Evaluation of a polysaccharide conjugate vaccine to reduce colonization by *Campylobacter jejuni* in broiler chickens

Douglas C Hodgins†, Neda Barjesteh†, Michael St. Paul†, Zuchao Ma, Mario A Monteiro and Shayan Sharif*

**Abstract**

**Background:** *Campylobacter jejuni* is a leading bacterial cause of food-borne illness in humans. Symptoms range from mild gastroenteritis to dysentery. Contaminated chicken meat is the most common cause of infection. Broiler chickens become colonized with high numbers of *C. jejuni* in the intestinal tract, but do not become clinically ill. Vaccination of broiler chicks to control colonization by *C. jejuni* is challenging because immune function is limited in the first 2 weeks post-hatch and immune suppressive maternal antibodies are common. In addition, there is little time for induction of immunity, since broilers reach slaughter weight by 5–6 weeks of age. In the current study the immunogenicity of a *C. jejuni* capsular polysaccharide—diphtheria toxoid conjugated vaccine (CPSconj), administered subcutaneously with various adjuvants was assessed and the efficacy of vaccination for reducing cecal colonization after experimental challenge was evaluated by determining colony-forming units (CFU) of *C. jejuni* in cecal contents.

**Results:** The CPSconj vaccine was immunogenic when administered as three doses at 3, 4 and 5 weeks of age to specific pathogen free chicks lacking maternal antibodies (seroconversion rates up to 75%). Commercial broiler chicks (having maternal antibodies) receiving two doses of CPSconj vaccine at 7 and 21 days of age did not seroconvert before oral challenge at 29 days, but 33% seroconverted post challenge; none of the placebo-injected, challenged birds seroconverted. Vaccinated birds had significantly lower numbers of *C. jejuni* in cecal contents than control birds at necropsy (38 days of age). CFU of *C. jejuni* did not differ significantly among groups of birds receiving CPSconj vaccine with different adjuvants. In two trials, the mean reduction in CFU associated with vaccination was 0.64 log₁₀ units.

**Conclusions:** The CPSconj vaccine was immunogenic in chicks lacking maternal antibodies, vaccinated beginning at 3 weeks of age. In commercial broiler birds (possessing maternal antibodies) vaccinated at 7 and 21 days of age, 33% of birds seroconverted by 9 days after challenge, and there was a modest, but significant, reduction in cecal counts of *C. jejuni*. Further studies are needed to optimize adjuvant, route of delivery and scheduling of administration of this vaccine.

**Keywords:** *Campylobacter jejuni*, Cecum, Capsular polysaccharide, Conjugate vaccine, Vaccination, Broiler chickens

---

**Background**

Food-borne illness due to infection with *Campylobacter* species has been estimated to cost 1.7 billion dollars a year in medical costs, lost productivity and “quality-adjusted life years” in the United States alone [1]. Reports compiled by the European Food Safety Authority demonstrate increasing numbers of cases in humans over the most recent 4 years of study, in contrast to a steady decrease in the incidence of food-borne *Salmonella* infections [2]. Contaminated chicken meat is considered the most important source of infection with *Campylobacter* in developed countries [3]. Broiler chickens typically become infected with *Campylobacter jejuni* after 3 weeks of age and can harbor 10⁸ colony-forming units (CFU) or more per gram of cecal contents [4].
by slaughter age (5–6 weeks of age). In contrast to the intense diarrhea and vomiting, and severe inflammation of intestinal tissues associated with C. jejuni infection in humans [5], chickens do not exhibit signs of clinical illness after colonization by C. jejuni, and inflammation of intestinal tissue is not evident histologically [6].

Enhanced biosecurity in poultry flocks and improved hygiene during processing of poultry products have potential to reduce contamination of meat at the retail level, but vaccination of broiler chickens will be needed in conjunction with these approaches to have a major impact on campylobacteriosis in humans [7]. At present there are no licensed vaccines for reduction of colonization of chickens by C. jejuni [8]. Various vaccine approaches have been explored in experimental studies in chickens (reviewed by de Zoete et al. [9]), including bacterins [10, 11], subunit vaccines [11], live Salmonella vectored vaccines [12–14], and encapsulated particle vaccines [8, 15], by parenteral, oral, and nasal routes. Putative virulence factors and potential vaccine antigens have included outer membrane proteins [8], flagellin [11, 16], and transport proteins [12–14].

Recent studies have investigated the role of the capsular polysaccharide of C. jejuni in virulence in some species, and its potential as a vaccine antigen [17–20]. The capsular polysaccharide of C. jejuni 81-176 has been shown to mediate adherence and invasion of a human embryonic epithelial cell line, and to play a role in induction of diarrhea in a ferret model [21]. Wong et al. [22] have reported that modifications of the structure of the capsule of C. jejuni NCTC 11168 are associated with significant impairment of cecal colonization of young chicks. Capsular polysaccharide conjugated to the diphtheria toxoid “cross-reacting material 197” (CRM197) has been reported to be immunogenic in Aotus monkeys, and to protect against clinical diarrhea, but not colonization, following experimental challenge [17]. Although purified capsular polysaccharides can induce protection against encapsulated bacteria, as T-independent antigens they typically are not immunogenic in young infants or chicks [23, 24], and IgG and memory responses are limited [25]. Conjugation of purified capsular polysaccharide to a protein carrier such as CRM197 induces T-dependent responses, and facilitates antibody responses at an earlier age, with isotype switching to IgG and induction of B cell memory [26].

Although vaccination of broiler chicks is an attractive approach to control colonization, there are immunological and logistical barriers that must be overcome. Immune function is limited in the first 2 weeks post-hatch [27, 28] and maternal antibodies to C. jejuni are common in the sera of young chicks [29]. In addition there is little time for induction of immunity, since broiler birds reach slaughter weight by 5–6 weeks of age.

In the current studies the immunogenicity of the capsular polysaccharide of C. jejuni conjugated to CRM197 was assessed by vaccinating specific pathogen free (SPF) chicks (lacking maternal antibodies), starting at 3 weeks of age (when immune function has matured) and following serum antibody responses. In contrast, the protective efficacy of this antigen for reducing cecal colonization of broiler chicks was assessed by vaccinating commercial broiler chicks (having maternal antibodies) with two doses of vaccine (at 1 and 3 weeks of age), followed by experimental challenge at 4 weeks of age. Thus immunogenicity was tested under conditions favorable to induction of antibody responses, but protective efficacy was tested in commercial chicks using earlier vaccination, consistent with the need to induce immunity as young as possible.

**Methods**

**Vaccine**

Capsular polysaccharide of C. jejuni strain 81-176 was purified and conjugated to diphtheria toxoid CRM197 to form capsular polysaccharide conjugate (CPSconj) as described previously [17].

**Immunogenicity in the absence of maternal antibodies**

Specific pathogen free chicks (SPF, N2 strain, Cornell University Center for Animal Resources and Education, Cornell, New York, USA, 5 chicks per experimental group), lacking detectable maternal antibodies at the time of vaccination, were raised in the isolation facilities of the Ontario Veterinary College and vaccinated subcutaneously at 3 and 4 weeks of age with 10 μg CPSconj with either 20 μg Quil A (Brenntag Biosector, Frederickssund, Denmark) or 10 μg CpG oligodeoxynucleotide (CpG ODN) 2007 (Eurofins Operon, Huntsville, AL, USA) as adjuvants, or received 20 μg CPSconj with 20 μg Quil A at 3, 4 and 5 weeks of age. Vaccination of these chicks thus occurred under favorable conditions (absence of maternal antibodies) beginning at an age when immune function is considered to be mature [27, 28]. Control (placebo) birds received phosphate buffered saline (PBS) in place of vaccine.

**Vaccination and challenge of commercial broiler chicks**

One-day-old female commercial broiler chicks (Ross 308 chicks from Stratford Chick Hatchery, Stratford, ON, Canada) were housed in isolation facilities at the Ontario Veterinary College. Chicks were allocated (8 chicks per group) to receive 25 μg CPSconj with 10 μg CpG or 100 μl Addavax™ (squalene based adjuvant, InvivoGen, San Diego, CA, USA) as adjuvant, or 25 μg CPSconj without adjuvant, or PBS as a placebo by subcutaneous injection in a 200 μl volume at 7 days post-hatch. Birds
received a booster dose of 10 μg of the same antigen preparation as previously, or PBS at 21 days post-hatch. In contrast to the chicks used in the immunogenicity experiment above, these broiler chicks were obtained from a commercial source and had detectable serum (maternal) antibodies at the time of primary vaccination. At 29 days post-hatch birds were challenged orally with 2 × 10^7 CFU of C. jejuni strain 81-176 prepared by the method of Davis and DiRita [30] (see below). Birds were necropsied at 9 days post-challenge (38 days post-hatch) and dilutions of the cecal contents were plated onto Mueller–Hinton agar containing Preston Campylobacter Selective Supplement (Oxoid, Basingstoke, Hampshire, UK) and incubated at 42°C for 48 h in a microaerobic environment to quantitate CFU of C. jejuni. CFU per gram of cecal contents were calculated based on plate counts, adjusting for dilutions. An additional eight birds (serving as negative controls for challenge) were housed in a separate isolation room, were not vaccinated and were not challenged, but were necropsied at the same time as challenged birds. Blood was collected at 7, 21, 29, 34 and 38 days post-hatch for serological testing. The experiment was repeated a second time using chicks from the same source, following the identical protocol in the same facilities, and the results were pooled for statistical analysis.

Animal ethics
All experiments were approved by the Animal Care Committee of the University of Guelph (Animal Utilization Protocol number 10R086-1836) and followed the guidelines of the Canadian Council on Animal Care.

Enzyme-linked immunosorbent assay (ELISA) for serum IgG antibodies
Purified capsular polysaccharide of C. jejuni diluted to 40 μg/ml in PBS buffer was coated onto Nunc 96 well (MicroWell™ untreated polystyrene, Thermo Fisher Scientific, Rochester, New York, USA) plates, 100 μl/well, at 37°C for 3 h. Plates were washed four times with wash buffer consisting of PBS with 0.5% fish skin gelatin (Sigma, St. Louis, MO, USA) and 0.05% Tween 20 (Sigma). Sera were diluted 1/20 in wash buffer and 100 μl was added to wells in duplicate, followed by a 2 h incubation at 37°C. After washing four times, bound antibodies were detected using rabbit anti-chicken IgG (Fc specific) horse radish peroxidase conjugate (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) diluted 1/350, incubating at 37°C for 1 h. After washing four times, ABTS (2,2′-azino-di (3-ethyl-benzthiazoline-6-sulfonate)) substrate (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA) was added, 100 μl/well; 1% sodium dodecylsulfate (Bio-Rad, Hercules, CA, USA) was added as stop solution after 1 h and the optical densities were evaluated at 405 nm. A dilution series of a positive control serum consisting of pooled high titre sera from mature hens was run on each plate. Titres were estimated using the method of Sacks et al. [31]. The limit of detection of the assay was 3.32 log_2 units.

Bacterial culture
Campylobacter jejuni strain 81-176 was cultured according to the method of Davis and DiRita [30] to provide bacteria for experimental oral challenge. Briefly, a 10-μl loop of frozen C. jejuni culture maintained at −80°C, was inoculated onto Mueller–Hinton agar (Oxoid) and incubated for 18 h in a sealed jar using gas packs (CampyGen, Oxoid) to maintain microaerobic conditions. Subsequently, several colonies of C. jejuni were inoculated into 100 mL fresh Mueller–Hinton broth and incubated at 41°C in microaerobic conditions for 40 h. The broth culture was centrifuged at 3,500×g for 10 min and the bacteria were diluted with PBS (pH 7.4) to attain an optical density corresponding to approximately 4.0 × 10^7 CFU/ml, based on previous analysis of growth curves. The number of viable C. jejuni received by the chickens at the time of challenge was established retrospectively by plating dilutions of the inocula onto Mueller–Hinton agar plates.

Statistical analysis
Data from the two challenge trials were pooled. CFU/gm of C. jejuni in cecal contents were log_{10} transformed before analysis using a mixed model (Proc Mixed) including trial as a random variable, in SAS version 9.2. Vaccine groups were subsequently compared using Tukey’s test. Antibody titres were expressed on a log_2 scale. Titres were not normally distributed for the majority of time points; a nonparametric test (Proc Npar1way [Kruskal–Wallis test]) was used to compare antibody titres among groups. Seroconversion (increase in titre by ≥2 log_2 units) rates were compared using Fisher’s exact test. Correlation between serum antibody titres on the day of necropsy and CFU of C. jejuni in cecal contents was evaluated using Pearson’s correlation coefficient using Proc Corr in SAS.

Results and discussion
Immunogenicity of the CPS conjugate vaccine in the absence of maternal antibodies
Mean serum IgG antibody titres increased in vaccinated SPF birds, but not in negative control birds that received PBS (Figure 1). Seroconversion rates were 20, 40 and 75% by 6 weeks of age in birds receiving two doses of CPSconj vaccine with CpG, two doses CPSconj with Quil A and three doses CPSconj with Quil A respectively. The seroconversion rate in birds receiving three doses of CPSconj
was significantly higher than the rate in birds receiving PBS as placebo (p < 0.05, one-tailed Fisher’s exact test).

**Antibody responses of commercial broiler chicks**

Maternal IgG antibodies were evident in serum at 7 days post-hatch at the time of primary vaccination, but declined considerably by day 21 (day of secondary vaccination, Figure 2). Maternal antibodies have been shown to mediate partial protection against colonization by *C. jejuni* [32], but the short half-life of maternal antibodies (about 5 days in young chicks [33]) suggests that titres of maternal antibodies would need to be considerably higher to provide protection up to slaughter age. Mean serum IgG antibody titres declined further by day of challenge (day 29) in all groups. IgG antibody titres did not differ significantly among the four experimental groups (three groups of vaccinates and one placebo group) at any of the time points (Kruskal–Wallis test). Data from the vaccinated groups (CPS conjugate without adjuvant, CPS conjugate with Addavax™ adjuvant and CPS conjugate with CpG as adjuvant) were pooled for further analysis since there were no significant differences among them.

In Figure 3 the distribution of serum IgG antibody titres in vaccinated broiler birds and birds receiving placebo, is contrasted by means of histograms. Seroconversion (an increase of titre ≥2 log₂ units) was not detected before day of challenge in vaccinates. This is in contrast to responses seen in SPF birds by 3 weeks post primary vaccination (Figure 1). Seroconversion occurred in 33% of vaccinated broiler birds by 9 days post-challenge, but in none of the birds that had received placebo before challenge (p < 0.01, Fisher’s exact two-tailed test). This suggests that although vaccination with two doses of vaccine did not induce increases in circulating antibody titres...
before the day of challenge, there was a degree of priming for anamnestic antibody responses (which occurred after exposure to live *C. jejuni*) in some of the birds. In a similar fashion, Siegrist et al. [34] have reported an absence of serum antibody responses to vaccination in neonatal mice with circulating maternal antibodies. In these mouse experiments anamnestic antibody responses became evident after subsequent antigen exposure. It is possible that if cecal CFU were monitored beyond 9 days post-challenge, that mean CFU would decline further as immune responses aided in clearance of *C. jejuni*.

Reduction in *C. jejuni* in cecal contents of vaccinated chicks

Colony-forming units of *C. jejuni* were significantly lower in the cecal contents of birds receiving CPSconj vaccine (with or without adjuvant) compared to control birds receiving PBS (Table 1). Mean CFU of *C. jejuni* did not differ significantly among groups of vaccinated birds and
were pooled for further analysis. Vaccinated birds had a mean reduction in cecal *C. jejuni* of 0.64 log₁₀ units compared to birds receiving PBS as placebo (p < 0.001). There was essentially no correlation between serum antibody titres and cecal counts of *C. jejuni* at necropsy (r value of −0.06 among vaccinates with p = 0.70, data not shown). Since *Campylobacter* colonize the mucus layer of the intestinal tract and are rarely found in extra-intestinal sites, a low correlation between serum antibody titres and protection could be anticipated. Assay of antibodies in intestinal mucus should be more informative. Vaccination with CPSconj by a mucosal route might improve vaccine efficacy compared to the subcutaneous route used in the present study, by inducing IgA antibodies at sites of colonization by *C. jejuni*. The subcutaneous route was used in the present study to assess immunogenicity of the vaccine construct and to provide a baseline for comparison as systems for delivery to mucosal sites are developed. Rosenquist et al. [35] have suggested, based on mathematical modeling, that a 2.0 log₁₀ unit reduction in *C. jejuni* in chickens at slaughter would reduce clinical disease in primates, the end goal of vaccination in order. A capsular polysaccharide—diphtheria toxoid conjugate vaccine administered subcutaneously at 7 and 21 days post-hatch with capsular polysaccharide conjugate vaccine with or without CpG or Addavax™ as an adjuvant, or received PBS as a placebo. Birds were challenged at 29 days post-hatch and necropsied at 38 days post-hatch.

### Alternative vaccination approaches

The current study assessed immunogenicity of the capsular polysaccharide antigen conjugated to diphtheria toxoid CRM197. This carrier protein has been used in vaccines for human infants and has been shown to enhance immune responses to polysaccharide antigens in the first months of life [26]. Other carrier proteins have been used in conjugate vaccines [36] and perhaps these or even *C. jejuni* surface proteins may also prove efficacious in chickens. Schijns et al. [37] have reported that some adjuvants enhance the immunogenicity of tetanus toxoid in immunologically mature chickens (3 weeks-old) but fail to induce antibodies to this antigen in day-old chicks, whereas other adjuvants are effective at both ages. Thus it may be necessary to optimize both carrier protein and adjuvant to produce conjugate vaccines immunogenic for day-old chicks.

In the present study vaccines were administered subcutaneously to evaluate immunogenicity of the capsular antigen. Mucosal vaccination, especially by the oral route may be more effective to induce intestinal immune responses and reduce intestinal colonization, but improved adjuvants and delivery systems will be required to make this approach practical.

Further studies will be needed to optimize adjuvants, route of delivery, and scheduling of administration for use of CPSconj antigen in broiler chickens. Studies using heterologous challenge strains of *C. jejuni* are also in order.

### Conclusions

A capsular polysaccharide—diphtheria toxoid conjugate vaccine administered subcutaneously, starting at 3 weeks

### Table 1 Colony-forming units of *C. jejuni* per gram of cecal contents 9 days post-challenge

| Vaccine group                  | n  | CFU per gram of cecal contents (log₁₀ transformed) | Standard error of the mean | p value for comparison with the PBS group |
|--------------------------------|----|--------------------------------------------------|---------------------------|------------------------------------------|
| PBS                            | 16 | 8.11                                             | 0.15                      |                                          |
| Conjugate alone                | 13 | 7.38                                             | 0.16                      | 0.01                                     |
| Conjugate + CpG                | 14 | 7.55                                             | 0.15                      | <0.05                                    |
| Conjugate + Addavax™           | 16 | 7.47                                             | 0.14                      | 0.01                                     |
| All vaccines (data pooled)     | 43 | 7.47                                             | 0.09                      | <0.001                                   |

Combined results from two experiments using Ross 308 broiler chicks from the same source and following the same experimental protocol. Chickens were vaccinated subcutaneously at 7 and 21 days post-hatch with capsular polysaccharide conjugate vaccine with or without CpG or Addavax™ as an adjuvant, or received PBS as a placebo. Birds were challenged at 29 days post-hatch and necropsied at 38 days post-hatch.
of age, induced serum IgG antibodies in SPF chicks lacking detectable maternal antibodies. Vaccination of broiler chicks (that had maternal antibodies) starting at 7 days post-hatch was not associated with seroconversion with IgG antibodies before challenge at 29 days post-hatch. Some (33%) of the vaccinated broiler birds seroconverted by 9 days post-challenge, but none of the PBS (placebo) birds, indicating that the vaccine primed for an earlier IgG antibody response following challenge. There was a modest (0.64 log10 colony forming units) but significant (p < 0.001) reduction in C. jejuni in cecal contents, 9 days post-challenge, in vaccinated birds compared to negative control (placebo) birds. Further studies are needed to optimize adjuvant, route of delivery and scheduling of administration to make the most effective use of this vaccine antigen.

Abbreviations

CFU: colony forming units; PBS: phosphate buffered saline; ODN: oligonucleotide; CPS-Com: capsular polysaccharide-diphtheria toxoid conjugate; SPF: specific pathogen free.

Authors’ contributions

DCH and NB contributed equally in the design, and execution of the work. DCH drafted the manuscript. MSP assisted with experimental procedures. ZM and MAM were responsible for the production of the capsular polysaccharide-conjugate vaccine, and with the revision of the manuscript. SS contributed to the design of the experiment, the analysis of results and revision of the manuscript. All authors read and approved the final manuscript.

Author details

1 Department of Pathobiology, University of Guelph, Guelph, ON N1G 2W1, Canada. 2 Department of Immunology, University of Toronto, Toronto, ON, Canada.

Acknowledgments

Research funding was provided by the Canadian Poultry Research Council, Poultry Industry Council and the Ontario Ministry of Agriculture and Food (OMAF), and by the Natural Sciences and Engineering Research Council of Canada (SS and MAM). The funding agencies were not involved in the study design, or the collection, analysis, or interpretation of the data, or in the writing of the manuscript, or in the decision to submit the manuscript for publication. The assistance of Dr Shahriar Orouji in immunogenicity testing and the assistance of Dr. Hodgins in reviewing the manuscript, or in the decision to submit the manuscript for publication. The authors declare that they have no competing interests.

Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests.

Received: 11 February 2015   Accepted: 20 May 2015
Published online: 02 June 2015

References

1. Hoffmann S, Batz MB, Morris JG (2012) Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. J Food Prot 75:1292–1302
2. European Food Safety Authority/European Centre for Disease Control (2013) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. EFSA J 11:3129
3. Young KT, Davis LM, DiRita VJ (2007) Campylobacter jejuni: molecular biology and pathogenesis. Nat Rev Microbiol 5:665–679
4. Sahin O, Morishita TY, Zhang Q (2002) Campylobacter colonization in poultry: sources of infection and means of transmission. Anim Health Res Rev 3:95–105
5. Li Y-P, Ingmer H, Madsen M, Bang DD (2008) Cytokine responses in primary chicken embryo intestinal cells infected with Campylobacter jejuni strains of human and chicken origin and the expression of bacterial virulence-associated genes. BMC Microbiol 8:107
6. Dhillion AS, Shivaprasad HL, Schaberg D, Wier F, Weber S, Bandli D (2006) Campylobacter jejuni infection in chickens. Avian Dis 50:53–58
7. Newell DG, Elvers KT, Dopler D, Hanson J, Jones P, James S et al (2011) Biosecurity-based interventions and strategies to reduce Campylobacter spp. on poultry farms. Appl Environ Microbiol 77:8605–8614
8. Annamalai T, Pina-Mimbela R, Kumar A, Bivawadagi B, Liu Z, Renukaradhya GJ et al (2013) Evaluation of nanoparticle-encapsulated outer membrane proteins for the control of Campylobacter jejuni colonization in chickens. Poult Sci 92:2201–2211
9. de Zoete MR, van Putten JPM, Wagenaar JA (2007) Vaccination of chickens against Campylobacter. Vaccine 25:5548–5557
10. Burr DH, Rollins D, Lee LH, Patterson DL, Walz SS, Tian JH et al (2005) Prevention of disease in ferrets fed an inactivated whole cell Campylobacter jejuni vaccine. Vaccine 23:4315–4321
11. Widders PR, Perry R, Muir WH, Hubzand AJ, Long KA (1996) Immunization of chickens to reduce intestinal colonization with Campylobacter jejuni. Br Poult Sci 37:765–778
12. Wyszynska A, Raczko A, Lec M, Juszynska-Krynica EK (2004) Oral immunization of chickens with avirulent Salmonella vaccine strain carrying C. jejuni 72Dz/92cpa gene elicits specific humoral immune response associated with protection against challenge with wild-type Campylobacter. Vaccine 22:1379–1389
13. Layton SL, Morgan MJ, Cole K, Kwon YM, Donoghue DJ, Hargis BM et al (2011) Evaluation of Salmonella-vector Campylobacter peptide epitopes for reduction of Campylobacter jejuni in broiler chickens. Clin Vaccine Immunol 18:449–454
14. Buckley AM, Wang J, Hudson DL, Grant AJ, Jones MA, Maskell DJ et al (2010) Evaluation of live-attenuated Salmonella vaccines expressing Campylobacter antigens for control of C. jejuni in poultry. Vaccine 28:1094–1105
15. Huang JY, Pan YX, Pan ZM, Zhang G, Zhih AP, Liu XG et al (2010) Intranasal immunization with chitosan-p/CAGGS-fla nanoparticles inhibits Campylobacter jejuni in a White Leghorn model. J Biomed Biotechnol. doi:10.1155/2010/589476
16. Khoury CA, Meinersmann RF (1995) A genetic hybrid of the Campylobacter jejuni flaA gene with LT-B of Escherichia coli and assessment of the efficacy of the hybrid protein as an oral chicken vaccine. Avian Dis 39:812–820
17. Monteiro MA, Baqar S, Hall ER, Chen Y-H, Porter CK, Bentzel DE et al (2009) Capsule polysaccharide conjugate vaccine against diarrheal disease caused by Campylobacter jejuni. Infect Immun 77:1128–1136
18. Guerry P, Poly F, Riddle M, Maue AC, Chen Y-H, Monteiro MA (2012) Campylobacter polysaccharide capsules: virulence and vaccines. Front Cell Infect Microbiol 2:7
19. Bertolo L, Guerry P, Poly F, Riddle M, Maue AC, Chen Y-H, Monteiro MA (2013) The design of a capsule polysaccharide conjugate vaccine against Campylobacter jejuni vaccine strain 72Dz/92 cpA. Carbohydr Res 365:45–49
20. Maue AC, Mohawk KL, Giles DK, Poly F, Ewing CP, Jiao Y et al (2013) The polysaccharide capsule of Campylobacter jejuni modulates the host immune response. Infect Immun 81:665–672
21. Bacon DJ, Szymanski CM, Burr DH, Rollins D, Lee LH, Kitts DM et al (2006) Salmonella typhimurium outer membrane proteins for the control of Campylobacter jejuni colonization in chickens. Poult Sci 85:765–778
22. Wong A, Lange D, Houle S, Arbatovsky NP, Valvano MA, Knir SK et al (2015) Role of capsular modified heptose in the virulence of Campylobacter jejuni. Mol Microbiol. doi:10.1111/mmi.12995
23. Jeunisse SM, Janse EM, van Rooijen N, Claassen E (1998) Inadequate anti-polysaccharide antibody responses in the chicken. Immunobiology 198:385–395
24. Murphy K (2012) Janeway’s immunobiology, 8th edn. Garland Science, London, p 407
25. Tizard IR (2013) Veterinary immunology, 9th edn. Elsevier Saunders, St. Louis, p 156
26. Klein Klouwenberg P, Bont L (2008) Neonatal and infantile immune responses to encapsulated bacteria and conjugate vaccines. Clin Dev Immunol. doi:10.1155/2008/628963
27. Mast J, Goddeeris BM (1999) Development of immune competence of broiler chickens. Vet Immunol Immunopathol 70:245–256
28. Bar-Shira E, Sklan D, Friedman A (2003) Establishment of immune competence in the avian GALT during the immediate post-hatch period. Dev Comp Immunol 27:147–157
29. Sahin O, Zhang Q, Metzler JC, Harr BS, Morishita TY, Mohan R (2001) Prevalence, antigenic specificity, and bactericidal activity of poultry anti-Campylobacter maternal antibodies. Appl Environ Microbiol 67:3951–3957
30. Davis L, DiRita V (2008) Growth and laboratory maintenance of Campylo-bacter jejuni. Curr Protoc Microbiol, Chapter 8, Unit 8A.1.1–8A.1.7
31. Sacks JM, Gillette KG, Frank GH (1988) Development and evaluation of an enzyme linked immunosorbent assay for bovine antibody to Pasteurella haemolytica: constructing an enzyme linked immunosorbent assay titer. Am J Vet Res 49:38–41
32. Sahin O, Luo N, Huang S, Zhang Q (2003) Effect of Campylobacter-specific maternal antibodies on Campylobacter jejuni colonization in young chickens. Appl Environ Microbiol 69:5372–5379
33. Hartle S, Magor KE, Gobbel TW, Davison F, Kaspers B (2014) Structure and evolution of avian immunoglobulins. In: Schat KA, Kaspers B, Kaser P (eds) Avian immunology, 2nd edn. Elsevier, Amsterdam, p 108
34. Siegrist CA, Barrios C, Martinez X, Brandt C, Berney M, Córdova M et al (1998) Influences of maternal antibodies on vaccine responses: inhibition of antibody but not T cell responses allows successful early prime-boost strategies in mice. Eur J Immunol 28:4138–4148
35. Rosenquist H, Nielsen NL, Sommer HM, Norrung B, Christensen BB (2003) Quantitative risk assessment of human campylobacteriosis associated with thermophilic Campylobacter species in chickens. Int J Food Microbiol 83:87–103
36. Goldblatt D (2000) Conjugate vaccines. Clin Exp Immunol 119:1–3
37. Schijns VE, Weining KC, Nuijten P, Rijke EO, Staeheli P (2000) Immunoadjuvant activities of E. coli- and plasmid-expressed recombinant chicken IFN-alpha/beta, IFN-gamma and IL-1beta in 1-day-and 3-week-old chickens. Vaccine 18:2147–2154