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REVIEW ARTICLE

Control strategies against *Campylobacter* at the poultry production level: biosecurity measures, feed additives and vaccination

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Summary

Campylobacteriosis is the most prevalent bacterial foodborne gastroenteritis affecting humans in the European Union, and ranks second in the United States only behind salmonellosis. In Europe, there are about nine million cases of campylobacteriosis every year, making the disease a major public health issue. Human cases are mainly caused by the zoonotic pathogen *Campylobacter jejuni*. The main source of contamination is handling or consumption of poultry meat. Poultry constitutes the main reservoir of *Campylobacter*, substantial quantities of which are found in the intestines following rapid, intense colonization. Reducing *Campylobacter* levels in the poultry chain would decrease the incidence of human campylobacteriosis. As primary production is a crucial step in *Campylobacter* poultry contamination, controlling the infection at this level could impact the following links along the food chain (slaughter, retail and consumption). This review describes the control strategies implemented during the past few decades in primary poultry production, including the most recent studies. In fact, the implementation of biosecurity and hygiene measures is described, as well as the immune strategy with passive immunization and vaccination trials and the nutritional strategy with the administration of organic and fatty acids, essential oil and plant-derived compound, probiotics, bacteriocins and bacteriophages.

Introduction

Campylobacter is a spiral-shaped Gram-negative microorganism which grows under microaerophilic conditions. Since 2005, it has been the leading cause of human bacterial gastroenteritis in the European Union (EFSA 2015), affecting approximately nine million people each year and costing around €2·4 billion per year (EFSA 2011).

Two species are mainly responsible for human diseases: *Campylobacter jejuni*, causing for approx. 90% of cases, and *Camp. coli* <10%. Other species, such as *Camp. lari* and *Camp. fetus* (*Camp. fetus* is often isolated from septicaemia), rarely cause human diseases (Gillespie *et al.* 2002; Wagenaar *et al.* 2014; http://www.cnrch.u-bordeaux2.fr/wp-content/uploads/2011/03/BilanCampylobacter20121.pdf). After contamination (a dose of 500–800 colony forming units (CFU) could be sufficient (Robinson 1981; Black *et al.* 1988)), *Campylobacter* colonizes the lower part of the intestines, including the ileum, jejunum and colon. The severity of the illness varies greatly between patients according to the strain's virulence and the host's receptivity. It can induce mild disease up to cases of dehydration. The main symptoms are diarrhoea, abdominal pain and fever. Vomiting, bloody diarrhoea and bacteraemia are also reported (Gillespie *et al.* 2002; Janssen *et al.* 2008; Dasti *et al.* 2010).

This bacterial infection can also lead to extra-intestinal manifestations with more serious sequels (Janssen *et al.*

2008) such as reactive arthritis (ReA), characterized by sterile articular inflammation, or the autoimmune Guillain–Barré syndrome (GBS), an acute auto-immune disorder affecting the peripheral nervous system (Nyati and Nyati 2013). This disease could be due to molecular mimicry between the lipo-oligosaccharides (LOS) of some *Camp. jejuni* strains and human gangliosides, inducing cross-immune reaction of anti-*Campylobacter* antibodies (Perera *et al.* 2007; Nyati and Nyati 2013). Moreover, *Campylobacter* strains have been isolated from a number of patients with inflammatory bowel disease (IBD), including Crohn's disease (Janssen *et al.* 2008). In some cases, *Campylobacter* infections can lead to death. This mortality rate was evaluated at about 0·02% in England and Wales (Adak *et al.* 2005) and at about 0·04% in Netherland in 2009 (Havelaar *et al.* 2012).

Most *Campylobacter* infections are not severe, are resolved in few days and do not require an antibiotic treatment. However, old, young and immune-compromised individuals can suffer from severe and prolonged infections needing antibiotic treatment. Erythromycin is used as a first-line treatment (Allos 2001). Fluoroquinolones are also frequently used due to their broad spectrum of activity against enteric pathogens. Tetracycline and gentamycin are dispensed for systemic infections, but cases of resistance to all these antimicrobial agents are constantly increasing, making campylobacteriosis a major public health concern (Luangtongkum *et al.* 2009).

Human infections are mainly due to handling and/or consumption of raw or undercooked poultry meat (EFSA 2015). Seasonal incidence is observed for human campylobacteriosis, with higher rates during the summer, when a higher incidence in poultry colonization is observed. *Campylobacter jejuni* and *Camp. coli* are zoonotic strains that can infect farm animals such as poultry, cattle, pigs and sheep in addition to wild birds and mammals. A report from the European Food Safety Authority (EFSA) states that contaminated broiler meat could account for 20–30% of human campylobacteriosis, while the chicken reservoir as a whole could be responsible for 50–80% of cases due to strains reaching humans by ways other than food (EFSA 2011); confirming Wilson *et al.* (2008) results. In contrast, wild animals, water or pets represent a minor source of human contamination (approx. 3%). Although C*ampylobacter* infections are typically sporadic, outbreaks mainly related to water (Jakopanec *et al.* 2008) or raw milk contamination (Heuvelink *et al.* 2009) can occur.

In Europe, the mean prevalence of *Campylobacter* in primary poultry production is very high, up to 70% of broiler batches being contaminated (EFSA 2010). Large differences of between 2 to 100% are observed between countries. Moreover, the prevalence of *Campylobacter* on broiler carcasses is much higher at the slaughterhouse due to cross contamination between infected and noninfected birds, standing at about 75% in Europe. On the whole, northern countries are less impacted than others.

Broiler chickens are commonly considered a natural host for *Campylobacter*. Colonized birds can carry high levels of *Campylobacter* (from 10^6 to 10^9 CFU g^{-1}) and remain infected until slaughter. This bacterium usually colonizes the mucus layer over the epithelial cells of the caecum and the small intestine (Meade *et al.* 2009; Hermans *et al.* 2012b). Meade *et al.* (2009) showed that the oesophagus was quickly colonized after an experimental infection. Dissemination to extra-intestinal organs such as the spleen, crop, gizzard or liver is also possible. Recent studies have suggested *Campylobacter*'s involvement in gut mucosa damage and problems with chicken feet and legs (Williams *et al.* 2013; Humphrey *et al.* 2014). These damages particularly affects fast-growing birds infected by *Campylobacter* reaching slaughter weight in only 35 days compared to slower-growing and/or noninfected chickens, and could be associated with a higher inflammatory response and a lower regulatory immune response compared to slower-growing breeds.

Many factors influence chicken colonization, including age, the infecting dose and the *Campylobacter* strain (Stas *et al.* 1999). As demonstrated by Messens *et al.* (2009), only one *Campylobacter* genotype can be found in flocks during rearing, but multiple genotypes may be recovered simultaneously or successively from broiler flocks. These results are in accordance with Bull *et al.* (2006) and Hue *et al.* (2011), and could be due to subsequent introduction or clone mutations.

In flocks, *Campylobacter* colonization naturally occurs by horizontal transmission from the environment in 2- or 3-week-old chicks due to the availability of protective maternal antibodies in chick sera in the first weeks posthatching (Sahin *et al.* 2003; Cawthraw and Newell 2010). Sahin *et al.* (2003) showed that anti-*Campylobacter* maternal antibodies significantly delay the onset of colonization in chicks obtained from hens already colonized by *Camp. jejuni* compared with chicks from specific-pathogen-free (SPF) laying hens. During the first weeks of life, maternal antibody levels progressively decrease until fully degraded at the end of the third week. In a more recent study, authors showed that antibodies grant chicks protection from homologous and heterologous colonization when experimentally challenged at 8 days old (Cawthraw and Newell 2010). After the first contamination, *Campylobacter* infection spreads very quickly in the flock by horizontal transmission from one bird to another. van Gerwe *et al.* (2009) estimated a transmission rate of 2·37 new infections per infected bird per day, confirming experimental results (Stern *et al.* 2001). This rate means that *Campylobacter* prevalence increases from one

infected bird to 95% of a whole flock of 20 000 broilers within a week. This very rapid *Campylobacter* transmission could be explained by high faecal shedding, the contamination of drinking water and litter, and the coprophagic behaviour of chickens.

In contrast to horizontal transmission, vertical transmission from breeding hens to their offspring is considered a minor source of *Campylobacter* infection (Bull *et al.* 2006). Its prevalence in eggs is virtually nonexistent or even nonexistent.

Campylobacter is ubiquitous in the environment, and broiler contamination sources are diverse (Hermans *et al.* 2012b). Wild and farm animals are a major risk for *Campylobacter* transmission to broiler flocks. The molecular typing of *Campylobacter*, for example, identified similar profiles on adjacent broiler and dairy farms (Ridley *et al.* 2011b). These results indicate a high risk of horizontal transmission between animals, particularly on farms with multiple species. Flies and rodents are also potential sources of contamination for broiler flocks. Flies act as a mechanical vector, and their abundance in summer could explain the higher prevalence of *Campylobacter* during this period, (Huneau-Salaun *et al.* 2007; Hue *et al.* 2010; Allain *et al.* 2014). Contaminated water from puddles and ditches could contribute to horizontal transmission. Strains isolated from puddles before the introduction of animals and those recovered later in flocks sometimes had the same genotype (Bull *et al.* 2006; Messens *et al.* 2009). Finally, vehicles, personal equipment and hauling crates are frequently contaminated by *Campylobacter* before arrival on the farm (Ridley *et al.* 2011a), making them a potential risk of broiler contamination, particularly during the thinning process aimed at partial depopulation, when equipment and workers are introduced into the flock. Recently, a publication studying *Campylobacter* contamination sources for farm poultry quoted all the above mentioned factors (Agunos *et al.* 2014). The authors concluded that the highest risk of contaminating a new flock appeared to be related to a persistently contaminated environment due to insufficient cleaning, disinfection and downtime between two flocks, and the second highest risk is the presence of adjacent poultry flocks.

Recently, EFSA provided a quantitative microbiological risk assessment of campylobacteriosis in Europe (Romero-Barrios *et al.* 2013). The assessment focused on the slaughterhouse and primary production. Controlling *Campylobacter* in broiler flocks could be highly beneficial to public health because of its impact all along the broiler food chain (slaughter, retail sales and consumption). It has been estimated that subjecting broiler carcasses to chemical treatment, a long or short freezing period or immersion in hot water could reduce human campylobacteriosis cases by 37–98% (Romero-Barrios *et al.* 2013). Irradiating or cooking meat on an industrial scale could even eliminate human campylobacteriosis. However, these processes are generally known to impact meat quality. In primary production, hygiene and biosecurity improvements and the restriction of the slaughter age could reduce risks. The most feasible and long-reaching measure is the reduction in caecal colonization between 2 and 3 $\log_{10}$ units, which could reduce the risk of human campylobacteriosis by 76–100% (Rosenquist *et al.* 2003). Measures could not be applied easily in all EU member states, and the lack of effective tools, particularly those aimed at reducing avian gut colonization, could limit the decrease in the risk of human campylobacteriosis.

In a recent review, Robyn *et al.* (2015) describe *Campylobacter* risk factors for broilers and measures trialled during the rearing period. However, other studies should also be taken into consideration. This present article reviews the control strategies implemented in the past few decades up to the present in order to reduce *Campylobacter* prevalence in primary poultry production to reduce the prevalence of human campylobacteriosis. Three control strategies are described: biosecurity, nutritional and immunization measures.

Control strategies

To limit human exposure to *Campylobacter,* the load on broiler carcasses needs to be decreased, mainly through the reduction in poultry colonization levels at the primary production stage. While carcass and meat treatment at the slaughterhouse impact *Campylobacter* levels in food, the public health benefits would be greater if bacteria in broiler chickens were reduced earlier in the production chain, as there are contamination pathways other than broiler meat consumption (EFSA 2011). Reducing intestinal colonization by *Campylobacter* appears to be the best strategy for reducing human campylobacteriosis, but also a real challenge because of the mainly commensal behaviour of this bacterium in the broiler gut. This strategy could be implemented through at least three measures: biosecurity measures to avoid flock contamination and transmission between different batches, nutritional measures through various substances, such as essential oils, pre- and probiotics, bacteriocins and bacteriophages, and immunization measures by passive immunization or vaccination (Fig. 1).

Several measures have already been tested with various results and are described in the following sections.

Security and hygiene measures

In European Union countries, several biosecurity control strategies aimed at reducing *Campylobacter* in broilers have

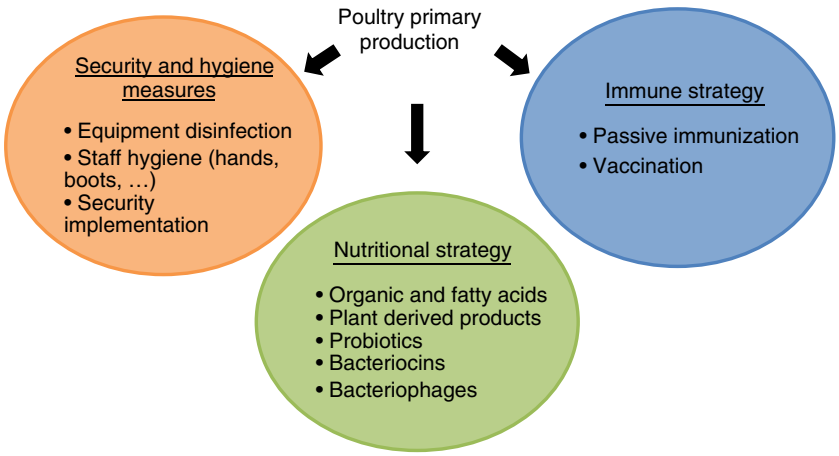

Figure 1 Control strategies implemented at the primary production level to limit the intestinal colonization of broilers by *Campylobacter* and reduce human campylobacteriosis cases.

been tested. In Denmark, the first initiatives date back to the '90s. They comprised hygiene measures, checking of broiler flocks and meat, and finally consumer information. In 2003, active strategies were developed that included initiatives in the production chain, meat treatment and consumer education (Rosenquist *et al.* 2009). This 5-year study led to a decrease from 43 to 27% of *Campylobacter*-positive flocks at slaughter. In Iceland, domestic campylobacteriosis cases in 2000 decreased from 116 to 33/100 000. This reduction was correlated with the implementation of various actions within the Icelandic poultry industry, including biological security measures, freezing of carcasses and increased consumer education (Stern *et al.* 2003). In this review, we focus on biosecurity measures applied to primary broiler production.

Biosecurity measures to reduce *Campylobacter* infection on farms can be applied at different levels. In Newell *et al.* (2011) reviewed *Campylobacter* transmission among poultry and biosecurity measures. As *Campylobacter* spreads rapidly throughout the flock mainly by horizontal transmission, the key goal is to prevent colonization of the first bird. There is a high risk of contamination by staff moving in and around the poultry farm. This risk could be reduced by biosecurity measures and hygienic practices such as the use of overshoes, disinfection dips for boots, boot changes between different poultry houses and washing hands before and after visits. A field study applying these measures reduced *Campylobacter* colonization by 50% (Gibbens *et al.* 2001). A British study showed that vehicle disinfection, hand washing and sanitization, dedicated footwear and personal equipment reduced the prevalence of *Campylobacter* on farm staff and transporters. The most marked improvement was observed for vehicles, prevalence dropping from 53% before disinfection measures to 18% afterwards. However, no effects were observed on chicken colonization: all the flocks were contaminated (Ridley *et al.* 2011a).

Nonpoultry livestock, wild and domestic animals can also act as a potential source of flock contamination, but their role remains unclear at best and studies are controversial (Newell *et al.* 2011). However, it has been shown that flies and other flying insects are involved in *Campylobacter* transmission, their impact varying according to the season and the country in relation to temperature and geographical location. In Denmark, the installation of fly screens on broiler houses significantly reduced the percentage of positive flocks from 51·4 to 15·4% (Hald *et al.* 2007). Another long-term study confirmed these initial results (Bahrndorff *et al.* 2013). It found a significant reduction in *Campylobacter* colonization of poultry after fly screens were added to the experimental poultry houses and indeed, a decrease in campylobacteriosis nationwide. Moreover, in houses fitted with fly screens, *Campylobacter* prevalence did not increase in the summer, remaining at the low winter prevalence level. It was estimated that 77% of broiler flocks could have avoided *Campylobacter* colonization if fly screens had been implemented nationwide throughout Denmark.

Rodents are also considered a potential vector for *Campylobacter* contamination. Allain *et al.* (2014) showed that rodent control around the broiler house led to a significant reduction in contamination: 92% of flocks were *Campylobacter* positive when no rodent control was implemented, whereas this percentage dropped to 66% on farms applying rodent control measures.

Air, litter, feed and water can be a potential source of passive *Campylobacter* transmission. Feed and litter are usually negative for *Campylobacter* but are likely to be contaminated during storage and transport, particularly in humid conditions, which are beneficial to *Campylobacter* development. It was also shown that transport crates are a potential source of *Campylobacter* infection for chickens. Cleaning appeared ineffective since it has been shown that broilers can be infected when exposed to

naturally contaminated and then washed crates, even after only three hours of contact (Ridley *et al.* 2011a).

Several of these measures could strongly impact *Campylobacter* colonization in poultry. Even if their implementation is feasible and could help reduce *Campylobacter* prevalence, implementation requires staff compliance and is difficult to maintain due to the cost and the constant pressure of environmental contamination.

Nutritional strategies

These strategies are applied at the primary broiler production level and consist in administering feed or water supplemented with various products or micro-organisms having an anti-*Campylobacter* activity. This section describes studies dealing with nutritional strategies and summarizes them in different tables.

Organic and fatty acids

Several studies have reported that fatty acids have antimicrobial activities against a large range of micro-organisms. As shown in Table 1, many studies have evaluated effectiveness of caprylic acid, a medium-chain fatty acid (MCFA), as a feed additive to reduce *Campylobacter* levels in broilers, but the findings diverge greatly. For example, Solis de Los Santos *et al.* (2008) showed the beneficial effect of several doses of caprylic acid added to feed, leading to a reduction up to 2 $\log_{10}$ CFU compared to the control group. Globally speaking, the lower caprylic acid doses trialled were more efficient than the three higher ones, but divergent results were observed between trials. The same researchers also demonstrated the beneficial effect of caprylic acid as a therapeutic treatment on market-aged broilers (Solis de los Santos *et al.* 2010). Added to feed from the day of hatching, a mixture of MCFA (C_8-C_{12}) reduced intestinal colonization by *Campylobacter* after an experimental challenge (van Gerwe *et al.* 2010). Contrary to previous studies, Hermans *et al.* (2010) did not detect any effect of three medium-chain fatty acids on caecal *Campylobacter* loads when added to chicken feed, despite their bactericidal activities demonstrated *in vitro* against two *Camp. jejuni* strains. This study also suggested that mucus played a protective role against *Campylobacter* elimination. Other organic and fatty acids were tested. After promising *in vitro* results, butyrate was micro-bead coated and added to chicken feed from hatching to the end of the experiment, but after an experimental *Camp. jejuni* infection, no reduction was observed in caecal colonization (Van Deun *et al.* 2008). Skanseng *et al.* (2010) demonstrated the beneficial impact of formic acid and sorbate on intestinal *Camp. jejuni* colonization when added to feed. Although formic acid and sorbate did not individually reduce *Campylobacter* load after an oral challenge, the combination of both significantly decreases *Camp. jejuni* load to total inhibition, giving results similar to the noninfected group.

Easier to apply by poultry producers, several studies have investigated the effects of products added to broiler drinking water (Table 1). MCFAs were tested in water as they had been previously in feed. Metcalf *et al.* (2011), for example, tested the administration of various doses of caprylic acid to broiler chicks. Only one of the tested doses led to a significant reduction in *Campylobacter* load compared to the control group. However, this result was not reproduced in the second trial, in which none of the tested doses impacted birds' intestinal colonization. Hermans *et al.* (2012a) showed that an MCFA emulsion was not able to reduce intestinal *Campylobacter* colonization and/or transmission when added to drinking water, whether as a therapeutic or a preventive treatment. Several commercial acidifying water additives have also been tested in broiler drinking water. Selko DWB® was administered to 11-day-old chickens until the end of the experiment. Although the drinking water remained *Campylobacter* free throughout the experiment, most chickens were colonized after an experimental challenge. Reductions in *Campylobacter* counts were observed in treated birds but remained insignificant (Chaveerach *et al.* 2004). Despite good *in vitro* results in water suspension, improved with higher doses and longer exposure, PWT—a commercial water acidification product—did not allow *in vivo* reduction in *Campylobacter* caecal loads (Haughton *et al.* 2013). In a recent field study including three similar trials, Selko® 4Health—a commercial additive based on organic acids and medium-chain fatty acids—was used to acidify poultry drinking water. For all three trials, caecal *Campylobacter* count means were significantly lower in the treated groups. However, some rearing cycles did not show significant results. On day 42, for example, treatment was observed to be effective in two out of three trials, highlighting the nonreproducible effectiveness of the treatment (Jansen *et al.* 2014).

A common field practice entails withdrawing feed a few hours before slaughter. Byrd *et al.* (2001) made use of this time to administer lactic acid to chickens via drinking water. This acid treatment reduced *Campylobacter* incidence both in the crops of treated birds compared to the control group and in carcasses rinsed before chilling.

The poor efficiency of acid supplements in drinking water to reduce *Campylobacter* loads is unclear. It could be due to changes in broilers' intestinal adsorption of the product compared to adsorption through feed, leading to ineffective concentrations reaching the gut. What is more,

Table1 Overview of the nutritional experiments using fatty and organic acids against *Campylobacter* in chickens

Administration				*Campylobacter* Challenge				
Compound	Route	Time	Dose	Strain	Time	Doses	*Campylobacter* colonization results	Ref
Caprylic acid	Feed	10 days (all)	0·35 –1·4%	*Camp. jejuni* – 5 strains from chickens	Day 3	10^6 CFU	• Divergent results between identical trials • Caecal reduction of >2 $\log_{10}$ CFU g^1 for the 4 lower doses • For the 3 higher doses, no significant reduction in one trial, and reduction of at least 2 $\log_{10}$ CFU g^1 in another one	Solis de Los Santos *et al.* (2008)
Caprylic acid	Feed	• 3/7 days before slaughter • with/without 12 h withdrawal	0·7%	*Camp. jejuni* – 5 strains from chickens	Day 14	6,5 10^7 CFU	• Caecal reduction of >3 $\log_{10}$ CFU g^1 on day 42 for 3 conditions • No significant reduction for the group treated 7 days before slaughter with a 12 h withdrawal.	Solis de los Santos *et al.* (2010)
Lodestar (MCFA C_8-C_{12})	Feed	Throughout the experiment	1%	*Camp. jejuni*, C356	Day 14/18	$10^{1,2}$–$10^{5,5}$ CFU	• Lower susceptibility of treated birds • *Camp. jejuni* dose for the colonization of 50% of the inoculated birds 200 times higher in the treated group: $10^{4·8}$ CFU *vs* $10^{2·5}$ CFU for the control group	van Gerwe *et al.* (2010)
Caproic, caprylic and capric acids	Feed	3 days before slaughter	1%	*Camp. jejuni*, KC40	Day 15	5 10^7 CFU	No reduction for any acids	Hermans *et al.* (2010)
Butyrate	Feed	Throughout the experiment	0·05%	*Camp. jejuni*, KC40	Week 2	10^9 CFU ml^1	No reduction 5 days postinfection	Van Deun *et al.* (2008)

Table 1 (*Continued*)

Administration				*Campylobacter* Challenge				
Compound	Route	Time	Dose	Strain	Time	Doses	*Campylobacter* colonization results	Ref
Formic acid + Sorbate	Feed	Throughout the experiment	1·0 –2·0% + 0·1%	*Camp. jejuni* – 5 strains from chickens, dogs	Day 13–15	1.5 10^4 CFU	• No reduction with individual administration or combined with formic acid 1% and sorbate 0·1% • Caecal count reduced to under the detection limit with combined treatment: formic acid (1·5 and 2%) and sorbate 0·1%. Results similar to the noninfected group	Skanseng *et al.* (2010)
Caprylic acid	Drinking water	3 days before slaughter (day 14)	0·175–2·8%	*Camp. jejuni* – 5 strains	Day 3	5 10^6 CFU	Significant caecal reduction by 2·9 $\log_{10}$ CFU g^1 only for the 0·175% dose	Metcalf *et al.* (2011)
			0·044–1·4%			$3{\cdot}10^3$ CFU	No reduction for any doses	
MCFA (caproic, caprylic, capric and lauric acids)	Drinking water	From day 25 From day 1	0·4%	*Camp. jejuni*, KC40	Day 14	10^6 CFU $3{\cdot}10^2$–$2{\cdot}10^4$°CFU	No reduction in 4-week-old birds • Fewer colonized birds in the treated groups on day 15 • No caecal reduction on day 20 (challenge on 3 seeders only)	Hermans *et al.* (2012a,b)
Lactic acid	Drinking water	10 h during feed withdrawal	0·44%	NA – Naturally preinfected birds			• Crop colonization signifi cantly reduced by >20% in treated group • *Campylobacter* incidence on carcass rinses prior to chilling significantly reduced by >10% in treated group	Byrd *et al.* (2001)*

Table 1 (*Continued*)

Administration				*Campylobacter* Challenge				
Compound	Route	Time	Dose	Strain	Time	Doses	*Campylobacter* colonization results	Ref
Selko DWB	Drinking water	From day 11 to day 20	ND	ND	Day 11	$10^3/10^5$ CFU	• Caecal reduction of 2 $\log_{10}$ CFU g^1 on day 20 with the lower challenge dose, 1 bird *Campylobacter* free in the treated group, 2 in the control one • Caecal reduction of >0·5 log10 CFU g^1 on day 20 with the higher challenge dose	Chaveerach *et al.* (2004)
PWT	Drinking water	First 7 days + 2 days before and after feed changes + prior to slaughter/Only 24 h prior to slaughter	ND	NA – Naturally preinfected birds			No significant caecal reduction for either treatment schedules on days 35 and 42	Haughton *et al.* (2013)*
Selko 4Health	Drinking water	From day 1 until the end of the experiment	0·075%	NA – Naturally preinfected birds			• Globally (3 trials), caecal reduction on days 35 and 42 by 1·4 and 4·3 $\log_{10}$ CFU g^1 respectively • Day 35, significant caecal reduction for trial 2 only of 6·9 $\log_{10}$ CFU g^1 • Day 42, significant caecal reduction for trials 2 and 3 of 4·2 and 6·5 $\log_{10}$ CFU g^1 respectively	Jansen *et al.* (2014)*

Table 1 (*Continued*)

Administration				*Campylobacter* Challenge				
Compound	Route	Time	Dose	Strain	Time	Doses	*Campylobacter* colonization results	Ref
Forticoat (birds housed together)	Drinking water	From day 1	0·2%	*Camp. jejuni* C356	Day 12 (10 birds)	10^5 CFU	All birds were rapidly colonized after exposition to infected birds	van Bunnik *et al.* (2012)
				On day 16, remaining birds (13) exposed to the 10 infected birds				
Forticoat (birds housed individually)				*Camp. jejuni* C356	Day 12 (5 birds/9)	10^5 CFU	• Delayed indirect transmission of *Campylobacter* between separated broilers • Significantly fewer colonized birds in treated groups	
Monocaprin + Polysorbate 40	Drinking water + feed	From day 23 until the end of the experiment (13 days after *Camp. jejuni* exposure)	0·12% +0·02%	*Camp. jejuni* strains from chickens	Day 19 (4 birds)	$10^{6,9}$ CFU	• Significant cloacal reduction on days 2, 5 and 10 postexposure in the first experiment of between 1·2 and 2·1 $\log_{10}$ CFU ml^{1}, but no difference on day 13 • Significant cloacal reduction on days 2 and 4 postexpo sure in the second experiment of between 1·7 and 2·1 $\log_{10}$ CFU ml^{1}, but no difference on day 10	Hilmarsson *et al.* (2006)
				Then, groups of 5 uninfected birds exposed to 2 infected birds on day 24				
		From day 37 for 3 days	0·24% +0·04%	NA – Naturally preinfected birds			Significant cloacal reduction on day 3 post treatment of between 1·5 and 2·2 $\log_{10}$ CFU g^{1}.	

ND, not determined; NA, not applicable.

*These studies refer to field experiment. All other experimental trials were done in controlled environments.

intestinal flora or physiological parameters could interact with or alter the active ingredient, which would then be no longer available to impact *Campylobacter* colonization. However, in spite of poor results on intestinal decrease, several of the studies mentioned observed that treated water remained *Campylobacter* free throughout the experimentation, suggesting that acidifying water could prevent *Campylobacter* spreading via drinking water in broiler flocks, thereby excluding water as a potential source of contamination. van Bunnik *et al.* (2012) showed that acidifying drinking water with Forticoat®, a product based on modified organic acids, decreased the indirect transmission of *Campylobacter* between spatially separate broilers.

The same authors investigated treatment of both drinking water and feed pellets with monocaprin and polysorbate 40 during the same experiment. *Campylobacter*-free 24-day-old treated chickens were exposed to experimentally infected chickens and the treatment was prolonged throughout the experiment. All the birds were colonized after only 2 days of contact with seeder birds, but cloacal *Campylobacter* loads were significantly lower in the treated group than in the control one. However, at the end of the experiment, no difference was observed between the two groups. The same products also allowed significant reductions in *Campylobacter* load when administered therapeutically to colonized birds. Used as preventive and therapeutic treatment, monocaprin is effective in reducing *Campylobacter* loads a few days after its application, suggesting its usefulness 2 or 3 days before slaughter (Hilmarsson *et al.* 2006).

Overall, although some promising load reduction results have been obtained in several experimentations by the use of fatty and organic acids, there are large discrepancies between the results of different studies and results remain by and large poor. Other acids could be tested. Moreover, the comparison results between the studies turn out to be very difficult since numerous parameters, such as avian and *Campylobacter* strains or the growing conditions, are different. A standardization of these ones should be a good way for effectively compare experiments but very difficult to implement. Lauric arginate, derived from lauric acid, ethanol and arginate, effectively decreased *Camp. jejuni* to undetectable levels during *in vitro* experiments both on broth and breast fillets (Nair *et al.* 2014), but no *in vivo* experiments have yet been conducted. Numerous variables determine an experiment's success. Recently, Molnar *et al.* (2015) showed that chickens' diet could impact their intestinal *Campylobacter* colonization. In their study, 14 days after an experimental challenge, jejunum and caecum colonization was significantly lower when chickens were fed with enzyme-supplemented wheat-based diets rather than a maize-based diet. However, colonization levels were similar 21 days postchallenge. This decrease was related to an increase in caecal SCFA concentration and a lower pH value.

Essential oils and plant-derived compounds

As described in Table 2, plant-derived compounds were also tested to reduce intestinal *Campylobacter* colonization. Promising *in vitro* results were obtained for cinnamon oil ingredient trans-cinnamaldehyde (CIN), leading researchers to test its *in vivo* bactericidal effect. Coated on microbeads and added to chick feed or directly injected into the caecum, CIN was not able to reduce caecal *Campylobacter* load in chickens orally challenged at 2 weeks (Hermans *et al.* 2011). Allicin, a compound extracted from garlic, has also been tested. Like cinnamon, although efficient when tested *in vitro*, and despite a tendency to reduce *Campylobacter* counts, allicin was not able to significantly reduce colonization (Robyn *et al.* 2013b). Administration of thymol-β-D-glucopyranoside, derived from thyme, decreased *Camp. jejuni* levels in chicken crops but not caecal contents (Epps *et al.* 2015), indicating that the product was adsorbed before reaching the intestines. Five other substances, including essential oils, plant extracts and secondary plant compounds, were recently investigated but none demonstrated marked effectiveness against *Camp. jejuni* (Kurekci *et al.* 2014).

However, a medicinal plant appears to be effective in reducing *Campylobacter* colonization in chickens. When added to feed from the day of hatching, Sangrovit® treatment led to a reduction in *Campylobacter* caecal counts after an oral challenge on day 21 without altering the feed intake and body weight of the treated chickens compared to the control group. Villi height and immune response were also improved by this treatment (Gharib Naseri *et al.* 2012).

Overall, few studies have focused on the *in vivo* experimentation of plant compounds, and no convincing results have yet been provided. For oral administration, encapsulation methods, protecting the active compound from degradation before it reaches it place of action at the intestinal level should improve *in vivo* efficiencies. However, particular formulations of active compounds imply increases in treatment costs.

Probiotics

The World Health Organization defines probiotics as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (WHO 2001). Naturally present in the intestinal flora of chick-

Table 2 Overview of the nutritional experiments using essential oils and plant-derived compounds against *Campylobacter* in chickens

Administration				*Campylobacter* Challenge				
Compound	Route	Time	Dose	Strain	Time	Doses	*Campylobacter* colonization results	Ref
Cinnamaldehyde (CIN)	Feed	From day 1	0·3%	*Camp. jejuni*, KC40	Day 15 (3 birds)	$1{\cdot}4 \times 10^8$ CFU	No reduction	Hermans *et al.* (2011)
	In 1 caecum	Day 18/21	100 mmol $l^{-1,}$ 200 μl	*Camp. jejuni*, KC40	Day 14	10^7–10^8 CFU	No reduction	
Allicin	Drinking water	From day 1	25 mg kg^{-1}*	*Camp. jejuni*, MB4185	Day 14	$0{\cdot}5$–1×10^3 CFU	No reduction	Robyn *et al.* (2013b)
Thymol	Oral gavage	Twice: 9 and 13 hours before slaughter	25 mmol l^{-1}, 3 ml	NA – Naturally infected birds			No caecal and crop reductions	Epps *et al.* (2015)
Thymol-β-D-glucopyranoside			25 mmol l^{-1}, 3 ml				Reduction in crops but not in caecal contents	
Acacia decurrens extract *Eremophila glabra* extract α-tops Terpinene-4-ol Lemon myrtle oil (LMO)	Feed	From day 4	0·5–1 g kg^{-1}* 100–200 mg kg^{-1}*	NA – Naturally infected birds			• No caecal reduction on day 45, whatever the compound • Significant faecal reduction on day 41 for α-tops treatment compared to the control group (≈ 1 $\log_{10}$ CFU g^{-1})	Kurekci *et al.* (2014)
Sangrovit®	Feed	From day 1	20–50 g $tone^{-1}$*	*Camp. jejuni*, ATCC 33291	Day 21	10^9 CFU	• Caecal reduction by >1 $\log_{10}$ CFU ml^{-1} on day 49 • Faecal reduction by 0·7 $\log_{10}$ CFU ml^{-1} on day 42	Gharib Naseri *et al.* (2012)

NA, not applicable.

*The doses are expressed in mg g^{-1} of compound per kg $tone^{-1}$ of feed. All experimental trials were done in controlled environments.

ens, the use of probiotics aims to create competitive exclusion between species.

Studies investigating probiotics have shown large discrepancies in terms of intestinal *Campylobacter* load reduction (Table 3). Ghareeb *et al.* (2012) tested the anti-*Campylobacter* activity of PoultryStar sol, a mixture of avian-specific probiotic bacteria isolated from chicken gut (*Enterococcus faecium, Pediococcus acidilactici, Bifidobacterium animalis, Lactobacillus salivarius* and *Lactobacillus reutiri*). After promising *in vitro* results, probiotics were administered to poultry via drinking water and birds were challenged by *Camp. jejuni* on the first day of life. After 8 and 15 days postchallenge, there was a significant reduction in caecal *Camp. jejuni* load in the probiotic-treated group (reduction of 6 $\log_{10}$ CFU g^{-1} compared to the control group). It has also been demonstrated that *Lactobacillus gasseri* SBT2055 is able to reduce *Campylobacter* colonization by more than 2 $\log_{10}$ CFU g^{-1} in daily treated 14-day-old chicks compared to the PBS control group (Nishiyama *et al.* 2014). However, the treatment's long-term efficiency has not been evaluated. Recently, four human probiotic isolates were tested in combination to reduce *Camp. jejuni* intestinal counts. By the end of the experiment, marked reductions were observed in the experimental groups. Results were similar regardless of the treatment's application (probiotic diet from the day of hatching or only during the last week of growth) (Cean *et al.* 2015). Several other studies have shown slight but significant reductions in *Campylobacter* loads after probiotic administration. Santini *et al.* (2010) identified in 2010 a *Bifidobacterium* strain with an anti-*Campylobacter* activity. Probiotic treatment led to a significant 1 $\log_{10}$ reduction in the intestinal count of *Camp. jejuni* in poultry. Broiler feed supplemented with PrimaLac® administered from the day of hatching was also able to reduce *Campylobacter* counts in intestinal contents. Some studies have also shown that probiotics improve villi height and immune response (Gharib Naseri *et al.* 2012). However, not all the probiotics proved effective in reducing intestinal *Campylobacter* loads. Robyn *et al.* (2013a), for instance, showed that *Enterococcus faecalis* strain MB5259 was not able to decrease *Campylobacter* load even when a daily dose of 10^8 CFU was administered daily.

Probiotic effectiveness varies greatly between studies, but they could be an easy, effective way of reducing intestinal *Campylobacter* load in poultry.

Bacteriocins

Several studies have used bacteriocins to control *Campylobacter* colonization in poultry (Table 3). In the chicken's digestive tract, bacteriocins are produced by lactic acid bacteria such as *Lactococcus*, *Lactobacillus* and *Pediococcus*. These are ribosomally synthesized peptides with a varying range of antimicrobial activity. They can be active against a broad or narrow spectrum of bacteria, so may be selected to kill a particular pathogen without altering the animal's microflora. Most bacteriocins either form pores in the outer membrane of susceptible bacteria, allowing inorganic ions to enter, or create disorders in the structure and synthesis of cell walls, leading in both cases to bacterial death (Svetoch and Stern 2010). Currently, few bacteriocins with an anti-*Campylobacter* activity have been purified. Most producer strains have been isolated from chicken ceca. Bacteriocin OR-7, isolated from *Lact. salivarius* strain NRRL B-30514 (Stern *et al.* 2006) and a bacteriocin from *Paenibacillus polymyxa* strain NRRL B-30509 (Stern *et al.* 2005) have been purified and added individually to chicken feed. *In vivo* trials revealed that bacteriocin treatment significantly reduced the caecal *Campylobacter* load by more than 4·5 $\log_{10}$ CFU g^{-1} compared to an untreated group of birds. However, feed supplementation with probiotics producing these two bacteriocins did not reduce caecal *Camp. jejuni* loads after a challenge (Stern *et al.* 2008). Svetoch *et al.* (2011) isolated the L-1077 bacteriocin from another *Lact. salivarius* strain and administered it as a therapeutic treatment to chickens not via feed but via drinking water. In the same way, the caecal load of *Camp. jejuni* was significantly lower in the experimental group than in the untreated group (Svetoch *et al.* 2011). More recently, Messaoudi *et al.* (2012) identified the SMXD51 bacteriocin produced by a *Lact. salivarius* strain. An agar well diffusion test showed this peptide's activity against several strains of *Camp. jejuni* and *Camp. coli*. *Campylobacter jejuni* NCTC 11168 populations were reduced *in vitro* by about 2 $\log_{10}$ when growth was performed with SMXD51. *In vivo* studies have not yet been performed.

Two other bacteriocins produced by *Enterococcus* species, which are not lactic acid bacteria, have also been identified: E-760, from the NRRL B-30745 strain, and E 50–52 from the *Ent. faecium* NRRL B-30746 strain. Both had antimicrobial activity against a broad spectrum of bacteria and reduced caecal *Camp. jejuni* load under the detection limit when added to chick feed after an experimental infection on the day of hatching. Furthermore, after natural infection, caecal *Campylobacter* counts were not detectable or greatly reduced when bacteriocins were added to drinking water or feed (Line *et al.* 2008; Svetoch *et al.* 2008).

The studies described above suggest that purified anti-*Campylobacter* bacteriocins are a generally more efficient way of decreasing chickens' intestinal load than are probiotic strains. This could be due by the fact that

Table 3 Overview of the nutritional experiments using probiotics and bacteriocins against *Campylobacter* in chickens

Administration				*Campylobacter* Challenge			*Campylobacter* colonization results	Ref
Compound	Route	Time	Dose	Strain	Time	Doses		
Probiotics								
PoultryStar sol	Drinking water	From day 1	2/20 mg per day	*Camp. jejuni*, 3015/2010	Day 1	10^4 CFU	Caecal reduction of 6 $\log_{10}$ on day 15	Ghareeb *et al.* (2012)
Lactobacillus gasseri SBT2055	Oral gavage	From day 2	10^8 CFU per day	*Camp. jejuni,* 81–176	Day 1	10^6 CFU	Caecal reduction of >2 $\log_{10}$ on day 14	Nishiyama *et al.* (2014)
Lactobacillus paracasei J.R and *Lactobacillus rhamnosus* 15b +/- *Lactococcus lactis* Y and *Lactococcus lactis* Foa	Feed	From day 1 Last week before slaugher	10^9 CFU kg^{-1}	NA – Naturally infected birds			• Significant duodenal reduction of ≈ 8 $\log_{10}$ CFU ml^{-1} whatever the administration timing and the probiotic strains. • Significant caecal reduction of <1 $\log_{10}$ CFU ml^{-1} whatever the administration timing and the probiotic strains	Cean *et al.* (2015)
Bifidobacterium longum PCB133	Oral gavage	From day 15–20 15 days	10^8 CFU per day	NA – Naturally preinfected birds			Faecal reduction of 1 $\log_{10}$ CFU on day 15	Santini *et al.* (2010)
PrimaLac®	Feed	From day 1	100 mg kg^{-1}*	*Camp. jejuni,* ATCC 33291	Day 21	10^9 CFU	• Caecal reduction of 0·9 $\log_{10}$ CFU ml^{-1} on day 49 • Faecal reduction of 0·8 $\log_{10}$ CFU ml^{-1} on day 42	Gharib Naseri *et al.* (2012)
Enterococcus faecalis MB5259	Oral gavage	From day 1	$10^4/10^8$ CFU per day	*Camp. jejuni,* MB4185	Day 15 (2 birds)	2·0 × 10^4 CFU	No reduction for either dose	Robyn *et al.* (2013a)
Bacteriocins								
OR-7 (*Lact. salivarus*, strain NRRL B-30514)	Feed	From day 1	250 mg kg^{-1}*	*Camp. jejuni*, AL-22/BH-6/BL-1/CL-11	Day 1	10^8 CFU	Significant caecal reduction of >6 $\log_{10}$ whatever the challenge strain	Stern *et al.* (2006)

Table 3 (*Continued*)

Administration				*Campylobacter* Challenge			*Campylobacter* colonization results	Ref
Compound	Route	Time	Dose	Strain	Time	Doses		
Paenibacillus polymyxa, strain B-30509 bacteriocin	Feed	From day 7 to 9	250 mg kg^{-1}*	*Camp. jejuni*, AL-22/BH-6/BL-1/CL-11	Day 1	10^8 CFU	Significant caecal reduction of between 4·6 and 6·3 log_{10} CFU g^{-1} (under the detection limit) whatever the challenge strain on day 10	Stern *et al.* (2005)
L-1077 (*Lact. salivarus*, strain NRRL B-50053)	Drinking water	All along the experiment	25/12·5/6·25 mg l^{-1}	NA – Naturally preinfected birds			Significant caecal reduction of >4 log_{10}	Svetoch *et al.* (2011)
E-760 (*Enterococcus*, strain NRRL B-30745)	Feed	From day 4	31·2–125 mg kg^{-1}*	*Camp. jejuni*, B1 and L4	Day 1	10^6 CFU	Significant caecal reduction of >6·5 log_{10} CFU g^{-1} (under the detection limit) on day 7	Line *et al.* (2008)
		4 days before slaugther	125 mg kg^{-1}*	NA – Naturally preinfected birds (39 days old)			Significant caecal reduction of >3·5 log_{10} CFU g^{-1} (under the detection limit)	
E50-52 (*Enterococcus faecium*, strain NRRL B-30746)	Feed	From day 4–7	31·2–125 mg kg^{-1}*	*Camp. jejuni*, B1 and L4	Day 1	10^6 CFU	Significant caecal reduction to nondetectable levels (8·40 log_{10} CFU g^{-1} in the control group) on day 15	Svetoch *et al.* (2008)
	Drinking water	1, 2 or 3 days before slaugther	12·5 mg l^{-1}	NA – Naturally preinfected birds			Significant caecal reduction of >5 log_{10} CFU	

NA, not applicable.

*The doses are expressed in mg/g of compound per kg/tone of feed. All experimental trials were done in controlled environments.

bacteriocins which are released by administrated probiotic strains should be at a lower concentration compared to directly administrated bacteriocins.

Bacteriophages

Discovered at the beginning of the 19th century, bacteriophages are natural bacterial killers ubiquitous in the environment. In some countries, they are today widely used in a health context for both veterinary and human medicine (Tiwari *et al.* 2014). Due to their host-specific nature, based on interactions between phage tail proteins and bacterial receptors, phage treatment could be the answer to the emergence of antibiotic-resistant bacterial strains.

As the avian gut represents the main reservoir of *Campylobacter*, most studies have used phages isolated from the chicken gastro-intestinal tract. Janez and Loc-Carrillo have described the isolation and characterization of *Campylobacter* phages (Janez and Loc-Carrillo 2013). Phages replicate only in the target bacterial cell, and due to their host specificity, bacteriophages applied to combat *Campylobacter* do not alter normal gut flora.

As described in Table 4, several scientific studies using phages to control animal diseases have given promising results and led researchers to consider *Campylobacter* bacteriophages as a tool to combat chicken colonization. Phages could be administered individually like in the study by Loc Carrillo *et al.* (2005) in which CP8 and CP34 phages were orally administered to broilers colonized by *Camp. jejuni* (HPC5 or GIIC8 strains). Results highlighted the high host specificity of phages since CP8 treatment substantially decreased *Camp. jejuni* levels in GIIC8-colonized chickens in the first days after phage application leading to a final significant difference of about 2 $\log_{10}$ in the caecum and lower intestine, whereas no significant *Campylobacter* reduction was observed in the HPC5-colonized chickens. The study also reveals that CP34 was more effective than CP8 at reducing *Camp. jejuni* loads in HPC5-colonized chickens, with significant results at all intestinal sites. Phage strain 71 was also tested individually on chickens as therapeutic or preventive treatment. At the end of the experiments, both treatments led to the same *Campylobacter* load decrease of about 1 $\log_{10}$ but a greater reduction can be observed for the first 48 h when the therapeutic measure was applied (Wagenaar *et al.* 2005). It was also shown that HPC5-colonized chickens showed a significant decrease in bacterial load in the three parts of the intestine 24 h after an oral treatment by phage CP22010 compared to the control group (El-Shibiny *et al.* 2009). After 3 days, the treatment is less effective. For *Camp. coli* OR12-infected birds, a higher dose was needed to obtain a significant load reduction in the caecum and the lower intestine 48 h after phage administration. These studies showed that individual phage administration could be effective in decreasing *Campylobacter* counts in chickens, but phage and bacterial strains determine the treatment's success. It may be more effective to administer several phages in combination to overcome host specificity.

Several studies have been conducted to investigate the administration of a bacteriophage cocktail. Wagenaar *et al.* (2005) showed that administration of both phage strains 69 and 71 as a therapeutic treatment first reduced *Campylobacter* by 1·5 $\log_{10}$ but finally led to a stabilized 1 $\log_{10}$ reduction. The cocktail phages including phiCcoIBB35, phiCcoIBB37 and phiCcoIBB12 have also been tested against *Camp. jejuni* 2140CD1 and *Camp. coli A11* either by oral gavage or by inclusion in the feed. The results showed a reduction in *Campylobacter* titres from 4 days post-treatment whatever the conditions of administration, a reduction maintained through to the end of the experiment. Incorporated in food, phage treatment was effective in reducing the *Camp. coli* load earlier (Carvalho *et al.* 2010). More recently, Fischer *et al.* (2013) demonstrated that a phage cocktail or single phage NCTC 12673 similarly decreased intestinal *Campylobacter* load in commercial broilers experimentally infected with *Camp. jejuni* field strains. Added to the drinking water of commercial broiler flocks, the same phage cocktail decreased *Campylobacter* load below the detection limit one day after application, and the bacterial load was reduced by 3·2 $\log_{10}$ at slaughter compared to the control group (Kittler *et al.* 2013). However, no significant reduction was observed in two other similar field trials. Inefficacy could be explained by the timing between *Campylobacter* colonization and phage application. Furthermore, the phage cocktail used may be suitable for some colonized strains but not for others due to the host-specific nature of phages.

Generally speaking, phage cocktails were no more effective than single phages in terms of reducing intestinal *Campylobacter* loads, but could target more *Campylobacter* species than a single target-specific phage.

Throughout these studies, the impact of phage application on the emergence of the resistance of *Campylobacter* strains to bacteriophages was evaluated. Although resistance levels increased after phage treatment, it had no significant impact on the colonization of resistant strains due to their loss of virulence and poor ability to compete with susceptible strains (Connerton *et al.* 2011).

Phage treatment has a beneficial impact on reducing intestinal *Campylobacter* loads, particularly immediately after application, indicating that it should be used just few days before slaughter. However, questions remain on

Table 4 Overview of the nutritional experiments using bacteriophages against *Campylobacter* in chickens

Administration				*Campylobacter* Challenge				
Compound	Route	Time	Dose	Strain	Time	Doses	*Campylobacter* colonization results	Ref
CP8 phage	Oral gavage	Day 25	$10^5/10^7/10^9$ PFU	*Camp. jejuni* HPC5/GIIC8	Day 18–20	10^3–10^8 CFU	• No reduction for HPC5 colonized chickens • Intestinal reduction for GIIC8 colonized chickens, until 5·6 $\log_{10}$ CFU g^{-1} for the first 3 days after the treatment • Intestinal reduction by 2 $\log_{10}$ CFU g^{-1} after 72 h in the caecum and lower intestine	Loc Carrillo *et al.* (2005)
CP34 phage				*Camp. jejuni* HPC5			• Globally more effective than CP8 phage treatment • Caecal reduction by 3·9 $\log_{10}$ CFU g^{-1} for HPC5 colonized chickens after 24 h for the 10^7 PFU dose • Same trend for the 10^5 PFU dose • No reduction after 24 h for the 10^9 PFU dose, reduction by 1·4 $\log_{10}$ CFU g^{-1} on day 4	
Phage strain 71	Oral gavage	From day 7 10 days	10^9–10^{10} PFU	*Camp. jejuni* C356	Day 10	10^5 CFU	Caecal reduction by >2 $\log_{10}$ CFU g^{-1} for the first 48 h after challenge and by 1 $\log_{10}$ CFU g^{-1} until the end	Wagenaar *et al.* (2005)
		From day 15 6 days	10^9–10^{10} PFU				Caecal reduction by 3 $\log_{10}$ CFU g^{-1} for the first 48 h after phage treatment and by 1 $\log_{10}$ CFU g^{-1} until the end	

Table 4 (*Continued*)

Administration				*Campylobacter* Challenge				
Compound	Route	Time	Dose	Strain	Time	Doses	*Campylobacter* colonization results	Ref
CP220 phage	Oral gavage	Day 25	$10^5/10^7/10^9$ PFU	*Camp. jejuni* HPC5	Day 20	10^8 CFU	• Caecal reduction of 2·1 $\log_{10}$ CFU g^{-1} 24 h after phage treatment for the 10^7 PFU dose. Less efficient after 3 days • Similar results for the 10^9 PFU dose • Small reduction for the 10^5 PFU dose	El-Shibiny *et al.* (2009)
				Camp. coli OR12			• Caecal reduction of 1·9 $\log_{10}$ CFU g^{-1} and lower intestinal reduction of 3·7 $\log_{10}$ CFU g^{-1} 48 h after phage treatment for the 10^9 PFU dose • Small or no significant reduction for the 10^5 and 10^7 PFU doses	
Phage cocktail Strain 69 and 71	Oral gavage	From day 39 4 days	10^9–10^{11} PFU each	*Camp. jejuni* C356	Day 32	10^5 CFU	Initial caecal reduction by 1·5 $\log_{10}$ CFU g^{-1} during treatment and by 1 $\log_{10}$ CFU g^{-1} until the end	Wagenaar *et al.* (2005)
Phage cocktail: phiCcoIBB35, phiCcoIBB37 and phiCcoIBB12	Oral gavage Feed/oral gavage	Day 7	10^6 PFU $1{\cdot}5 \times 10^7$ PFU/10^6 PFU	*Camp. jejuni* 2140CD1 *Camp. coli* A11	Day 1	10^6 CFU	Faecal reduction by about 2 $\log_{10}$ CFU g^{-1} • Faecal reduction by >1·5 $\log_{10}$ CFU g^{-1} for phage administration by gavage on days 4 and 7 postadministration • Faecal reduction by about 2 $\log_{10}$ CFU g^{-1} for phage administration in feed on days 2 and 7 postadministration	Carvalho *et al.* (2010)

Table 4 (*Continued*)

Administration				*Campylobacter* Challenge				
Compound	Route	Time	Dose	Strain	Time	Doses	*Campylobacter* colonization results	Ref
Phage cocktail: NCTC 12673, NCTC 12674, NCTC 12678, NCTC 12672	In crop	From day 9	10^7 PFU	*Camp. jejuni* 1474-06	Day 6	10^4 CFU	Average caecal reduction of 1·3 $\log_{10}$ CFU g^{-1} for both cocktail and single phage.	Fischer *et al.* (2013)
NCTC 12673 phage							Significant reduction from 1 to 4 weeks after phage treatment, maximal reduction of 2·8 $\log_{10}$ CFU g^{-1} on day 21	
Phage cocktail: NCTC 12673, NCTC 12674, NCTC 12678, NCTC 12672	Drinking water	From day 31/32/36	$10^{5·8}$–$10^{7·6}$ PFU	NA – Naturally preinfected birds (field study)			• No significant reduction for 2 out of 3 field trials • Reduction under the detection limit 1 day after the phage treatment, and of 3·2 $\log_{10}$ CFU g^{-1} at slaughter age	Kittler *et al.* (2013)*

NA, not applicable.

*The study refers to field experiment. All other experimental trials were done in controlled environments.

the effects of releasing phages in the environment and the acceptability of phage-treated food for consumers.

Immunization strategies

These strategies are also applied at primary broiler production level and consist in administering antibodies or vaccines. The goal of the experiments is to develop a specific anti-*Campylobacter* immune response, particularly at a mucosal level to neutralize and eliminate colonizing, but not invasive, *Campylobacter* and to limit intestinal load before slaughter. In the following section, studies addressing immunization strategies are described and vaccine experiments summarized in Table 5.

Passive immunization

Campylobacter colonization naturally occurs in 2- or 3-week-old chicks due to the availability of protective maternal antibodies in chick sera in the first weeks posthatching. This passive protection can be maintained by administration of *Campylobacter*-specific immunoglobulin type Y (IgY). When orally administered at the same time as the challenge strain, bovine or chicken immunoglobulins protect the chicks from colonization by *Camp. jejuni* during the 5 days of the experiment, but just 3 days later, *Campylobacter* was recovered from all the treated animals. In the same study, a therapeutic experiment showed a marked decrease in *Camp. jejuni* loads after the oral administration of immunoglobulins, but pretreatment load levels were reached once the treatment was stopped (Tsubokura *et al.* 1997). Recently, Hermans *et al.* (2014) vaccinated laying hens with a *Camp. jejuni* whole-cell lysate. A hyperimmune egg yolk was collected from these hens and added to the broiler feed. Three chicks in each group were challenged by a homologous strain. The results showed that 3 days postchallenge, caecal *Camp. jejuni* load was reduced by 2·9 to more than 5 $\log_{10}$ CFU g^{-1} compared to control groups, and bacterial transmission to nonchallenged birds in the treated group was greatly reduced or completely stopped depending on the challenge dose (Hermans *et al.* 2014). There are currently no studies demonstrating the long-term effect of passive immunization. The lack of knowledge on the efficacy period of this strategy suggests it could be used just a few days before slaughter to impact the contamination level of carcasses by *Campylobacter*.

Vaccination

Since *Campylobacter* is a major public health issue in developed countries, poultry vaccination remains one of the best strategies to impact human campylobacteriosis incidence. To date, many vaccination studies have been conducted using various strategies, including whole-cell or subunit vaccines and micro-organism-vectored vaccines. Combined strategies have also been studied. The studies described below, and summarized in Table 5, investigated the protective efficiency of vaccines in reducing *Campylobacter* load in the intestinal tract of chickens to limit meat contamination during slaughter processing and finally to decrease human contamination.

Whole-cell vaccines (WCV)

Whole-cell vaccines were the first to be investigated. They consist in administering killed or attenuated bacteria devoid of virulent and/or colonizing abilities. In the following paragraph, only poultry vaccination experiments targeting *Campylobacter* are described.

Vaccination using formalin-inactivated *Camp. jejuni* strain F1BCB reduced caecal *Camp. jejuni* loads from 16 to 93% in the vaccinated groups compared to the unvaccinated control group. Furthermore, IgA titres in serum or bile were generally higher in vaccinated birds than in the control group, and with more immune-responding birds. In this study, the heat labile toxin (LT) adjuvant did not impact vaccine efficacy (Rice *et al.* 1997). Contrary to these results, other teams did not obtain consistent results. For example, Glünder *et al.* (1997) showed that although specific antibodies were generated in chicken serum after subcutaneous immunization of formol-inactivated *Camp. jejuni* and complete Freund's adjuvant, vaccination had little effect on intestinal colonization after a homologous challenge and none at all after a heterologous inoculation. In another experiment, the vaccination of chicks with four viable but noncolonizing *Camp. jejuni* strains did not give protective immunity despite the chicks' immunological competence, and all the birds were colonized like the positive control group, regardless of the tested experimental conditions (Ziprin *et al.* 2002). Widders *et al.* (1998) tested WCV but combined with purified flagellin. A significant reduction in caecal *Camp. jejuni* loads was observed only when birds were immunized twice intraperitoneally, and not when the second vaccination was performed by the oral route. Also, another study in which chicks were first immunized *in ovo* then boosted orally after hatching demonstrated the generation of a strong immune response since IgY, IgA and IgM were detected in serum and IgA in intestinal contents and bile. The oral booster led to a higher increase in secreted IgA levels in the bile and intestines. These results indicate the development of an immune response before hatching, but the protective potential of this vaccine was not evaluated (Noor *et al.* 1995).

Table 5 Overview of vaccination experiments against *Campylobacter* in chickens

Administration								
Antigens	Dose	Vaccine strain	Adjuvant	Immunization scheme	Challenge (strain, dose)	Immunization Results	*Campylobacter* colonization results	Ref
Whole-Cell Vaccines								
Camp. jejuni, formalin-inactivated	10^9 CFU	*Camp. jejuni*, F1BCB	NA	2 doses, oral (D2, D8) Challenge D11	*Camp. jejuni*, F1BCB mix of vaccinated birds to experimentally colonized seeders ($4{\cdot}1 \times 10^4$ CFU)	• Higher percentage of IgA responding birds for 4 out of 5 time points (over the 3 trials) • Higher or equal specific IgA titres in serum or bile *Camp. jejuni*, F1BCB mix of vaccinated birds to experimentally colonized seeders ($4{\cdot}1 \times 10^4$ CFU)	Colonization reduced by 50% on day 17 Colonization reduced by 16 and 93% on days 31 and 46 respectively Colonization reduced by 81 and 25% on days 31 and 50 respectively	Rice *et al.* (1997)
			Labile toxin of *E. coli*, 25 μg	3 doses, oral (D2, D9, D16) Challenge D19				
			Labile toxin of *E. coli*, 50 μg	3 doses, oral (D2, D4, D6) Challenge D10				
Camp. jejuni, formol-inactivated	10^{10} CFU	*Camp. jejuni*, LIO 1 *Camp. jejuni*, LIO 6	Complete Freund's adjuvant	1 dose, SC (week 7)/2 doses, SC (weeks 4 and 7) Challenge week 10 1 dose, SC (week 1)/2 doses, SC (weeks 1 and 3) Challenge week 5	*Camp. jejuni*, LIO 1/LIO 6 $0{\cdot}6 \times 10^4$ CFU *Camp. jejuni*, LIO 6 $0{\cdot}6 \times 10^4$ CFU	• Neither the vaccination time nor the number of doses had an impact on the humoral response by the end of the experiment • The specific antibody titres were higher in the vacci nated groups • There was a slight increase in antibody titres after the booster in the negative and infected control groups	• Generally no differences between vaccinated and nonvaccinated groups 3 weeks postchallenge • Lower *Campylobacter* excretion after 1 vaccination in week 7 and after a homologous challenge	Glünder *et al.* (1997)
Camp. jejuni mixture	10^8 CFU 10^8 CFU (IM) + 10^9 CFU (oral) 10^8 CFU 10^8 CFU (IM) + 10^9 CFU (oral)	*Camp. jejuni*, 4 noncolonizing mutant strains	NA Ribi's adjuvant R-700	1 dose, IM (D5) Challenge D11 1 dose, IM + oral (D5) Challenge D11 1 dose, IM (D5) Challenge D11 1 dose, IM + oral (D5) Challenge D11	*Camp. jejuni*, F38011 10^4 CFU	NE	• No significant caecal reduction compared to the unvaccinated group • No differences between groups	Ziprin *et al.* (2002)

Table 5 (*Continued*)

Administration								
Antigens	Dose	Vaccine strain	Adjuvant	Immunization scheme	Challenge (strain, dose)	Immunization Results	*Campylobacter* colonization results	Ref
WC + Flagellin	ND	*Camp. jejuni*, Isolate #V2	NA	2 doses, IP/oral (D16: IP and D29: IP/oral) Challenge D36	*Camp. jejuni*, Isolate #V2 Exposure to infected birds	• Induction of specific antibodies in serum and intestinal secretion • Significantly higher specific anti-flagellin IgY in serum • Significantly higher specific anti-C.jejuni IgY in serum and intestinal secretions	• Significant caecal reduction for birds immunized IP twice by 2 $\log_{10}$ CFU g^{-1} • No significant caecal reduction for birds immunized IP and orally	Widders *et al.* (1998)
Camp. jejuni, heat-killed	10^8 CFU	ND	NA	1 dose, *in ovo* (D16 of incubation)/2 doses, in ovo/oral (D16 of incubation, D7 posthatching)	NA	• Significantly higher specific angi-flagellin IgY, IgA and IgM titres in serum, IgA in bile and intestinal scraping for both immunized groups • Higher specific IgA titres in bile and intestinal scraping for the boosted group • Significantly more IgM- and IgA-producing cells in the spleen of immunized birds • More IgM- and IgA-producing cells in the duodenum, ileum and spleen for twice-immunized birds, the difference being significant for IgM-cells in the duode num and spleen, and for IgA-cells in the ileum • Significantly more IgY-pro ducing cells in the spleen of twice-immunized birds	NA	Noor *et al.* (1995)

Table 5 (*Continued*)

Administration								
Antigens	Dose	Vaccine strain	Adjuvant	Immunization scheme	Challenge (strain, dose)	Immunization Results	*Campylobacter* colonization results	Ref
Subunit Vaccines								
Flagellin protein	ND	*Camp. jejuni*, Isolate #V2	NA	2 doses IP (D16, D29) Challenge D36	*Camp. jejuni*, Isolate #V2 Exposure to infected birds	• Induction of specific antibodies in serum and intestinal secretion • Significantly higher specific anti-flagellin IgY in serum	No significant caecal reduction	Widders *et al.* (1998)
Flagellin protein fused to LT-B	250/500/ 1000 μg	*Camp. jejuni*, A74/0	Labile toxin, B subunit of *E. coli*	2 doses, oral/IM (weeks 1 and 3)	NA	Significantly higher specific anti-flagellin antibodies only for the higher dose at 1 mg by the oral route.	NA	Khoury and Meinersmann (1995)
	1000 μg			2 doses, oral (weeks 2 and 4) Challenge on week 3	*Camp. jejuni*, A74/C, $2{\cdot}10^8$ CFU	NE	Significantly fewer colonized birds in vaccinated groups for 3 independent trials	
Flagellin DNA	150 μg	*Camp. jejuni*, ALM-80	Chitosan nanoparticules	3 doses (D1, D15, D29), IN Challenge on D42, oral	*Camp. jejuni*, ALM-80 5×10^7 CFU	• Significantly higher serum IgY and mucosal IgA titres after the second and third vaccination • Significantly higher level of CD4 + cells in caecal tonsils and spleen after the third vaccination	• Caecal reduction of 2 $\log_{10}$ CFU g^{-1} • Reduction of 2–3 $\log_{10}$ CFU g^{-1} in the large intestine • Totally clear in the small intestine on day 60	Huang *et al.* (2010)

Table 5 (*Continued*)

Administration								
Antigens	Dose	Vaccine strain	Adjuvant	Immunization scheme	Challenge (strain, dose)	Immunization Results	*Campylobacter* colonization results	Ref
FlaA/CadF/FlpA/ CmeC proteins or CadF-FlaA-FlpA fusion protein	240 μg	*Camp. jejuni*, F38011	Montanide ISA 70 VG	2 doses IM (D6: 90mer peptides, D16: full-length peptides) Challenge D20	*Camp. jejuni*, F38011 2×10^8 CFU	• Significantly higher sera reactivity for all vaccinated groups • Poor sera reactivity for the FlpA antigen	• Significant caecal reduction of about 3 $\log_{10}$ CFU g^{-1} for FlaA and FlpA antigens and the fusion protein on day 27 • Caecal reduction of about 1 $\log_{10}$ CFU g^{-1} for CadF antigen on day 27 • Slight caecal reduction for CmeC antigen on day 27. Large inter-individual variations	Neal-McKinney *et al.* (2014)
CjaA protein	14 μg	*Camp. jejuni*, M1	TiterMax®	2 doses, SC (D1/D15; D15/D29) Challenge 2 weeks after the second dose	*Camp. jejuni*, M1 10^7 CFU	• Significantly higher anti-CjaA IgY titres in serum for both experimental groups than in the control group serum • No significant difference between the two experi mental groups (first vaccination on day 1 or on day 15)	• Significant caecal reduction for birds first vaccinated on day 1 by 1·9 $\log_{10}$ and 2·3 $\log_{10}$ CFU g^{-1} on days 21 and 28 postchallenge respectively • Significant caecal reduction for birds first vaccinated on day 15 of 1·6 $\log_{10}$ and 3·0 log10 CFU g^{-1} on days 21 and 28 postchallenge respectively	Buckley *et al.* (2010)

Table 5 (*Continued*)

Administration								
Antigens	Dose	Vaccine strain	Adjuvant	Immunization scheme	Challenge (strain, dose)	Immunization Results	*Campylobacter* colonization results	Ref
Outer Membrane Proteins	25–250 μg orally/125 μg SC	*Camp. jejuni*, 81–176	PLGA nanoparticles	2 doses SC/orally (D7, D21) Challenge 14 days later (D35), oral	*Camp. jejuni*, 81–176 $2{\cdot}10^7$ CFU	• Oral: Low levels of IgY and IgA • SC: Higher IgY and IgA titres in serum and faeces, earlier response in serum	• Oral: slight caecal reduction for all OMP doses • SC: Significant reduction under the detection limit in caecal and cloacal loads	Annamalai *et al.* (2013)
Dsp protein	0·2 mg	*Camp. jejuni*, NCTC11168	Complete Freund's adjuvant	2 doses SC (D10, D24) Challenge 10 days later (D34)	*Camp. jejuni*, NCTC11168 10^5 CFU	NE	No protection against colonization	Theoret *et al.* (2012)
Antigens vectored by micro-organisms								
CjaA protein via *Salmonella enterica* strain χ3987	10^8 CFU	*Camp. jejuni*, 72Dz/92	NA	2 doses orally (D1, D14) Challenge 2 weeks after the second dose	*Camp. jejuni*, pUOA18 $2{\cdot}10^8$ CFU	• Moderate increase in specific anti-*Campylobacter* OMPs IgY titres in serum in week 6 and significant increase in week 8 • Increase in specific anti-Campylobacter OMPs intestinal IgA in week 4, decrease and stabilization in weeks 6 and 8	• All nonvaccinated birds colonized from $>10^6$ to $>10^9$ CFU g^{-1} • Only 3/20 vaccinated birds colonized by $>1{\cdot}10^3$ CFU g^{-1}. Caecal reduction of $>10^6$ CFU g^{-1} on day 12 post challenge	Wyszynska *et al.* (2004)

Table 5 (*Continued*)

Administration								
Antigens	Dose	Vaccine strain	Adjuvant	Immunization scheme	Challenge (strain, dose)	Immunization Results	*Campylobacter* colonization results	Ref
CjaA protein via *Salmonella* Typhimurium strain χ9718	10^8 CFU	*Camp. jejuni*, 81–176	NA	2 doses orally (D1, Week 2) Challenge 2 weeks after the second dose	*Camp. jejuni*, Wr-1 10^5 CFU	• Significantly higher anti-CjaA serum IgY and mucosal IgA titres in the vaccinated group especially on days 35 and 42 • Higher B-cell percentage in caecal tonsils of vaccinated birds on days 22, 31 and 35	No significant caecal reduction on days 7 and 14 postchallenge.	Laniewski *et al.* (2014)
CjaA protein fused to the tetanus toxin fragment C, via *Salmonella* Typhimurium 4/74 nal^R Δ*aroA*	10^8 CFU	*Camp. jejuni*, M1	NA	2 doses orally (D1, D14) Challenge 2 weeks after the second dose	*Camp. jejuni*, M1 10^7 CFU	• Significantly higher specific anti-CjaA IgY level in serum from day 8 to the end of the experiment (except for 1 time point) • Higher specific anti-CjaA IgA level in bile from day 22 to the end of the experiment. Significant for 5 time points out of 7.	Significant caecal reduction of 1·38 and 1·42 $\log_{10}$ CFU g^{-1} on days 21 and 28 postchallenge respectively	Buckley *et al.* (2010)

Table 5 (*Continued*)

Administration								
Antigens	Dose	Vaccine strain	Adjuvant	Immunization scheme	Challenge (strain, dose)	Immunization Results	*Campylobacter* colonization results	Ref
Omp18/CjaD or CjaA or ACE393 via attenuated *Salmonella* Enteritidis	$2{\cdot}5 \times 10^7$ CFU	ND	NA	1 oral dose (D1) Challenge on day 21	Mixture of 3 isolated *Camp. jejuni*, $3{\cdot}33 \times 10^7$ CFU	• Significantly higher anti-*Campylobacter* serum IgY titres for the 3 antigens on days 21 and 32 in vaccinated groups • Significantly higher anti-Campylobacter serum IgY and mucosal IgAs titres for Omp18/CjaD group compared to the other vaccinated groups	• Intestinal reductions of 1 and 2 $\log_{10}$ CFU g^{-1} for ACE393 and CjaA antigens respectively • Significant reduction to under the detection limit (of >4·8 $\log_{10}$ CFU g^{-1}) for Omp18/CjaD • Reproducible experiment for Omp18/CjaD	Layton *et al.* (2011)
CjaA/Peb1A/GlnH/ChuA proteins fused to the tetanus toxin fragment C via S*almonella* Typhimurium 4/74 nal^R Δ*aroA*	10^8 CFU	*Camp. jejuni*, M1	NA	2 doses orally (D1, D14) Challenge 2 weeks after the second dose	*Camp. jejuni*, M1 10^7 CFU	Significantly higher specific anti-TetC IgY levels in serum on days 29 and 43 for all vaccinated birds regardless of the antigen	• Caecal reduction of 1·64 $\log_{10}$ CFU g^{-1} in Peb1A vaccinated birds compared to the control group. Comparable to CjaA vaccinated birds • No caecal reduction for GlnH and ChuA vaccinated birds compared to the control group	Buckley *et al.* (2010)
Dsp proteins via *Salmonella* Typhimurium strain χ9088	ND	*Camp. jejuni*, NCTC11168	NA	3 doses orally (D3 D10, D16) Challenge 10 days later (D34)	*Camp. jejuni*, NCTC11168 10^5 CFU	NE	• All chickens colonized in both treated and nontreated groups • Significant caecal reduction of 2·48 $\log_{10}$ CFU compared to the control group receiving the empty vector	Theoret *et al.* (2012)

Table 5 (*Continued*)

Administration								
Antigens	Dose	Vaccine strain	Adjuvant	Immunization scheme	Challenge (strain, dose)	Immunization Results	*Campylobacter* colonization results	Ref
CjaA protein via *Eimeria tenella* oocyst	300 parasites 100, 500, 3000 and 5000 parasites	ND	NA	1 oral dose on day 2 Challenge on day 28 4 oral doses (100–5000) on days 1, 3, 7, 20 Challenge on day 28	*Camp. jejuni*, 02M6380 10^5 CFU	NE	• No difference between single and multiple immunizations. • Significant caecal reduction of ≈ 1 $\log_{10}$ CFU g^{-1} compared to the control groups	Clark *et al.* (2012)

IM, intra-muscular; IP, intra-peritoneal; SC, subcutaneous; ND, not defined; NA, not applicable; NE, not experimented.
All experimental trials were done in controlled environments.

Subunit vaccines

In chickens, the first subunit vaccine experiments were based on the immunodominant antigen of *Campylobacter*, flagellin. This is the main component of bacterial flagella, which play a crucial role in bacterial colonization. Subunit flagellin vaccination gave inconsistent results from one study to another. Widders *et al.* (1998) used purified native flagellin for subunit vaccination. Although this led to the development of a specific humoral immune response at both systemic and mucosal level, no significant reduction in *Campylobacter* loads was observed after the challenge. Fused to the B subunit of the *E. coli* labile toxin, and orally administered twice at the higher dose of 1 mg, flagellin induced specific antibodies in more than two-thirds of the vaccinated birds. The vaccinated birds had lower *Camp. jejuni* counts than the control group after an oral challenge (Khoury and Meinersmann 1995). More recently, Neal-McKinney *et al.* (2014) demonstrated that birds vaccinated with flagellin combined with the Montanide adjuvant showed a 3 $\log_{10}$ CFU g^{-1} intestinal reduction compared to the control group, in addition to a higher specific sera reactivity. Huang *et al.* (2010) tested flagellin vaccination using DNA by the intranasal route with chitosan nanoparticles in which pCAGGS-flaA, a DNA plasmid used as the flagellin A vector, was incorporated. After the second and third immunizations, significant higher specific antibody titres were detected for both serum IgY and intestine mucosal IgA compared to the control groups, along with a decrease in bacterial loads of 2–3 and 2 $\log_{10}$ CFU g^{-1} in the large intestine and caecum, respectively, after an oral challenge. Interestingly, *Camp. jejuni* was absent from the small intestine at the end of the study, confirming the immunization power of the *Campylobacter* flagellin.

However, despite promising results, flagellin cannot be used as an antigen for large-scale poultry vaccination for several reasons. The first is because of the differences in flagellin between *Campylobacter* strains and a lack of cross protection against various strains susceptible to colonize broilers. Next, many anti-flagellin antibodies are directed against nonsurface exposed epitopes, and consequently do not neutralize the bacterium during infection (Widders *et al.* 1998). Finally, some antibodies recognize glycosylated patterns with variable phases, allowing *Campylobacter* to evade the immune system thanks to its ability to vary the amount and nature of these residues.

Other antigens were tested in subunit vaccine experiments. The CjaA protein, known as the binding protein component of an ABC transporter system (Muller *et al.* 2005), was inoculated on day one or day 15 posthatching. In both experimental groups, significantly higher specific

IgY titres were detected than in the control group and were the same for both inoculation periods. Caecal *Campylobacter* loads were also similar in both groups on day 21 postchallenge, slightly higher on day 28 postchallenge when birds were first vaccinated on day 15 and always significantly lower than in the infected control group, indicating the immunization potential of the CjaA protein (Buckley *et al.* 2010). A study using nanoparticles was conducted to decrease *Camp. jejuni* colonization in chickens. Outer membrane proteins (OMP) were extracted and encapsulated in poly (lactide-co-glycolide) (PLGA) nanoparticles. When administered orally, there was no significant reduction in *Campylobacter* load regardless of the tested doses. However, subcutaneous vaccination was more efficient since the intestinal colonization level dropped below the detection limit, unlike the control group, and was accompanied by development of a strong immune response (Annamalai *et al.* 2013). Another study, investigating the role of the *dps* gene in biofilm formation, showed its involvement in *Camp. jejuni* colonization in its host and suggested it as a potential vaccine antigen. However, recombinant Dps subunit vaccination subcutaneously did not protect chickens from *Camp. jejuni* colonization after a challenge (Theoret *et al.* 2012). More recently, CadF, FlpA and CmeC proteins, having a role in *Campylobacter* adherence during poultry colonization, were tested as antigens in subunit vaccination experiments. Using the Montanide adjuvant, all of them induced an increase in sera reactivity of vaccinated birds. Although caecal reductions were not significant for both CadF- and CmeC-vaccinated groups, FlpA immunization significantly reduced caecal load by about 3 $\log_{10}$ CFU g^{-1}. Also, vaccination with the fused CadF-FlaA-FlpA protein and a mixture of the three full-length individual proteins as a booster led to a significant intestinal decrease of about 3 $\log_{10}$ CFU g^{-1} (Neal-McKinney *et al.* 2014). All these studies revealed the potential immunization power of certain *Campylobacter* antigens and need to be thoroughly investigated and repeated to confirm their vaccine features.

Antigens vectored by micro-organisms

In recent years, a new method of vaccine delivery has been studied. This consists in delivering antigens using micro-organisms harbouring plasmids with the DNA of interest. For *Campylobacter* antigens, attenuated *Salmonella* strains have been widely used as a vector with the CjaA protein as the main focus of study. Large discrepancies between studies have been observed despite similar conditions for the immunization scheme based on two vaccinations, the first on day 1 and the second 2 weeks later, followed by an oral challenge. Wyszynska *et al.* (2004) showed an increase in the specific anti-*Campylobacter* response for both IgY in serum and intestinal IgA compared to the unvaccinated group, along with a marked decrease in caecal *Campylobacter* load, particularly on day 12 postchallenge with a reduction of more than 6 $\log_{10}$ CFU g^{-1}. In contrast, Laniewski *et al.* (2014) did not find any significant reduction in caecal load, although they did demonstrate development of a humoral immune response and an increase in B-cell population in the caecal tonsils of the vaccinated group. Interim results have also been observed with the development of a specific immune response and the decrease by approx. 1·4 $\log_{10}$ CFU g^{-1} of the intestinal *Campylobacter* count (Buckley *et al.* 2010). Similar results were obtained after a single vaccination with an attenuated *Salmonella*-vectored CjaA protein followed by an oral challenge 3 weeks later (Layton *et al.* 2011). In these studies *Campylobacter*, *Salmonella* and the avian strains differed, which could explain discrepancies in colonization results. It was also shown that the specific immune response development was not necessary correlated with the decrease in intestinal level of *Campylobacter*. According to these experiments, all using the same immunization scheme, the choice of strains seemed to be essential in determining the vaccination's success.

Other antigens vectored by attenuated *Salmonella* strains have been trialled to decrease *Campylobacter* colonization in poultry. Layton *et al.* (2011) showed that after a single vaccination of chicks on the day of hatching, ACE393 vectored antigen, encoding a probable periplasmic protein, led to significantly higher IgY levels than the control groups, and to an approximately nonsignificant 1 $\log_{10}$ CFU g^{-1} *Campylobacter* reduction in the ileum after an oral challenge on day 21. With the same immunization scheme, Omp18/CjaD vectored antigen gave more promising results with significantly higher specific serum IgY and mucosal IgA titres, along with a significant drop in intestinal counts below the detection limit. The latter results were confirmed in repeated experiments (Layton *et al.* 2011). Buckley *et al.* (2010) showed that after two vaccinations, the Peb1A antigen significantly reduced the caecal load of *Camp. jejuni* by 1·6 $\log_{10}$ CFU g^{-1} when fused to the tetanus toxin and *Salmonella*-vectored, whereas no decreases were observed for GlnH and ChuA. The Dsp protein was also *Salmonella*-vectored and after three oral vaccinations followed by a challenge 10 days after the last vaccination, all the treated birds were found to be colonized, although the caecal load was significantly reduced by 2·48 $\log_{10}$ CFU g^{-1} compared to the group vaccinated with the empty vector (Theoret *et al.* 2012).

Besides *Salmonella*, oocysts of *Eimeria tenella* were used as a vector for the expression of the *Camp. jejuni* CjaA protein in immunization experiments. Single and multiple

oral vaccinations in young chickens were trialled, and after an oral challenge, the intestinal *Camp. jejuni* load was significantly lower, by approx. 1 $\log_{10}$ CFU g^{-1}, than that of the control groups with no difference between the single- and multiple-vaccination groups (Clark *et al.* 2012).

Some promising studies aimed at reducing *Campylobacter* colonization in broiler flocks through antigen-vectored micro-organisms have been described. However, as for other live vaccines and in spite of attenuated characteristics, a reversion process cannot be excluded. Antigen vectors used in the experiments could become virulent again with a gene acquired from the environment and therefore become pathogenic for the vaccinated birds, especially as *Salmonella* and *Eimeria* are species particularly pathogenic for poultry.

Combined strategies

Few studies to date have investigated the combination of several control strategies. For example, as *in vivo* experiments revealed that a *Bifidobacterium* strain and galactooligosaccharide (GOS) CUP Oligo P were individually efficient at reducing *Campylobacter* load in faeces, and that GOS was also able to increase *Bifidobacterium* spp. loads, Baffoni *et al.* (2012) trialled microencapsulated *Bifidobacterium longum* and GOS as additives to the normal feed. Compared to the control group, the symbiotic mixture significantly reduced the *Camp. jejuni* level after 14 and 21 days of administration, whereas the *Campylobacter* spp. population remained stable.

These studies combined only different nutritional compounds. It could be beneficial to trial a combined control strategy associating immunization and nutritional measures. Due to the different mechanisms involved in eliminating *Campylobacter*, we can expect a cumulative effect from the combination of a vaccine and a feed additive which could reduce by about 3 log units the caecal content as estimated by Romero-Barrios *et al.* (2013) to reduce the risk of human campylobacteriosis by over 90%. However, to date, there is no study which uses both nutritional and immunization strategies to induce synergy aimed at reducing *Campylobacter* load.

Conclusion

Despite all the studies conducted over the past few decades, *Campylobacter* remains one of the major bacterial causes of human intestinal diseases throughout the world. It has been proven to be mainly related to the handling and consumption of chicken meat. Some experiments have shown promising results, which suggest that it is possible to reduce *Campylobacter* loads in the chicken gut. Effective studies need to be further investigated to obtain reproducible results, and measures need to be applied on a larger scale, for instance, on chicken flocks intended for retail, in order to be able to evaluate their impact on human campylobacteriosis prevalence.

For both nutritional and immunization strategies, large discrepancies may be observed between studies. Several factors could explain these observations. The specific avian strains used during the experiments could impact the results because such strains can be more or less sensitive to *Campylobacter* and to treatment (Guyard *et al.* 2014; Humphrey *et al.* 2014). *Campylobacter* strains, doses and virulence could also explain these varied results as well as administration doses, routes and timings. For the nutritional control strategy, several studies highlighted discrepancies between *in vitro* and *in vivo* results which could be explained by degradation of the active product before reaching the intestinal tract of chickens, which is the desired site of action. Furthermore, products could act on other bacterial species *in vivo* yet be present in insufficient amounts to be effective on the target micro-organism. Indeed, differences between *in vivo* and *in vitro* experiments have recently been highlighted for several feed additives (Guyard *et al.* 2015). To counteract the early deterioration of active products by the digestive tract's acidic environment, one solution could be to protect them by encapsulation. This strategy was mentioned in an experiment using probiotic strains (Arsi *et al.* 2015). However, to test the strain's efficiency against *Campylobacter in vivo* and select the most promising strains, another strategy entailed first evaluating probiotic efficiency by intracloacal inoculations. Concerning the vaccination strategy, despite all the experimental studies and promising results, there is currently no vaccine available on the market to reduce the intestinal *Campylobacter* load in chickens. Some experiments with promising results have not yet been followed through. It may be necessary to identify and characterize new antigens. Recently, the flagellar capping protein FliD from *Camp. jejuni* D1-39 was investigated, focusing on its characterization and antigenicity. Immunoblotting tests showed that sera from 4-week- old chickens reacted strongly with the FliD protein, suggesting that it could be a potential vaccine antigen. This should be further evaluated through *in vivo* immunization experiments (Yeh *et al.* 2014). Similarly, several other recombinant flagellar proteins have been identified as potential vaccine antigens (Yeh *et al.* 2015). Recent novel strategies designed to identify new vaccine antigens based in particular on bioinformatics genome analysis could also prove useful in developing new, more powerful vaccines.

It should be remembered that primary poultry production is not the only step to target in order to reduce the

number of human campylobacteriosis cases. The following links along the poultry meat production chain must be taken into account. Many studies have focused on the slaughtering level, including both chemical and physical treatment to decontaminate carcasses. Meat retailing, consumer education and good hygiene practices at home have also been investigated.

However, the effectiveness of measures is not the only criteria to evaluate. The final appearance of the meat, the cost involved, and the consumer's acceptance of decontamination measures are all essential to the implementation of control strategies. A recent study evaluated consumer acceptability through a printed survey (MacRitchie *et al.* 2014), which revealed that irradiation or chemical treatment of chicken meat are the least acceptable measures, even if they could decrease human campylobacteriosis cases by 90%. In contrast, better hygiene practices on farms are the most acceptable to consumers. Four other treatments—including vaccination, steaming, freezing and feed additives—give mixed results. The study also demonstrated that prior awareness about *Campylobacter* and food poisoning did not impact acceptability, indicating the difficulty involved in increasing consumer acceptability.

New efforts need to be made to test *in vivo* components such as new nutritional additives, vaccine antigens or a combination of both at experimental and farm levels, with biosecurity measures being implemented and maintained throughout the poultry rearing process, supported by educational initiatives among farmers. Consumers also need to be informed on good hygiene practices, in addition to how and why it is necessary to thoroughly cook poultry meat.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this review.

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