it must be safe for animals as well as for humans.

Table 1 gives an overview of studies on vaccination against *Campylobacter* in broilers. Vaccines tested were based on killed whole cells, live attenuated strains, flagellin, an ABC transporter protein (CjaA = Cj0398c), an outer membrane protein (CjaD = Cj0113 = Omp18), an aspartate/glutamate-binding ABC transporter protein (Peb1A), the putative glutamine-binding ABC transporter protein (GlnH), a hemin-uptake outer-membrane receptor (ChuA), an outer-membrane component of the CmeABC multidrug efflux pump (CmeC), and a probable periplasmatic protein (ACE393 = Cj0420). The developed vaccines also differ in the vectors used and in the use of an adjuvant to increase immune response. Tests in poultry with killed whole cell vaccines or flagellum-based vaccines have been carried out, but results indicated that only partial protection against *Campylobacter* was obtained, with the best results being a 1.5 $\log_{10}$ reduction of CFU of

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Table 1–Overview of studies on vaccination against *Campylobacter* in chickens.

Type of vaccine	Administration	Effect	Ref
Experimental colonization with wildtype *C. jejuni*	Orally	Approximately 1 log reduction upon homologous challenge	Cawthraw and others (1998)
Experimental colonization with noncolonizing mutantof *C. jejuni*	Orally	No effect upon homologous challenge	Ziprin and others (2002)
Formalin-inactivated *C. jejuni* +/− LT	Orally with boosters	Approximately 1.5 log reduction upon homologous challenge no additional effect of LT	Rice and others (1997)
Formalin-inactivated *C. jejuni* +/− LT or CT	Orally with boosters	No effect upon homologous challenge	Cawthraw and others (1998)
Formalin-inactivated *C. jejuni* complete Freund's adjuvant	SC with booster	Some reduced shedding during first 2 weeks upon homologous challenge only	Glünder and others (1998)
Heat-killed *C. jejuni*	*In ovo* with oral booster	Generation of flagellin-specific serum IgG, IgM and IgA, and IgA in bile and intestine	Noor and others (1995)
Native flagellin +/− heat-killed *C. jejuni*	IP with IP or oral booster	Approximately 1 to 2 log reduction upon homologous challenge	Widders and others (1996, 1998)
Recombinant flagellin fused to LT	Orally with booster	Reduction of *C. jejuni* positive chickens (40/145 compared with 70/142 in control group)	Khoury and Meinersmann (1995)
Plasmid DNA containing the flagellin gene	IM with booster	2 log reduction upon homologous challenge	Newell and Cawthraw (2006)
67, 73.5, and 77.5 kDa immunogenic *C. jejuni* proteins	IP	No effect upon homologous challenge	Widders and others (1998)
Attenuated *Salmonella* expressing CjaA	Orally	>6 logs reduction upon homologous challenge	Wyszynska and others (2004)
Attenuated *Salmonella* expressing CjaA or Peb1A	Orally	Approximately 1,5 log reduction in cecal load of *C. jejuni*	Buckley and others (2010)
Attenuated *Salmonella* expressing GlnH or ChuA	Orally	no significant reduction in cecal load of *C. jejuni*	Buckley and others (2010)
Transgenic *E. tenella* population expressing CjaA and the fluorescent reporter mCitrine	Orally	91% and 86% immune protection against *C. jejuni*	Clark and others (2012)
CmeC vaccine +/− LT	Orally	No effect upon homologous challenge	Zeng and others (2010)
CmeC vaccine +/− LT	Subcutaneous	No effect upon homologous challenge	Zeng and others (2010)
Live *Salmonella* vectors expressing 3 linear peptide epitopes from *Campylobacter* proteins (Cj0420, Cj0113, and Cj0982c)	Orally	Cj0420 and Cj0982: 1-log and 2-log reductions Cj0113: 4.8-log reduction in the ileal samples	Layton and others (2011)

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homologous *Campylobacter* per gram cecal feces (Rice and others 1997; Meeusen and others 2007). Best results were obtained when CjaA and CjaD proteins expressed in live *Salmonella* vectors were tested as possible vaccines, with 4 to 6 $\log_{10}$ reductions of CFU of homologous *Campylobacter* per gram of ileum content (Layton and others 2011) or cecal content (Wyszynska and others 2004). When broilers were orally challenged with an attenuated *Salmonella* strain expressing the *C. jejuni* CjaA protein, heterologous *C. jejuni* colonization was also reduced (Wyszynska and others 2004). Western blots have already demonstrated that the CjaA protein is antigenically conserved among different serotypes of *C. jejuni* and *C. coli* (Pawelec and others 2000), implying it might fulfill one of the important standards for being an effective vaccine. The CjaD/Omp18 protein, on the other hand, was assessed to be highly conserved among *C. jejuni* strains isolated from humans, dogs, cats, calves, and chickens, but was not conserved in other *Campylobacter* species (Burnens and others 1995), indicating the vaccine will probably not be cross-protective. Further investigation of new subunit vaccines should be directed toward conserved proteins (like CjaA) and the mode of vaccine delivery. Moreover, futher studies should elucidate the mechanisms of vaccination and *in vivo* large-scale studies under working conditions should be done.

Hermans and others (2014) studied the use of passive immunization to reduce *Campylobacter* colonization in broiler chicks. Laying hens were immunized with either a whole-cell lysate or the hydrophobic protein fraction of *C. jejuni* and their eggs were collected. *In vitro* specific immunoglobulinY (IgY) against *C. jejuni* was induced significantly in the egg yolks of immunized hens. When the hyperimmune egg yolk was administered preventively, bacterial counts of seeder animals were significantly ($P < 0.01$) reduced 3 d after oral inoculation with approximately 10^4 CFU *C. jejuni*, compared to control birds. Transmission to nonseeder birds was dramatically reduced (hydrophobic protein fraction) or even completely prevented (whole-cell lysate). The *in vivo* mode of action was supposed to be enhanced mucosal clearance as purified IgY promoted bacterial binding to chicken intestinal mucus. Immunodominant antigens of *C. jejuni* reacting with the hyperimmune egg yolk IgY are involved in a variety of cell functions, including chemotaxis and adhesion. Some are highly conserved proteins (ATP synthase F1, alpha subunit AtpA, translation elongation factor thermo unstable EF-Tu, Co-chaperonin GroEL, and putative secreted carboxyl-terminal protease CtpA) and could be studied as subunit vaccines.

Bacteriophages

Because of the dramatic rise in multidrug-resistant bacteria, bacteriophage therapy has been investigated more closely as an alternative to combat infectious bacteria (Monk and others 2010). Bacteriophages are natural predators/viruses that can infect, multiply, and kill susceptible bacteria. The total number of bacteriophages in the biosphere has been estimated to be in the region of 10^{31} (Hendrix and others 1999). They are ubiquitous in the environment, have high host-specificity, are self-limiting, and are self-replicating in their target bacterial cell (Sulakvelidze and others 2001; Labrie and others 2010). As the activity of bacteriophages is maximal at the optimal growth temperature of their host (Hudson and others 2005), which is about 41.5 °C in the case of *C. jejuni*, bacteriophage therapy is best used during rearing, although it can also reduce the *Campylobacter* load on chicken skin after slaughter, by 1.1 to 2.0 $\log_{10}$ CFU/cm^2 of chicken skin, depending on bacteriophage titer applied (Atterbury and others 2003; Goode and others 2003). Atterbury and others (2003) even demonstrated a reduction of 2.5 $\log_{10}$ CFU/cm^2 of chicken skin when chicken skin was frozen.

These traits indicate that bacteriophage therapy can become a promising tool in combatting *Campylobacter* colonization in broilers, but it should meet a few requirements. First, phages used should have a broad host spectrum and, thus, should be able to kill multiple *C. jejuni* and *C. coli* strains. Table 2 shows the reduction of *C. jejuni* and *C. coli* obtained in broiler chickens when bacteriophage therapy was applied in *in vivo* experiments. Depending on the mode of administration, in most cases, the *in vivo* experiments resulted in a 2 $\log_{10}$ decrease in *Campylobacter* numbers in either cecal content or feces. Additionally, Atterbury and others (2005) demonstrated that in an on-farm study in UK flocks the natural presence of bacteriophages resulted in a reduction of *Campylobacter* numbers by about 100-fold. It was also assessed that viable *Campylobacter* cells could be recovered from only 29% of the phage-positive ceca as opposed to 71% of the phage-negative ceca. Second, bacteriophages selected for use against *Campylobacter* in broilers should also have an obligate lytic life cycle, lysing bacterial host cells. If bacteriophages follow a lysogenic life cycle, they can integrate their DNA into the host genome and, besides leaving the host bacterium intact, may render it immune to infection through the production of a phage-encoded repressor (Connerton and others 2011). Third, phage therapy should be safe and cost-effective. On the subject of safety issues, it is believed that oral consumption of phages by humans is harmless. Furthermore, due to their host specificity, bacteriophages will have only minimal effects on other microbial populations (Wagenaar and others 2005). As to cost-effectiveness, Havelaar and others (2007) calculated in a *Campylobacter* risk management assessment that, based on *in vivo* results by Wagenaar and others (2005), applying bacteriophage therapy on broilers prior to slaughter was fairly cost-effective compared to other intervention measures, especially when only positive flocks were phage-treated. A comparable result was obtained by Gellynck and others (2008) who demonstrated a positive cost-benefit ratio of 2.54 for phage therapy. Fourth, in response to concerns that *Campylobacter* will become resistant to phages (Barrow 2001) and efficiency of phage therapy would be lost, Labrie and others (2010) reported that phages are able to overcome bacterial resistance. El-Shibiny and others (2009) also stated that phages constantly evolve to evade host infection barriers. Several *in vivo* studies with phage therapy could only identify a low resistance (El-Shibiny and others 2009; Carvalho and others 2010), although these studies cannot predict long-term outcome. Additionally, it has been demonstrated that phage-resistant mutations in bacteria are correlated with reduced virulence *in vivo* (Connerton and others 2004; Loc Carillo and others 2005). On a negative note, Scott and others (2007) demonstrated that the presence of bacteriophages could constitute a strong selective pressure granting a competitive advantage to a *C. jejuni* strain insensitive to phages. Normally this strain would have been dominated by *C. jejuni* strains sensitive to phages. Another study also demonstrated the succession of phage-insensitive *Campylobacter* types within broiler flocks that naturally harbor phages (Connerton and others 2004).

Wagenaar and others (2005) demonstrated in small-scale *in vivo* experiments that the concentration of *Campylobacter* in the feces could be reduced 100 times by using therapeutic or preventive bacteriophage therapy, although after 5 to 7 d bacterial counts stabilized 10 times lower than that of the control group. According to Havelaar and others (2007), this could reduce the risk for the Dutch consumer by approximately 75%. This would

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Table 2–Overview of studies on bacteriophage therapy against *Campylobacter* in chickens.

Type of bacteriophage therapy	Administration	Effect	Ref
Group II *Campylobacter* phage CP220	Orally in therapeutic design[a] 5, 7, or 9 log PFU[b] of CP220 given once	In *C. jejuni* challenge: approximately 2 log reduction in cfu/g cecal content with 7 log PFU during 48 h. From day 3 on: less effective. In *C. coli* challenge: approximately 2 log reduction in cfu/g cecal content with 9 log PFU during 48 h. Resistance: 2% of *C. jejuni* isolates from treated birds	El-Shibiny and others (2009)
Bacteriophages 69 (NCTC 12669) and 71 (NCTC 12671)	Orally in therapeutic and preventive design[c] given daily	Therapeutic: 3 log decline of *C. jejuni in ceca* initially after 5 d; 1 log decline preventive: delayed cecal colonization and 2 log reduction after 7 d; 1 log reduction	Wagenaar and others (2005)
Bacteriophages CP8 and CP34	Orally administered in antacid suspension (therapeutic) 5, 7, or 9 log PFU given once	0.5 and 5 log reduction in cfu/g cecal content over 5 d period dependending on phage-Campylobacter combination, phage dose applied and time elapsed after administration.	Loc Carillo and others (2005)
Phage cocktail (phiCcoIBB35, phiCcoIBB37, phiCcoIBB12)	Orally In feed	*C. jejuni and C. coli*: 2 log cfu reduction per g feces *C. jejuni* and *C. coli*: 2 log cfu reduction per g feces 2 d earlier then in oral administration resistance: frequency of 13%. No reduced ability to colonize the chicken guts	Carvalho and others (2010)

[a] Therapeutic design: phage administered after *Campylobacter* inoculation. [b] PFU: plaque forming units. [c] Preventive design: phage administered before *Campylobacter* inoculation.

lead to approximately 7000 prevented cases of campylobacteriosis. Even when reduction of the concentration in the feces is only 1 log-unit, the risk of campylobacteriosis would be reduced by approximately 45%. However, when there is also a reduction of the exterior contamination of chickens by 1 log-unit, the risk reduction for consumers could be approximately 90% (Havelaar and others 2007). These results should, however, still be confirmed in practice. Furthermore, applying phage therapy should not select *C. jejuni* and *C. coli* strains insensitive to phages, thereby shifting the problem to other strains. This effect might be counteracted by using a cocktail of a wide spectrum of phages that broadens the host spectrum as much as possible.

Influence of other feed and drinking water properties on *Campylobacter* colonization

Other feed and drinking water properties can also influence *Campylobacter* colonization in broilers. Broilers fed plant protein-based feed had lower cecal *Campylobacter* colonization levels, compared to broilers fed animal or animal/plant protein-based feed, although there was no statistically significant difference between the feed groups in the shedding of *C. jejuni* (Udayamputhoor and others 2003). This plant protein-based feed can contain undigestible carbohydrates, which might influence pH, lactic acid content, and other properties influencing survival of microorganisms. Hinton and others (2002) demonstrated that broilers given drinking water supplemented with 4% sucrose had significantly lower *Campylobacter* numbers in their crop (4.22 ± 3.87 log CFU/g crop tissue) than control broilers (7.31 ± 0.12 log CFU/g crop tissue). This was probably due to the decrease in pH because of the elevated presence of lactic acid bacteria thriving on the sucrose.

Moen and others (2012) offered broilers a diet supplemented with 15% oat/barley hulls for structure. They demonstrated that *C. jejuni* spread in a broiler group was delayed and that the relative amount of *C. jejuni* in the cecum was reduced. A comparable study researching interference of *Campylobacter* adhesion to the host cell due to addition, in feed, of large molecules, namely glucuronic acid-enriched polysaccharides from immature okra fruits, had no effect. This was probably due to metabolic breakdown of these molecules in the broiler chicken gastrointestinal tract (Wittschier and others 2007).

Probiotics, competitive exclusion, and prebiotics

Probiotic bacteria are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Rijkers and others 2010). The precise mode of action of probiotics is not always known, but they are presumed to act in different ways. They can interact directly with gut microbiota and thus influence the microbial ecosystems in the gut lumen. They can also interact with the gut mucus and the epithelium and influence barrier effects, digestive processes, the mucosal immune system, and the enteric nervous system or provide signaling to the host and influence activity of the immune system and various organs. Competitive exclusion (CE) was first described by Nurmi and Rantala (1973) and is based on supplying nonpathogenic microorganisms to the host. These microorganisms may in turn occupy the same ecological niche as the undesired/pathogenic bacteria and reduce or remove these bacteria. This can happen due to competition for nutrients or production of inhibitors such as lactic acid, hydrogen peroxide, and bacteriocins (see below). Prebiotics are nondigestible food ingredients and carbohydrates that stimulate the growth and/or activity of bacteria, probiotic ones and other ones, in a way that is beneficial

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to the host (Gibson and Roberfroid 1995). The term synbiotics is used when both prebiotic and probiotic concepts are put together.

Feeding CE preparations to broilers has given variable results. Some studies demonstrated a reduction of *Campylobacter* colonization (Soerjadi and others 1982; Soerjadi-Liem and others 1984; Stern and others 1988; Mulder and Bolder 1991; Aho and others 1992; Schoeni and Doyle 1992; Stern 1992, 1994; Scupham and others 2010), while other studies demonstrated only limited success in preventing cecal colonization (Stern and others 2005, 2008). These CE preparations can be defined or undefined. Early studies collected strains from adult chickens free of pathogens and administered these to young chicks. These CE organisms were originally isolated from feces with later isolations from ceca (Soerjadi-Liem and others 1984). After Beery and others (1988) determined *Campylobacter* was located primarily in mucus-rich cecal crypts, bacteria were isolated from intestinal mucus (Stern 1994; Mead and others 1996). Unfortunately, cecal and mucosal microbiota was not more efficient in preventing *Campylobacter* colonization of chickens than previously used CE organisms. Mead and others (1996), however, showed that when chicks were given anaerobic preparations of cecal mucus from *Campylobacter*-free adult hens, they were partly protected against *C. jejuni*. Hakkinen and Schneitz (1999) have tested Broilact® (Orion Corporation, Helsinki, Finland), a commercially available lyophilized CE product, containing 32 different bacterial strains, that is already used in the prevention of *Salmonella* in broilers. Broilact® was able to reduce *C. jejuni* counts 10^8- to 10^9-fold and to lower the percentage of colonized birds by 38 to 100% in 2 separate *in vivo* trials. In a third trial, however, the counts and the proportion of colonized chicks in the treated groups were higher. Aho and others (1992) reported Broilact® was not capable of inhibiting *Campylobacter*. However, when Broilact® was combined with bacteria, identified as K-bacteria, a 1.5 to 2.0 $\log_{10}$ reduction in CFU/g cecal content could be demonstrated at slaughter.

In vitro studies have identified different probiotic strains, belonging to diverse genera like *Bacillus*, *Paenibacillus*, *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Megamonas*, *Enterococcus,* or species like *Escherichia coli* to be effective against *C. jejuni* by bacteriocin production or other mechanisms of action. Different authors (Fooks and Gibson 2002; Chaveerach and others 2004; Nazef and others 2008; Svetoch and others 2008; Svetoch and Stern 2010; Robyn and others 2012) have demonstrated the bactericidal effect of a selected anti-*Campylobacter probiotic* strain in an agar diffusion assay, a co-culture study, or in a batch fermentation study in the presence of a *C. jejuni* strain. In the agar diffusion assays, anti-*Campylobacter* activity was assessed by observing a zone of *Campylobacter* growth inhibition formed around the selected anti-*Campylobacter* strain (Nazef and others 2008; Svetoch and others 2008; Svetoch and Stern 2010). In the co-culture studies or in batch fermentation studies *C. jejuni* numbers dropped between 1 $\log_{10}$ CFU/mL to under the detection limit in the co-culture (Fooks and Gibson 2002; Chaveerach and others 2004; Robyn and others 2012).

In vivo experiments have given mixed results, which could be due to the protective effect of mucus, different niches inhabited by the probotioc strain and *C. jejuni*, and so on. Line and others (1998) supplemented feed with *Saccharomyces boulardii* and could not prove a significant cecal *Campylobacter* reduction. Neither could probiotic *Lactobacillus salivarius* or *Paenibacillus polymyxa* strains, although they are both able to produce bacteriocins. The use of their purified bacteriocin, on the other hand, could influence cecal *C. jejuni* numbers (see below) (Stern and others 2008). Robyn and others (2013b) tested an *E. faecalis* strain for *in vivo* anti-*C. jejuni* activity. Despite *in vitro* activity, no inhibition was observed in the *in vivo* experiments independent of the inoculum size. Other probiotic strains tested showed a reductive or preventive effect on *C. jejuni* colonization levels or fecal shedding: *Lactobacillus acidophilus* and *Enterococcus faecium* (Morishita and others 1997), *Citrobacter diversus*, *Klebsiella pneumoniae,* and *E. coli* (Schoeni and Wong 1994), *L. acidophilus*, *L. fermentum*, *L. crispatus,* and *L. brevis* (Chang and Chen 2000). A low, although significant, reduction in *C. jejuni* numbers in feces (one $\log_{10}$ CFU) was also obtained when a *Bifidobacterium longum* strain was administred daily for 2 weeks to broilers (Santini and others 2010).

Other studies tested chicken *C. jejuni* strains not associated with human disease to control the colonization of *C. jejuni* in broilers (Barrow and Page 2000; Chen and Stern 2001). Results obtained during these studies showed that pre-inoculation of a *C. jejuni* strain could prevent cecal establishment of a *C. jejuni* strain inoculated later. They demonstrated that strains showing superior colonizing ability were the dominant strains in the *Campylobacter* populations in chicken ceca. Theoretically this would mean that when nonpathogenic, strongly colonizing *C. jejuni* strains are identified, these strains can potentially be used to exclude pathogenic *C. jejuni* from broiler ceca. Calderon-Gomez and others (2009) identified a highly colonizing strain that could not be displaced by other colonizing strains. However, this measure does not take into account that *Campylobacter* species are capable of exchanging virulence properties (Zhou and others 2013) detrimental to humans with other *Campylobacter* species.

Not many prebiotics have been tested for use as anti-*Campylobacter* additives in feed or drinking water. When mannose-oligosaccharide or xylanase were added to feed supplied to naturally and artificially infected broilers, respectively, a significant, though small, decrease in cecal C. *jejuni* counts was observed (Fernandez and others 2000; Baurhoo and others 2009). Prebiotics, however, have been tested more often as part of synbiotics, meaning the combined use of pre- and probiotics. Fooks and Gibson (2002) identified different prebiotics (oligofructose [FOS] and mixtures of inulin:FOS and FOS: xylo-oligosaccharide) that inhibit pathogen growth (*E. coli*, *C. jejuni*, and *S.* Enteritidis) strongly when combined with *L. plantarum* or *Bifidobacterium bifidum in vitro*. An *in vivo* protective effect against *C. jejuni* broiler colonization was observed by Schoeni and Wong (1994) who administred CE cultures of *Citrobacter diversus*, *Klebsiella pneumoniae,* and *E. coli* in combination with mannose to the chickens. Baffoni and others (2012) reported that the combined use of a *Bifidobacterium longum* subsp. *longum* strain with a galacto-oligosaccharide, both microencapsulated in a lipid matrix and added to feed, was able to significantly reduce *C. jejuni* in broiler feces by $\pm$ 1 $\log_{10}$ CFU.

Although CE, probiotics, prebiotics, and synbiotics have generated promising results, further investigation is needed. Undefined CE products can still contain strains detrimental to humans. Unraveling the mechanisms of *Campylobacter* protection by these products is of primary importance. Ganan and others (2012) have already stated that the human probiotic strains *L. rhamnosus* GG, *Propionibacterium freudenreichii* ssp. *shermanii* JS, and the starter culture bacteria *Lactoccoccus lactis* ssp. *lactis* strain were able to adhere well to chicken intestinal mucus. This adhesion reduced the binding of any *Campylobacter* spp. to mucus when aforementioned strains colonized the mucus prior to the pathogen. This mechanism might theoretically influence mucosal *Campylobacter* clearance as well as the interaction of any

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Campylobacter species with antibacterial components, the activity of which can be hindered by the presence of mucus.

Bacteriocins

Bacteriocins are a heterogeneous group of bacterial peptides active against other bacteria (mostly closely related ones). They can be ribosomally or nonribosomally synthesized and can be postribosomally modified. Additionally, the producer has a specific immunity mechanism against these active peptides. They vary in spectrum of activity, mode of action, molecular weight, genetic origin, and biochemical properties (Klaenhammer 1993; Abee and others 1995). They are mainly cationic, hydrophobic, or amphiphilic peptides, with molecular weights of <5 to >60 kDa and between 20 to >700 amino acids (Nissen-Meyer and Nes 1997; Riley and Wertz 2002). They are produced by Gram-positive as well as Gram-negative microorganisms. There are 4 major classes of bacteriocins: Class I: lantibiotics, Class II: small heat-stable peptides, Class III: large heat-labile proteins, and Class IV: complex proteins, the activity of which requires the association of carbohydrate or lipid moieties (Klaenhammer 1993; Abee and others 1995).

Research into bacteriocins displaying activity against pathogens has been ongoing for several years. Nazef and others (2008) identified 2 bacteriocins produced by *Enterococcus faecalis* and *L. reuteri* having *in vitro* anti-*C. jejuni* activity, while Messaoudi and others (2011) isolated 3 *L. salivarius* strains from chicken ceca which produced a bacteriocin with antagonistic activity against *C. jejuni* and *C. coli*. Jones and others (2008) identified lactic acid bacteria from meat products able to inhibit *C. jejuni* growth *in vitro*. Inhibition appeared to be mediated by cell-associated molecules or by molecules released extracellularly with bacteriocin-like properties. Svetoch and others (2005) reported the identification of bacteriocins produced by *Bacillus circulans* and *Paenibacillus polymyxa* isolates (Svetoch and others 2005). When the purified *P. polymyxa*-produced bacteriocin (B 602) was added to feed and given to already colonized broilers from day 8 to 10 post hatch, a 6 to 8 $\log_{10}$ CFU reduction in cecal *C. jejuni* numbers was found (Stern and others 2005). Providing broilers with feed or drinking water supplemented with a purified bacteriocin (OR 7 or L-1077, respctively) produced by 2 different *L. salivarius* strains resulted in a significant decrease of *C. jejuni* numbers in the chicken gut (Stern and others 2006; Svetoch and others 2011). OR 7 was given from day 7 to day 10 post hatch, while L-1077 was provided for 3 d to naturally *Campylobacter*-infected broilers at market age. Bacteriocin L-1077 reduced cecal *C. jejuni* numbers by 4 $\log_{10}$ CFU in market-aged broilers (41 to 43 days old), while OR 7 reduced cecal *C. jejuni* numbers by 6 $\log_{10}$ CFU in broilers aged 10 d. Cole and others (2006) used both bacteriocins in a turkey *in vivo* experiment and detected no *C. coli* in turkey ceca, with a detection limit of 10^2 CFU/g. Later studies identified bacteriocins produced by *E. durans/E. faecium/E. hirae* (E 760) and *E. faecium* (E 50–52) (Line and others 2008; Svetoch and others 2008). In *in vivo* surveys, the E 760 bacteriocin was supplied in feed to market-age broilers during the 4 d leading up to euthanasia, while E 50–52 was given in feed from day 4 to 7 post hatch with analysis at day 15. E 50–52 was also given in drinking water for a 3-d period to market-age broilers (41 d). In the first 2 surveys, *C. jejuni* numbers were reduced below detectable levels, while in the third trial *C. jejuni* numbers were reduced 5 $\log_{10}$ CFU/g cecal content.

The safety of these bacteriocins for humans and animals was also validated in experiments with Vero and Hela cells and in mice and chickens (Svetoch and Stern 2010).

Results obtained with purified bacteriocin in feed and drinking water indicate their preferred use to live bacterial cells, although possible mechanisms for resistance to bacteriocins have already been proposed (Kaur and others 2011).

Summary

Measures directed toward lowering the *Campylobacter* prevalence in flocks during rearing should be integrated in a wider set of control measures covering the entire poultry meat production process, including consumption. Measures should not only focus on rearing, but also on lowering or avoiding external contamination of broiler and broiler carcasses during transport and slaughtering. Steps can also be undertaken to lower *Campylobacter* contamination on broiler meat products and on informing consumers. However, when internal colonization is lower, the effectiveness of measures to avoid or remove external *Campylobacter* contamination will probably increase.

As several recent studies have shown, measures aimed at reducing *C. jejuni* colonization of broilers during rearing has resulted in contradictory results (Stern and others 2005; Solis de Los Santos and others 2009; Hermans and others 2010; 2011a; Baffoni and others 2012). Based on different studies, lowering or delaying *Campylobacter* colonization should be done by applying a combination of measures directed against both introduction of *Campylobacter* into a broiler flock, but also directed towards lowering *Campylobacter* survival in broilers, in the broiler house and in its surroundings. Table 3 gives an overview of measures with an effect on *C. jejuni* colonization in broilers that need to be confirmed in on-farm studies.

Controlling *Campylobacter* introduction into a broiler flock during rearing starts with good biosecurity measures. Studies have indicated that increasing biosecurity can have a positive effect (Hald and others 2007b; Katsma and others 2007). Several measures can be taken to lower the infection pressure of *Campylobacter* or to delay *Campylobacter* colonization. Installing fly screens and concrete aprons and strictly following biosecurity measures by both thinning crew and farm workers can influence *Campylobacter* prevalence. Adequate cleaning and disinfecting of broiler houses, to avoid carry-over to successive flocks, should always be accompanied by adequate management and biosecurity practices, as well as pest control Stopping partial depopulation can influence the prevalence of *Campylobacter*-positive broilers, as partial depopulation is an important risk factor for *Campylobacter* introduction (Rasschaert and others 2007; Ridley and others 2011b). In Denmark, abandoning partial depopulation is now part of the overall strategy to lower campylobacteriosis cases and has generated good results (Rosenquist and others 2009). The presence of multiple broiler houses on the farm, other neighboring livestock on the farm or on nearby farms results in an increased risk of *C. jejuni*-positive flocks when an effectieve hygiene barrier is absent. Hald and others (2000) stated that the presence of a hygiene barrier was the single-most important biosecurity measure for production of *Campylobacter*-free broilers.

Although fresh litter, dry used litter and drinking water in most cases are not directly responsible for the introduction of *Campylobacter* in flocks, they can play a role in the spread of *Campylobacter* throughout the flock and in maintaining persistent *Campylobacter* colonization as they can be a source of a constant *C. jejuni* colonization pressure after *C. jejuni* introduction. Once *Campylobacter* infection is established in a part of the flock, further *Campylobacter* transmission can thus be delayed or partly avoided by applying a number of measures like acidifying drinking

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Table 3–Overview of investigated measures with an effect on *C. jejuni* colonization in broilers that need to be confirmed in on-farm studies.

Type of measure/risk factor	Type of study	Effect	Ref
Presence of effective hygiene barrier	Longitudinal study	Hygiene barrier absent: 30% to 50% higher *campylobacter* flock positivity	Hald and others (2000)
Good use of footdips in hygiene barrier	Longitudinal study	Hazard ratio of 1,58 with inadequate food dip use	Evans and Sayers (2000)
Use of fly nets	Field study	Number of *Campylobacter*-positive flocks dropped by 36%	Hald and others (2007bb)
Old broiler houses	Longitudinal study	Hazard ratio of 1,60 to 2,45 if houses in need of repair	Hald and others (2000), Chowdhury and others (2012)
Adding lactic acid to drinking water during feed-withdrawal period	*In vivo* study	Crop and pre-chill carcass contamination reduced by 20% and 15%, respectively	Byrd and others (2001)
Adding mixture of MCFA to drinking water	*In vivo* study	Reduces colonization susceptibility and prevents *C. jejuni* survival in drinking water	Hermans and others (2012a)a
coated or uncoated caprylic and capric acid in feed used prophylactic	*In vivo* study	0,1 to 4,1 log CFU/g cecal content reduction up to 4 d after *C. jejuni* inoculation	Molatová and others (2011)
Therapeutic use of caprylic acid in feed	*In vivo* study	3 to 4 log CFU reduction of *C. jejuni* in broilers	Solis de los Santos and others (2008, 2009, 2010)
1% MCFA mixture in feed	*In vivo* study	Increase (200×) in *C. jejuni* inoculation dose necessary to colonize 50% of broilers	van Gerwe and others (2010)
2% formic acid + 0,1% sorbate in feed used prophylactic	*In vivo* study	Prevention of *C. jejuni* colonization in broilers	Skånseng and others (2010)
0.24% monocaprin + 0.04% polysorbaat in feed an drinking water, therapeutic use	*In vivo* study	1 to 2 log CFU/ml reduction of *C. jejuni* in cloacal swabs of 36-day-old naturally colonized birds	Hilmarsson and others (2006)
5.7% lactic acid + 0.7% acetic acid in feed	*In vivo* study	Hazard of infection reduced by 0,31	Heres and others (2004)
Therapeutic or prophylactic use of bacteriophages	*In vivo* study	1 $\log_{10}$ recution of *Campylobacter* in feces	Wagenaar and other (2005)
Prophylactic use of live probiotic strains, i.e. *L. acidophilus*, *L. fermentum*, *L. brevis*, *E. faecium*, *L. crispatus*,*B. longum*, . . .	*In vivo* study	Reduction/prevention of C. jejuni colonization or fecal shedding	Chang and Chen (2000), Schoeni and Wong (1994), Santini and others (2010)
Purified *P. polymyxa* bactericocin in feed given therapeutically to orally inoculated birds	*In vivo* study	6–8 $\log_{10}$ reduction in cecal numbers at 10 d of age	Stern and others (2005)
Purified *L. salivarius* bacteriocin in feed given therapeutically for 7 to 9 d	*In vivo* study	6 $\log_{10}$ reduction in cecal numbers at 10 d of age	Stern and others (2006)
Purified *L. salivarius* bacteriocin in drinking water given therapeutically for 1 to 3 d	*In vivo* study	4 $\log_{10}$ reduction in cecal numbers at 42 to 43 d of age	Svetoch and others (2011)
Purified *Enterococcus* bacteriocin in feed given therapeutically for 4 d	*In vivo* study	reduction below detectable levels in ceca	Line and others (2008)
Purified *Enterococcus faecium* bacteriocin in feed given therapeutically for 4 d	*In vivo* study	reduction below detectable levels in ceca at 15 d of age	Svetoch and others (2008)
Purified *Enterococcus faecium* bacteriocin in feed given therapeutically for 3 d	*In vivo* study	5 $\log_{10}$ reduction in cecal numbers in market-aged broilers	Svetoch and others (2008)

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water or litter to lower *Campylobacter* survival and transmission in the flock (Hermans and others 2012a; Line and Bailey 2006).

On the other hand, bacteriophages, bacteriocins, and passive immunization seem to be the most promising, though still experimental, measures to combat *Campylobacter* survival in broilers. Especially the use of passive immunization has resulted in marked decreases in broilers in preliminary research (Hermans and others 2014). However, passive immunization is expensive. Recent studies have focused on using purified bacteriocins, produced by lactic acid bacteria, as feed additives to primarily lower *C. jejuni* numbers in the broiler cecum (Svetoch and others 2005; Stern and others 2005; Stern and others 2006; Svetoch and others 2011). Most of these bacteriocins with demonstrated anti-*C. jejuni* activity in broilers belonged to class IIa bacteriocins. Results obtained with bacteriocins in market-aged broilers showed reductions up to 5 log in the ceca (Svetoch and others 2008; 2011). This indicates that the use of bacteriocins can be a very promising *C. jejuni* combatting strategy. The price of producing and coating bacteriocins on a large scale can, however, pose a problem and using bacteriocins can result in either resistant strains or a shift in the *Campylobacter* strains colonizing broilers. However, based on unpublished results, Svetoch and Stern (2010) stated that the development of resistance to one bacteriocin does not provoke bacterial cross-resistance to other bacteriocins. They also explained that class IIA bacteriocins probably do not generate or generate a low frequency of bacteriocin-resistant mutants.

Based on different studies, lowering or delaying *Campylobacter* colonization should probably be done by a combination of the described measures.

In a second phase, controlling external contamination of broilers during transport and slaughter should be increased. The most obvious control measures lower cross-contamination due to contaminated transport crates and equipment. This can be done by applying strict sanitation of both transportation equipment and workers' clothing. It has been demonstrated that standard crate-washing procedures do not effectively remove *Campylobacter* from crate surfaces (Slader and others 2002; Ramabu and others 2004; Rasschaert and others 2007).

In Sweden, Norway, and Denmark, flocks are all sampled prior to slaughter, at slaughter, or at both points (Hofshagen and Kruse, 2005; Hansson and others 2007a; Rosenquist and others 2009; Sears and others 2011). Based on these results, both logistic slaughter and scheduling of contaminated meat for heat treatment or for use in frozen products is done. This has all contributed to the decline in the number of campylobacteriosis cases.

Lastly, public education should be 2-fold. It should focus on correct packaging of meat products at the retail store, but also on correct handling of the product, on proper kitchen hygiene, and on correct food preperation at the consumer or restaurant kitchen level.

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