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1 ***Campylobacter* control in poultry by current intervention measures ineffective: urgent**
2 **need for intensified fundamental research**

3

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6

7 Running title: *Campylobacter* control in poultry

8

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14

15 **Abstract**

16 *Campylobacter*-contaminated poultry meat is an important source of foodborne gastroenteritis
17 and poses a serious health burden in industrialized countries. Broiler chickens are commonly
18 regarded as a natural host for this pathogen and infected birds carry a very high
19 *Campylobacter* load in their gastrointestinal tract, especially the ceca. This results in
20 contaminated carcasses during processing. While hygienic measures at the farm and control
21 measures during carcass processing can have some effect on the reduction of *Campylobacter*
22 numbers on the retail product, intervention at the farm level by reducing colonization of the
23 ceca should be taken into account in the overall control policy. This review gives an up-to-

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1 date overview of suggested on-farm control measures to reduce the prevalence and
 2 colonization of *Campylobacter* in poultry.

3

4 **Keywords:** *Campylobacter*; poultry; cecal colonization; on-farm control measure

5

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1 **1. Introduction**

2

3 Today, *Campylobacter* infections are the leading cause of human bacterial gastroenteritis
4 in many developed countries (EFSA, 2010b). Broiler chickens are a potential reservoir for
5 *Campylobacter* strains pathogenic to human (Friis et al., 2010) and broiler chicken meat
6 contaminated with this pathogen is believed to be responsible for up to 40% of human
7 campylobacteriosis cases (EFSA, 2010a).

8 *Campylobacter* is highly prevalent among broiler flocks with on average 60% to 80% of
9 the analyzed flocks being colonized with the bacterium at slaughter age in the EU (Evans and
10 Sayers, 2000; Herman et al., 2003; Rasschaert et al., 2006; Reich et al., 2008; EFSA, 2010c).
11 Primary infection of broilers probably occurs through horizontal transmission from the
12 environment (Jacobs-Reitsma et al., 1995). Potential sources and vectors for contamination
13 are infected livestock and free-living animals (van de Giessen et al., 1996; Zweifel et al.,
14 2008; Ellis-Iversen et al., 2009), rodents and flies (Hald et al., 2008; Hazeleger et al., 2008),
15 contaminated surface water (Messens et al., 2009) and personnel and farm equipment
16 (Ramabu et al., 2004) at the farm. Also partial thinning of broiler flocks has been implicated
17 as a potential risk factor for *Campylobacter* colonization of the remainder of the animals, due
18 to difficulties in maintaining biosecurity during thinning (Allen et al., 2008). Most flocks
19 become colonized at an age of two to four weeks only (Jacobs-Reitsma et al., 1995; Evans
20 and Sayers, 2000; Herman et al., 2003; van Gerwe et al., 2009). The majority of the birds in a
21 flock are colonized within only a few days after the first chick is infected (van Gerwe et al.,
22 2009). These broiler chickens carry high *C. jejuni* numbers in their intestinal tract, especially
23 in the ceca (between 10^6 to 10^8 CFU/g or higher), and remain colonized until slaughter (Beery
24 et al., 1988; Jacobs-Reitsma et al., 1995; Evans and Sayers, 2000).

1 Intestinal colonization of broiler chickens with *Campylobacter* during rearing is
2 responsible for the contamination of the carcasses after processing (Herman et al., 2003;
3 Rasschaert et al., 2006; Rosenquist et al., 2006; Reich et al., 2008). Worldwide, an average
4 prevalence of *Campylobacter* contamination on poultry carcasses is reported to be in the
5 range of 60% to 80% (Suzuki and Yamamoto, 2009; EFSA, 2010c). Carcass contamination
6 occurs during defeathering and evisceration, by contaminated feces leaking from the cloaca
7 and visceral rupture of the ceca carrying a high *Campylobacter* load (Berrang et al., 2001;
8 Smith et al., 2007; Allen et al., 2008; Boysen and Rosenquist, 2009). In addition, carcasses
9 can become contaminated by cross-contamination of *Campylobacter* strains between
10 slaughtered flocks (Allen et al., 2008; Normand et al., 2008).

11

12 **2. *Campylobacter* control in poultry**

13

14 In the past few years, several quantitative risk assessments for *Campylobacter* in poultry
15 meat have been developed as a guidance tool to control the presence of this zoonotic pathogen
16 throughout the poultry meat production chain (Nauta et al., 2009). Although there is
17 considerable variation between countries in the approach of these models, all risk assessments
18 conclude that aiming to reduce the *Campylobacter* levels on broiler carcasses after
19 evisceration is the most effective intervention measure, rather than reducing its prevalence.
20 Besides reducing external surface contamination of broiler carcasses from *Campylobacter*-
21 colonized flocks directly, by physical or chemical means (Rosenquist et al., 2006; Boysen and
22 Rosenquist, 2009), reduced *Campylobacter* numbers on carcasses can also be obtained
23 indirectly. On-farm intervention measures aimed to prevent *Campylobacter* introduction and
24 transmission in poultry flocks or to reduce intestinal *Campylobacter* counts in colonized
25 animals could lead to reduced contamination levels of the carcasses of these animals after

1 processing. Moreover, because the intestine of living poultry is the only amplification site for
2 *Campylobacter* throughout the entire food chain, reducing the cecal *Campylobacter* load in
3 poultry during primary production is expected to significantly reduce the incidence of human
4 campylobacteriosis (Lin, 2009).

5 In Denmark, a quantitative microbial risk assessment of human campylobacteriosis
6 associated with thermotolerant *Campylobacter* spp. in broiler chickens was developed. The
7 simulations showed that reducing the number of *Campylobacter* bacteria on chicken carcasses
8 by 2 logs causes a 30-fold reduction in the incidence of campylobacteriosis in humans
9 (Rosenquist et al., 2003). A Belgian risk assessment showed that the incidence in Belgium
10 would be reduced by 48%, 85% and 96% when respectively a one log, two log or three log
11 reduction of the *Campylobacter* contamination on carcasses would be achieved (Messens et
12 al., 2007).

13 Theoretically, controlling *Campylobacter* colonization in poultry on-farm may be
14 achieved in a number of different ways, including hygienic and biosecurity measures (2.1.),
15 water treatment (2.2.), supplementing plant-derived additives to the feed (2.3.), bacteriophage
16 application (2.4.), vaccination (2.5.), passive immunization (2.6.) and application of pre- and
17 probiotics/competitive exclusion microflora (2.7.) or bacteriocins (2.8). It is important to
18 differentiate between prevention and colonization-reducing measures, which intervene at a
19 different stage of the colonization process. Preventive measures, summarized in Table 1, aim
20 at reducing the probability of birds to become colonized by *Campylobacter*, while
21 colonization-reducing measures, presented in Table 2, strive for a reduced cecal
22 *Campylobacter* load in colonized birds prior to slaughter, thereby reducing surface
23 contamination of the carcasses. Moreover, also by improving health and welfare of the
24 animals colonization might be reduced (Bull et al., 2008). Finally, genetic selection could also
25 contribute in combating *Campylobacter* colonization in poultry (Kapperud et al., 1993), when

1 poultry lines with improved overall immunological responsiveness, being more resistant to
2 colonization by this pathogen, are developed (Swaggerty et al., 2009). Some antibiotics
3 efficiently reduce *C. jejuni* counts in the broiler chick GI tract (Farnell et al., 2005; Hermans
4 et al., 2010), but their use is controversial due to concerns on development of antibiotic
5 resistance in *C. jejuni*, which may compromise treatment of human campylobacteriosis
6 (Dibner and Richards, 2005; Zhu et al., 2006).

7

8 2.1. Hygienic and biosecurity farming practices

9

10 Good hygienic farming practices constitute a strategy aiming at preventing the
11 introduction of *Campylobacter* into a flock by a combination of hygiene and biosecurity
12 measures. A Belgian quantitative microbial risk assessment showed that the incidence of
13 human campylobacteriosis in Belgium would be reduced by 32%, 53% and 77% when the
14 *Campylobacter* flock prevalence is reduced by 25%, 50% or 75% respectively (Messens et al.,
15 2007). Application of specific hygienic measures during the rearing period, such as washing
16 hands before entering the chicken house, the use of separate boots for each broiler house,
17 footbath disinfection when entering a broiler house and a high standard of cleaning and
18 disinfection of the drinking water equipment may significantly reduce the risk of
19 *Campylobacter* infections in broiler flocks (van de Giessen et al., 1996; Evans and Sayers,
20 2000). After introduction of hygienic and biosecurity measures, including the control of
21 rodents and insects, in two Dutch broiler farms, the percentage of *Campylobacter*-colonized
22 flocks decreased from 66% at one farm and 100% at the second farm to 22% and 42%,
23 respectively (van de Giessen et al., 1998). In the UK, the implementation of an intervention
24 trial, based on a standard hygiene protocol for personnel and proper disinfection of the broiler
25 house prior to stocking, reduced the prevalence of *Campylobacter* infection in the broiler

1 population from 80% to < 40% (Gibbens et al., 2001). It has been demonstrated that the
2 prevalence of broiler flocks colonized with *Campylobacter* can be reduced from 51.4% to
3 15.4% by placing fly screens in broiler houses (Hald et al., 2007). In Denmark, strategies to
4 control *Campylobacter* were intensified in 2003 (Rosenquist et al., 2009). Focus was on
5 biosecurity, allocation of meat from colonized flocks to the production of frozen meat
6 products (having reduced *Campylobacter* counts on their surface due to the freezing
7 procedure) as much as possible and campaigns to inform the consumer. This implemented
8 control strategy lead, at least in part, to a decrease of *Campylobacter*-colonized flocks from
9 43% in 2002 to 27% in 2007, a reduction in *Campylobacter*-positive samples of chilled
10 broiler meat after processing from 18% in 2004 to 8% in 2007 and a drop in registered human
11 campylobacteriosis cases by 12% from 2002 to 2007. These findings suggest that proper
12 application of biosecurity measures can lead to reduced colonization in poultry. However,
13 because broiler chickens are under a constant contamination pressure, biosecurity measures
14 alone will not be sufficient to solve the problem.

15

16 2.2. Drinking water treatment

17

18 By treating the drinking water of poultry flocks, the risk of the animals to become infected
19 might be reduced, probably through a reduction in bacterial numbers both in the drinking
20 water and the crop. In this way, *Campylobacter* is less likely to reach the ceca and
21 transmission throughout the flock might be reduced or prevented.

22 *In vitro* studies have demonstrated that organic acids have a strong bactericidal effect on
23 *Campylobacter* spp. and addition of these acids to the drinking water on poultry farms could
24 prevent transmission through broiler flocks (Chaveerach et al., 2002; Chaveerach et al.,
25 2004b). Addition of 0.44% (vol/vol) lactic acid in the drinking water during pre-slaughter

1 feed withdrawal reduced both crop and pre-chill carcasses contamination (Byrd et al., 2001).
2 Moreover, addition of monocaprin, the mono-acylglycerol of capric acid (Thormar et al.,
3 2006), to drinking water from the last three days before slaughter, resulted in a reduced *C.*
4 *jejuni* count on cloacal swabs of both artificially and naturally infected birds (Hilmansson et
5 al., 2006). This treatment did, however, not prevent *Campylobacter* spread from artificially
6 infected to non-infected birds. Also chlorinating the drinking water is helpful as it reduces the
7 risk for *Campylobacter* colonization (Ellis-Iversen et al., 2009). Chlorination of flock
8 drinking water (with 2-5 ppm chlorine) under commercial production practices in the US in
9 2002 did, however, not result in a reduced *Campylobacter* prevalence in the birds receiving
10 treated water (Stern et al., 2002).

11

12 2.3. Plant-derived feed additives

13

14 Changes in the composition of the feed can promote gastrointestinal health and thus
15 contribute to the control of *Campylobacter* in poultry. Plant-derived antimicrobial feed
16 additives can be administered from day-of-hatch to prevent broiler chickens to become
17 colonized and to reduce *Campylobacter* transmission throughout the flock. Also in this
18 application, the observed effect is largely due to the anti-*Campylobacter* effect in the crop of
19 the animals.

20 Next to their application in drinking water, organic acids might also be used as feed
21 additives to reduce *Campylobacter* prevalence in poultry. However, *in vivo* trials
22 demonstrated only a limited effect of feed acidification on *C. jejuni* prevalence in broiler
23 flocks. At most it could delay the onset of colonization (Heres et al., 2004; Line and Bailey,
24 2006). Broilers that were fed fermented liquid feed, i.e. a moistened feed with a high number
25 of lactobacilli, a high concentration of lactic/acetic acid and a pH of 4, were less likely to shed

1 *Campylobacter* after oral infection (Heres et al., 2003). However, at the end of the trial no
2 significantly different *C. jejuni* counts in the ceca could be observed compared to chickens on
3 a standard feed. The higher level of lactic acid in combination with a low pH in the crop was
4 suggested to reduce the probability for *Campylobacter* to reach the ceca. In a later
5 experiment, individually housed chickens that were fed acidified feed were found to be less
6 susceptible to *Campylobacter* infection compared to control birds, as less chickens became
7 colonized at equal inoculation doses (Heres et al., 2004). Also caprylic acid leads to reduced
8 colonization in 10-day-old chicks when given preventively (Solis de los Santos et al., 2008).
9 In contrast, addition of butyrate to the feed was not able to reduce cecal *Campylobacter*
10 colonization in a seeder model using two-week-old broilers (Van Deun et al., 2008). Skanseng
11 et al. (2010) found little effect when supplementing only formic acid to the feed, but a
12 combination of 2% formic acid with 0.1% sorbate prevented *C. jejuni* colonization in chicks.
13 Finally, it was demonstrated that the addition of a medium-chain fatty acid mixture to the feed
14 at 1% reduces the probability of broilers becoming colonized (van Gerwe et al., 2010).

15 Several other plant-derived compounds are known to possess antimicrobial properties.
16 Thousands of phytochemicals have already been identified to be inhibitory toward
17 microorganisms, including phenolics and essential oils (Cowan, 1999). Friedman et al. (2002)
18 analyzed the *in vitro* bactericidal activity of 96 essential oils and 23 isolated oil compounds
19 against *C. jejuni*. Lots of these analyzed compounds were capable of killing the bacterium at
20 relatively low concentrations, especially the cinnamon-oil *trans*-cinnamaldehyde. The
21 potential use of in-feed *trans*-cinnamaldehyde to prevent colonization, and/or to reduce the
22 cecal *Campylobacter* numbers in broilers, has been examined very recently (Hermans et al.,
23 2011). In this study it was shown that, despite its marked activity *in vitro*, *trans*-
24 cinnamaldehyde was ineffective in preventing or reducing cecal colonization by *C. jejuni* in a
25 broiler seeder model, where the compound was administered at 0.3% (wt/wt) to the feed, from

1 day-of-hatch until euthanasia. Also when directly injected in the ceca of broilers, no reduction
2 in *Campylobacter* numbers was observed after two or 24 hours.

3 Administration of large molecules that interfere with *Campylobacter* adhesion to the host
4 cell is successful *in vitro* but suffers from premature metabolic breakdown in the broiler
5 chicken gastrointestinal tract (Wittschier et al., 2007). Finally, cecal colonization of birds
6 receiving plant-protein-based feed was significantly lower compared to birds receiving
7 animal-protein-based feed or a combination of plant- and animal-protein sources
8 (Udayamputhoor et al., 2003).

9 Alternatively, colonized broiler chickens might be fed pulse doses of the additives for a
10 certain period, just before slaughter, aiming at reducing the cecal *Campylobacter* load and
11 reducing carcass contamination after slaughter. Thus, in this application one aims to reduce
12 the *Campylobacter* numbers in the ceca of already colonized birds. To efficiently reach the
13 cecum, additives are often coated on/encapsulated in carrier material that will prevent
14 premature degradation along the gastrointestinal tract and assure efficient release of the active
15 compound into the gut (Van Immerseel et al., 2004).

16 Hermans et al. (2010), however, found no effect in cecal *Campylobacter* numbers of
17 broilers fed medium-chain fatty acids (caproic, caprylic or capric acid) from three days before
18 euthanization in 28-day-old broilers. Also direct injection in the broiler cecum of a
19 concentrated sodium caprate solution did not prevent colonization, nor was it able to reduce
20 cecal *Campylobacter* numbers. These authors showed that intestinal mucus is likely to protect
21 *C. jejuni* in the broiler cecum against the bactericidal effects of organic acids seen *in vitro*. In
22 contrast, another research group observed a considerable reduction (several logs) in cecal
23 *Campylobacter* numbers when caprylic acid was given from three days before slaughter, in
24 already colonized market-aged broilers (Solis de los Santos et al., 2010). This reduction was
25 strikingly not accompanied by an altered cecal microbial population. Moreover, addition of

1 monocaprin to the feed from the last three days before slaughter, resulted in a reduced *C.*
2 *jejuni* count on cloacal swabs of both artificially and naturally infected birds (Hilmansson et
3 al., 2006).

4 As the available *in vivo* results are limited and moreover contradictory, it cannot be
5 univocally be determined what the contribution of feed additives will be to control cecal
6 *Campylobacter* colonization. Preventive supplementation from day-of-hatch, rather than to
7 aim for reduced cecal *Campylobacter* numbers in already colonized birds, seems most
8 promising. The ineffectiveness of butyrate and the very promising *trans*-cinnamaldehyde,
9 however, puts the use of in-feed organic acids and plant-derived antimicrobial compounds to
10 combat cecal *Campylobacter* colonization in poultry in question.

11

12 2.4. Bacteriophage application

13

14 Bacteriophage application to reduce cecal *Campylobacter* colonization in poultry is
15 promising (Carrillo et al., 2005; Wagenaar et al., 2005). Results indicate an immediate drop of
16 approximately three logs in the number of *Campylobacter* in already-colonized chicken ceca
17 (Wagenaar et al., 2005). After five days, however, bacterial counts stabilized at a level one
18 log lower compared to control birds, an effect also observed when phages were given
19 prophylactically. Also El-Shibiny et al. (2009) observed an immediate (after two days) two-
20 log CFU/g reduction in cecal *Campylobacter* levels. Despite the fact that *Campylobacter*,
21 after a sudden drop, seems to re-establish itself to nearly its original counts, results indicate
22 that bacteriophages can possibly be successfully applied in broilers just before slaughter to
23 reduce the cecal bacterial load. Further research in this area showed that administering phages
24 in the feed is more efficient than oral gavage (Carvalho et al., 2010). This study revealed an
25 initial drop, already after two days, of approximately two logs in the numbers of *C. jejuni* in

1 the fecal material of infected one-week-old birds. Moreover, *C. jejuni* did not regain its
2 original counts throughout the experimental period, which was ended seven days after phage
3 administration had started.

4 Although the use of phage products in broilers seems to be a promising way to reduce
5 cecal colonization with *C. jejuni*, questions regarding both immediate and long-term efficacy,
6 consumer safety and application methods arise (Hagens and Loessner, 2010). Safety concerns
7 should not be a main obstacle as phages are highly specific and can only infect a limited range
8 of host bacteria. Moreover, their oral consumption, even at very high levels, is believed to be
9 completely harmless to humans. Answers concerning the efficacy seem to be more complex,
10 especially if long-term efficacy of the phage product has to be ensured. In the study of El-
11 Shibiny et al. (2009) it was shown that 2% of the *Campylobacter* population exposed to
12 virulent phages in the chicken, developed phage-resistance. These resistant types remained a
13 minor component of the population. Carvalho et al. (2010) isolated phage-resistant
14 *Campylobacter* strains from phage-administered chicks at a frequency of 13%. Strikingly,
15 also before phage application resistance was observed, although at a lower frequency (6%),
16 indicating that *Campylobacter* can acquire phage resistance naturally. Nevertheless, an
17 increase in the resistant *Campylobacter* population was observed after applying phages,
18 suggesting that phages might have selected for resistant strains. Because further information
19 on this topic is lacking, long-term efficacy of phages to control *C. jejuni* in poultry cannot be
20 ensured.

21

22 2.5. Vaccination

23

24 Several vaccination studies aiming at reducing the susceptibility of broiler chickens for
25 *Campylobacter* colonization have been reported, although with variable results. *In ovo*

1 vaccination by injection of heat-killed *C. jejuni* in the amniotic fluid resulted in an increase in
2 Immunoglobulin A (IgA) antibodies (Noor et al., 1995). However, the consequences on a
3 subsequent challenge were not studied. Intraperitoneal immunizations of chickens with killed
4 *C. jejuni* whole cells at 16 and 29 days of age reduced the intestinal colonization, which was
5 associated with an increase in specific IgY in intestinal secretions (Widders et al., 1996). In
6 addition, Rice et al. (1997) demonstrated some reduction of *Campylobacter* colonization of
7 chicks orally vaccinated with formalin-killed *C. jejuni* whole cells in combination with
8 *Escherichia coli* heat-labile toxin when compared to non-vaccinated control birds.

9 For subunit vaccines, flagellin and outer membrane proteins have been tested and are
10 considered useful candidates. In a study involving immunization of chickens with heat-killed
11 *C. jejuni*, intestinal colonization upon challenge was reduced, with flagellin and a 67 kDa
12 protein showing up as the immunodominant antigens (Widders et al., 1998). Vaccination of
13 chickens with a hybrid protein containing part of the *C. jejuni* FlaA and the B-subunit of *E.*
14 *coli* heat-labile toxin elicited specific antibodies against *C. jejuni* flagellin and reduced
15 colonization of the chickens after challenge (Khoury and Meinersmann, 1995). Chickens
16 orally immunized with an avirulent recombinant *Salmonella* strain carrying the
17 *Campylobacter cjaA* gene, encoding a highly immunogenic lipoprotein which is conserved
18 among different *Campylobacter* serotypes, developed serum IgY and mucosal IgA antibody
19 responses against *Campylobacter* and *Salmonella* outer membrane proteins and were
20 protected against cecal colonization with a heterologous wildtype *C. jejuni* strain (Wyszynska
21 et al., 2004). A more recent study evaluated the potential use of a heterologous vaccine for
22 *Campylobacter* control in poultry using substantially more animals (Buckley et al., 2010).
23 Upon vaccination with a *Salmonella* Typhimurium Δ aroA mutant, expressing CjaA as a
24 plasmid-encoded fusion to tetanus toxin, birds had significantly reduced cecal *C. jejuni* counts
25 of approximately \log_{10} 1.4 CFU/g three and four weeks after *C. jejuni* inoculation, compared

1 to unvaccinated control birds. This protection was associated with increased levels of CjaA-
2 specific serum IgY and biliary IgA in the vaccinated chicks. Also in this study, a group of
3 chicks receiving a vaccine strain containing the non-recombinant plasmid was incorporated.
4 These animals were not protected, indicating that the protective effect observed in the birds
5 receiving the heterologous vaccine, expressing CjaA, is due to responses directed against
6 CjaA rather than competitive or cross-protective effects mediated by the carrier. Broiler
7 chicks orally gavaged with live *Salmonella*-vectors expressing *Campylobacter* Omp18/CjaD,
8 CjaA and ACE393 at day-of-hatch and inoculated with *C. jejuni* at 21 days of age, had higher
9 serum IgG and mucosal sIgA levels as well as reduced ileal *C. jejuni* counts at day 32,
10 compared with control birds (Layton et al., 2010). Vaccination with the Omp18/CjaD peptide-
11 expressed vector was most effective and *Campylobacter* could not be recovered from ileal
12 samples. However, the cecal *Campylobacter* load, a better indicator for the colonization level
13 in broiler chicks (Beery et al., 1988), was not determined.

14 Zeng et al. (2009) showed that specific CfrA antibodies can block the function of this
15 protein, diminishing ferric enterobactin-mediated growth promotion under iron-restricted
16 conditions in a dose-dependent way. As inactivation of the *cfrA* gene completely eliminates
17 *Campylobacter* colonization in chicks and CfrA is both expressed and immunogenic in
18 chickens experimentally infected with *C. jejuni*, CfrA could be a promising candidate for a
19 subunit vaccine for *Campylobacter* control in poultry (Zeng et al., 2009), but this hypothesis
20 has yet to be tested.

21 Despite all this research, an effective vaccine to combat cecal *Campylobacter* colonization
22 in poultry is not yet available.

23

24 2.6. *Passive immunization*

25

1 Experimental studies have shown that chick colonization can be inhibited by using
2 antibodies. *Campylobacter*-specific maternal antibodies protect young chickens from
3 colonization (Sahin et al., 2003). Pre-incubation of *Campylobacter* with rabbit hyper-immune
4 antiserum or chicken bile antibodies increased the dose required to colonize the chicken
5 cecum (Stern et al., 1990). Oral administration of bovine or chicken Ig preparations from
6 respectively milk or eggs of hyper-immunized animals, conferred a marked protection against
7 challenge with *C. jejuni* in chickens (Tsubokura et al., 1997). Fecal bacterial counts were
8 reduced by >99% (prophylaxis) or 80%-95% (post-colonization) using an antibody
9 preparation. The mean number of bacteria quickly increased, however, after ending the
10 colonization-reducing addition with antibodies. This strategy might thus be applied to reduce
11 cecal numbers of bacteria immediately before slaughter.

12

13 2.7. Prebiotics and probiotics/competitive exclusion

14

15 Although the exact exclusion mechanism is not fully understood, experiments have shown
16 that competitive exclusion microflora can prevent *Campylobacter* colonization of the chicken
17 gut. Competitive exclusion is a prophylactic measure that aims at increasing the resistance of
18 chicks to *Campylobacter* infection.

19 Undefined bacterial mixtures have been demonstrated to effectively control
20 *Campylobacter* infections in young chicks artificially challenged with a chicken *C. jejuni*
21 isolate (Soerjadi et al., 1982; Soerjadi-Liem et al., 1984). In another study, however, this
22 protective effect was not observed (Stern et al., 1988). The efficacy of competitive exclusion
23 depends on cultivation methods and storage of the microbiota. It was found that the efficacy
24 of using competitive exclusion microflora decreased with storage of the cultures (Stern,
25 1994). Different culture preparation techniques, with respect to the level of anaerobic culture,

1 degree of epithelial scraping of the ceca, media used for subculturing and incubation
2 temperature, resulted in different degrees of protection against colonization by *Campylobacter*
3 spp. (Stern et al., 2001). However, Schoeni and Wong (1994) concluded that protection by
4 aerobically grown cultures was not statistically different from that obtained with anaerobically
5 grown cultures.

6 Later, attempts have been made to develop defined microbiota. A standard feed
7 supplemented with the yeast *Saccharomyces boulardii* did not significantly affect cecal
8 *Campylobacter* colonization of experimentally challenged chickens (Line et al., 1998). The
9 use of a probiotic containing *Lactobacillus acidophilus* and *Enterococcus faecium* in chicks,
10 during the first three days of rearing, reduced both *C. jejuni* fecal shedding and jejunal
11 colonization in colonized market-aged broilers, experimentally infected with *C. jejuni* six
12 hours after the first oral administration of the probiotic, with 70% and 27%, respectively
13 (Morishita et al., 1997). Administration of competitive exclusion cultures of *Citrobacter*
14 *diversus*, *Klebsiella pneumoniae* and *E. coli* effectively prevented or reduced *C. jejuni*
15 colonization in chickens after *Campylobacter* inoculation (Schoeni and Wong, 1994). This
16 protection was enhanced by feeding mannose to the chickens. In a simulated chicken
17 digestive tract model, addition of *L. acidophilus*, *L. fermentum*, *L. crispatus* and *L. brevis*
18 exerted an antagonistic effect on *C. jejuni* (Chang and Chen, 2000). Svetoch and Stern (2010)
19 have screened thousands of isolates of *Bacillus*, *Paenibacillus*, *Lactobacillus*, *Streptococcus*,
20 *Enterococcus* and *Escherichia* and selected hundreds of strains that were active against *C.*
21 *jejuni in vitro*. A *Lactobacillus* strain was isolated from an adult chicken gut that showed
22 bactericidal effects against *Campylobacter in vitro*, probably by the production of organic
23 acids and an anti-*Campylobacter* peptide (Chaveerach et al., 2004a). Two promising
24 antagonistic isolates (*L. salivarius* NRRL B-30514 and *Paenibacillus polymyxa* NRRL-B-
25 30509), acting as probiotics, were ineffective to control *Campylobacter*, whether the isolates

1 were fed to chicks before or after artificial challenge with *C. jejuni* (Stern et al., 2008). These
2 isolates were, however, able to produce bacteriocins which are able to reduce the
3 *Campylobacter* load in the gut of colonized birds (see further).

4 It has been demonstrated that it is possible to use combinations of (heterologous) *C. jejuni*
5 chicken isolates for the competitive exclusion of human pathogenic *C. jejuni* strains in poultry
6 (Chen and Stern, 2001). Circulation of uncharacterized environmental *Campylobacter* strains
7 in commercial poultry flocks could possibly be biologically controlled by a characterized
8 hyper-colonizing *C. jejuni* strain. Australian researchers identified such a strain that was
9 capable of displacing other colonizing strains and maintain itself in the chicken GI tract for
10 the entire 56-day broiler production cycle, without being displaced by other (hyper-
11)colonizing strains, once colonization was established (Calderon-Gomez et al., 2009).

12 With an approach called antibiotic dissection, day-old turkey poults were inoculated with
13 cecal contents of *Campylobacter*-free adult turkeys after which the microbial communities in
14 these poults were modified by different antibiotic treatments. It was investigated which
15 modified intestinal microbiota was able to outcompete a *Campylobacter* challenge. Molecular
16 examination of the constituents of these communities detected a subtype I of *Megamonas*
17 *hypermegale* to be specific for a *C. jejuni*-suppressive application (Scupham et al., 2010). *In*
18 *vivo* competition experiments with *M. hypermegale* isolates of both subtypes will be
19 necessary to prove *C. jejuni* exclusion in poultry.

20 Finally, addition of mannanoligosaccharide to the feed of naturally infected birds and
21 xylanase to the feed of artificially infected broilers, as prebiotics, resulted both in a minor,
22 although significant decrease in cecal *C. jejuni* counts in these animals (Fernandez et al.,
23 2000; Baurhoo et al., 2009).

24

25 2.8. Bacteriocin application

1

2 Svetoch and Stern (2010) recently reviewed bacteriocin application to reduce the cecal
3 *Campylobacter* counts in broiler chickens of colonized flocks. Applying purified encapsulated
4 bacteriocin from either *L. salivarius* NRRL B-30514 or *P. polymyxa* NRRL-B-30509 to the
5 feed during three days before euthanization led to a reduction of cecal *Campylobacter*
6 colonization in broiler chickens, orally gavaged with *C. jejuni* at day-of-hatch, by at least six
7 logs. However, birds were only seven to ten days of age and birds at slaughter age have not
8 been examined in this study. Further research by these authors led to the identification of two
9 more bacteriocin-producing isolates with marked anti-*Campylobacter* activity: *E.*
10 *durans/faecium/hirae* (NRRL B-30745) producing bacteriocin BCN E 760 and *E. faecium*
11 (NRRL B-30746) producing BCN E 50-52. Both bacteriocins were able to tremendously
12 lower ($> 6 \log_{10}$ CFU/g or below detectable levels) the cecal *C. jejuni* load in inoculated
13 broilers. Also in market-aged broilers naturally infected with *C. jejuni*, these bacteriocins
14 were effective. BCN E 760 reduced the cecal *Campylobacter* load in these animals from an
15 average of \log_{10} 6.2 CFU/g to undetectable levels when added to the feed four days before
16 slaughter. BCN E 50-52 at 10.8 mg per bird was able to reduce cecal colonization by $> 5 \log_{10}$
17 CFU/g when added to the drinking water three days before slaughter. Supplementing BCN
18 760 in drinking water at 3.5 to 25 mg per bird for three days before slaughter was most
19 effective, resulting in a complete elimination of *C. jejuni* in 90% of the cases or else, a
20 reduction of over six logs. The safety of these bacteriocins was confirmed by conducting
21 experiments on monkey and human cell cultures as well as in treated mice and chickens.
22 Italian researchers (Santini et al., 2010) very recently reported both marked *in vitro* and *in*
23 *vivo* activity for *Bifidobacterium longum* PCB 133 toward *Campylobacter*. After two weeks
24 of daily administration, excreted *B. longum* PCB 133 counts were still high in the feces of

1 orally gavaged chicks, even after a wash-out period of six days, and *C. jejuni* numbers were
2 significantly reduced by one log after this administration period.

3

4 **3. Concluding remarks**

5

6 Despite all efforts during the past decade there is still no effective, reliable and practical
7 intervention measure available to prevent or to reduce *Campylobacter* colonization in broilers
8 (Lin, 2009). As a consequence, neither the overall prevalence of this pathogen in chicken
9 retail products, nor the number of reported poultry meat consumption-related human
10 campylobacteriosis cases have been reduced in recent years (Moran et al., 2009; EFSA,
11 2009). The incomplete understanding of the chick immune system hampers vaccine
12 development, although the subunit (Omp18/CjaA) *Salmonella*-vectored vaccine seems a
13 promising candidate for further evaluation. Therefore, increased knowledge about the
14 interaction between *C. jejuni* and the chicken immune system is needed to identify
15 colonization factors of *C. jejuni* in the broiler chick which might act as potential targets for
16 vaccine development. The use of bacteriocins and bacteriophages is highly promising and
17 possibly commercially applicable, since safety concerns should not be a main obstacle and
18 their use is ergonomic since they can be easily and efficiently administered to the feed or
19 drinking water. Their potential use, however, still needs further research concerning long-term
20 efficacy. Also, large-scale field trials need to be performed to examine the practical effect of
21 such applications in a commercial poultry production environment. Moreover, successful
22 application of these methods (as well as competitive exclusion, probiotics and even
23 vaccination) might be affected by genomic instability in *C. jejuni* (Ridley et al., 2008)
24 possibly affecting long-term efficacy. Therefore, further research on the abovementioned

1 topics must be encouraged to demonstrate the genuine contribution of bacteriocin and
2 bacteriophage application in commercial poultry settings.

3 To conclude, *Campylobacter* control in poultry faces many hurdles to overcome and
4 probably several strategies will have to be combined if one wants to develop a suitable,
5 reliable and effective strategy to eradicate this human pathogen from poultry flocks.

6

7 **Conflict of interest**

8

9 None to declare

10

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12

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16

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Table 1Preventive on-farm control measures to reduce *Campylobacter* prevalence and transmission in poultry flocks.

| Intervention measure | Intervening stage in colonization process | Points of attention | References |
|--|--|--|---|
| <i>Hygiene and biosecurity farming practices</i> | Reduced prevalence of <i>Campylobacter</i> -colonized flocks | Need to be properly applied; not enough as a single intervention measure due to constant contamination pressure | van de Giessen et al. (1998) Gibbens et al. (2001) Hald et al. (2007) Rosenquist et al. (2009) |
| <i>Drinking water treatment</i> | Reduced transmission as well as reduced crop and pre-chill carcass contamination by adding organic acids | Only a limited effect on <i>Campylobacter</i> prevalence in broiler chickens | Chaveerach et al. (2002) Chaveerach et al. (2004b) Byrd et al. (2001) |
| | Reduced risk for <i>Campylobacter</i> colonization when chlorinating drinking water | No reduced <i>Campylobacter</i> prevalence observed under commercial settings | Ellis-Iversen et al. (2009) Stern et al. (2002) |
| <i>Feed additives</i> | Reduced crop contamination and reduced probability of chickens to become colonized when organic acids are supplemented to the feed | Contradictory observations | Heres et al. (2004) Van Deun et al. (2008) Solis de los Santos et al. (2008) Skanseng et al. (2010) van Gerwe et al. (2010) |
| | Phytochemicals ineffective? | <i>In vitro</i> and <i>in vivo</i> discrepancy | Unpublished results |
| <i>Vaccination</i> | Increased resistance to colonization by <i>Campylobacter</i> upon treating with killed whole cells and subunit vaccines | Incomplete understanding of the chicken immune system hampers development of an effective vaccine; genomic instability of <i>C. jejuni</i> ? | Widders et al. (1996) Rice et al. (1997) Widders et al. (1998) Khoury and Meinersmann (1995) Wyszynska et al. (2004) Buckley et al. (2010) |
| | No <i>Campylobacter</i> could be recovered from ileum with subunit (Omp18/CjaD) <i>Salmonella</i> -vectored vaccine | Cecal <i>Campylobacter</i> counts were not determined | Layton et al. (2010) |
| <i>Passive immunization</i> | Major reduction (>99%) in fecal <i>C. jejuni</i> counts upon treating with Immunoglobulin preparations | Optimization and experimental trials in industrial or farm settings are needed to prove efficacy | Stern et al. (1990) Tsubokura et al. (1997) |
| <i>Competitive exclusion microflora</i> | Increased resistance to <i>Campylobacter</i> colonization by feeding animals (un-)defined microbiota | Contradictory observations | Line et al. (1998) Morishita et al. (1997) Schoeni and Wong (1994) |

| | | | |
|------------------------------|---|--|---|
| | Competitive exclusion of human pathogenic <i>C. jejuni</i> strains by characterized hyper-colonizing <i>C. jejuni</i> strains | Needs further research regarding applicability under commercial settings | Svetoch and Stern (2010) Chen and Stern (2001) Calderon-Gomez et al. (2009) |
| <i>Prebiotics</i> | Reduction in cecal <i>Campylobacter</i> load in animals fed mannanoligosaccharide or xylanase | Limited reducing potential | Baurhoo et al. (2009); Fernandez et al. (2000) |
| <i>Antibiotic dissection</i> | A subtype I of <i>Megamonas hypermegale</i> specific for a <i>C. jejuni</i> -suppressive treatment | <i>In vivo</i> experiments are needed to prove <i>C. jejuni</i> exclusion in poultry | Scupham et al. (2010) |

Table 2On-farm intervention measures to reduce the cecal *Campylobacter* load of already-colonized broiler chickens

| Intervention measure | Effect on cecal <i>Campylobacter</i> numbers | Points of attention | References |
|---------------------------------|---|--|---|
| <i>Drinking water treatment</i> | Reduced <i>C. jejuni</i> count on cloacal swabs of broilers receiving monocaprin | Correlation between cecal <i>C. jejuni</i> load and numbers on cloacal swabs? | Hilmarrsson et al. (2006) |
| <i>Feed additives</i> | Reduced cecal <i>Campylobacter</i> load in broilers fed organic acids | Contradictory observations | Hermans et al. (2010) Solis de los Santos et al. (2010) Hilmarrsson et al. (2006) |
| <i>Bacteriophages</i> | Reduced cecal <i>Campylobacter</i> load by 2 log when phages are administered through the feed | Long-term efficacy? Genomic instability? | Wagenaar et al. (2005) El-Shibiny et al. (2009) Carvalho et al. (2010) |
| <i>Passive immunization</i> | Moderate reduction (80%-95%) in fecal bacterial counts upon treatment with Ig preparations | Optimization and experimental trials in industrial or farm settings are needed to prove efficacy | Tsubokura et al. (1997) |
| <i>Bacteriocins</i> | Supplementation of BCN 760 in drinking water capable of complete eradicating <i>C. jejuni</i> in 90% of the cases | Safety? Long-term efficacy? | Svetoch and Stern (2010) |