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(54) Title: CAMPYLOBACTER IMMUNOGENIC COMPOSITIONS AND USES THEREOF

(57) Abstract: The present invention provides immunogenic compositions against Campylobacter and methods for using the immunogenic composition to generate an immune response against Campylobacter and/or reduce intestinal colonization by Campylobacter.

Campylobacter Immunogenic Compositions and Uses Thereof

Cross Reference

This application claims priority to U.S. Provisional Patent Application Serial Numbers 61/632,888 filed February 1, 2012 and 61/689,078 filed May 29, 2012, incorporated by reference herein in their entirety.

Statement of Government Support

This invention was made with government support under grant number 127032 awarded by United States Department of Agriculture Hatch Funding. The government has certain rights in the invention.

Background

Campylobacteriosis is primarily a food-borne disease with the handling and consumption of poultry considered to be the most significant risk factor in transmission. Infection by *Campylobacter* spp. is one of the leading causes of bacterial gastroenteritis, causing an estimated 1.3 million cases annually in the U.S. (Scallan et al, 2011), resulting in health care costs of $ 0.8-5.6 billion per year (Buzby et al., 1997). Serious complications such as arthritis occur in an estimated 1-5% of cases (Pope et al., 2007)) and Guillain-Barre Syndrome, a form of neuromuscular paralysis, occurs at a rate of 1.0 per 1,000 patients (Altekruse and Tollefson, 2003). Due to the emergence and persistence of antibiotic resistance coupled with increasing regulatory restrictions on the industry, control strategies such as vaccination are urgently needed. To date, there is no intervention method or vaccine available to the producer to reduce numbers of *Campylobacter* in poultry going to processing.

Beginning July 1, 2011, USDA-FSIS (Food Safety and Inspection Service) has implemented new performance standards for *Campylobacter* for young chicken chilled carcasses at slaughter establishments (FSIS NOTICE, 31-11, 6/30/11). These standards will allow no more than 8 positive *Campylobacter* samples out of a 51-sample set, with plans to initially warn the companies and fines to be imposed in 2013. A large baseline study was conducted in our laboratory (funded by the USDA) to quantify *Campylobacter* levels in slaughtering plants from 2007-2009. Our studies demonstrated 21.9% (213/972) of post-

chilled carcass rinse samples were positive for *Campylobacter,* which amounts to 11.17 per 51-sample set. Clearly, this is over the allowable number of *Campylobacter* from chilled carcass samples and will precipitate the issuing of fines for both the producer and processing plant unless a reduction of *Campylobacter* in poultry can be obtained. Currently, there are no available intervention methods or vaccines available for producers to use to reduce the *Campylobacter* load in poultry.

Summary of the Invention

In a first aspect, the present invention provides immunogenic compositions, comprising one or more expression vectors comprising:

(a) at least one polynucleotide encoding a protein selected from the group consisting of proteins comprising an amino acid sequence at least 80 percent identical to SEQ ID NO:2 (*Cj0998c* protein), SEQ ID NO:4 (*Cj0588* protein), and SEQ ID NO:6 (*Cj0248* protein), or antigenic portions thereof; and

(b) a promoter operatively linked to the polynucleotide, wherein the promoter region is capable of directing expression of the encoded protein(s).

In a second aspect, the present invention provides immunogenic compositions, comprising

(a) one or more isolated proteins selected from the group consisting of proteins comprising an amino acid sequence at least 80 percent identical to SEQ ID NO:2 (*Cj0998c* protein), SEQ ID NO:4 (*Cj0588* protein), and SEQ ID NO:6 (*Cj0248* protein), or antigenic portions thereof; and

(b) a pharmaceutically acceptable carrier.

In a third aspect, the present invention provides methods for stimulating an immune response against *Campylobacter*, comprising administering to a subject an effective amount of the immunogenic composition according to any embodiment of the invention to generate an immune response against *Campylobacter*.

In a fourth aspect, the present invention provides methods for reducing *Campylobacter* intestinal colonization in a subject, comprising administering an amount effective of the immunogenic composition according to any embodiment of the invention to reduce *Campylobacter* intestinal colonization in the subject.

Description of the Figures

Figure 1 is a schematic drawing of the pYA3493 plasmid.

Detailed Description of the Invention

All references cited are herein incorporated by reference in their entirety. Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), "Guide to Protein Purification" in *Methods in Enzymology* (M.P. Deutshcer, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press, San Diego, CA), *Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed.* (R.I. Freshney. 1987. Liss, Inc. New York, NY), *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX).

As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. "And" as used herein is interchangeably used with "or" unless expressly stated otherwise.

All embodiments of any aspect of the invention can be used in combination, unless the context clearly dictates otherwise.

In a first aspect, the present invention provides immunogenic compositions, comprising one or more expression vectors comprising:

(i) at least one polynucleotide encoding a protein selected from the group consisting of proteins comprising an amino acid sequence at least 80 percent identical to SEQ ID NO:2 (*Cj0998c* protein), SEQ ID NO:4 (*Cj0588* protein), and SEQ ID NO:6 (*Cj0248* protein), or antigenic portions thereof; and

(ii) a promoter operatively linked to the polynucleotide , wherein the promoter region is capable of directing expression of the encoded protein(s).

The inventors have identified three putative virulence genes (*Cj0248*, *Cj0588*, and *Cj0998c*) from *C. jejuni* encoding novel proteins from the outer-membrane of the bacterium. The inventors have further discovered that each of the *Cj0998c* protein, the *Cj0588* protein, and the *Cj0248* protein are potent immunogens for stimulating an effective immune response against *Campylobacter jejuni* ("*C. jejuni*"). For example, as disclosed in detail herein, two separate vaccination trials of chickens with a vector expressing the *Cj0988c* protein demonstrated reduced numbers of *C. jejuni* in birds after challenge an average of 2.5 logs

CFU (geomean 3 logs) when compared to the cecal numbers of non-vaccinated control birds. Furthermore, vaccination trials demonstrated a significant reduction (1-4 logs) (1 log with heterologous strain and 4 logs with homologous strain) of *C. jejuni* in cecal contents of chickens vaccinated with vectors expressing the *Cj0588* protein and challenged with *Campylobacter jejuni*. Each of the proteins was initially isolated from an outer-membrane (OMP) extraction of a *C. jejuni* biofilm.

Thus, the immunogenic compositions of the invention can be used, for example for stimulating an immune response in subjects at risk of *Campylobacter* infection and/or colonization, including but not limited to vertebrates such as chickens, turkeys, birds, cattle, sheep, pigs, dogs, cats, and humans.

In one embodiment, the one or more expression vectors encode one of the recited proteins, or antigenic fragments thereof. In another embodiment, the one or more expression vectors encode two proteins comprising an amino acid sequence at least 80 percent identical to the recited amino acid sequences (i.e.: SEQ ID NO:2 and SEQ ID NO:4; SEQ ID NO:2 and SEQ ID NO:6; or SEQ ID NO:6 and SEQ ID NO:8), or antigenic fragments thereof. In this embodiment, a single expression vector may encode both proteins, or antigenic fragments thereof, or the composition may comprise two expression vectors, with each expression vector encoding one of the recited proteins, or antigenic fragments thereof. In this embodiment, the expression vector used may be the same (other than the protein coding sequence) or different.

In a further embodiment, the one or more expression vectors encode all three of the proteins comprising an amino acid sequence at least 80 percent identical to the recited amino acid sequences (i.e.: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6) , or antigenic fragments thereof. In this embodiment, a single expression vector may encode all three proteins, or antigenic fragments thereof. Alternatively, the composition may comprise two expression vectors, with one expression vector encoding one of the recited proteins, or antigenic fragments thereof, and the other expression vector encoding two of the recited proteins, or antigenic fragments thereof. In a further alternative of this embodiment, the composition may comprise three expression vectors, with each expression vector encoding one of the recited proteins, or antigenic fragments thereof. In each of these alternative embodiments, the multiple expression vectors used may be the same or different. Non-limiting examples of expression vectors encoding the one or more recited proteins are provided herein. Based on the present disclosure, it is well within the level of those of skill in the art to prepare expression vectors according to all embodiments of the invention.

The *Cj0998c, Cj0588*, and *Cj0248* proteins are present in highly conserved variants between different strains of *C. jejuni*, and thus the proteins encoded by the one or more expression vectors can be at least 80% identical or similar (residues with similar properties, i.e.: hydrophobic, hydrophilic, etc.) over the full length of the recited amino acid sequences, or antigenic fragments thereof. In various further embodiments, the proteins encoded by the one or more expression vectors are at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% percent identical over the full length of the recited amino acid sequence(s), or antigenic fragment thereof.

In one embodiment, the at least one polynucleotide encodes a protein selected from the group consisting of the following, or an antigenic fragment thereof:

(a) **SEQ ID NO:7 (*Cj0998c* genus)**

MKK(I/V/F)(L/V)(V/A/L)S(V/I)(L/F)S(S/F)CLLASALSAVSFKEDSLK(I/V)SFEGYKTKD M(I/V)G(T/V/I/A)(K/R)GEFKNVEY(K/N)FSK(N/S)(I/T)KD(L/F)ASYLKGAKATI(K/E)P S(N/D)AFM(G/S)EG(N/L)D(I/V)ITNNITKVFFPALLG(D/N)(T/A)DIKVVFQD(V/A/M)I(A /V)GE-

X1ITMDKKSTI(V/I)PLTYTIKD(N/D)KFEAKGQ(L/F)DLH(T/A)FKN(G/A)SKALKALSD (V/A)A(A/T/P)GHGGISWPLVDISFNADL(A/T/V)E

wherein X1 is absent or is

NKGVISAK (SEQ ID NO: 135);

(b) **SEQ ID NO:8: (*Cj0588* genus)**

X1-

(L/M)(D/N/E)LL(S/R)EIY(V/I)SRAALKLK(K/N)FLEEN(D/G/N)IE(I/V)(K/N)(H/Q/N)K NCLDIGSSTGGFVQILLEN(Q/K)ALKIT(A/T)LDVG(S/N)NQLH(P/S/L)(S/N)LR(V/A/T) NE(K/I)(I/V)IL(H/Y)EN(T/I)DLR(A/T/V)FKSEEKFE(L/F)(V/I)TCDVSFISL(I/V)NLLYY (I/V)(D/N)NLAL(K/R)EIILLFKPQFEVGKN(I/V)KRDKKGVLKD(D/G)(K/R)(A/V)ILK A(R/K)MDFEK(A/E)CAKL(G/S)W(L/F/I)LKNTQKS(S/C)IKGKEGNVEYFYYYIKN

wherein X1 is absent or is

M(R/I)(F/-)(D/-)FF(V/I)SKRL(N/D)ISRNKALELIE(N/S)EE(I/V)LLNGK(S/N)FKAS(F/C)DV KN(F/L)LENLKK(T/A/K)QDLN(P/L/S)E(D/E)(I/V)(L/Y)L(A/T/S)(N/D/K)(E/G)L(K/N) (SEQ ID NO: 136); and

(c) **SEQ ID NO:9 (*Cj0248* genus)**

(M/-)I(G/-)DMNELLLKSVEVLPPLPDTVSKLRKYVSEANSNIETMKV(A/V)EIISSDPL
MTAKLLQLANSPYYGFTREITTI(N/S)QVITLLG(V/I)GNIINIV(M/T)ADSI(R/K)D(N/S)F
KIDVSPYGL(N/D)T(Q/K)(N/V)FL(K/R)(T/N)CN(E/D)EATFI(A/V/T)NWLNDEDKKLSH
LLVPCAMLLRLGIVIFSNFLIQN(H/Y/F)(K/R)-**X1**

wherein **X1** is absent, or is
(D/E)K(D/E)FL(A/T)FLN(-/E/K)(-/T)K(N/S/I))EN(L/I)ALAENEFLGVDHISFLGFLL(H/Y)
RWNFDD(V/I)LIESICFV(R/H)TPHAARE(K/E)VKKSAYALAITDHLF(A/T)PHDGSSPF
N
(A/V/T)KAAVALL(K/E)EAK-**X2** (SEQ ID NO: 137 or 138); and

wherein **X2** is absent or is selected from the group consisting of
TQGINFDL(N/D)NLLSKLP(N/S)KAKENL(N/D)(K/E)ED (SEQ ID NO: 139) and
NSRN (SEQ ID NO: 140).

In various further embodiments, the at least one polynucleotide encodes a protein selected from the group consisting of SEQ ID NOS: 10-106, or an antigenic fragment thereof. SEQ ID NOS, 44-82 are each *Cj0998c* protein homologs from other *C. jejuni* strains; SEQ ID NOS, 83 to106 are each *Cj0588* protein homologs from other *C. jejuni* strains; and SEQ ID NOS, 10 to 43 are each *Cj0248* protein homologs from other *C. jejuni* strains.

The expression vectors may encode "antigenic portions" of the recited protein. As used herein, and "antigenic portion" is any fragment of 10 or more contiguous amino acids in the recited amino acid sequence. In various further embodiments, the antigenic portion is any fragment of any of the embodiments of the invention is 15, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, or 275 contiguous amino acids of the recited amino acid sequence.

The one or more isolated polynucleotides may be single or double stranded DNA, RNA, genomic DNA, or cDNA. The one or more isolated polynucleotides may be any nucleic acids encoding the recited one or more proteins, or antigenic fragments thereof. In one embodiment, the one or more isolated polynucleotides are one or more of SEQ ID NO:1 (*Cj0998c* gene), SEQ ID NO:3 (*Cj0588* gene), and SEQ ID NO:5 (*Cj0248* gene), or portions thereof encoding antigenic portions of the recited proteins. It will be apparent to those of skill in the art, based on the teachings herein, what polynucleotide sequences will encode the recited polypeptides or antigenic fragments thereof.

As used herein, "isolated polynucleotides" are those that have been removed from their normal surrounding nucleic acid sequences in the genome or in cDNA sequences. Such isolated nucleic acid sequences may comprise additional sequences useful for promoting

expression and/or purification of the encoded protein, including but not limited to polyA sequences, modified Kozak sequences, and sequences encoding epitope tags, export signals, secretory signals, nuclear localization signals, and plasma membrane localization signals, as appropriate for a given use.

Any expression vector suitable for an intended use can be used in the immunogenic compositions of the present invention. Such expression vectors can be of any type known in the art, including but not limited to plasmid and viral-based expression vectors. The construction of expression vectors for use in transfecting prokaryotic cells is also well known in the art, and thus can be accomplished via standard techniques. (See, for example, Sambrook, Fritsch, and Maniatis, in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989; *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX). The expression vector must be replicable in the host organisms either as an episome or by integration into host chromosomal DNA. In a preferred embodiment, the expression vector comprises a plasmid. However, the invention is intended to include other expression vectors that serve equivalent functions, such as viral vectors. Specifics of the expression vector will depend on the ultimate desired use. Designing appropriate expression vectors for an intended use is well within the level of those of skill in the art based on the teachings herein.

Any suitable promoter may be used that can direct expression (i.e.: is “operatively linked”) of the encoded proteins. The term "promoter" includes any nucleic acid sequence sufficient to direct expression of the encoded protein(s), including inducible promoters, repressible promoters and constitutive promoters. If inducible, there are sequences present which mediate regulation of protein expression so that the polynucleotide is transcribed only when an inducer molecule is present. Such cis-active sequences for regulated expression of an associated polynucleotide in response to environmental signals are well known to the art. The expression vector may comprise any other control sequences as may be suitable for an intended use. The control sequences need not be contiguous with the nucleic acid sequences, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the nucleic acid sequences and the promoter sequence can still be considered "operably linked" to the coding sequence. Other such control sequences include, but are not limited to, polyadenylation signals, enhancers, termination signals, and ribosome binding sites.

The immunogenic compositions of the present invention may further comprise any other suitable components as may be useful for a given purpose. In various non-limiting embodiments, the compositions may further comprise one or more expression vectors comprising at least one polynucleotide encoding a protein selected from the group consisting of proteins comprising an amino acid sequence at least 80 percent identical to an amino acid sequence of SEQ ID NO:142 (*Cj1534c* protein; encoded, for example, by SEQ ID NO:141), SEQ ID NO:108 (1656c protein; encoded, for example, by SEQ ID NO:107), SEQ ID NO:110 (0428 protein; encoded, for example, by SEQ ID NO:109), SEQ ID NO:112 (0168c protein; encoded, for example, by SEQ ID NO:111), SEQ ID NO:114 (0427 protein; encoded, for example, by SEQ ID NO:113), SEQ ID NO:116 (Cj0113 protein; encoded, for example, by SEQ ID NO:115),SEQ ID NO:118 (Cj0982c protein; encoded, for example, by SEQ ID NO:117), SEQ ID NO:120 (Cj0921c protein; encoded, for example, by SEQ ID NO:119), SEQ ID NO:122 (Cj1259 protein; encoded, for example, by SEQ ID NO:121), SEQ ID NO:124 (Cj1339c protein; encoded, for example, by SEQ ID NO:123), SEQ ID NO:126 (Cj0034c protein; encoded, for example, by SEQ ID NO:125), SEQ ID NO:128 (Cj0404 protein; encoded, for example, by SEQ ID NO:127), SEQ ID NO:130 (Cj0365c protein; encoded, for example, by SEQ ID NO:129),
SEQ ID NO:132 (Cj0755 protein; encoded, for example, by SEQ ID NO:131), and SEQ ID NO:134 (Cj0420 protein; encoded, for example, by SEQ ID NO:133), or antigenic fragments thereof.

The one or more expression vectors may be the same or different one or more expression vectors that comprise the at least one polynucleotide encoding SEQ ID NO:2 (*Cj0998c* protein), SEQ ID NO:4 (*Cj0588* protein), SEQ ID NO:6 (*Cj0248* protein), or fragments thereof. All embodiments of the one or more expression vectors disclosed above apply equally for these additional components. By way of non-limiting example, the one or more expression vectors may be 1, 2, 3, or 4 additional vectors that encode SEQ ID NO:142 (*Cj1534c* protein), SEQ ID NO:108 (1656c protein), SEQ ID NO:110 (0428 protein), SEQ ID NO:112 (0168c protein), and SEQ ID NO:114 (0427 protein), or antigenic fragments thereof. Those of skill in the art will understand the variety of other combinations that can be employed in accordance with the methods of the invention. Based on the present disclosure, it is well within the level of those of skill in the art to prepare expression vectors according to all embodiments of the invention.

Similar to the immunogens recited above, the optional protein immunogens are present in highly conserved variants between different strains of *Campylobacter jejuni*, and

thus the proteins encoded by the one or more expression vectors can be at least 80% identical over the full length of the recited amino acid sequences, or antigenic fragments thereof. In various further embodiments, the proteins encoded by the one or more expression vectors are at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% percent identical over the full length of the recited amino acid sequence(s), or antigenic fragment thereof.

The immunogenic compositions of the present invention may be used for inducing an immune response by administration as naked DNA using standard methods, such as by parenteral delivery. Alternatively, the expression vectors may comprise viral expression vectors, including but not limited to a recombinant adeno-associated virus (AAV) gene delivery vector. In this embodiment, the expression vector is bounded on the 5' and 3' end by functional AAV inverted terminal repeat (ITR) sequences. By "functional AAV ITR sequences" is meant that the ITR sequences function as intended for the rescue, replication and packaging of the AAV virion. Recombinant AAV (rAAV) virions encapsidating the expression vectors of the invention may be produced using standard methodology. In one embodiment, an AAV expression vector according to the invention is introduced into a producer cell, followed by introduction of an AAV helper construct, where the helper construct includes AAV coding regions capable of being expressed in the producer cell and which complement AAV helper functions absent in the AAV vector. This is followed by introduction of helper virus and/or additional vectors into the producer cell, wherein the helper virus and/or additional vectors provide accessory functions capable of supporting efficient rAAV virus production. The producer cells are then cultured to produce rAAV. These steps are carried out using standard methodology. Replication-defective AAV virions encapsulating the recombinant AAV vectors of the instant invention are made by standard techniques known in the art using AAV packaging cells and packaging technology. Examples of these methods may be found, for example, in U.S. Pat. Nos. 5,436,146; 5,753,500, 6,040,183, 6,093,570 and 6,548,286, expressly incorporated by reference herein in their entirety. Further compositions and methods for packaging are described in Wang et al. (US 2002/0168342), also incorporated by reference herein in its entirety. Any suitable method for producing viral particles for delivery can be used.

In another embodiment, the one or more expression vectors are present in a carrier cell, including but not limited to an avirulent, non-*Campylobacter* bacterial carrier cell. Live bacterial vaccine “vectors” (i.e.: bacterial cells comprising immunogenic compositions) have been used successfully to elicit effective immune responses in order to prevent infection.

Recombinant attenuated bacterial cell delivered vaccines have been adapted to stably express protective antigens at high levels. They are capable of stimulating strong primary humoral, mucosal and lasting memory immune responses without significant tissue damage or other performance reducing effects. In various non-limiting embodiments, the bacterial carrier cell is an avirulent bacterial cell selected from the group consisting of attenuated *L. monocytogenes,* attenuated *Salmonella* spp., attenuated *V. cholerae,* attenuated *Shigella* spp., attenuated *M. bovis* BCG, attenuated *Y. enterocolitica,* attenuated *B. anthracis, S. gordonii, Lactobacillus* spp., and *Staphylococcus* spp. As used herein, "attenuated" means that the bacteria is reduced in causing disease symptoms in a host it is delivered to compared to a non-attenuated bacterial vector. Suitable attenuated bacteria can be any species or strain that is or can be sufficiently attenuated to allow for its non-pathological administration to humans and/or animals in live and/or dead form. In one embodiment, an attenuated *Salmonella* species is used. In exemplary embodiments, *Salmonella* that can be used include, but are not limited to *Salmonella enterica* strains selected from the group consisting of *S.* Typhimurium, *S.* Enteriditis, *S.* Heidelberg, *S.*Gallinarum, *S.* Hadar, *S.* Agona, *S.* Kentucky, *S.* Typhi, *S.* Paratyphi and *S.*Infantis. *S.* Typhimurium is especially useful for vaccination purposes because the genome sequence is fully characterized and many animal studies confirm its safe medical use. Recombinant attenuated *Salmonella* vaccines (RASVs) have been constructed to deliver antigens from other pathogens to induce immunity to those pathogens in vaccinated hosts; see, for example, Curtiss et al., Crit Rev Immunol. 2010;30(3):255-70; 2010; Qiu et al., J. Virological Methods 188:108; Strugnell et al., Infect. Immunol. 1992, 60:3994; Layton et al., Clinical and Vaccine Immunology march 2011, 449-454; Al-Ojali et al., Microbial Pathogenesis 52:326 (2012); and (Wyszynska et al., 2004) Wyszyńska et al. (2004). In one embodiment, the *RASV* comprises attenuating mutations in the *pmi* (mannose-6-phosphate isomerase), *fur* (ferric uptake regulator) and *crp* (cAMP regulatory protein) genes (see, for example, (Li et al., PNAS 106:592-597 2009, Curtiss et al., 2009) and US 8,133,493. In another embodiment, the RASV comprises the χ9992 vector disclosed in US 8,133,493. In another embodiment, the RASV is one that is commercially available, such as Megan®Vac1 (Lohman Animal Health, US).

Attenuated bacterial cells can be transfected with the one or more expression vectors using standard techniques in the art.

In a second aspect, the present invention provides an immunogenic composition, comprising

(a) one or more isolated proteins selected from the group consisting of proteins comprising an amino acid sequence at least 80 percent identical to SEQ ID NO:2 (*Cj0998c* protein), SEQ ID NO:4 (*Cj0588* protein), and SEQ ID NO:6 (*Cj0248* protein), or antigenic portions thereof; and

(b) a pharmaceutically acceptable carrier.

As disclosed above for the first aspect of the invention, the immunogenic compositions of the second aspect of the invention can be used, for example for stimulating an immune response in subjects at risk of *C. jejuni* infection or colonization, including but not limited to vertebrates such as chickens, turkeys, cattle, sheep, pigs, and humans.

In one embodiment, the immunogenic composition comprises one of the recited proteins, or antigenic fragments thereof. In another embodiment, the immunogenic composition comprises two of the proteins comprising an amino acid sequence at least 80 percent identical to the recited amino acid sequences (i.e.: SEQ ID NO:2 and SEQ ID NO:4; SEQ ID NO:2 and SEQ ID NO:6; or SEQ ID NO:6 and SEQ ID NO:8), or antigenic fragments thereof. In a further embodiment, the immunogenic composition comprises all three of the proteins comprising an amino acid sequence at least 80 percent identical to the recited amino acid sequences (i.e.: (i.e.: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6) , or antigenic fragments thereof.

The *Cj0998c, Cj0588*, and *Cj0248* proteins are present in highly conserved variants between different strains of *C. jejuni*, and thus the proteins can be at least 80% identical over the full length of the recited amino acid sequences, or antigenic fragments thereof. In various further embodiments, the proteins are at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% percent identical over the full length of the recited amino acid sequence(s), or antigenic fragment thereof.

In one embodiment, the at least one protein is selected from the group consisting of the following, or an antigenic fragment thereof:

(a) **SEQ ID NO:7 (*Cj0998c* genus)**

MKK(I/V/F)(L/V)(V/A/L)S(V/I)(L/F)S(S/F)CLLASALSAVSFKEDSLK(I/V)SFEGYKTKD M(I/V)G(T/V/I/A)(K/R)GEFKNVEY(K/N)FSK(N/S)(I/T)KD(L/F)ASYLKGAKATI(K/E)P S(N/D)AFM(G/S)EG(N/L)D(I/V)ITNNITKVFFPALLG(D/N)(T/A)DIKVVFQD(V/A/M)I(A /V)GE-

X1ITMDKKSTI(V/I)PLTYTIKD(N/D)KFEAKGQ(L/F)DLH(T/A)FKN(G/A)SKALKALSD (V/A)A(A/T/P)GHGGISWPLVDISFNADL(A/T/V)E

wherein X1 is absent or is

NKGVISAK (SEQ ID NO: 135);

(b) **SEQ ID NO:8: (*Cj0588* genus)**

X1-

(L/M)(D/N/E)LL(S/R)EIY(V/I)SRAALKLK(K/N)FLEEN(D/G/N)IE(I/V)(K/N)(H/Q/N)K NCLDIGSSTGGFVQILLEN(Q/K)ALKIT(A/T)LDVG(S/N)NQLH(P/S/L)(S/N)LR(V/A/T) NE(K/I)(I/V)IL(H/Y)EN(T/I)DLR(A/T/V)FKSEEKFE(L/F)(V/I)TCDVSFISL(I/V)NLLYY (I/V)(D/N)NLAL(K/R)EIILLFKPQFEVGKN(I/V)KRDKKGVLKD(D/G)(K/R)(A/V)ILK A(R/K)MDFEK(A/E)CAKL(G/S)W(L/F/I)LKNTQKS(S/C)IKGKEGNVEYFYYYIKN

wherein X1 is absent or is

M(R/I)(F/-)(D/-

)FF(V/I)SKRL(N/D)ISRNKALELIE(N/S)EE(I/V)LLNGK(S/N)FKAS(F/C)DV KN(F/L)LENLKK(T/A/K)QDLN(P/L/S)E(D/E)(I/V)(L/Y)L(A/T/S)(N/D/K)(E/G)L(K/N) (SEQ ID NO: 136); and

(c) **SEQ ID NO:9 (*Cj0248* genus)**

(M/-)I(G/-)DMNELLLKSVEVLPPLPDTVSKLRKYVSEANSNIETMKV(A/V)EIISSDPL MTAKLLQLANSPYYGFTREITTI(N/S)QVITLLG(V/I)GNIINIV(M/T)ADSI(R/K)D(N/S)F KIDVSPYGL(N/D)T(Q/K)(N/V)FL(K/R)(T/N)CN(E/D)EATFI(A/V/T)NWLNDEDKKLSH LLVPCAMLLRLGIVIFSNFLIQN(H/Y/F)(K/R)-**X1**

wherein **X1** is absent, or is

(D/E)K(D/E)FL(A/T)FLN(-/E/K)(-/T)K(N/S/I))EN(L/I)ALAENEFLGVDHISFLGFLL(H/Y) RWNFDD(V/I)LIESICFV(R/H)TPHAARE(K/E)VKKSAYALAITDHLF(A/T)PHDGSSPF N

(A/V/T)KAAVALL(K/E)EAK-**X2** (SEQ ID NO: 137 or 138); and

wherein **X2** is absent or is selected from the group consisting of TQGINFDL(N/D)NLLSKLP(N/S)KAKENL(N/D)(K/E)ED (SEQ ID NO: 139) and NSRN (SEQ ID NO: 140).

In various further embodiments, the at least one polynucleotide encodes a protein selected from the group consisting of SEQ ID NOS: 10-106, or an antigenic fragment thereof. SEQ ID NOS, 44-82 are each *Cj0998c* protein homologs from other *C. jejuni* strains; SEQ ID NOS, 83-106 are each *Cj0588* protein homologs from other *C. jejuni* strains; and SEQ ID NOS, 10-43 are each *Cj0248* protein homologs from other *C. jejuni* strains.

The immunogenic composition of any embodiment of this second aspect of the invention may comprise "antigenic portions" of the recited proteins. As used herein, and "antigenic portion" is any fragment of 10 or more contiguous amino acids in the recited amino acid sequence. In various further embodiments, the antigenic portion is any fragment of 15, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, or 275 contiguous amino acids of the recited amino acid sequence.

The immunogenic compositions of the second aspect of the invention may comprise proteins modified in any suitable way. In one embodiment, the protein component(s) of the composition are treated to extend *in vivo* half-life by, for example, such as by PEGylation, HESylation, PASylation, or glycosylation. The proteins may also be glycosylated as deemed appropriate, using standard techniques in the art. In another embodiment, those protein components in the immunogenic compositions that possess N-glycosylation sequences (NXS or NXT) may be glycosylated, to help further stimulate the immune response.

The immunogenic compositions of the second aspect of the invention may further comprise any other suitable components as may be useful for a given purpose. In various non-limiting embodiments, the compositions may further comprise one or more additional proteins selected from the group consisting of proteins comprising an amino acid sequence at least 80% identical to SEQ ID NO:142 (*Cj1534c* protein), SEQ ID NO:108 (Cj1656c protein), SEQ ID NO:110 (Cj0428 protein), SEQ ID NO:112 (Cj0168c protein), SEQ ID NO:114 (Cj0427 protein), SEQ ID NO:116 (Cj0113 protein),SEQ ID NO:118 (Cj0982c protein), SEQ ID NO:120 (Cj0921c protein), SEQ ID NO:122 (Cj1259 protein), SEQ ID NO:124 (Cj1339c protein), SEQ ID NO:126 (Cj0034c protein), SEQ ID NO:128 (Cj0404 protein), SEQ ID NO:130 (Cj0365c protein), SEQ ID NO:132 (Cj0755 protein), and SEQ ID NO:134 (Cj0420 protein), or antigenic fragments thereof. Similar to the immunogens recited above, the optional protein immunogens are present in highly conserved variants between different strains of *Campylobacter jejuni*, and thus the proteins can be at least 80% identical over the full length of the recited amino acid sequences, or antigenic fragments thereof. In various further embodiments, the proteins encoded by the one or more expression vectors are at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% percent identical over the full length of the recited amino acid sequence(s), or antigenic fragment thereof.

In another embodiment, the immunogenic compositions of the second aspect of the invention may further comprise a *C. jejuni* pilus protein as described in WO 2008/008092, incorporated by reference herein in its entirety. The pilus protein described in WO

2008/008092 was shown to stimulate an immune response against *C. jejuni*, and thus it is suitable for inclusion in the immunogenic compositions of the second aspect of the present invention. Methods for isolating the *C. jejuni* pilus protein are described in WO 2008/008092.

The immunogenic compositions of the invention may comprise any suitable amount/dosage of the composition as determined most appropriate. In one embodiment, the immunogenic composition comprises about $10^6 - 10^{10}$ avirulent bacterial cells per dose. In another embodiment where the composition comprises immunogenic proteins, the composition may comprise about 0.1 ug/kg-100 mg/kg body weight of the proteins; alternatively, it may be 0.5 ug/kg to 50 mg/kg; 1 ug/kg to 25 mg/kg, or 5 ug/kg to 10 mg/kg body weight of the proteins.

The immunogenic compositions of the present invention (i.e.: any embodiment or combination of embodiments of the first and second aspects of the invention) can be formulated by any of the means known in the art. The immunogenic compositions are typically formulated as a pharmaceutical composition, such as those disclosed above, and can be formulated for administration via any suitable route, including orally, as injectables, parentally, by inhalation spray, intranasally, rectally, mucosally, topically, or for administration by oral gavage or *ad libitum* feeding, for example, in drinking water, either as liquid solutions or suspension, in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intra-arterial, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or intraperitoneally. Solid forms suitable for solution in, or suspension in, liquid prior to injection or other administration may also be prepared. The compositions may also, for example, be emulsified, or the encapsulated in liposomes or microparticles. The immunogenic compositions may also be present in and/or expressed by transgenic plants.

The immunogenic compositions according to the present invention may further comprise any suitable adjuvant. Immunological adjuvants in general comprise substances that boost the immune response of the host in a nonspecific manner. A number of different adjuvants are known in the art. Examples of adjuvants are Freunds Complete and Incomplete adjuvant, vitamin E, non-ionic block polymers and polyamines such as dextransulphate, carbopol and pyran, oligopeptide, emulsified paraffin-Emulsigen™ (MVP Labs, Ralston, Nebr.), L80 adjuvant containing aluminum hydroxide (Reheis, N.J.), Quil A™ (Superphos); surface active substances such as Span™, Tween™, hexadecylamine, lysolecitin, methoxyhexadecylglycerol and saponins; peptides such as muramyldipeptides,

dimethylglycine, and tuftsin; immune-stimulating complexes (ISCOMS), mineral oil e.g. Bayol or Markol, vegetable oils or emulsions thereof, aluminum hydroxide; N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP); N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-s- n-glycero-3hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE); and RIBI, which contains three components extracted from bacteria: monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. In embodiments designed for mucosal administration, the immunogenic compositions may further comprise an adjuvant, including but not limited to such as the nontoxic cholera toxin B subunit (Sigma Chemical Company, St. Louis, Mo.) and organometallopolymers including linear, branched or cross-linked silicones which are bonded at the ends or along the length of the polymers to the particle or its core. Such polysiloxanes can vary in molecular weight from about 400 up to about 1,000,000 daltons; the preferred length range is from about 700 to about 60,000 daltons. Suitable functionalized silicones include (trialkoxysilyl) alkyl-terminated polydialkylsiloxanes and trialkoxysilyl terminated polydialkylsiloxanes, for example, 3-(triethyoxysilyl) propyl terminated polydimethylsiloxane. See U.S. Pat. No. 5,571,531, incorporated by reference herein. Phosphazene polyelectrolytes can also be incorporated into immunogenic compositions for mucosal administration (See e.g., U.S. Pat. No. 5,562,909).

The immunogenic compositions according to the present invention may also comprise preservatives such as sodium azide, thimersol, gentamicin, neomycin, and polymyxin.

The immunogenic compositions may be mixed with excipients or carriers which are pharmaceutically acceptable and compatible with the protein immunogen(s) to be used. Suitable excipients include, but are not limited to, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. Such an immunogenic composition can easily be prepared by admixing the protein with a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier is understood to be a compound that does not adversely affect the health of the animal to be vaccinated, at least not to the extent that the adverse effect is worse than the effects seen when the animal is not vaccinated. A pharmaceutically acceptable carrier can be e.g. sterile water or a sterile physiological salt solution. In a more complex form, the carrier can e.g. be a buffer.

The immunogenic composition may further comprises stabilizers, e.g. to protect degradation-prone components from being degraded, to enhance the shelf-life of the composition, or to improve freeze-drying efficiency. Useful stabilizers include, without

limitation, SPGA, skimmed milk, gelatin, bovine or other serum albumin, carbohydrates e.g. sorbitol, mannitol, trehalose, starch, sucrose, dextran or glucose, proteins such as albumin or casein or degradation products thereof, and buffers, such as alkali metal phosphates. Where an albumin is used, it is desirably from the same species as the animal (or human) to which the immunogenic composition containing it will be administered. Freeze-drying is an efficient method for conservation. Freeze-dried material can be stored stable for many years. Storage temperatures for freeze-dried material may well be above zero degrees, without being detrimental to the material. Freeze-drying can be done according to all well-known standard freeze-drying procedures. However, the immunogenic compositions of the invention may be stored in any suitable manner. For example, the immunogenic compositions could be lyophilized or otherwise stabilized and stored in food or water for delivery.

In an exemplary embodiment when the immunogenic compositions are designed for administration to a non-human subject, such as chickens, turkeys, birds, sheep, pigs, cattle, dogs, or cats, the immunogenic composition is formulated for mucosal administration, such as by admixing of the composition with drinking water or food, or admixing for use as a spray, to mist over the animals to uptake the immunogenic composition during grooming behavior. However, any suitable method of administration can be used for any subject. Thus, in various embodiments the immunogenic compositions are formulated for intraocular, intranasal, or transdermal administration.

In a third aspect, the present invention provides methods for stimulating an immune response against *Campylobacter*, comprising administering to a subject an effective amount of the immunogenic composition according to any embodiment or combination of embodiments of the first or second aspects of the present invention to generate an immune response against *Campylobacter*.

In a fourth aspect, the present invention provides methods for reducing *Campylobacter* intestinal colonization in a subject, comprising administering an amount effective of the immunogenic composition according to any embodiment or combination of embodiments of the first or second aspects of the present invention to reduce *Campylobacter* intestinal colonization in the subject.

As disclosed herein, the inventors have identified three novel putative virulence genes (*Cj0248*, *Cj0588*, and *Cj0998c*) from C. *jejuni* encoding novel proteins from the outer-membrane of the bacterium. The inventors have further discovered that each of the *Cj0998c* protein, the *Cj0588* protein, and the *Cj0248* protein are potent immunogens for stimulating an effective immune response against *Campylobacter jejuni* ("*C. jejuni*"). For example, as

disclosed in detail herein, two separate vaccination trials of chickens with a vector expressing the *Cj0988c* protein demonstrated reduced numbers of *C. jejuni* in birds after challenge an average of 2.5 logs CFU (geomean 3 logs) when compared to the cecal numbers of non-vaccinated control birds. Furthermore, vaccination trials demonstrated a significant reduction (1-4 logs) (1 log with heterologous strain and 4 logs with homologous strain) of *C. jejuni* in cecal contents of chickens vaccinated with vectors expressing the *Cj0588* protein and challenged. Each of the proteins was initially isolated from an outer-membrane (OMP) extraction of a *C. jejuni* biofilm.

Campylobacteriosis is a food-borne disease primarily generally caused by *C. jejuni*. The major risk factor in acquiring the disease is the handling and consumption of poultry. However, the epidemiology of poultry colonization with *Campylobacter* such as *C. jejuni* is extremely complex. Birds become colonized within 14 days of hatching, spreading the infection throughout the flock by the end of the grow-out period. Strain differences, based on subtyping and/or genotyping assays, exist between and within flocks. Although some flocks remain *Campylobacter*-free, most flocks have 50-100% of the birds colonized by grow-out. Nevertheless, broilers (i.e., chickens of either gender that will be slaughtered for meat at about 5 to 8 weeks old depending on weight) become contaminated with *Campylobacter*, such as *C. jejuni* and although this microorganism acts as normal flora in the chicken, undercooked chicken is a primary vehicle for transmission of *Campylobacter*, such as *C. jejuni* to humans and remains a significant public health concern.

The methods may be used on any suitable subject at risk of *Campylobacter* infection, including but not limited to vertebrates such as chickens, turkeys, birds, cattle, sheep, pigs, dogs, cats, and humans. In one non-limiting embodiment, a human subject may be anyone that consumes chicken, beef, turkey, or pork. In another embodiment, a human subject may be one that works with animals (i.e.: farm workers, workers at slaughterhouses and meat processing plants, etc.) such as chickens, turkeys, cattle, sheep, and pigs.

In another embodiment, the subject is a feed animal such as chickens, turkeys, cattle, sheep, and pigs. In a preferred embodiment, the subject is a chicken. In these embodiments, the feed animal may be of any suitable age. Birds become colonized with *C. jejuni* within 14 days of hatching, spreading the infection throughout the flock by the end of the grow-out period. Thus, in one embodiment, the methods are initially carried out by about 14 days after hatching. It will be understood by those of skill in the art that additional booster administrations may be desirable after the initial administration; such booster administrations can be carried out at any suitable time, such as by about 21 days after hatching.

In another non-limiting embodiment where the subject is a feed animal, the methods are carried out before slaughtering. However, it will be understood that the methods can be used on chickens or turkeys at any suitable time, as appropriate for a given use.

Campylobacteriosis is currently one of the most common bacterial food-borne diseases in humans in the U.S. and is responsible for causing an estimated 1.3 million cases annually in the U.S., often accompanied by acute gastroenteritis. The infectious dose in the development of the disease is variable and ranges from 500 to 10^6 organisms. Variation in infectious dose is thought to be due to either individual susceptibility or to the relative virulence of the organism. The incubation period is one to seven days with clinical symptoms including fever, severe abdominal cramps, and a watery diarrhea or a dysentery-like syndrome typical of shigellosis. The disease is usually self-limiting, lasting from two to seven days but occasionally is fatal (120-360 deaths per year), mainly in infants and young adults. Serious complications such as arthritis occur in an estimated 1-5% of cases and Guillain-Barre Syndrome, a form of neuromuscular paralysis occurs at a rate of 1 per 1,000 patients.

Conversely, *Campylobacter*, such as *C. jejuni,* colonizes poultry as a commensal, that is, without producing any overt signs of disease. Similarly, *Campylobacter*, such as *C. jejuni* may colonize other feed animals as a commensal as well. However, infected cattle may suffer from diarrhea, weight loss, and suffer from fever and increased heart rate.

The methods of the third and fourth aspects of the invention may be used to stimulate an immune response against or to reduce intestinal colonization of any species of *Campylobacter.* In preferred embodiments, the methods of the third and fourth aspects of the invention are used to stimulate an immune response against or to reduce intestinal colonization of varied species of *Campylobacter* including but not limited to *C. jejuni*, *C. coli, C. lari* and/or *C. upsaliensis*.

As used herein, methods for "stimulating an immune response" result in one or more effects (e.g., maturation, proliferation, direct- or cross-presentation of antigen, gene expression profile) on cells of either the innate or adaptive immune system. For example, the immune response may involve, effect, or be detected in innate immune cells such as, for example, dendritic cells, monocytes, macrophages, natural killer cells, and/or granulocytes (e.g., neutrophils, basophils or eosinophils). The immune response may also involve, effect, or be detected in adaptive immune cells including, for example, lymphocytes (e.g., T cells and/or B cells). The immune response may be observed by detecting such involvement or

effects including, for example, the presence, absence, or altered (e.g., increased or decreased) expression or activity of one or more immunomodulators. The immune response may stimulate a de novo or previously undetected antibody response, or enhance or suppress an existing response against the immunogen by, for example, causing an increased antibody response (e.g., amount of antibody, increased affinity/avidity) or an increased cellular response (e.g., increased number of activated T cells, and/or increased affinity/avidity of T cell receptors. In certain embodiments, the immune response may be protective, meaning that the immune response may be capable of preventing initiation or continued infection of or growth within a host and/or by eliminating *C. jejuni* from the host. In some instances, elimination of an agent from the host may mean that the method is therapeutic, in that the method is used to treat a subject already infected with *C. jejuni.* When the method is therapeutic, the method may comprise treating a *C. jejuni* infection, wherein "treating" means accomplishing one or more of the following: (a) reducing the severity of the infection; (b) limiting or preventing development of symptoms characteristic of the infection; (c) inhibiting worsening of symptoms characteristic of the infection; (d) limiting or preventing recurrence of the disorder(s) in subjects that have previously had the infection; and (e) limiting or preventing recurrence of symptoms in subjects that were previously symptomatic for the infection.

As used herein, "reducing *Campylobacter* intestinal colonization" means reducing a level of intestinal colonization that would be observed in the subject in the absence of administering the one or more immunogenic compositions of the invention. Any level of reduction is beneficial in reducing *Campylobacter* transmission, for example, from poultry to a human consuming the poultry. In one embodiment, the reduction comprises at least 10% reduction in intestinal colonization compared to intestinal colonization in the absence of treatment; in various further embodiments, the intestinal colonization is reduced by at least 20%, 25%, 50%, 75%, 80%, 85%, 90%, 95%, or more compared to intestinal colonization in the absence of treatment. Techniques for quantifying a level of *Campylobacter* intestinal colonization are well known in the art and include, but are not limited to, examining cecal or fecal levels of *Campylobacter.* Techniques for determining fecal contamination of *Campylobacter* in production to quantify the level of *Campylobacter* organisms from a carcass rinse of feed animals, such as poultry, at various points of processing, such as after chilling are also established.

In preferred embodiments of the third and fourth aspects of the invention, the one or more expression vectors are present in a carrier cell, including but not limited to a non-

Campylobacter bacterial carrier cell. In various non-limiting embodiments, the bacterial carrier cell is an avirulent bacterial cell selected from the group consisting of attenuated *L. monocytogenes,* attenuated *Salmonella* spp., attenuated *V. cholerae,* attenuated *Shigella* spp., attenuated *M. bovis* BCG, attenuated *Y. enterocolitica,* attenuated *B. anthracis, S. gordonii, Lactobacillus* spp., and *Staphylococcus* spp. In one embodiment, an attenuated *Salmonella* species is used. In exemplary embodiments, *Salmonella* that can be used include, but are not limited to *Salmonella* enterica strains selected from the group consisting of *S.* Typhimurium, *S.* Enteriditis, *S.* Heidelberg, *S.*Gallinarum, *S.* Hadar, *S.* Agona, *S.* Kentucky, *S.* Typhi, *S.* Paratyphi and *S.* Infantis. *S.* Typhimurium is especially useful for vaccination purposes because the genome sequence is fully characterized and many animal studies confirm its safe medical use. Recombinant attenuated *Salmonella* vaccines (RASVs) have been constructed to deliver antigens from other pathogens to induce immunity to those pathogens in vaccinated hosts; see, for example, Curtiss et al., Crit Rev Immunol. 2010;30(3):255-70; 2010; Qiu et al., J. Virological Methods 188:108; Strugnell et al., Infect. Immunol. 1992, 60:3994; Layton et al., Clinical and Vaccine Immunology march 2011, 449-454; Al-Ojali et al., Microbial Pathogenesis 52:326 (2012); and Wyszyńska et al. (2004). In one embodiment, the RASV comprises attenuating mutations in the *pmi*, *fur* and *crp* genes and US 8,133,493. In another embodiment, the RASV comprises the χ9992 vector disclosed in US 8,133,493. In another embodiment, the RASV is one that is commercially available, such as Megan®Vac1 (Lohman Animal Health, US).

The immunogenic compositions can be administered via any suitable route, including orally, as injectables, parentally, by inhalation spray, intranasally, rectally, mucosally, topically, or for administration by oral gavage or *ad libitum* feeding, for example, in drinking water, either as liquid solutions or suspension, in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intra-arterial, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or intraperitoneally. Solid forms suitable for solution in, or suspension in, liquid prior to injection or other administration may also be prepared. The compositions may also, for example, be emulsified, or the encapsulated in liposomes.

In an exemplary embodiment when the subject is a non-human subject, such as chickens, turkeys, birds, sheep, pigs, cattle, dogs, or cats, the immunogenic composition are administered orally or mucosally, such as by admixing of the composition with drinking water or food, or admixing for use as a spray, to mist over the animals to uptake the

immunogenic composition during grooming behavior. In exemplary embodiments where the subject is a human, the immunogenic compositions are administered orally or mucosally. However, any suitable method of administration can be used for any subject.

The immunogenic compositions are administered in a manner compatible with the dosage formulation, and in such amount and manner as will be prophylactically and/or therapeutically effective, according to what is known to the art. The quantity to be administered depends on the subject to be treated, the functional capacity of the subject's immune system, the degree of protection desired, and other factors. Precise amounts of the active ingredient required to be administered may depend on the judgment of the individual administering the immunogenic compositions and may be peculiar to each individual, but such a determination is within the level of those of skill in the art based on the teachings herein. In one embodiment, about $10^6 - 10^{10}$ avirulent bacterial cells per dose can be administered. In another embodiment where immunogenic proteins are administered, a suitable dosage range may, for instance, be 0.1 ug/kg-100 mg/kg body weight; alternatively, it may be 0.5 ug/kg to 50 mg/kg; 1 ug/kg to 25 mg/kg, or 5 ug/kg to 10 mg/kg body weight.

The immunogenic compositions may be administered in a single dose or in multiple dosages as determined most appropriate, such as a two dose schedule, for example two to eight weeks apart; or a multiple dose schedule or in combination with other vaccines. A multiple dose schedule is one in which a primary course of vaccination may include 1 to 10 or more separate doses, followed by other doses administered at subsequent time intervals as required to maintain and/or reinforce the immune response, e.g., at 1 to 4 months for a second dose, and if needed, a subsequent dose(s) after several months.

Example 1 Immunogenic compositions based on *Cj0988c*

The DNA and protein sequence of *Cj0988c* are provided in SEQ ID NO:1 and SEQ ID NO:2, respectively.

Salmonella Typhimurium vector χ9992:

The vector used was a *Salmonella* Typhimurium vector χ9992 (Curtiss et al., 2009) which contains eight mutations, with three of the mutations attenuating: the *pmi, fur,* and *crp* genes.

1) Δ*pmi*-2426-Eliminates phosphomannose isomerase which ceases LPS-O antigen synthesis in the absence of mannose;

2) Δ*gmd-fcl*-Reduces biofilm formation and prevents the formation of colonic acid which aids in the survival of *asdA* mutants;

3) Δ*asdA27*-Deletion of a gene encoding an enzyme necessary for diaminopimelic acid (DAP). Strain requires supplemental DAP until the introduction of the plasmid. Eliminates need for antibiotic resistance markers;

4) ΔP_{fur77}::TT *araC* P_{BAD}*fur*::TT *araC* P_{BAD} c2 ΔPcrp527::TT *araC* P_{BAD} *crp* -Allows *fur* and *crp* gene expression only in the presence of arabinose, producing pYA3493 (**Figure 1**), a strain which is maximally invasive prior to display of attenuated phenotype following cell division;

5) ΔrelA198::*araC*P_{BAD}*lacI*TT - eliminates *relA* gene to uncouple growth from protein synthesis and provides arabinose-dependent synthesis of the LacI repressor to confer regulated delayed *in vivo* synthesis of recombinant proteins, and

6) Δ*araE25* Δ*araBAD23*- Eliminates the ability of *Salmonella* to metabolize arabinose. Allows retention of arabinose in the cytoplasm without use.

The vector plasmid pYA3493 contains the *asd* gene to compliment the chromosomal Δ*asdA27* mutation and ensure that the plasmid will not be lost *in vivo*. The plasmid also fuses the expressed product to a β-lactamase signal sequence for periplasmic secretion of the protein. Finally, the plasmid has a strong promoter (-35 region of trp promoter/-10 lac) in P_{trc} (**Fig. 1**). Use of this plasmid does <u>not</u> confer antibiotic resistance to the *Salmonella* vaccine.

The vector that we used for the amplification of the plasmid with the inserted *C. jejuni* gene is *E. coli* χ6212. This strain is Asd- allowing amplification of the plasmid, which is then extracted and electroporated into *Salmonella* χ9992.

Gene *Cj0998c* expresses a hypothetical protein that is part of the outer membrane of the bacterium. Gene *Cj0998c* was cloned individually into plasmid pYA3493 and expressed from the *Salmonella* vector χ9992.

A. Cloning of the *Cj0998c* gene into the *Salmonella* vector

The *Salmonella* strain (χ9992) used as the vector has three different mutations which results in its attenuation. The strain is safe and immunogenic and acts as a phenotypically wild-type strain at the time of immunization but becomes attenuated after colonization of host tissues. The plasmid contains the *asd* gene which provides a marker for laboratory differentiation using media not containing DAP to grow plasmid–containing strains. Since this gene is required for the survival of the vector without DAP, the plasmid is maintained in

the vector during *in vivo* colonization. Gene *Cj0998c* was cloned into *Salmonella* χ9992. Essentially, primers to each gene were designed and the gene amplified using PCR. Each gene product was inserted into plasmid pYA3493 (*asd*+), cloned into *E. coli* χ6212 (*asd*-) and cultured for amplification. Following growth, the plasmid was extracted from the *E. coli* strain, cloned into the *Salmonella* Typhimurium vector, and the transformants plated on LB media minus DAP, cultured at 37°C on LB agar, harvested, and stored frozen at -80°C. Confirmation that the gene was present in the vector was accomplished through a plasmid extraction and sequence analysis of genes following PCR and gel electrophoresis.

B. *Salmonella* growth *in vitro*

The *Salmonella* vector expressing the Cj0998c protein and the *Salmonella* empty vector were grown in Luria Bertani (LB) broth supplemented with 0.1% glucose, 0.05% mannose, and 0.1% arabinose for 24 h at 37°C with shaking. Following incubation, the overnight cultures were pelleted and re-suspended in PBS to a final concentration of $1x10^{10}$ CFU/ml.

C. Vaccination of poultry

Vaccination of chickens, twice, with the vector expressing the Cj0988c protein reduced cecal numbers of *C. jejuni* in birds an average of 2.5 logs CFU (geomean of 3.0 logs CFU) when compared to the cecal numbers of non-vaccinated control birds (Table 1). One outlier of 1.00E+07 (highlighted cell, Table 3) was present in 31 birds examined. Cornish X Rock chickens were obtained from McMurray Hatchery (IA) and randomly assigned to isolated groups. The chickens were screened for the fecal presence of *C. jejuni* and/or *Salmonella*. The chickens were vaccinated orally, at days 10 and 16, with 1 ml of the *Salmonella* vector expressing the Cj0988 protein (~10^{10} CFU/ml) or the *Salmonella* empty vector and challenged with 10^5 CFU/ml of *C. jejuni* strain NCTC11168 10 days after the second vaccination. Prior to vaccination and challenge, chickens were fasted for 8 hours. Food was returned one hour post vaccination/challenge. Aside from this period, food and water were available *ad libitum*. The chickens were necropsied 10 days after challenge infection. Cecal contents were ten-fold diluted and direct plated onto modified Campy Cefex agar plates incubated for 48 h at 10% CO_2 microaerophilic conditions for enumeration of *Campylobacter*.

Table 1. Combined data of *C. jejuni* vaccination trials 1 & 2

C. jejuni NCTC11168 Challenge-Trials 1&2			
	Cj0998c	Control-EV	Controls
Average	6.11E+05	6.75E+07	1.15E+08
Geomean	2.60E+04	4.41E+07	4.83E+07

Table 2. Vaccination Trial 1 – Ten day old chickens vaccinated twice with the vector expressing gene *Cj0998c*, or the *Salmonella* empty vector only (Control-EV), or non-vaccinated controls

C. jejuni NCTC11168 Challenge - Trial 1			
	Cj0998c	Control-EV	Controls
	1.0E+03	1.0E+07	3.0E+08
	7.0E+04	1.2E+07	6.0E+07
	7.4E+04	1.0E+08	1.0E+07
	8.2E+04	2.0E+08	1.0E+07
	1.0E+04	7.0E+07	2.0E+08
	2.0E+04	9.0E+07	2.0E+07
	3.0E+04	1.0E+08	4.0E+08
	2.0E+03	1.0E+08	1.2E+08
	6.0E+04	2.0E+07	1.0E+07
	3.0E+04	1.0E+07	2.0E+07
Average	**3.79E+04**	**7.12E+07**	**1.15E+08**
Geomean	**1.98E+04**	**4.45E+07**	**4.83E+07**

Table 3. Vaccination Trial 2 – Ten day old chickens vaccinated twice with the vector expressing gene *Cj0998c* (two groups), or the *Salmonella* empty vector only (Control-EV)

C. jejuni NCTC11168 Challenge - Trial 2			
	Cj0998c	*Cj0998c*	Control-EV
	1.40E+04	2.00E+05	5.00E+07
	3.20E+03	1.70E+05	3.00E+07
	7.00E+04	3.00E+04	2.00E+07
	1.00E+02	2.70E+05	1.70E+08
	7.00E+04	3.00E+05	3.00E+07
	9.00E+03	2.40E+04	
	1.05E+04	1.50E+04	
	1.00E+00	5.00E+06	
	6.00E+03	2.30E+06	
	3.00E+03	1.00E+07	
	6.00E+04		
Average	**2.23E+04**	**1.83E+06**	**6.00E+07**
Geomean	**3.78E+03**	**2.84E+05**	**4.33E+07**

Example 2. Immunogenic compositions based on *Cj0588 and Cj0248*

1. The DNA and protein sequence of *Cj0588* are provided in SEQ ID NO:3 and SEQ ID NO:4, respectively.

2. The DNA and protein sequence of *Cj0248* are provided in SEQ ID NO:5 and SEQ ID

NO:6, respectively.

3. Mutation of *C. jejuni* genes

Genes *Cj0588 and Cj0248* were cloned and mutated in *C. jejuni* by insertion of a chloramphenicol cassette in the center of each gene. Essentially, two sets of primers were designed for each gene to yield fragments consisting of the initial and terminal 15 bases of the gene and their associated flanking regions. These fragments were cloned into a suicide vector and positioned around the chloramphenicol acetyltransferase (CAT) gene, such that the antibiotic cassette was between the initial and terminal regions of the gene, taking up ~255 bases of each gene. The vector containing the CAT gene and the flanking bases of each gene was then introduced by electroporation into *C. jejuni* strain M129 (*Cj0588*) or strain NCTC11168 (*Cj0248*), and the mutation transferred into the genome via a double crossover. Following electroporation the mutant strain was plated on Mueller Hinton agar containing chloramphenicol. Colonies that grew on the selective media were then confirmed by PCR to contain the cassette in the chromosome in the proper direction. Colonies were then harvested and stored at -78°C.

4. Effects of the Cj0588 mutation on broiler colonization.

The Δ*Cj0588* mutant and M129 *C. jejuni* parent strain were each examined for colonization traits in poultry. Four separate studies were conducted. The detection limit is less than 10 organisms. There was a significant reduction in the colonization of birds inoculated with the M129::*tlyA* mutant, demonstrating that the *C. jejuni Cj0588* gene is important in the colonization of poultry.

Study 1:

Chickens were obtained and housed as above. At 12 days, chickens were orally inoculated with 1.0 ml of approximately 1.0 x 10^5 per ml of viable *C. jejuni* M129 and the M129::*tlyA* mutant. At day 22 the birds were necropsied, and *Campylobacter* was enumerated as above. There was a significant reduction in the colonization of birds inoculated with the M129::*tlyA* mutant.

Table 4. Colonization of chickens with a *C. jejuni* parent M129 and M129::*tlyA* strain.

M129::tlyA	M129::tlyA	M129	M129

52	ND	73	1.00E+02
53	ND	74	ND
54	ND	75	2.00E+02
55	ND	76	ND
56	ND	77	4.20E+03
57	ND	78	ND
58	ND	79	ND
59	ND	80	ND
60	ND	81	1.20E+04
61	ND	82	1.00E+04
TOTAL	**ND**	**Average**	**5.30E+03**

Study 2:

Chickens were obtained and housed as above. At 14 days, birds were orally inoculated with 1.0 ml of approximately 1.0 x 10^6 per ml of viable *C. jejuni* M129 and the M129::*tlyA* mutant. At day 28, the birds were necropsied, and *Campylobacter* was enumerated as above. There was a significant reduction in the colonization of birds inoculated with the M129::*tlyA* mutant.

Table 5. Colonization of chickens with a *C. jejuni* parent M129 and M129::*tlyA* strain.

M129::tlyA	M129::tlyA	M129	M129
1	ND	1	2.30E+05
2	ND	2	1.40E+05
3	ND	3	1.50E+03
4	ND	4	ND
5	ND	5	ND
6	ND	6	1.30E+06
7	ND	7	ND
8	ND	8	1.90E+03
9	ND	9	1.80E+03
10	ND	10	ND
11	ND	11	8.70E+03
12	ND	12	ND
13	ND	13	1.70E+03
14	ND	14	1.70E+03
15	ND	15	ND

16	ND	16	3.60E+03
17	ND	17	3.10E+03
TOTAL	**ND**	**Average**	**1.54E+05**

Study 3:

Chickens were obtained and housed as above. At 14 days, birds were orally inoculated with 1.0 ml of approximately 1.0 x 10^8 per ml of viable *C. jejuni* M129 and the M129::*tlyA* mutant. At day 28, the birds were necropsied, and *Campylobacter* was enumerated as above. There was a significant reduction in the colonization of birds inoculated with the M129::*tlyA* mutant.

Table 6. Colonization of chickens with a *C. jejuni* parent M129 and M129::*tlyA* strain.

M129::tlyA	M129::tlyA	M129	M129
1	ND	1	9.40E+04
2	ND	2	2.10E+03
3	ND	3	2.10E+03
4	ND	4	1.00E+02
5	ND	5	4.70E+03
6	ND	6	2.60E+07
7	ND	7	3.70E+03
8	ND	8	1.10E+03
9	ND	9	4.00E+03
10	ND	10	2.70E+03
11	ND	11	2.80E+03
12	ND	12	1.50E+04
13	ND	13	2.10E+03
14	ND	14	1.10E+03
15	ND	15	1.50E+05
16	ND	16	5.10E+03
		17	2.00E+02
TOTAL	**ND**	**Average**	**1.55E+06**

Study 4

Chickens were obtained and housed as above. At 14 days, birds were orally inoculated with 1.0 ml of approximately 1.0 x 10^8 per ml of viable *C. jejuni* M129 and the

M129::*tlyA* mutant. At day 28, the birds were necropsied, and *Campylobacter* was enumerated as above. There was a significant reduction in the colonization of birds inoculated with the M129::*tlyA* mutant.

Table 7. Colonization of chickens with a *C. jejuni* parent M129 and M129::*tlyA* strain.

M129::tlyA	M129::tlyA	M129	M129
1	ND	1	1.10E+03
2	ND	2	2.00E+04
3	ND	3	ND
4	ND	4	1.00E+02
5	ND	5	4.30E+03
6	ND	6	2.30E+03
7	ND	7	1.00E+02
8	ND	8	3.00E+02
9	ND	9	1.00E+02
10	ND	10	ND
11	ND	11	2.30E+03
12	ND	12	2.80E+03
13	ND	13	1.70E+06
14	ND	14	1.10E+03
15	ND	15	1.50E+03
16	ND	16	2.80E+03
17	ND	17	1.10E+04
18	ND	18	1.10E+03
19	ND	19	7.00E+02
20	ND	20	2.50E+05
21	ND	21	1.10E+06
22	ND	22	ND
23	ND	23	4.80E+03
TOTAL	**ND**	**Average**	**1.35E+05**

5. Effects of the Δ*Cj0248* mutation on broiler colonization.

The Δ*Cj0248* mutant and NCTC11168 *C. jejuni* parent strain were examined for colonization traits in poultry. Chickens were obtained and housed as above. At day 14, the birds were orally inoculated with 0.5 ml of approximately 1.0 x 10^5 per ml of viable *C. jejuni*

strain NCTC11168 (n=31), the Δ*Cj0248* mutant strain (n=30), or served as negative controls. At day 24, the birds were necropsied, and *Campylobacter* was enumerated as above. There was a significant reduction in the colonization of birds inoculated with the Δ*Cj0248* mutant strain. All the birds inoculated with the wild-type strain NCTC11168 were colonized at an average of 7.42E+07 CFU/g, whereas, 3 of 30 birds (<10 CFUs) were colonized at an average of 7.33E+01 CFU/g with the *C. jejuni* mutant strain Δ*Cj0248* (Table 8).

Table 8. Colonization of Broilers with *C. jejuni* NCTC11168 Wild-type and Mutant

	NCTC11168 Wild Type			**NCTC11168 Mutant *Cj0248***		
Controls	**Group 1**	**Group 2**	**Group 3**	**Group 1**	**Group 2**	**Group 3**
ND	3.30E+07	2.30E+08	4.60E+05	ND	ND	2.00E+02
ND	4.10E+08	3.20E+07	8.00E+03	ND	ND	ND
ND	1.60E+07	4.20E+05	1.00E+04	ND	1.90E+03	ND
ND	2.40E+07	7.10E+07	1.20E+04	ND	ND	ND
ND	9.30E+05	8.20E+04	3.40E+07	ND	ND	1.00E+02
ND	2.00E+06	9.50E+07	3.30E+05	ND	ND	ND
ND	1.40E+07	5.00E+06	3.10E+07	ND	ND	ND
ND	9.50E+08	5.30E+07	1.50E+06	ND	ND	ND
ND	5.90E+07	8.30E+07	1.00E+02	ND	ND	ND
ND	2.40E+07	2.30E+04	3.10E+03	ND	ND	
-	1.30E+08			ND		
		-				
Average	**1.51E+08**	**5.70E+07**	**6.73E+06**	**ND**	**1.90E+02**	**3.33E+01**
	Average: Wild type= 7.42E+07; Mutant= 7.33E+01; ND=not detected,<10CFU					

6. *Salmonella* Typhimurium vector χ9992:

The vector used is a *Salmonella* Typhimurium vector χ9992, as described above.

7. Cloning and expressing *C. jejuni* genes into the *Salmonella* vector.

Both genes *Cj0588* and *Cj0248* were cloned into the vector plasmid pYA3493 and expressed from the attenuated *Salmonella* Typhimurium vector χ9992, as described above.

8. Vaccination of chickens with the attenuated *Salmonella* vector expressing *Cj0588* from plasmid pYA3493.

Chickens were obtained, housed, and vaccinated as above. Trial 1 chickens were orally challenged with 1.0 ml of the homologous *C. jejuni* strain M129 (~1x10^7 CFU/ml) and trial 2 chickens were orally challenged with 1.0 ml of a heterologous strain *C. jejuni* NCTC11168 (~1x10^5 CFU/ml) at 10 days after the final vaccination. Ten days post challenge, the chickens were necropsied, and *Campylobacter* enumerated as above.

In trial 1, a 4-log reduction of *C. jejuni* in cecal contents of chickens vaccinated with the vector expressing Cj0588 protein following challenge with the homologous M129 strain was observed, as compared to chickens receiving the empty vector (EV) vaccine. A 2-log reduction in *C. jejuni* was observed when the Cj0588 vaccinates were compared to the normal controls. In trial 2, an overall 1-log reduction of *C. jejuni* in cecal contents of chickens vaccinated with the Cj0588 protein was observed following challenge with a heterologous strain NCTC11168, as compared to chickens receiving the EV vaccine. These trials demonstrated a significant reduction of *C. jejuni* in cecal contents of chickens vaccinated with the vector expressing the Cj0588 protein and challenged with the homologous strain. In addition, a 1-log reduction was seen in chickens challenged with a heterologous strain. A greater reduction would have occurred in trial 2 if not for three outliers (shaded boxes) (Table 9).

Table 9. Cecal numbers of *C. jejuni* in vaccinated (*Cj0588*) and control chickens

***C. jejuni* M129 Challenge - Trial 1**				***C. jejuni* NCTC11168 Challenge -Trial 2**				
	Cj0588	**Control-EV**	**Controls**		***Cj0588***	***Cj0588***	***Cj0588***	**Control-EV**
	6.00E+03	1.00E+04	2.00E+04		1.10E+03	5.00E+03	1.20E+05	5.00E+07
	7.00E+02	2.00E+04	7.00E+04		1.50E+03	9.00E+04	1.00E+00	3.00E+07
	7.00E+03	1.00E+04	1.00E+05		1.00E+02	3.00E+03	1.20E+04	2.00E+07
	3.30E+03	1.00E+05	3.00E+04		1.00E+02	1.00E+00	6.00E+04	1.70E+08
	2.00E+02	3.00E+04	3.00E+04		5.00E+03	4.00E+01	1.00E+02	3.00E+07
	7.00E+02	3.00E+04	7.00E+04		1.00E+03	3.00E+07	4.00E+07	
	8.00E+03	1.00E+04	1.00E+04		2.00E+03	5.00E+01	2.70E+04	
	3.00E+03	3.00E+04	1.00E+06		7.00E+02	1.70E+04	6.00E+02	
	3.00E+03	3.00E+04	1.00E+04		2.00E+01	5.00E+07	7.00E+04	
	3.20E+03	5.00E+04	1.00E+04		5.00E+04	2.00E+02	1.20E+05	
	1.00E+03	2.30E+04	3.50E+05		7.00E+01	1.00E+00	2.00E+04	
	4.00E+03	1.00E+04	7.00E+04		5.60E+03	1.00E+00	2.00E+04	
	6.80E+03	1.00E+04	2.10E+06	**Average**	**5.60E+03**	**6.68E+06**	**3.37E+06**	**6.00E+07**
	2.30E+03	3.00E+08	7.00E+04	**Geomean**	**7.73E+02**	**1.24E+03**	**1.28E+04**	**4.33E+07**
	1.40E+03	1.00E+04	3.00E+05					
	7.00E+03		6.00E+05	**Combined Trial 2 data**				
					Cj0588			**Control-EV**
Average	**3.60E+03**	**2.00E+07**	**3.03E+05**	**Average**	**3.35E+06**			**6.00E+07**

Geomean	2.47E+03	3.83E+04	8.36E+04	Geomean	2.31E+03			4.33E+07

Example 3. Dual vaccination using Cj0998c and Cj0588

Chickens were obtained, housed, and vaccinated as above, with the exception that each chicken received 2 ml of vaccine, 1 ml per antigen, at each vaccination. Chickens were orally challenged with 1.0 ml of the homologous *C. jejuni* strain NCTC11168 (~$1x10^5$ CFU/ml) at 10 days after the final vaccination. Ten days post challenge the chickens were necropsied, and *Campylobacter* enumerated as above. One vaccinate group showed a near total ~7 log reduction in *Campylobacter* colonization, while colonization was reduced by ~1 log in the other group, as compared to empty vector and positive control groups.

Example 4. Water vaccination using Cj0998c

Chickens were obtained and housed as above, with the exception that no fasting period was observed prior to vaccination and that water was removed for two hours prior to vaccination. On days 10 and 16, ~$1x10^{10}$ cfu of *Salmonella* expressing Cj0998c (prepared as above) was added to ~2 L of water in commercially available fountain waterers. Water was returned when all vaccine had been consumed (8-12 hours). Controls received ~1x10^10 cfu empty vector *Salmonella* or no vaccine orally. Chickens were orally challenged with 1.0 ml of the homologous *C. jejuni* strain NCTC11168 (~$1x10^5$ CFU/ml) at 10 days after the final vaccination. At day 36, the chickens were necropsied and *Campylobacter* enumerated as above. *Campylobacter* colonization was reduced in vaccinate groups by approximately 2.5 logs as compared to empty vector and positive control groups.

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We claim

1. An immunogenic composition, comprising one or more expression vectors comprising:

(a) at least one polynucleotide encoding a protein selected from the group consisting of proteins comprising an amino acid sequence at least 80 percent identical to SEQ ID NO:2 (*Cj0998c* protein), SEQ ID NO:4 (*Cj0588* protein), and SEQ ID NO:6 (*Cj0248* protein), or antigenic portions thereof; and

(b) a promoter operatively linked to the polynucleotide , wherein the promoter region is capable of directing expression of the encoded protein(s).

2. The immunogenic composition of claim 1, wherein the composition comprises one or more polynucleotide encoding two or more of the recited proteins, or antigenic portions thereof.

3. The immunogenic composition of claim 1, wherein the composition comprises one or more polynucleotide encoding all three of the recited proteins, or antigenic portions thereof.

4. The immunogenic composition of claim 1, wherein the at least one polynucleotide encodes one or more proteins comprising an amino acid sequence selected from the group consisting of SEQ ID. NO. 7, SEQ ID NO:8, and SEQ ID NO:9, or antigenic portions thereof.

5. The immunogenic composition of claim 1, wherein the at least one polynucleotide encodes one or more proteins comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10-106.

6. The immunogenic composition of any one of claims 1-5, wherein the at least one polynucleotide encodes a protein comprising an amino acid sequence selected from the group consisting SEQ ID NO:2 (*Cj0998c* protein), SEQ ID NO:4 (*Cj0588* protein), and SEQ ID NO:6 (*Cj0248* protein).

7. The immunogenic composition of any one of claims 1-6, further comprising one or more expression vectors comprising at least one polynucleotide encoding a protein selected from the group consisting of proteins comprising an amino acid sequence at least 80 percent identical to an amino acid sequence of SEQ ID NO:142 (*Cj1534c* protein), SEQ ID NO:108 (Cj1656c protein), SEQ ID NO:110 (Cj0428 protein), SEQ ID NO:112 (Cj0168c protein), SEQ ID NO:114 (Cj0427 protein), SEQ ID NO:116 (Cj0113 protein),SEQ ID NO:118 (Cj0982c protein), SEQ ID NO:120 (Cj0921c protein), SEQ ID NO:122 (Cj1259 protein), SEQ ID NO:124 (Cj1339c protein), SEQ ID NO:126 (Cj0034c protein), SEQ ID NO:128

(Cj0404 protein), SEQ ID NO:130 (Cj0365c protein), SEQ ID NO:132 (Cj0755 protein), and SEQ ID NO:134 (Cj0420 protein), or antigenic fragments thereof.

8. The immunogenic composition of any one of claims 1-6, further comprising one or more expression vectors comprising at least one polynucleotide encoding a protein selected from the group consisting of an amino acid sequence of SEQ ID NO:142 (*Cj1534c* protein), SEQ ID NO:108 Cj1656c protein), SEQ ID NO:110 (Cj0428 protein), SEQ ID NO:112 (Cj0168c protein), SEQ ID NO:114 (Cj0427 protein), SEQ ID NO:116 (Cj0113 protein),SEQ ID NO:118 (Cj0982c protein), SEQ ID NO:120 (Cj0921c protein), SEQ ID NO:122 (Cj1259 protein), SEQ ID NO:124 (Cj1339c protein), SEQ ID NO:126 (Cj0034c protein), SEQ ID NO:128 (Cj0404 protein), SEQ ID NO:130 (Cj0365c protein), SEQ ID NO:132 (Cj0755 protein), and SEQ ID NO:134 (Cj0420 protein).

9. An immunogenic composition, comprising one or more expression vectors comprising:

(a) at least one polynucleotide encoding a protein selected from the group consisting of proteins comprising an amino acid sequence at least 80 percent identical to SEQ ID NO:10-106; and

(b) a promoter operatively linked to the polynucleotide, wherein the promoter region is capable of directing expression of the encoded protein(s).

10. The immunogenic composition of any one of claims 1-9, wherein the immunogenic composition is present in an avirulent, non-*Campylobacter* bacterial carrier cell.

11. The immunogenic composition of claim 10, wherein the avirulent, non-*Campylobacter* bacterial carrier cell is selected from the group consisting of attenuated *L. monocytogenes,* attenuated *Salmonella*, attenuated *V. cholerae,* attenuated *Shigella* spp., attenuated *M. bovis* BCG, attenuated *Y. enterocolitica,* attenuated *B. anthracis, S. gordonii, Lactobacillus* spp., and *Staphylococcus* spp.

12. The immunogenic composition of claim 10, wherein the avirulent, non-*Campylobacter* bacterial carrier cell is an attenuated *Salmonella.*

13. An immunogenic composition, comprising

(a) one or more isolated proteins selected from the group consisting of proteins comprising an amino acid sequence at least 80 percent identical to SEQ ID NO:2 (*Cj0998c* protein), SEQ ID NO:4 (*Cj0588* protein), and SEQ ID NO:6 (*Cj0248* protein), or antigenic portions thereof; and

(b) a pharmaceutically acceptable carrier.

14. The immunogenic composition of claim 13, wherein the composition comprises two or more of the recited proteins, or antigenic portions thereof.

15. The immunogenic composition of claim 13, wherein the composition comprises all three of the recited proteins, or antigenic portions thereof.

16. The immunogenic composition of any one of claims 13-15, wherein the one or more isolated proteins comprises an amino acid sequence selected from the group consisting of SEQ ID. NO. 7, SEQ ID NO:8, and SEQ ID NO:9, or antigenic portions thereof.

17. The immunogenic composition of any one of claims 13-15, wherein the one or more isolated proteins comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 10-106.

18. The immunogenic composition of any one of claims 13-16, wherein the one or more isolated proteins are have an amino acid sequence selected from the group consisting of SEQ ID NO:2 (*Cj0998c* protein), SEQ ID NO:4 (*Cj0588* protein), and SEQ ID NO:6 (*Cj0248* protein).

19. The immunogenic composition of any one of claims 13-18, further comprising one or more proteins having an amino acid sequence at least 80 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:142 (Cj1534c protein), SEQ ID NO:108 (Cj1656c protein), SEQ ID NO:110 (Cj0428 protein), SEQ ID NO:112 (Cj0168c protein), SEQ ID NO:114 (Cj0427 protein), SEQ ID NO:116 (Cj0113 protein),SEQ ID NO:118 (Cj0982c protein), SEQ ID NO:120 (Cj0921c protein), SEQ ID NO:122 (Cj1259 protein), SEQ ID NO:124 (Cj1339c protein), SEQ ID NO:126 (Cj0034c protein), SEQ ID NO:128 (Cj0404 protein), SEQ ID NO:130 (Cj0365c protein), SEQ ID NO:132 (Cj0755 protein), and SEQ ID NO:134 (Cj0420 protein), or antigenic fragments thereof.

20. The immunogenic composition of any one of claims 13-18, further comprising one or more proteins having an amino acid sequence selected from the group consisting of SEQ ID NO:142 (Cj1534c protein), SEQ ID NO:108 (Cj1656c protein), SEQ ID NO:110 (Cj0428 protein), SEQ ID NO:112 (Cj0168c protein), SEQ ID NO:114 (Cj0427 protein), SEQ ID NO:116 (Cj0113 protein),SEQ ID NO:118 (Cj0982c protein), SEQ ID NO:120 (Cj0921c protein), SEQ ID NO:122 (Cj1259 protein), SEQ ID NO:124 (Cj1339c protein), SEQ ID NO:126 (Cj0034c protein), SEQ ID NO:128 (Cj0404 protein), SEQ ID NO:130 (Cj0365c protein), SEQ ID NO:132 (Cj0755 protein), and SEQ ID NO:134 (Cj0420 protein).

21. The immunogenic composition of any one of claims 1-20, wherein the immunogenic composition is formulated for oral delivery or mucosal delivery.

22. The immunogenic composition of any one of claims 1-21, further comprising an adjuvant.

23. A method for stimulating an immune response against *Campylobacter*, comprising administering to a subject an effective amount of the immunogenic composition according to any one of claims 1-22 to generate an immune response against *Campylobacter*.

24. A method for reducing *Campylobacter* intestinal colonization in a subject, comprising administering an amount effective of the immunogenic composition according to any one of claims 1-22 to reduce *Campylobacter* intestinal colonization in the subject.

25. The method of claim 23 or 24, wherein the subject is selected from the group consisting of chickens, turkeys, birds, cattle, sheep, pigs, dogs, cats, and humans.

26. The method of claim 23 or 24, wherein the subject is selected from the group consisting of chickens and turkeys.

27. The method of claim 26, wherein the administering is initially carried out by 14 days after the chicken or turkey hatches.

28. The method of any one of claims 23-27, wherein the administering comprises oral or mucosal administration.

29. The method of claim 26 or 27, wherein the administering comprises oral administration in drinking water.

30. The method of claim 26 or 27, wherein the administering comprises administering the immunogenic composition in a spray on the subject's body.

31. The method of any one of claims 23-30, wherein the method is for inducing an immune response against, or reducing intestinal colonization of *Campylobacter jejuni, Campylobacter coli, Campylobacter lari*, and/or *Campylobacter upsaliensis*.

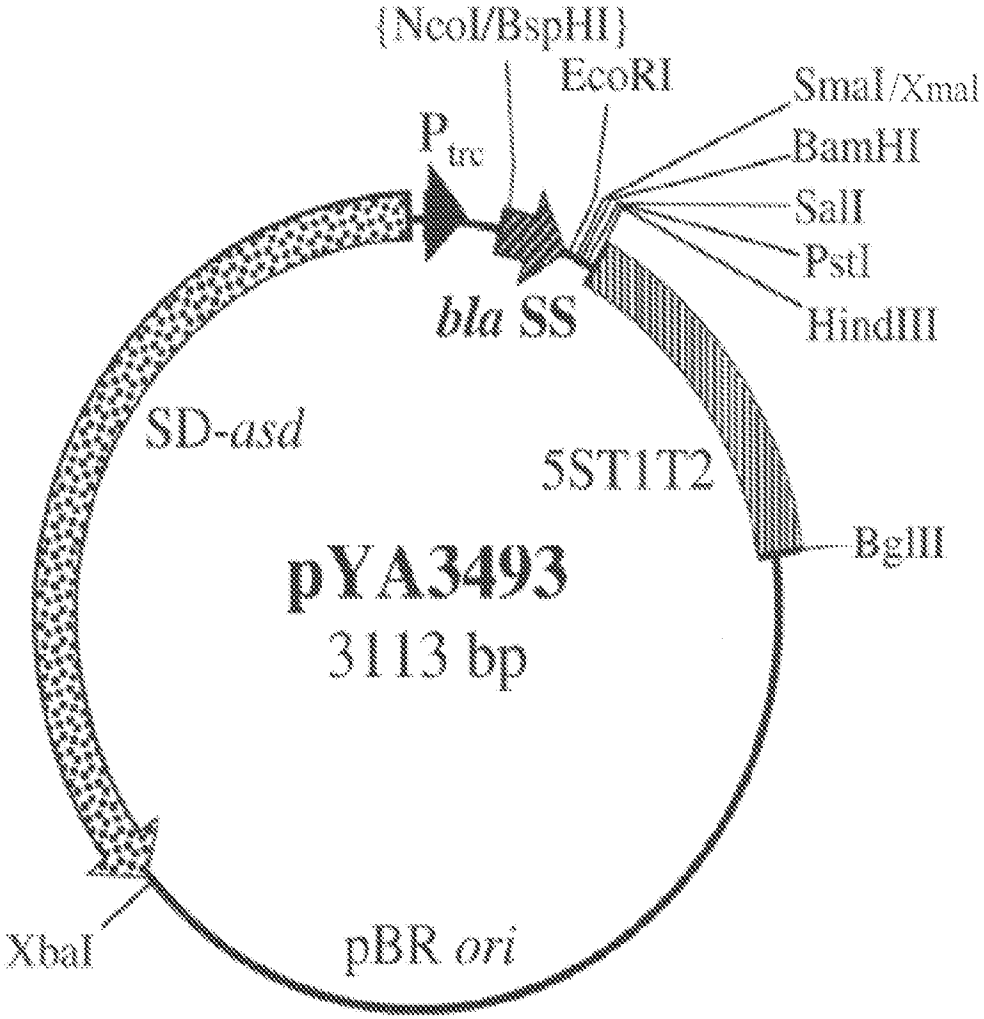
(NcoI/BspHI)
EcoRI
SmaI/XmaI
BamHI
SalI
PstI
HindIII
P_{trc}
bla SS
SD-*asd*
5ST1T2
BglII
pYA3493
3113 bp
XbaI
pBR *ori*

FIGURE 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/024332

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/02 A61P31/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/049641 A2 (ACE BIOSCIENCES AS [DK]; SCHROTZ-KING PETRA [DK]; SKAARUP CRAWFORD JAN) 2 June 2005 (2005-06-02) page 5, lines 5-29 page 9, line 30 - page 10, line 17 page 11, lines 6-48 page 13, lines 20-21 page 16, line 1 - page 17, line 3 page 21, lines 7-15 page 22, line 10 - page 23, line 11 page 42, line 10 - page 43, line 14 page 44, lines 1-21 page 61, line 32 - page 62, line 26 claims 1-3,9,12,36,37 page 17, line 28 - page 18, line 21 ----- -/--	1-31

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 April 2013	08/07/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Noë, Veerle

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/024332

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	NIELSEN LENE N ET AL: "Identification of immunogenic and virulence-associated Campylobacter jejuni proteins.", CLINICAL AND VACCINE IMMUNOLOGY : CVI FEB 2012, vol. 19, no. 2, February 2012 (2012-02), pages 113-119, XP002694959, ISSN: 1556-679X the whole document	1-31
A	US 2001/038844 A1 (NACHAMKIN IRVING [US]) 8 November 2001 (2001-11-08) paragraphs [0005], [0011]; examples 4,5	1-31
A	WO 2008/008092 A2 (UNIV ARIZONA [US]; JOENS LYNN A [US]; THEORET JAMES R [US]; REESER RYA) 17 January 2008 (2008-01-17) page 5, lines 1-29 page 11, line 25 - page 12, line 2	1-31

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/024332

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   [ ] on paper

   [X] in electronic form

   b. (time)

   [X] in the international application as filed

   [ ] together with the international application in electronic form

   [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/024332

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-31(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-31(partially)

Immunogenic composition comprising an expression vector comprising a polynucleotide encoding a protein comprising an amino acid sequence at least 80% identical to SEQ ID No:2 or an antigenic portion thereof; immunogenic composition comprising a polynucleotide encoding a protein comprising an amino acid sequence at least 80% identical to SEQ ID No:44-82 or an antigenic portion thereof; immunogenic composition comprising a protein comprising an amino acid sequence at least 80% identical to SEQ ID No:2 or an antigenic portion thereof; method for stimulating an immune response against Campylobacter or for reducing Campylobacter intestinal colonization by administering the immunogenic composition.

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2. claims: 1-31(partially)

Immunogenic composition comprising an expression vector comprising a polynucleotide encoding a protein comprising an amino acid sequence at least 80% identical to SEQ ID No:4 or an antigenic portion thereof; immunogenic composition comprising a polynucleotide encoding a protein comprising an amino acid sequence at least 80% identical to SEQ ID No:83-106 or an antigenic portion thereof; immunogenic composition comprising a protein comprising an amino acid sequence at least 80% identical to SEQ ID No:4 or an antigenic portion thereof; method for stimulating an immune response against Campylobacter or for reducing Campylobacter intestinal colonization by administering the immunogenic composition.

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3. claims: 1-31(partially)

Immunogenic composition comprising an expression vector comprising a polynucleotide encoding a protein comprising an amino acid sequence at least 80% identical to SEQ ID No:6 or an antigenic portion thereof; immunogenic composition comprising a polynucleotide encoding a protein comprising an amino acid sequence at least 80% identical to SEQ ID No:10-43 or an antigenic portion thereof; immunogenic composition comprising a protein comprising an amino acid sequence at least 80% identical to SEQ ID No:6 or an antigenic portion thereof; method for stimulating an immune response against Campylobacter or for reducing Campylobacter intestinal colonization by administering the immunogenic composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/024332

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2009285821 A1	19-11-2009
		WO 2008008092 A2	17-01-2008