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(54) **MODIFIED GENE VACCINES AGAINST AVIAN CORONAVIRUSES AND METHODS OF USING THE SAME**

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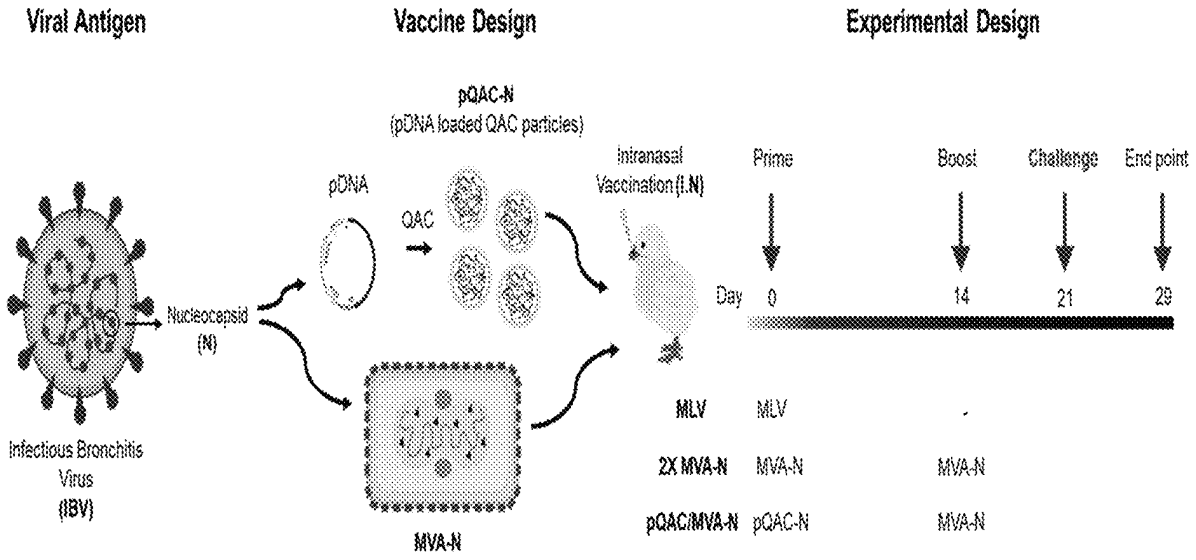
(57) **ABSTRACT**

The present invention provides both QuilA-loaded chitosan (QAC)-encapsulated NA vaccine compositions and viral vaccine compositions that encode an Infectious Bronchitis Virus (IBV) spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. Additionally, the present invention provides methods in which the disclosed vaccines are administered to a subject to induce an immune response against IBV or to vaccinate the subject against IBV.

Specification includes a Sequence Listing.



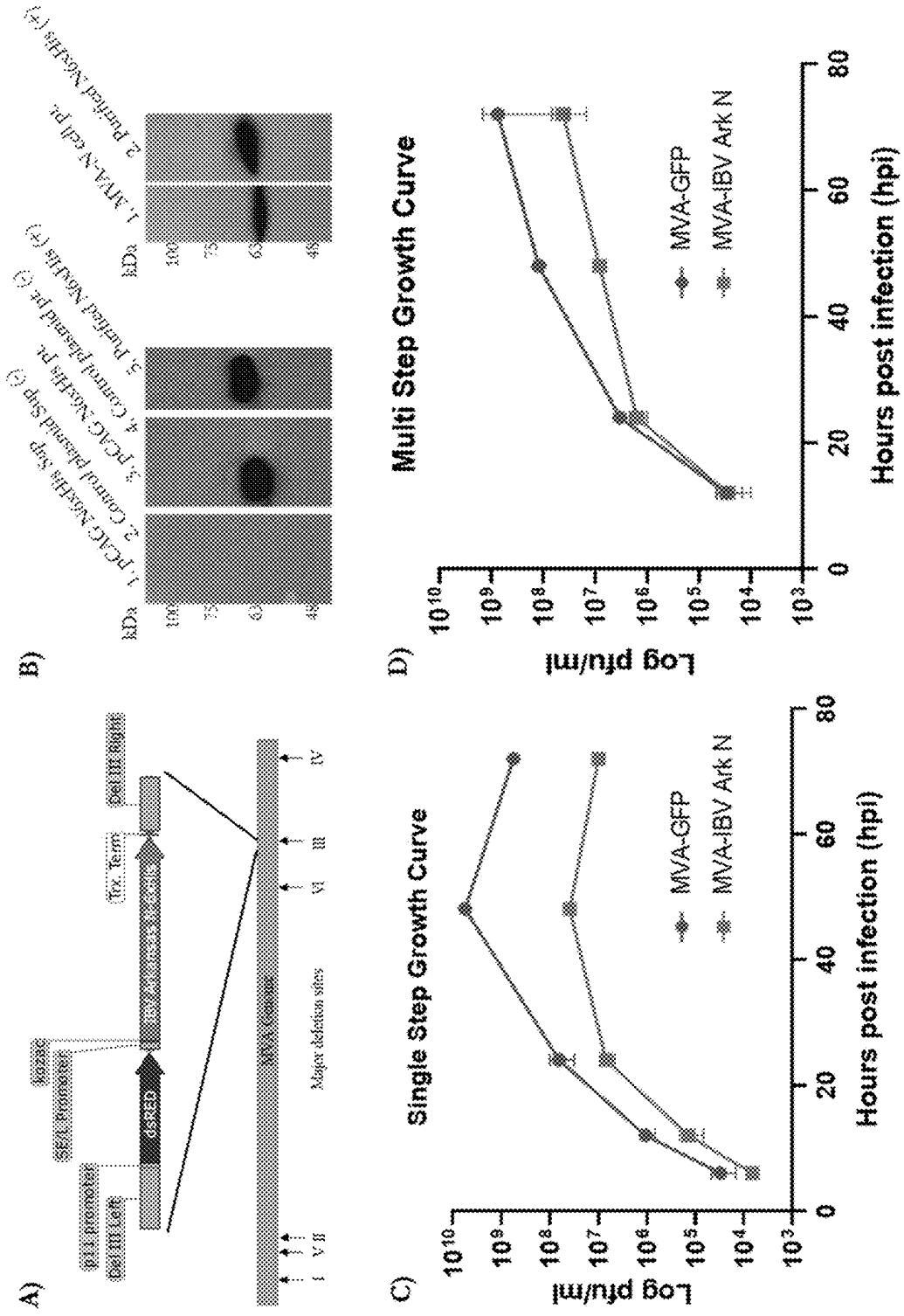

A)
Del III Left
P11 promoter
SE/L promoter
kozac
dsRED
Trx Term
Del III Right
Major deletion sites
I V II VI III IV
B)
kDa
100
75
63
48
1. rDCIG NotAfe Sup
2. Control plasmid Sup (-)
3. rDCIG NotAfe Pr.
4. Control plasmid Pr. (-)
1. MVA-N cell Pr.
2. Purified NotAfe (+)
C)
Single Step Growth Curve
Log pfu/ml
Hours post infection (hpi)
MVA-GFP
MVA-IBV Ark N
D)
Multi Step Growth Curve
Log pfu/ml
Hours post infection (hpi)
MVA-GFP
MVA-IBV Ark N

Fig. 1

Fig. 2

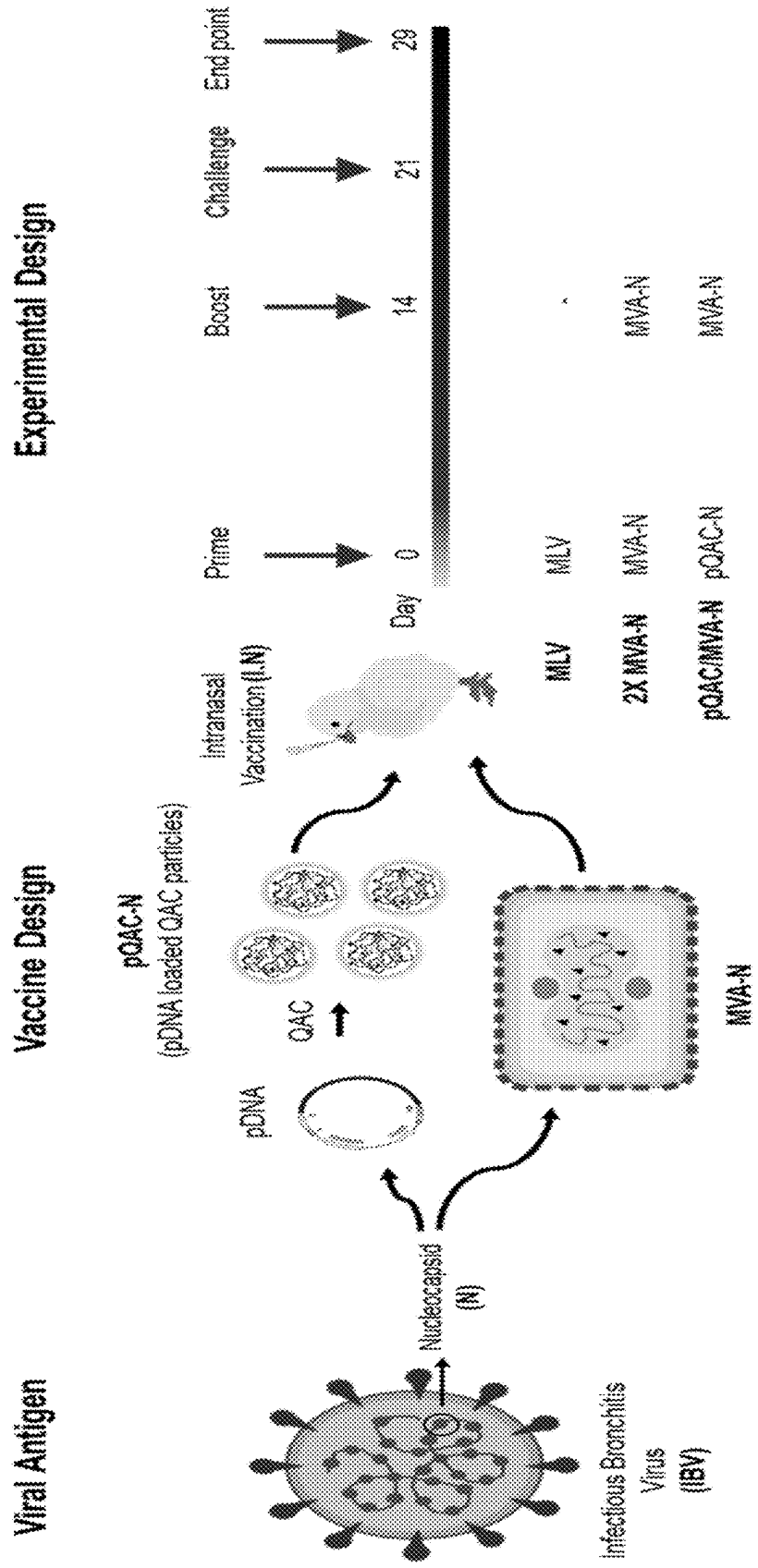
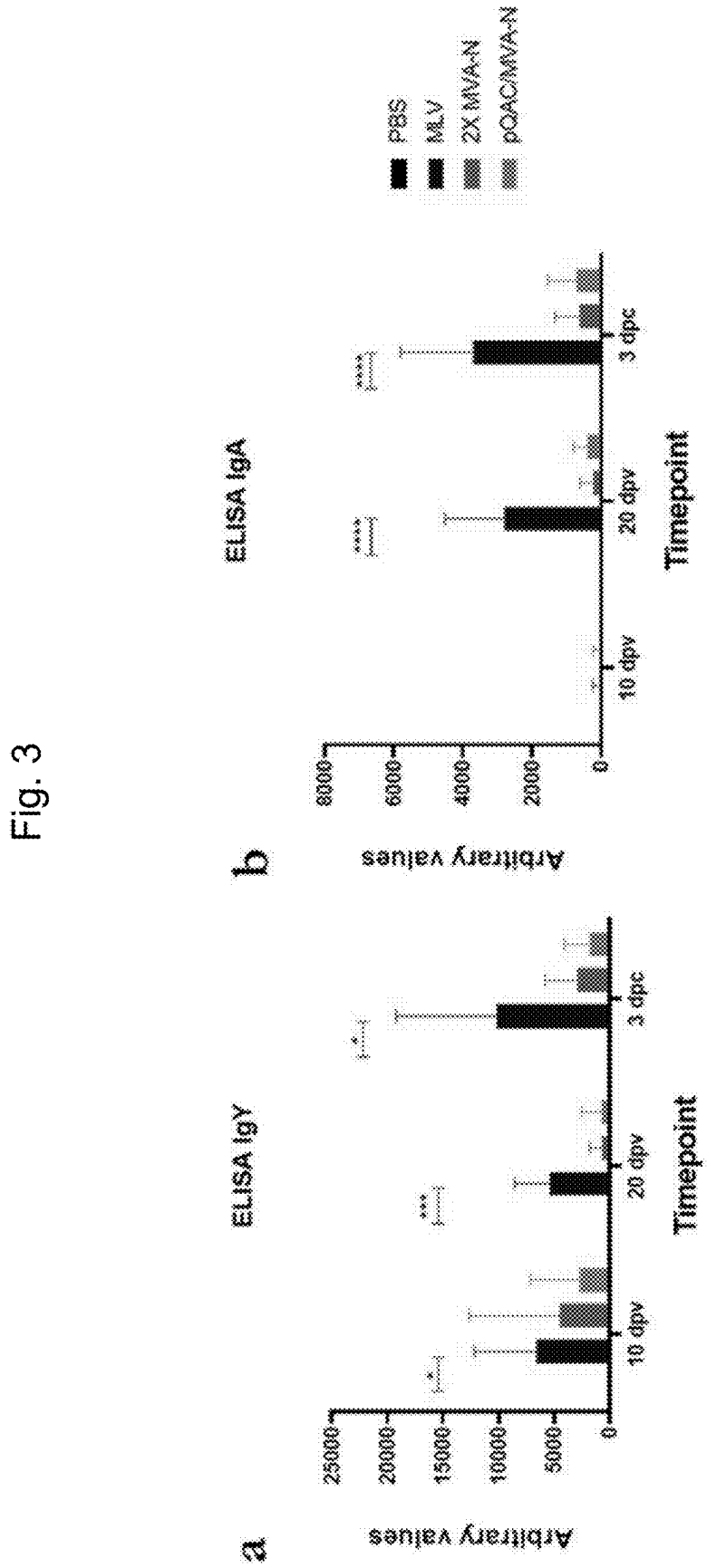

Viral Antigen
Infectious Bronchitis Virus (IBV)
Nucleocapsid (N)
Vaccine Design
pDNA
QAC
pQAC-N
(pDNA loaded QAC particles)
MVA-N
Intranasal Vaccination (I.N)
Experimental Design
Prime
Boost
Challenge
End point
Day 0
14
21
29
MLV
MLV
2X MVA-N
MVA-N
pQAC/MVA-N
pQAC-N
MVA-N

Fig. 3


a
ELISA IgY
Arbitrary values
25000
20000
15000
10000
5000
0
10 dpv
20 dpv
3 dpc
Timepoint
b
ELISA IgA
Arbitrary values
8000
6000
4000
2000
0
10 dpv
20 dpv
3 dpc
Timepoint
PBS
MLV
2X MVA-N
pOAC/MVA-N

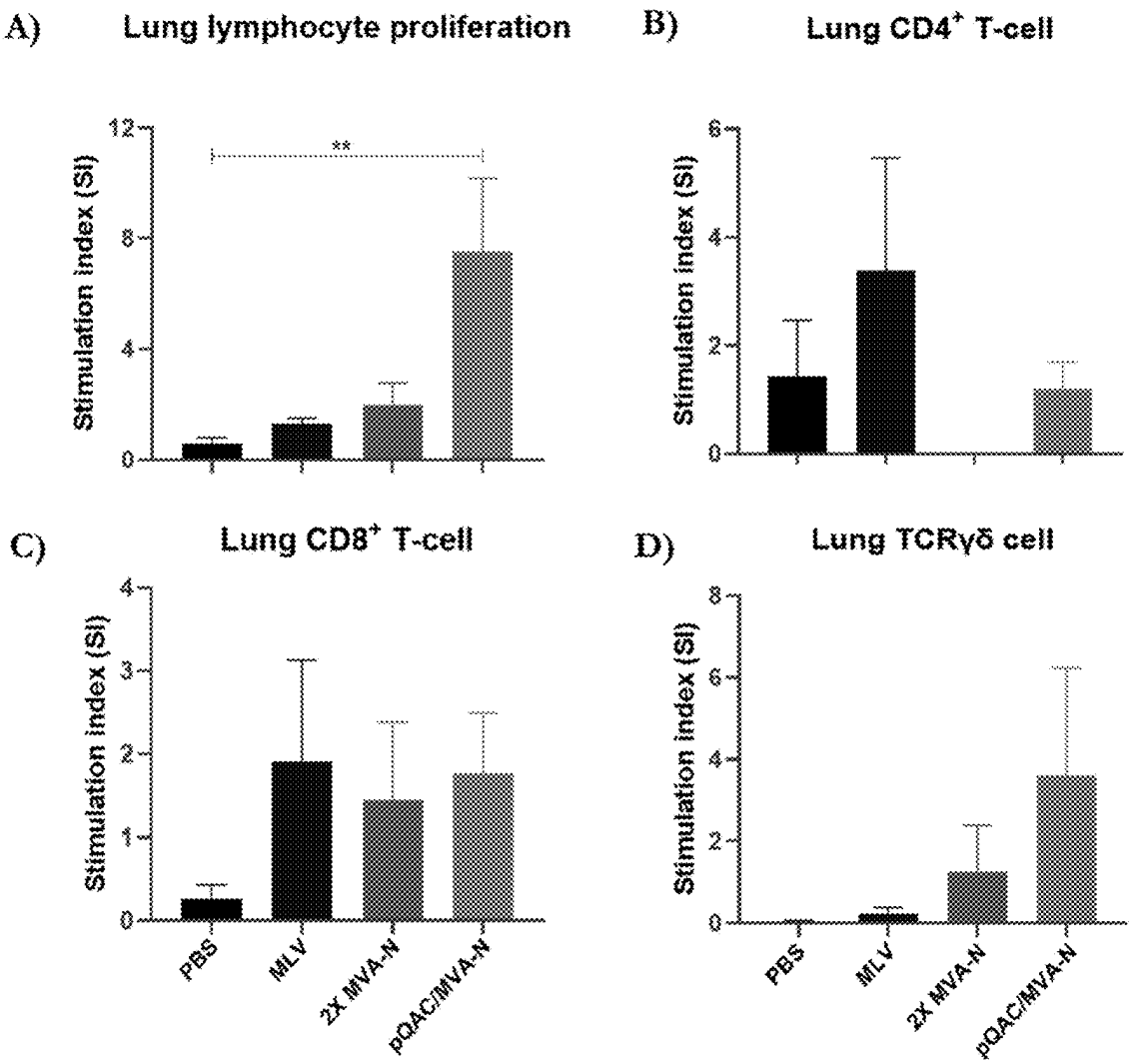
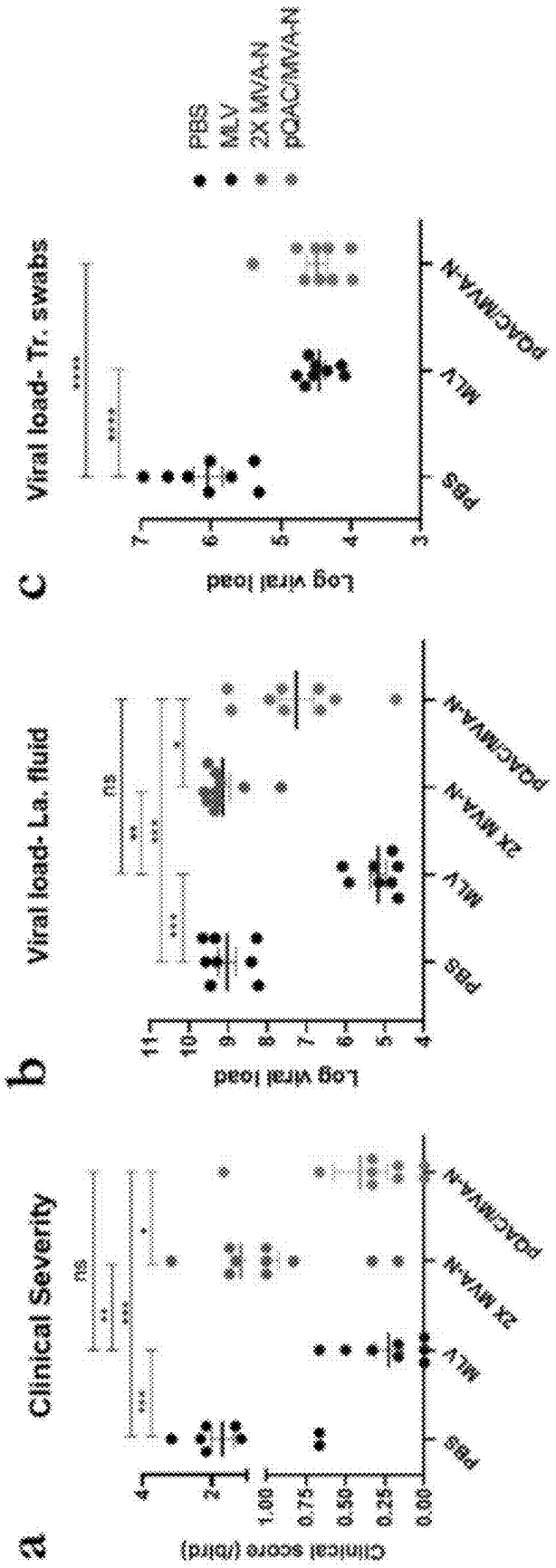

A)
Lung lymphocyte proliferation
Stimulation index (SI)
**
B)
Lung CD4+ T-cell
Stimulation index (SI)
C)
Lung CD8+ T-cell
Stimulation index (SI)
D)
Lung TCRγδ cell
Stimulation index (SI)
PBS
MLV
2X MVA-N
pQAC/MVA-N

Fig. 4

Fig. 5


a
Clinical Severity
Clinical score (bird)
b
Viral load- La. fluid
Log viral load
c
Viral load- Tr. swabs
Log viral load
PBS
MLV
2X MVA-N
pQAC/MVA-N

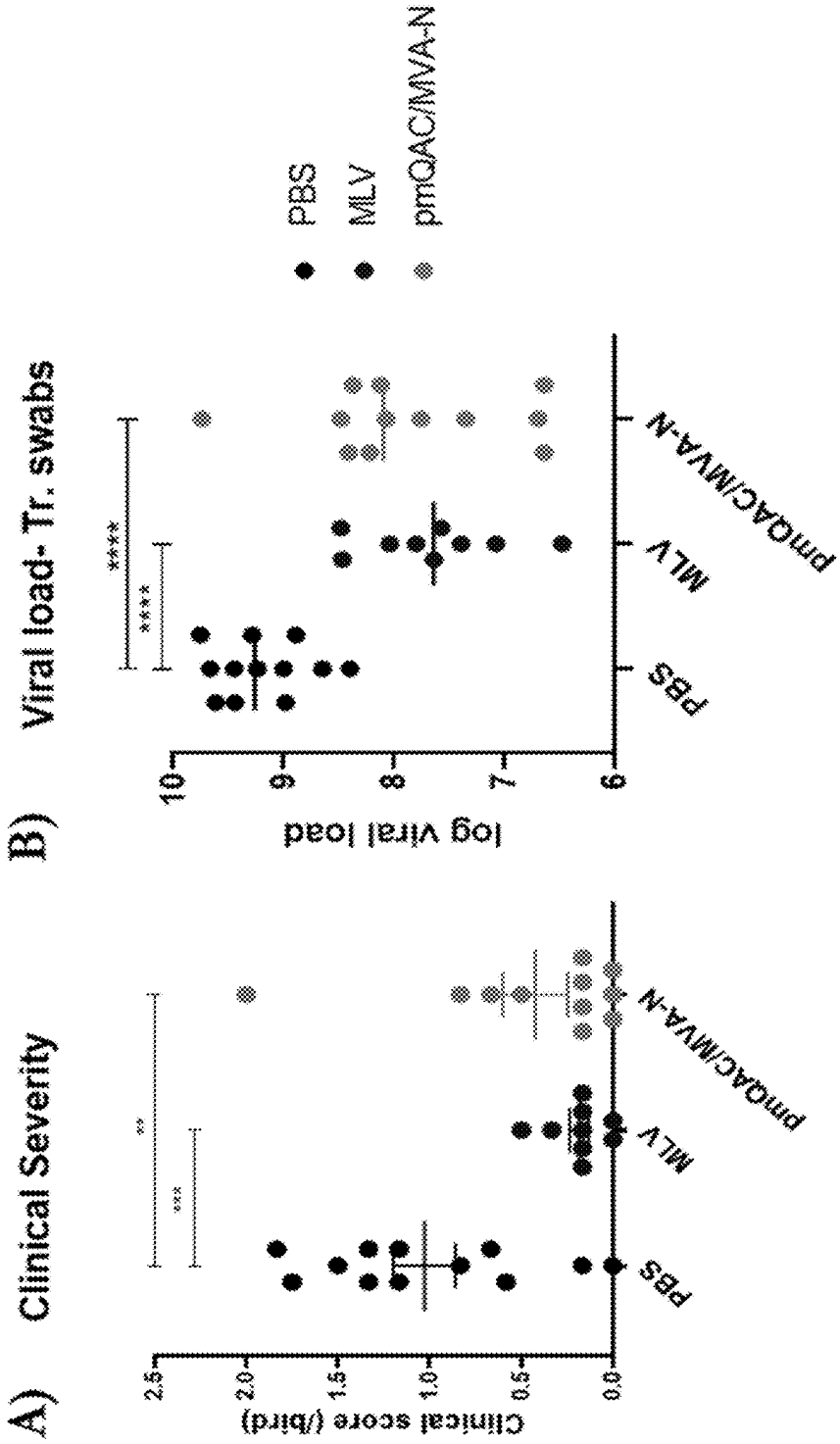

A)
Clinical Severity
Clinical score (/bird)
2.5
2.0
1.5
1.0
0.5
0.0
**
***
PBS
MLV
pmOAC/MVA-N
B)
Viral load- Tr. swabs
log viral load
10
9
8
7
6
****
****
PBS
MLV
pmOAC/MVA-N
PBS
MLV
pmOAC/MVA-N

Fig. 6

Fig. 7

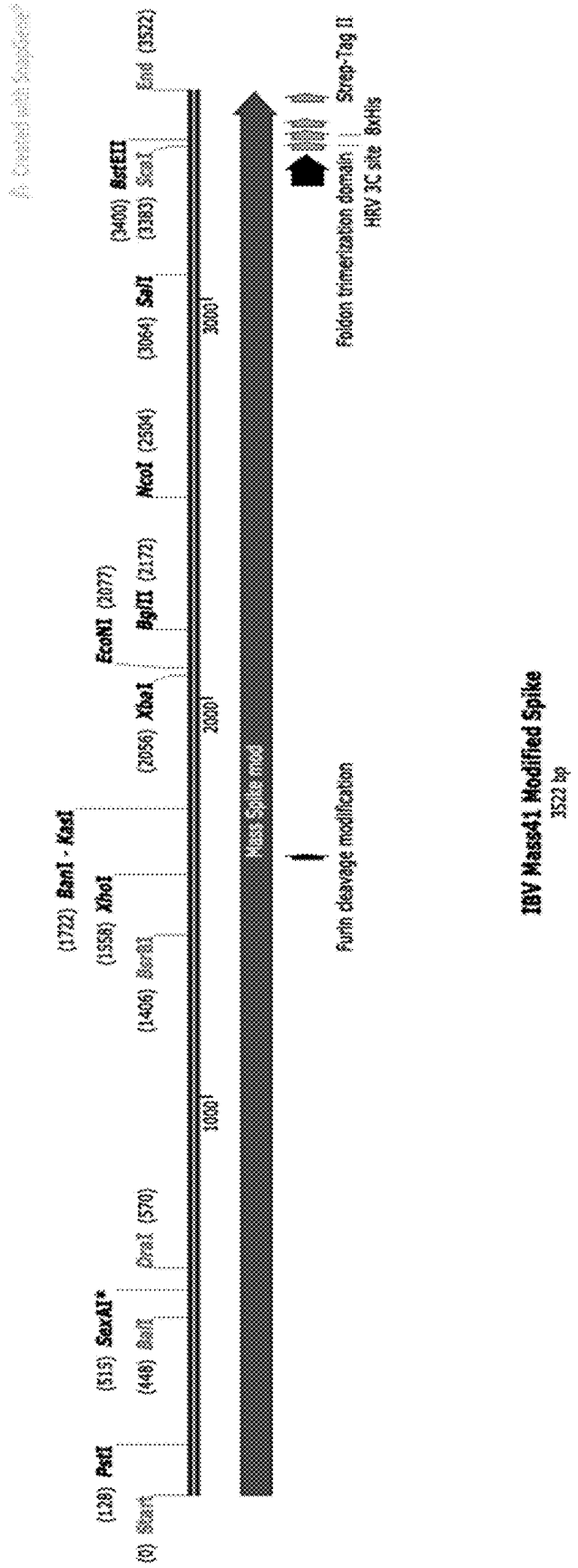
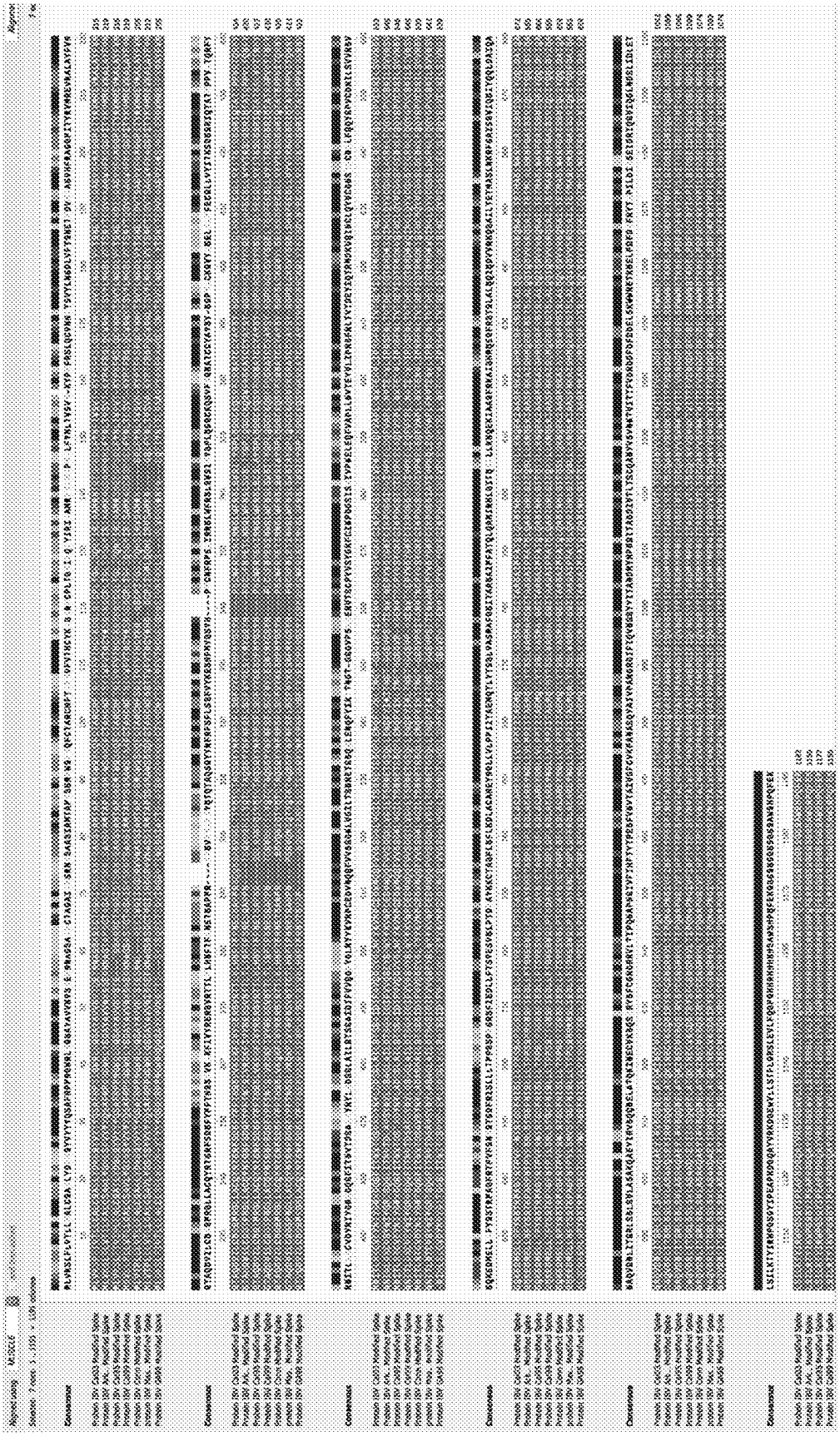

(0) Start
(128) PstI
(448) BamH
(515) SacII*
(570) ZraI
(1406) BsrBI
(1556) XhoI
(1722) BanI - KasI
(2056) XbaI
(2077) EcoNI
(2172) BglII
(2384) NcoI
(3064) SalI
(3400) BstEII
(3383) ScaI
(3522) End
1000
2000
3000
RBD S1/S2 mod
Furin cleavage modification
Foldon trimerization domain
HRV 3C site
8xHis
Strep-Tag II
IBV Mass41 Modified Spike
3522 bp

Fig. 8



MODIFIED GENE VACCINES AGAINST AVIAN CORONAVIRUSES AND METHODS OF USING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Patent Application No. 63/282,482 that was filed Nov. 23, 2021, the entire contents of which are hereby incorporated by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under 2016-67021-25042 and 2020-67021-31256 awarded by the USDA/NIFA. The government has certain rights in the invention.

SEQUENCE LISTING

[0003] This application is being filed electronically via Patent Center and includes an electronically submitted Sequence Listing in .xml format. The .xml file contains a sequence listing entitled "960296_04361" created on Nov. 23, 2022 and is 122,399 bytes in size. The Sequence Listing contained in this .xml file is part of the specification and is hereby incorporated by reference herein in its entirety.

BACKGROUND

[0004] Coronavirus infections, such as infection by infectious bronchitis virus (IBV) in poultry, cause significant health problems for avian subjects as well as economic losses to the poultry industry. A major hurdle to combat these infections is the diversity of viral antigens that can be present in a given outbreak. In addition, a critical failure in preparation for coronavirus infections in avian subjects is the absence of effective vaccines that can be delivered to thousands of animals at the same time. Consequently, there is a dire need for an objective vaccination method that effectively, yet parsimoniously, encompasses existing and emerging isolates of coronavirus, e.g., IBV, to protect against coronavirus infection in avian subjects.

SUMMARY

[0005] In a first aspect of the current disclosure, vaccine compositions are provided. In some embodiments, the vaccine compositions comprise a polynucleotide that encodes an infectious bronchitis virus (IBV) spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. The polynucleotide may be a DNA or RNA and maybe codon optimized for expression in the subject targeted for vaccination. The compositions may further comprise an adjuvant and the adjuvant may include disaggregated spherical nanostructures comprising Quil-A and chitosan.

[0006] In another aspect of the current disclosure, vaccine compositions comprising a viral vector are provided. In some embodiments, the viral vector comprises a polynucleotide encoding an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein.

[0007] In still another aspect, a vaccine composition comprising an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. The S and N proteins may include one or more of SEQ ID NOs: 11-17, 21, 23, 25, 27, 29, 31, 33, 10, 18, 35, and 37. The vaccine compositions may further comprise an adjuvant such as the Quil-A-chitosan adjuvant.

[0008] In another aspect of the current disclosure, methods of inducing an immune response against infectious bronchitis virus (IBV) in a subject are provided. In some embodiments, the method comprises: administering the vaccine compositions of current disclosure in an amount effective to induce the immune response against at least one IBV antigen in the subject.

[0009] In another aspect of the current disclosure, methods of inducing an immune response against infectious bronchitis virus (IBV) in a subject are provided. In some embodiments, the method comprises: administering a first vaccine composition comprising a polynucleotide that encodes an infectious bronchitis virus (IBV) spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein and a viral vector comprising a polynucleotide encoding an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein, wherein administration of the first vaccine composition and the second vaccine composition induces the immune response against at least one IBV antigen in the subject. The first and second vaccine compositions may be administered at separate times with at least two weeks separating the two administrations. In one embodiment the first vaccine composition comprising a polynucleotide is administered prior to the second vaccine composition comprising a viral vector expressing a polypeptide encoded by the polynucleotide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. **1**. Design and characterization of MVA-IBV vaccine constructs. a) MVA vaccine construct expressing N protein with the addition of C-terminal 6×His tag. Gene map was generated using Snapgene software. b) Western blot analysis with anti 6×His-HRP antibody for pCAG-N plasmid (left) and MVA-N (right) confirming expression of N protein from vaccine constructs. Lanes are as follows: Left, supernatant (lane 2) CEF cells transfected with control plasmid, supernatant (lane 1) CEF cells transfected with pCAG-N plasmid. Cell pellet (lane 4) CEF cells transfected with control plasmid, cell pellet (lane 3) CEF cells transfected with pCAG-N plasmid and control purified N6×His protein (lane 5). Right, cell pellet (lane 1) from CEF cells infected with MVA-TrN and control purified N6×His protein (lane 2). Cell pellet (lane 2) from CEF cells infected with MVA-N. c) Single step and d) Multi step growth curve of parental MVA-GFP and recombinant MVA-N vaccine vectors.

[0011] FIG. **2**. Vaccine experimental design. Experimental design of IBV immunization and challenge studies. Outline for vaccine construct and immunization protocol using groups of white leghorn SPF birds vaccinated with 2 doses of MVA-N (IN) or pQAC-CoV (I.N) at day-0 followed by boost with MVA-CoV (IN) day-14. Control groups include unvaccinated PBS group and commercial MLV vaccination at day-0 (IN).

[0012] FIG. **3**. Humoral responses in vaccinated SPF chicks. IBV specific a) IgY in serum and b) IgA in lachrymal

fluid, significance (*, P<0.05; ***, P<0.001; ****, P<0.0001) was determined by two-way ANOVA. Data show means±SEM.

[0013] FIG. **4**. Localized T-cell immune responses in vaccinated chicks. Lung cell proliferative capacity measured by CellTrace Violet dye dilution in unvaccinated, MLV, 2×MVA-N and pQAC/MVA-N vaccinated chickens. Proliferation was measured in a) total lung cells, (b) CD4+, (c) CD8+ and (d) TCRγδ+ lung T cells after 4 days in culture post antigen stimulation. Non-significance, ns or significance (*, P<0.05; **, P<0.01) was determined by one-way ANOVA with multiple comparisons. Data show means±SEM.

[0014] FIG. **5**. Increased protection with heterologous vaccine strategy against IBV. a) Clinical sign severity represented as average score/bird over 8 days post challenge in each group. b) IBV log viral load/10 ul lachrymal fluid at 6 days post challenge. c) IBV log viral load in tracheal swab at 6 days post challenge. Non-significance, ns or significance (***, P<0.001; ****, P<0.0001) was determined by one-way ANOVA with multiple comparisons. Data show means±SEM.

[0015] FIG. **6**. Protective efficacy of the MPLA-QAC triple adjuvant system. a) Clinical sign severity represented as average score/bird over 8 days post challenge in each group. b) IBV log viral load in tracheal swab at 6 days post challenge Significance (***, P<0.001; ****, P<0.0001) was determined by one-way ANOVA with multiple comparisons. Data show means±SEM.

[0016] FIG. **7**. Shows a map of the Mass41 S antigen with the modified 7 features (codon optimization is not shown with an arrow). All of the other sequences below have the same features.

[0017] FIG. **8**. Alignment of 7 IBV S protein amino acid sequences. Sequences correspond to, from top to bottom, SEQ ID NOs: 39-45.

DETAILED DESCRIPTION

[0018] The present invention provides nucleic acid-based vaccine compositions (DNA vaccines), protein subunit based vaccines and viral vaccine compositions encoding an infectious bronchitis virus (IBV) spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. Further, the present invention provides methods in which the disclosed vaccines are administered to a subject to induce an immune response directed against IBV.

[0019] Compositions:

[0020] In a first aspect, the present invention provides vaccine compositions. In some embodiments, the vaccine composition comprises a polynucleotide that encodes an infectious bronchitis virus (IBV) spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. Alternatively, the compositions may comprise a viral vector encoding an infectious bronchitis virus (IBV) spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. AS another alternative, protein subunit vaccine compositions comprising an infectious bronchitis virus (IBV) spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein are also provided. The nucleic acids encoding the proteins may be RNA or DNA and may be codon optimized for expression in the subject targeted for vaccination. The S and N proteins and nucleic acids encoding the same may be modified to allow for increased inducement of the immune response after administration.

[0021] As used herein, the terms "DNA vaccine," "nucleic acid vaccine," "NA vaccine" and "plasmid vaccine" are used interchangeably to refer to a polynucleotide encoding at least one antigen. Following immunization, a subject's cells take up the polynucleotide and express the encoded antigen from it, inducing an immune response against the antigen. NA vaccines offers several potential advantages over traditional vaccine strategies, including the stimulation of both B- and T-cell responses, improved storage stability, the absence of any infectious agent, and the relative ease of large-scale manufacture. However, NA vaccines also come with several challenges, including in vivo degradation of the construct by DNases or RNases, inefficient uptake by antigen presenting cells, and low immunogenicity. See, for example, P. Cai, X. Zhang, M. Wang, Y. L. Wu, X. Chen, Combinatorial Nano-Bio Interfaces. ACS Nano 12, 5078-5084 (2018); and D. H. a. M. Bros, DNA Vaccines—How Far From Clinical Use? Int J Mol Sci. 19, (2018), both of which are incorporated by reference herein. Nucleic acid-based vaccines generally contain additional elements in addition to the polynucleotide encoding the antigen such as a promoter functional in cells of the subject to be immunized or may be altered to offer increased stability or resistance to degradation in the host cell.

[0022] As used herein, "antigen" refers to a substance that induces a targeted immune response in a subject. For example, in some embodiments, the compositions disclosed herein comprise one or more polynucleotides that encode an infectious bronchitis virus (IBV) spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. Therefore, in the foregoing example, the antigens are the IBV S and N proteins that are encoded by the one or more polynucleotides. In some embodiments, the S proteins are encoded by one or more of the group consisting of SEQ ID NOs: 1-7, 22, 24, 26, 28, 30, 32, and 34. The S proteins encoded by these polynucleotides are provided as SEQ ID NOs: 11-17, 21, 23, 25, 27, 29, 31, and 33, and any polynucleotide encoding SEQ ID NO: 11-17, 21, 23, 25, 27, 29, 31, and 33, is included, as the coding sequence for the proteins may be optimized for expression in particular cell types. In some embodiments, the N proteins are encoded by one or more of SEQ ID NOs: 8, 9, 36 and 38. The N proteins encoded by these polynucleotides are provided as SEQ ID NOs: 10, 18, 35, and 37, respectively, and any polynucleotide encoding SEQ ID NO: 10, 18, 35, or 37 is also encompassed herein. The polynucleotides provided herein may be altered to optimize codon usage for maximal expression in a particular host such as a poultry. Thus, the sequences provided herein also include sequences with 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the sequences of SEQ ID NO: 1-9, 22, 24, 26, 28, 30, 32, 34, 36, and 38. The proteins encoded by the polynucleotides may also encompass changes especially as these proteins are known to exist in various isoforms and be antigenically diverse in outbreaks of IBV. The sequences provided herein also include sequences with 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequences of SEQ ID NO: 10-18, 21, 23, 25, 27, 29, 31, 33, 35 or 37. In some embodiments, the polynucleotide encodes both the S and N proteins on a single molecule. As such, in some embodiments, the polynucleotide comprises sequences linking the S and N pro-

teins. The N and S sequences may be linked via a polynucleotide of any length but should be in frame or contain independent regulatory regions such as an internal ribosome entry site to allow for expression of both proteins from the polynucleotide.

[0023] As used herein, a "fragment" is a portion of an amino acid sequence which is identical in sequence to, but shorter in length than a reference sequence. A fragment may comprise up to the entire length of the reference sequence, minus at least one amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous amino acid residues of a reference polypeptide, respectively. In some embodiments, a fragment may comprise at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 250, or 500 contiguous amino acid residues of a reference polypeptide. Fragments may be preferentially selected from certain regions of a molecule. A fragment may include an N-terminal truncation, a C-terminal truncation, or both N-terminal and C-terminal truncations relative to the full-length reference polypeptide.

[0024] The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, expression cassette, or vector, indicates that the cell, nucleic acid, protein, expression cassette, or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, have higher than normal expression, are under-expressed, or not expressed at all.

[0025] The polynucleotide vaccine compositions provided herein may be DNA or RNA and may include regulatory regions to allow for transcription and/or translation of the polynucleotides into polypeptides once in a cell of a vaccinated subject. The polynucleotides may be operably linked to promoters that are capable of recruiting transcriptional machinery in target cells of the vaccinated subject, e.g., cells of the upper respiratory tract, or, in some embodiments, any somatic cell of the subject.

[0026] However, as discussed above, NA vaccines can suffer from several drawbacks including in vivo degradation of the construct by DNases or RNases, inefficient uptake by antigen presenting cells, and low immunogenicity. In some embodiments, the vaccine composition further comprises an adjuvant. In some embodiments, the adjuvant comprises disaggregated spherical nanostructures comprising Quil-A and chitosan, which are present at a ratio between 1:15 and 1:100. As used herein, the term "adjuvant" or "vaccine adjuvant" refers to any substance that enhances the immune response to an antigen. The inventors envision that the use of articulate delivery systems, such as QuilA-loaded Chitosan (QAC) nanoparticles used with the present invention, may overcome these challenges by facilitating a prolonged release of active plasmid. See, for example, S. S. Chandrasekar, B. A. Kingstad-Bakke, C. W. Wu, M. Suresh, A. M. Talaat, A Novel Mucosal Adjuvant System for the Immunization Against Avian Coronavirus Causing Infectious Bronchitis. J Virol, (2020), which is incorporated by reference herein. An exemplary adjuvant used with the vaccine compositions disclosed herein is a Quil-A chitosan (QAC) complex, in which Quil-A and chitosan are combined to form distinct disaggregated spherical nanostructures. The QAC complexes are loaded with one or more payload molecules (in this case, the antigen-encoding polynucleotide) with which the QAC complex stimulates an immune response. The QAC complex adjuvant was previously described in International Application No. PCT/US2020/037438, which is incorporated by reference, and Chandrasekar et al. 2020, supra. Advantageously, QAC-adjuvanted vaccines appear to target local mucosal immunity, which results in a more effective immune response to IBV given that airway epithelium T cells and IgA humoral responses have been shown to be critical for restricting respiratory viral pathogens. See, for example, N. v. D. Emmie de Wit, Darryl Falzarano and Vincent J. Munster, SARS and MERS: recent insights into emerging coronaviruses. Nature Reviews Microbiology 14, (2016), which is incorporated by reference herein.

[0027] "Quil-A" refers to the powdered saponin fraction isolated from extract of the bark of *Quillaja saponaria* trees. Quil-A is commercially available, for example from Desert King sold under the product name Vet-Sap™.

[0028] "Chitosan" refers to a linear polysaccharide composed of randomly distributed β-linked D-glucosamine and N-acetyl-D-glucosamine. Chitosan can be obtained from the chitin shells of shrimp and other crustaceans by treatment of the shells with an alkaline substance. Chitosan is a nontoxic, naturally occurring cationic polymer that readily complexes with DNA and negatively charged proteins that is biocompatible and biodegradable. Compositions incorporating chitosan have sustained release kinetics and are immunomodulatory, enhancing the T-cell response. In some embodiments, chitosan is deacetylated chitosan, for example deacetylated chitosan (>75%). Deacetylated chitosan is available commercially from Sigma (C3646). Higher deacetylation percentages, for example about 90%, will meditate stronger binding with nucleic acids resulting in slower release kinetics from the nanoparticle structures of the QAC complex. In some embodiments, the chitosan is at least 70%, 75%, 80%, 85%, 90%, or 95% deacetylated. In some embodiments, the chitosan is between about 60% and about 90% deacetylated.

[0029] In some embodiments, the chitosan is functionalized. Chitosan may be functionalized with negatively charged sulfonate groups by reaction of the amino group of chitosan with 5-formyl-2-furan sulfonic acid (FFSA) followed by treatment using sodium borohydride to form a negatively charged chitosan surface. Use of the negatively charged chitosan in the formation of the QAC complex will generally be favorable for loading of positively charged payload molecules.

[0030] The QAC complex is loaded with the antigen-encoding polynucleotide by mixing a solution of Quil-A and polynucleotide into a solution of chitosan to form a final mixed solution containing a QAC-polynucleotide complex. In the final mixed solution, the Quil-A and the chitosan are present at a ratio of between 1:15 to 1:100. In some embodiments, the Quil-A and the chitosan are present at a ratio of about 1:20 (e.g., 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:21, 1:22, 1:23, 1:24, or 1:25) in the final mixed solution. In some embodiments, in the final solution Quil-A is at a concentration of 0.001% and chitosan is at a concentration between about 0.02% and about 0.1%.

[0031] In some embodiments, the QAC complex nanostructures are less 100 nm in diameter when measured in the absence of any payload molecules. For example, the nano-

structures may be between about 5 nm and about 100 nm, between about 10 nm and about 95 nm, between about 15 nm an about 90 nm, between about 20 nm and about 90 nm, or between about 25 nm and about 85 nm in the absence of a payload molecule. The QAC complex may be loaded with one or more payload molecules such as the polynucleotides described herein encoding an IBV spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. The nucleotide-QAC complex may be between about 20 nm and about 1000 nm in diameter. The specific size of the nucleotide-QAC complex will vary depending on the size and amount of payload in the nanostructure. As used herein, "disaggregated," refers to the formation of discrete observable particles as opposed to aggregated non-discrete assemblies with non-distinct boundaries and "spherical" means roughly spherical in nature and is not meant to be a precise definition of the structure.

[0032] Though the QAC adjuvant strategy significantly improves the immunogenicity and protective immune response generated by the NA vaccine compositions of the current disclosure, the inventors hypothesized that a heterologous vaccine approach may further increase the effectiveness of the compositions. As used herein, "heterologous vaccine approach" refers to practice of inducing a first immune response with a first vaccine composition, then inducing a second immune response with a second different vaccine composition. Accordingly, a "heterologous vaccine" may also refer to the "second different vaccine composition" in the preceding example.

[0033] Therefore, in a second aspect, vaccine compositions comprising a viral vector are provided. In some embodiments, the viral vector comprises a polynucleotide encoding an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein.

[0034] As used herein, a "viral vector" refers to a virus or viral particle that comprises a polynucleotide encoding at least one antigen. The viral vector delivers the polynucleotide into a subject's cells. Within the cell, the polynucleotide is transcribed and translated, producing the encoded antigen. Depending on the cell that is expressing the viral antigen, the antigen may be presented on major histocompatibility complex I or II (MHC-I or MHC-II). Thus, the adaptive immune system, e.g., T and B cells, may recognize the antigen and become activated. The viral vectors may be used to induce an immune response to the S or N protein of IBV. The viral vectors of the present invention are "recombinant viruses," in which foreign genetic material encoding an antigenic protein (i.e., from infectious bronchitis virus) has been inserted into the viral genome.

[0035] The viral vectors may be a weakened or killed version of a virus. For example, the viral vector can be based on an attenuated virus, which does not replicate or exhibits very little replication in a host but is able to introduce and express a foreign gene in infected cells. As used herein, an "attenuated virus" is a strain of a virus whose pathogenicity has been reduced compared to its natural counterpart. A virus may be attenuated using serial passaging, plaque purification, or other means. The viruses used herein may be viral like particles (VLP) that are not capable of replication in the subject but do carry the antigenic proteins.

[0036] In some embodiments, the viral vector is selected from an adeno-associated virus or a poxvirus. Suitable poxviruses for use with the present invention include, without limitation, canary poxvirus, raccoon poxvirus, vaccinia virus, fowl poxvirus, turkey herpes virus (HVT), and myxoma virus (MYXV). Poxviruses are advantageous for transferring genetic material into new hosts due to their relatively large genome size (approximately 150-200 kb) and because of their ability to replicate in the infected cell's cytoplasm rather than the nucleus, thereby minimizing the risk of integrating genetic material into the genome of the host cell. Of the poxviruses, the vaccinia and variola species are the two that are most studied. Vaccinia virus is highly immunogenic, provoking strong B-cell (humoral) and T-cell mediated (cellular) immune responses against its encoded gene products. Of these viruses, the modified vaccinia virus Ankara (MVA) is particularly safe, as it has diminished virulence while maintaining good immunogenicity. Thus, in some embodiments, the viral vector is a modified vaccinia Ankara (MVA) virus. Exemplary MVA virus strains include MVA 572, MVA 575, and MVA-BN, which have been deposited at the European Collection of Animal Cell Cultures (ECACC), Salisbury (UK) with the deposition numbers ECACC V94012707, ECACC V00120707 and ECACC V00083008, respectively, and are described in U.S. Pat. Nos. 7,094,412 and 7,189,536, incorporated herein by reference in their entireties.

[0037] In yet another embodiment, a vaccine composition including IBV spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein is provided. These proteins may be modified from those found natively in the IBV virus such that the protein subunit vaccine composition comprising these proteins induces an immune response in a subject after administration of the vaccine composition.

[0038] Both the NA vaccine compositions and the viral vaccine compositions of the present invention comprise a polynucleotide encoding an IBV spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. The protein subunit vaccine compositions provided herein comprise the IBV S protein, the N protein or a combination thereof. The vaccine compositions may also include more than one of the S proteins or N proteins or nucleic acids encoding more than one S protein or more than one N protein provided herein. Such vaccine compositions would be considered as multivalent vaccine compositions and any combination of S and N protein may be combined and the combination may vary depending on the circulating IBV virus in a particular area or at a particular point in time.

[0039] IBV S protein is the major antigen against which neutralizing and protective antibodies are produced. The S protein is partially or completely cleaved into the amino-terminal 51 and into the carboxy-terminal S2 subunits post translationally by a host furin-like protease. Furthermore, the 51 subunit is highly variable among different isolates of IBV and is responsible for viral attachment to host cell and contains major neutralizing epitopes. In some embodiments, the compositions of the current disclosure comprise polynucleotides encoding the S protein selected from the group consisting of SEQ ID NOs: 1-7, 22, 24, 26, 28, 30, 32, and 34 (DNA) or SEQ ID NOs: 11-17, 21, 23, 25, 27, 29, 31, and 33 (amino acid), sequences with 90% or more identity to SEQ ID NO: 1-7, 22, 24, 26, 28, 30, 32, and 34 or SEQ ID NOs: 11-17, 21, 23, 25, 27, 29, 31, and 33 or fragments or portions thereof. The S2 subunit is highly conserved among IBV strains and contributes to viral fusion activity and elicits some minor but broadly reactive neutralizing antibodies.

See, for example, Shirvani et al., "A Recombinant Newcastle Disease Virus (NDV) Expressing S Protein of Infectious Bronchitis Virus (IBV) Protects Chickens against IBV and NDV", Scientific Reports volume 8, Article number: 11951 (2018), incorporated by reference herein in its entirety.

[0040] IBV N protein is associated with the RNA genome and forms the ribonucleoprotein. In some embodiments of the disclosed compositions, the N protein is encoded by a sequence selected from the group consisting of SEQ ID NOs: 8, 9, 36, and 38 (DNA) or SEQ ID NOs: 10, 18, 35, and 37 (amino acid), sequences with 90% or more identity to SEQ ID NO: 8-9, 36, and 38, SEQ ID NO: 10, 18, 35, and 37 or fragments or portions thereof.

[0041] The compositions of the current disclosure are administered, in some embodiments, intranasally, intramuscularly, or are administered in ovo. In some embodiments, the compositions are administered to greater than one subject at a time through means known in the art, for example, through mass intranasal administration of a group of animals. In some embodiments, the compositions of the current disclosure are administered by aerosol delivery to a flock of birds, for example, chickens.

[0042] The vaccine compositions of the present invention may be used as a prophylactic, e.g., to prevent or ameliorate the effects of a future infection by IBV, or may be used as a therapeutic, e.g., to treat IBV. The vaccines provided herein are expected to induce and enhance the immune response of the subject to IBV. The immune response enhanced is suitably a polyfunctional response. As used herein, a "polyfunctional response" refers to an immune response comprising both B and T cells directed to the pathogen.

[0043] The vaccine compositions may further comprise other suitable agents or ingredients. Suitable agents may include a suitable carrier or vehicle for delivery. As used herein, the term "carrier" refers to a pharmaceutically acceptable solid or liquid filler, diluent or encapsulating material. A water-containing liquid carrier can contain pharmaceutically acceptable additives such as acidifying agents, alkalizing agents, antimicrobial preservatives, antioxidants, buffering agents, chelating agents, complexing agents, solubilizing agents, humectants, solvents, suspending and/or viscosity-increasing agents, tonicity agents, wetting agents or other biocompatible materials. A tabulation of ingredients listed by the above categories may be found in the U.S. Pharmacopeia National Formulary, 1857-1859, (1990).

[0044] The vaccine formulation may be separated into vials or other suitable containers. The vaccine formulation herein described may then be packaged in individual or multi-dose ampoules or be subsequently lyophilized (freeze-dried) before packaging in individual or multi-dose ampoules. The vaccine formulation herein contemplated also includes the lyophilized version. The lyophilized vaccine formulation may be stored for extended periods of time without loss of viability at ambient temperatures. The lyophilized vaccine may be reconstituted by the end user and administered to a patient.

[0045] Methods:

[0046] In another aspect of the current disclosure, methods of inducing an immune response against infectious bronchitis virus (IBV) in a subject are provided. In some embodiments, the method comprises: administering a first vaccine composition and administering a second vaccine composition wherein administration of the first vaccine composition and the second vaccine composition induces the immune response against at least one IBV antigen in the subject. In some embodiments, a first vaccine compositions comprises a polynucleotide that encodes an infectious bronchitis virus (IBV) spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. In some embodiments, the vaccine composition further comprises an adjuvant. In some embodiments, the adjuvant comprises disaggregated spherical nanostructures comprising Quil-A and chitosan, and wherein the Quil-A and chitosan are present at a ratio between 1:15 and 1:100. In some embodiments, the chitosan is functionalized by treatment with 5-formyl-2-furan sulfonic acid and sodium borohydride, such that the chitosan surface is negatively charged. In some embodiments, the vaccine composition comprises spherical nanostructures between about 5 nm and about 100 nm in diameter in the absence of a payload molecule.

[0047] The vaccine composition may be a polynucleotide. In some embodiments, the S protein is encoded by one or more of the group consisting of SEQ ID NO:1-7, 22, 24, 26, 28, 30, 32, and 34 or a sequence capable of encoding at least one of SEQ ID NO: 11-17, 21, 23, 25, 27, 29, 31, and 33. In some embodiments, the N protein is encoded by SEQ ID NO:8, 9, 36 or 38, or a sequence capable of encoding at least one of SEQ ID NO: 10, 18, 35, or 37. In some embodiments, the polynucleotide encodes both an S protein and an N protein.

[0048] In some embodiments, the vaccine composition comprises a viral vector. In some embodiments, the viral vector comprises a polynucleotide encoding an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. In some embodiments, the viral vector is selected from an adeno-associated virus or a poxvirus. In some embodiments, the viral vector is a modified vaccinia Ankara (MVA) virus or turkey herpes virus (HVT). In some embodiments, the sequence encoding the S protein comprises one or more of the group consisting of SEQ ID NO:1-7, 22, 24, 26, 28, 30, 32, and 34 or a sequence encoding SEQ ID NO: 11-17, 21, 23, 25, 27, 29, 31. In some embodiments, the sequence encoding the N protein comprises SEQ ID NO:8, 9, 36 or 38 or a sequence encoding SEQ ID NO: 10, 18, 35, or 37. In some embodiments, the viral vector encodes both the S protein and the N protein.

[0049] In other embodiments, the vaccine composition comprises a protein subunit vaccine composition. The protein subunits in the vaccine composition may include one or more of a IBV S protein or an IBV N protein or portion thereof. The vaccine compositions may further comprise an adjuvant and the adjuvant may be a Quil-A chitosan adjuvant. In one embodiment the the vaccine composition may include both an S protein and an N protein or combinations of more than one S protein and more than on N protein. The S protein may be selected from SEQ ID NO: 11-17, 21, 23, 25, 27, 29, 31 or combinations thereof. The N protein may be selected from SEQ ID NO: 10, 18, 35, or 37 or combinations thereof.

[0050] The methods of the current disclosure comprise administration of vaccine composition that elicits an immune response against IBV. The timing of the administration of the vaccine compositions may be varied. Accordingly, in some embodiments, administration of the second vaccine composition occurs at least three weeks after admin-

istration of the first vaccine composition. In some embodiments, administration of the second vaccine composition occurs at least six weeks after administration of the first vaccine composition. A hallmark of the QAC adjuvant system is slow release of payload with continual priming of the immune system. Thus, the inventors hypothesize that release of DNA payload can be sustained up to six weeks after which another immunization will further boost immune responses.

[0051] The inventors disclose herein that heterologous vaccine strategies for eliciting an immune response against IBV are highly successful. Therefore, in some embodiments, the first vaccine composition comprises a NA vaccine composition, and the second vaccine composition comprises a viral vector or protein subunit vaccine composition.

[0052] The methods of the current disclosure comprise administering two vaccine compositions. In some embodiments, both the administration events comprise administering the vaccine compositions via the same route. In other embodiments, the first and second vaccine compositions are administered via different routes. For example, in some embodiments, the vaccine compositions are administered intranasally, intramuscularly, or administered in ovo. Thus, in some embodiments, the first vaccine composition is administered in ovo and the second vaccine composition is administered intranasally. In some embodiments, the first vaccine composition is administered in ovo and the second vaccine composition is administered in ovo. In some embodiments, the first vaccine composition is administered in ovo and the second vaccine composition is administered intramuscularly. In some embodiments, the first vaccine composition is administered intranasally and the second vaccine composition is administered intranasally. In some embodiments, the first vaccine composition is administered intranasally and the second vaccine composition is administered intramuscularly. In some embodiments, the first vaccine composition is administered intramuscularly and the second vaccine composition is administered intramuscularly. In some embodiments, the first vaccine composition is administered intramuscularly and the second vaccine composition is administered intranasally.

[0053] As used herein, "subject" refers to avian and non-avian animals. An "avian subject" may be any member of the class Ayes including, but not limited to, chickens, turkeys, ducks, or other fowl. The term "poultry" refers generally to any avian subject that is agriculturally relevant, e.g., chickens, ducks, ostriches, guinea fowl, turkeys, quail, pheasants, Muscovy ducks, and the like. The term "subject" does not denote a particular age or sex. In one embodiment, the subject is a chicken. In a preferred embodiment, the chicken is at risk of being infected IBV.

[0054] The phrase "amount effective to induce the immune response," as used herein, refers to an amount of a vaccine composition that would induce a humoral immune response against at least one IBV antigen (e.g., the spike or nucleocapsid protein encoded by the disclosed vaccines) and suitably also induces a polyfunctional T cell response as well. Humoral immunity or cell mediated immunity or both humoral and cell mediated immunity may be induced. The immunogenic response of an animal to a vaccine may be evaluated, e.g., indirectly through measurement of antibody titers, lymphocyte proliferation assays, or directly through monitoring signs and symptoms after challenge with the virus. The protective immunity conferred by a vaccine may also be evaluated by measuring, e.g., clinical signs such as mortality, morbidity, temperature, overall physical condition, overall health, and the performance of the subject. The amount of a vaccine that is therapeutically effective may vary depending on the particular strain of virus used, the antigen used in the vaccine, the species of the subject, the condition of the subject (e.g., age, body weight, gender, health), and should be determined by a veterinarian or physician. The therapeutically effective amount may be administered in one or more doses and is preferably in the range of about 0.01-10 mL, most preferably 0.05-1 mL, containing 1-200 micrograms, most preferably 1-100 micrograms of vaccine formulation/dose.

[0055] The present disclosure is not limited to the specific details of construction, arrangement of components, or method steps set forth herein. The compositions and methods disclosed herein are capable of being made, practiced, used, carried out and/or formed in various ways that will be apparent to one of skill in the art in light of the disclosure that follows. The phraseology and terminology used herein is for the purpose of description only and should not be regarded as limiting to the scope of the claims. Ordinal indicators, such as first, second, and third, as used in the description and the claims to refer to various structures or method steps, are not meant to be construed to indicate any specific structures or steps, or any particular order or configuration to such structures or steps. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to facilitate the disclosure and does not imply any limitation on the scope of the disclosure unless otherwise claimed. No language in the specification, and no structures shown in the drawings, should be construed as indicating that any non-claimed element is essential to the practice of the disclosed subject matter. The use herein of the terms "including," "comprising," or "having," and variations thereof, is meant to encompass the elements listed thereafter and equivalents thereof, as well as additional elements. Embodiments recited as "including," "comprising," or "having" certain elements are also contemplated as "consisting essentially of" and "consisting of" those certain elements.

[0056] While some claims provided herein are directed to methods of treating a subject, both human and non-human subjects are envisioned. In addition, use of the compositions provided herein as medicaments for uses in therapy or for treating disease are also provided herein. Use of the compositions provided herein in the manufacture of a medicament for the treatment of a disease or condition are also encompassed.

[0057] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this disclosure. Use of the word "about"

to describe a particular recited amount or range of amounts is meant to indicate that values very near to the recited amount are included in that amount, such as values that could or naturally would be accounted for due to manufacturing tolerances, instrument and human error in forming measurements, and the like. All percentages referring to amounts are by weight unless indicated otherwise.

[0058] No admission is made that any reference, including any non-patent or patent document cited in this specification, constitutes prior art. In particular, it will be understood that, unless otherwise stated, reference to any document herein does not constitute an admission that any of these documents forms part of the common general knowledge in the art in the United States or in any other country. Any discussion of the references states what their authors assert, and the applicant reserves the right to challenge the accuracy and pertinence of any of the documents cited herein. All references cited herein are fully incorporated by reference, unless explicitly indicated otherwise. The present disclosure shall control in the event there are any disparities between any definitions and/or description found in the cited references.

[0059] The following examples are meant only to be illustrative and are not meant as limitations on the scope of the invention or of the appended claims.

EXAMPLES

Example 1—Heterologous and Homologous DNA Confer Protection Against Avian Coronavirus

[0060] Infectious bronchitis (IB) is an acute respiratory disease of chicken caused by the avian coronavirus, Infectious Bronchitis Virus (IBV). Modified Live Virus (MLV) vaccines commercially used can revert to virulence in the field, recombine with circulating serotypes and can cause tissue damage in vaccinated birds. Previously, we showed that a mucosal adjuvant system, QuilA-loaded Chitosan (QAC) nanoparticles encapsulating plasmid vaccine encoding for IBV Nucleocapsid (N) is protective against IBV. Here, we report a heterologous strategy using QAC encapsulated plasmid vaccine followed by a Modified Vaccinia Ankara (MVA) expressing the same IBV N antigen. Heterologous vaccination led to the development of robust T-cell responses. Heterologous vaccine immunized birds had reduced clinical severity and >2-fold reduction viral burden in lachrymal fluid and tracheal swabs post-challenge in contrast to homologous MVA vaccination where no protection was observed. Outcomes of this study indicate that the heterologous vaccine strategy is more immunogenic and protective than homologous vaccination.

[0061] Coronaviruses (CoVs) are enveloped, large viruses with a positive-sense, single-strand, RNA genome ranging from 27-31 Kb in length. They are broadly classified into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus CoVs can infect a wide range of hosts, including humans, poultry, mice, pigs, cats, camels, bats, etc. CoVs infections usually cause acute diseases, primarily in the respiratory and gastrointestinal tract [1]. Human CoVs like 0C43, 229E, HKU1, and NL63 cause mild respiratory disease. Other CoVs like SARS-CoV-2, SARS, MERS in humans, and Avian CoV like Infectious Bronchitis Virus (IBV) in chickens can cause more acute severe respiratory disease [1-3]. IBV is classified within the genus gammacoronavirus encoding for major structural proteins, spike glycoprotein (S), envelope (E), membrane (M), and nucleocapsid (N) [4] and is the etiological agent of infectious bronchitis in chickens. In a typical infectious bronchitis infection, chickens develop respiratory signs, including sneezing, tracheal rales, nasal discharge, and labored breathing[5]. Mortality associated with infectious bronchitis is low; however, concomitant secondary bacterial infections can increase mortality[3]. Infectious bronchitis has a significant economic impact on the commercial US poultry industry, valued at over $35 billion in the US [6]. Infectious bronchitis infections in broilers can lead to reduced weight gain, and low feed conversion and infections in layers can lead to a drop in egg production and quality[7]. Typically, losses of around $450,000 per week can be expected due to IB outbreaks in facilities producing about 1 million broilers per week, which is unsustainable in the poultry industry characterized by low-profit margins[8]. IBV control currently revolves around extensive vaccination and acceptable flock management practices like optimal stocking densities, house temperature, water and air quality, etc. to prevent increased mortality due to secondary bacterial infections. Modified live virus (MLV) and inactivated vaccines are the leading vaccine types used against D3. Although effective, MLVs have an inferior safety profile. MLVs have a propensity to persist, revert to virulence in the field, and readily recombine with other circulating serotypes, leading to novel serotypes' emergence due to single mutations as a consequence of lack of polymerase proof-reading activity [9-11]. The emergence of GA98 serotype has been linked to the extensive use of DE072 vaccine [12]. Moreover, current vaccines do not cross-protect against multiple circulating serotypes because of variations in the S protein [13-15]. Unfortunately, safer inactivated vaccines are poorly immunogenic underscoring the need to develop an effective and safe vaccine for IBV control [8].

[0062] Experimental plasmid DNA vaccines have been developed against multiple poultry pathogens, and most recently, conditional approval for a DNA vaccine against H5 avian flu was given [16]. Varying protection levels are observed with experimental plasmid DNA vaccines expressing IBV S1, N, and M genes delivered via the intramuscular, intranasal and in ovo routes[17-25]. DNA vaccines offer several advantages over traditional vaccine approaches; they are safe, thermostable, comparatively inexpensive, and can be rapidly developed in the face of a novel serotype field outbreak [26]. A significant problem with DNA vaccines is their low immunogenicity owing to in vivo degradation leading to reduced cellular uptake and bioavailability. Vaccine hostile surfaces like the nasal mucosa can degrade DNA vaccine before target immune cell uptake[27, 28]. Nanoparticle adjuvant systems like QAC can protect against DNA degradation and boost immune responses observed with DNA vaccines as described by our group previously for the intranasal delivery of DNA immunogens[29, 30].

[0063] Similarly, viral vector vaccines against IBV based on Newcastle disease virus, Herpesvirus of turkeys and avian metapneumovirus backbones have been developed with great promise [31, 32]. However, none of them have been licensed for use owing to limited efficacy and regulatory concerns. The heterologous vaccine has been evaluated against viral pathogens like HIV-1, HPV, HCV, and Influenza [33-36]. Although the concept of heterologous vaccine for the poultry industry refers to a broadly cross-protective vaccine, for the purpose of this paper the heterologous

vaccination refers to the concept of using a different vaccine platform for boosting from the vaccine that was used for priming. Particularly in this study, we evaluated DNA priming followed by viral vector boosting in comparison to viral vector homologous priming and boosting. The efficacy of heterologous vaccine strategies has been shown with different routes and viral vectors for boosting like vaccinia (e.g., Modified Vaccinia Ankara-MVA), adeno, and VSV (Vesicular Stomatitis Virus)[35]. Heterologous vaccination compared to homologous immunization can lead to a 4 to 10 fold increase in T-cell responses[35]. Previously, we have shown that a heterologous vaccination involving QAC encapsulated plasmid DNA priming followed by MVA boosting was shown to be immunogenic and protective against SARS-CoV-2 challenge in transgenic mice[37, 38]. Although the heterologous vaccine approach has been characterized and extensively evaluated for human viral pathogens, not much work has been done in the context of viral poultry pathogens.

[0064] We have previously shown that a two-dose QAC encapsulated plasmid DNA (pQAC-N) encoding the N protein was protective against IBV challenge to levels seen with MLV vaccination[30]. We hypothesized that a heterologous vaccine strategy with pQAC-N prime followed by an MVA viral vector boost expressing the N protein (MVA-N) would also protect immunized chicks against IBV challenge similar to our findings with human coronavirus, SARS-CoV-2[37, 38]. The prime/boost of the experimental vaccines were delivered via the intranasal route and hereafter referred to as either heterologous vaccine or pQAC/MVA-N. Our results indicate that pQAC/MVA-N vaccine elicits a robust IBV specific CD8+ and TCRγδ+ T-cells which protect vaccinated birds against IBV challenge. Levels of protection in vaccinated birds were higher when compared to homologous 2×MVA-N vaccine. Our data demonstrate that intranasal immunization with pQAC/MVA-N protected vaccinated birds with a significant reduction in clinical signs and viral load in trachea and lachrymal fluid to levels on par with commercial MLV vaccinated birds. Also, addition of another adjuvant MPLA (Synthetic Monophosphoryl Lipid A), did not significantly improve protection observed with pQAC/MVA-N.

[0065] Materials and Methods:

[0066] Ethics Statement

[0067] All the animals used in this study were cared for in accordance with established guidelines, and the experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Wisconsin at Madison.

[0068] Cells and Viruses

[0069] Chicken Embryonic Fibroblasts (CEF) were prepared from 9-day-old specific pathogen free (SPF) white leghorn eggs (Charles River Laboratories, Inc., WA, USA) as described previously[39] and used for confirming expression of IBV Ark N6×His protein from vaccine constructs. The cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) at 37° C., 5% CO2 atmosphere in plastic flasks with ventilated caps. The virulent IBV Arkansas DPI strain (a kind gift from Dr. Ladman and Dr. Gelb) was propagated in 9-day old SPF ECEs and allantoic fluid harvested four days after infection. The stock virus titer was determined using RT-qPCR (see below) and also titrated and expressed as 50% embryo infectious dose (EID_{50})[40]. IBV 51 gene sequence of Ark DPI challenge isolate is AF006624.

[0070] Preparation of IBV Vaccine Constructs

[0071] pCAG-N encoding IBV Arkansas N protein was constructed and loaded into QAC nanoparticles as described previously[30]. To confirm insertion of genes in the correct orientation, DNA sequencing was performed at the UW-Madison Biotechnology Center with an ABI Prism 3730XL DNA analyzer using BigDye terminators (Applied Biosystems, CA). To confirm expression of N protein, CEF cells seeded in 6-well format was transfected with an optimized ratio of DNA (4 ug): TransIT PRO transfection reagent (2 ul) according to manufacturer's instructions (Minis Bio, WI, USA). Three days post transfection, cells and supernatant (separately) were harvested for western blot analysis. The MVA expressing N was generated as described before in CEF cells[41]. The cell and supernatant fractions were boiled in Laemmli sample buffer (BioRad, Hercules, Calif., USA) and resolved on a 4-20% SDS-PAGE gel by electrophoresis using a Mini-PROTEAN 3 system (BIO-RAD, CA). Polyacrylamide gels were electroblotted onto nitrocellulose membranes using a Turboblot® system. Membranes were blocked in 5% (W/V) skim milk and probed with polyclonal anti-6×His HRP antibody (ThermoFisher Scientific, MA1-21315-HRP). Membranes were developed using a solid phase 3, 30, 5, 50-tetramethylbenzidine (TMB) substrate system.

[0072] Vaccine Efficacy Study.

[0073] The protective efficacy of pQAC/MVA-N construct was evaluated in 1-day-old white leghorn SPF chicks (Charles River Laboratories). A total of 40 chicks was divided equally into 4 groups (N=10 each) and used for the efficacy study, first 2 groups were inoculated with PBS (negative control) or commercial Arkansas MLV (Mildvac-Ark®, Merck Animal Health USA, positive control) via direct intranasal instillations (dose according to manufacturer's instructions). The other groups were either vaccinated with MVA-N (10^8 pfu/bird) at day-1 and followed by a booster dose at day-14 via intranasal (IN) route or pQAC-N (100 ug/bird) at day-1 and followed by a booster MVA-N (10^8 pfu/bird) dose at day-14 via intranasal (IN) route. Birds were challenged with a dose of 6.5E9 genome copy no or 10^{65} EID_{50}/bird of virulent IBV Arkansas DPI strain via direct intranasal instillations at day-21 of age. The challenge dose was determined in an independent infection experiment wherein the challenge dose resulted in discernable clinical signs as early as 3 dpc and peak viral load replication was observed at 6 dpc. At 10, 20 dpv & 3 days post challenge (DPC) serum and lachrymal fluid samples were harvested for ELISA and at 6 DPC for viral load estimation (see below). Lachrymation was induced by placing sodium chloride (salt) crystals on the eyes and lachrymal fluid were collected using micropipettes [42]. Clinical severity was noted every day post challenge for 8 days, as described before [31]. The severity scores of clinical signs of IBV were as follows; 0=normal, 1=Infrequent sneezing (single event during observation), 2=frequent sneezing (more than one event during observation), 1=mild rales, 2=severe rales, 2=presence of nasal exudate. The severity scores of IBV clinical signs, described in the figure legends were recorded once a day for each chicken for 8 days after challenge. Lachrymal fluid and tracheal swabs harvested at 6 dpc was analyzed for viral RNA using IBV N gene specific qRT-PCR. A similar experimental design was used to test the efficacy of the pmQAC/MVA-N vaccine candidate in a follow-up trial. 10 ug MPLA/bird (PHAD®, Avanti® Polar

Lipids) was added to QAC-pCAG-N formulation before IN inoculation and followed by a booster MVA-N (10^8 pfu/bird) dose at day-14 via intranasal (IN) route. Birds were challenged with a dose of 6.5E9 genome copy no or $10^{6.5}$ EID_{50}/bird of virulent IBV Arkansas DPI strain via direct intranasal instillations at day-21 of age. Vaccine efficacy read outs including viral shedding and clinical severity scoring as detailed for the previous primary trial were evaluated.

[0074] IBV Specific ELISA

[0075] Sera and lachrymal fluid from different time-points were screened for humoral response against IBV Arkansas serotype. In order to measure IgY and IgA antibody levels in plasma and lachrymal fluid of chicken respectively, an IBV-specific enzyme-linked immunosorbent assay (ELISA) was developed as described previously with modifications [43]. Briefly, ELISA plates were coated with inactivated IBV Arkansas (100 ng/well, IgY) or IBV Arkansas S1 and N6×His protein (50 ng total/well, IgA) diluted in carbonate/bicarbonate buffer, pH 9.6 and incubated overnight at 4C followed by blocking with 5% Skim milk to reduce background. A 50 ul of diluted serum (1/200) or lachrymal fluid (1/50) harvested at different time-points from immunized chickens was added to the wells and incubated at 37 C for 1 hour. Post washing (PBS-TritonX 100, 0.1%), either HRP conjugated anti-chicken IgY (NBP1-74778, NOVUS Bio) or anti-chicken IgA (NB7284, NOVUS Bio) at dilutions of 1/1000 was added to the wells and incubated at 37° C. for 1 hr. Post washing, 50 ul of TMB substrate solution was added and incubated for 20 minutes or until color developed. The reaction was stopped by the addition of 1M sulphuric acid and plates are read at 450 nm. To generate standard curves, sera and lachrymal fluid from severely IBV infected chickens from previous experiments was used. Two-fold serial dilutions was assigned and arbitrary value and used for analysis.

[0076] Flow Cytometric Assessment of IBV Specific Proliferation

[0077] In a separate follow-up study, 16 chicks were divided equally into 4 groups (N=10 each) and used for the flow cytometric assessment, first 2 groups were inoculated with PBS (negative control) or commercial Arkansas MLV (Mildvac-Ark®, Merck Animal Health USA, positive control) via direct intranasal instillations (dose according to manufacturer's instructions). The other groups were either vaccinated with MVA-N (10^8 pfu/bird) at day-1 and followed by a booster dose at day-14 via intranasal (IN) route or pQAC-N (100 ug/bird) at day-1 and followed by a booster MVA-N (10^8 pfu/bird) dose at day-14 via intranasal (IN) route. All chicks were euthanized at 20 dpv and single cell suspensions from lungs were prepared using standard techniques and used for T-cell proliferation assay. Briefly, lungs were excised and placed in a gentleMACS dissociator M tube (Miltenyi 130-093-236) with 5 mL collagenase B (2 mg/ml, Roche). Lung tissue was processed using the gentleMACS dissociator followed by incubation for 30 min at 37° C. with gentle shaking. Single-cell suspensions lung were prepared by gently squeezing through a 70-mm cell strainer (Falcon) after lysing RBCs using ix BD Biosciences BD Pharm Lyse™. Total of 10' cells/ml were stained with CellTrace™ Violet Cell Proliferation dye (Thermo Scientific C34557) according to manufacturer's instructions and 100 ul of cells plated/well in RPMI 1640 with 10% chicken immune serum. After overnight incubation at 41° C., 5% CO_2, cells were stimulated with 130 ng of IBV Arkansas N6×His protein complexed with chitosan per well in 100 ul of RPMI 1640 with 10% chicken immune serum. Four days post stimulation, cells were stained for surface markers, CD4-AF647 (clone CT-4), CD8α-FITC (clone 3-298) together and TCRγδ-FITC (clone TCR-1) independently for flow cytometry analysis. All antibodies were purchased from SouthernBiotech (Birmingham, Ala., USA). All samples were acquired on an BD LSR Fortessa flow cytometer. Data were analyzed with FlowJo software (BD Biosciences). The strategy for gating on proliferating CD4+ and CD8a+ T cells was debris exclusion on the Forward Scatter (FSC)—Side Scatter (SSC) dot plot followed by exclusion of dead cells by fixable viability dye eFluor 780 (Invitrogen™, #65-0865-14) staining. Out of the live cells, total proliferated cells were gated positive using a histogram plot with ef450 on the x-axis (for CellTrace™ Violet). Finally, CD4 cells were gated positive at the AF647 axis and CD8a cells were gated positive at the FITC axis in a FITC-AF647 dot plot. A similar approach was used for identifying proliferating TCRγδ+ T-cells. The output, stimulation index (SI) is the ratio of % proliferating cells post stimulation to the % proliferating cells in unstimulated condition. The chicks from different groups used here were part of another bigger study and the data for only the control groups (PBS and MLV) have already been published[30].

[0078] Viral Load Measurement

[0079] RNA was extracted from lachrymal fluid (10 μl) or Tracheal swabs (100 μl) collected from chickens using Zymo Direct-Zol™ RNA mini prep kit (Zymo Research, CA, USA) according to manufacturer's instructions. RT-qPCR was conducted in two steps: cDNA synthesis (Invitrogen™ SuperScript™ III First-Strand Synthesis System) and qPCR reactions. cDNA synthesis was performed with 0.5 μl (50 ng/μl) random hexamers, 0.5 μl of 10 mM dNTPs, and 4 μl RNA and heated at 65° C. for 5 min and chilled on ice followed by addition of 1 μl of 10×RT buffer, 1 μl of 0.1 M DTT, 1 μl of 25 mM $MgCl_2$, 0.5 μl of RNaseOUT and 0.5 μl of SuperScript III enzyme in final volume of 10 μl. The reaction conditions include 25° C. for 5 min, 50° C. for 60 min and 70° C. for 15 min. SYBR green RT-qPCR was performed using an IBV N gene specific primer pair set forward primer: 5' ATGCTCAACCTAGTCCCTAGCA 3' (SEQ ID NO: 46) and reverse primer: 5' TCAAACTGCGGATCATCACGT 3' (SEQ ID NO: 47) amplifying 128 nt of N gene of IBV Arkansas DPI. PCRs were performed using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, Calif., U.S.A) under the following conditions: one cycle 95 C for 2 min followed by 40 cycles of 95 C for 3 sec and 60 C for 30 sec. Each 20 μl reaction was carried out using 1 μl of diluted cDNA (1/10), 10 μl of GoTaq® qPCR mastermix (Promega), 2 μl of forward and reverse primers and 7 μl of nuclease free water. A serial 10-fold dilution of pCAG-IBV Ark N6×His plasmid was used to establish the standard curve. Temperature melt curve analysis was used to confirm the specificity of the product. The challenge dose as estimated with the above-described method was 6.5E9 genome copy no which roughly translated to $10^{6.5}$ EID_{50}.

[0080] Statistical Analysis

[0081] Statistical analyses were performed using GraphPad software (La Jolla, Calif.). Cellular immune assays, clinical severity scoring, viral loads were compared using an ordinary one-way ANOVA test with multiple comparisons

where *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001 were considered significantly different among groups. Antibody titers were compared using a two-way ANOVA test where *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001 were considered significantly different among groups.

[0082] Results:

[0083] Design and Construction of MVA-IBV Constructs

[0084] The expression of recombinant N from the plasmid DNA vaccine (pCAG-N) was confirmed using western blot analysis on cells and supernatant harvested from transfected chicken embryonic fibroblast (CEF) cells (FIG. **1**B). The SE/L promoter controls the expression of the recombinant N-6×His protein in the MVA vaccine candidate (MVA-N, FIG. **1**A). As observed with pCAG-N construct, expression of N-6×His antigen was also confirmed using western blot analysis with anti-6×His antibody staining in the cell pellets from MVA-N infected CEF cells (FIG. **1**B). To characterize and understand if the expression of IBV N-6×His protein affects MVA replication in cell culture, we evaluated the growth kinetics of MVA-N and parental MVA-GFP in permissive CEF cells. CEF cells were either infected at a MOI of 1 (single step) or 0.1 (multi-step) and viral titers subsequently determined on CEF cells (FIGS. **1**C and **1**D). MVA-N replicated at rates similar to parental MVA-GFP, although the final titers of the recombinant MVA were about 100-fold lower than that of the parental virus (FIGS. **1**C and **1**D).

[0085] Heterologous Vaccine Strategy Elicits Robust Localized T-Cell Responses

[0086] We have previously reported the safety, and protective efficacy of QAC complexed pCAG-N DNA vaccine (pQAC-N) in chickens against IBV Arkansas challenge although no humoral responses were observed [30]. We hypothesized that a heterologous mucosal strategy of priming with pQAC-N followed by boosting with MVA-N would offer a similar or better level of protection than observed with 2-dose intranasal (IN) pQAC-N vaccination with complementing humoral responses. We examined the ability of our experimental vaccines to elicit local and systemic IBV— specific immune responses following IN immunization (FIG. **2**). Lachrymal fluid samples and serum harvested at different time points, 10, 20 days post-vaccination (dpv, pre-challenge) and three days post-challenge (dpc) were examined for IBV specific IgA (lachrymal fluid, local) and IgY (serum, systemic) using ELISA. IBV specific IgA and IgY were significantly higher in the MLV groups when compared to the unvaccinated PBS group (FIGS. **3**A and **3**B). Although detectable at multiple time points, both IgA and IgY levels were not significantly high in birds vaccinated with either the homologous or heterologous vaccine strategy (FIGS. **3**A and **3**B).

[0087] We next evaluated the ability of the experimental vaccines to elicit local (lung) IBV N specific cellular immune responses. Antigen-specific T-cell proliferation assay based on CellTrace™ Violet Cell dye staining of lung cells to trace proliferating T cells was used as described previously[30]. The stimulation index (SI), which is the fold increase in stimulated to unstimulated cells was calculated. Total lung cells from pQAC/MVA-N vaccinated birds had significantly higher proliferation in response to N antigen stimulation which was higher than the control and 2×MVA-N groups (FIG. **4**A). An increase in the stimulation of proliferating TCRγδ+ and CD8+ T-cells was observed in pQAC/MVA-N vaccinated birds in comparison to control birds (FIGS. **4**C & **4**D) while CD4+ T-cell proliferation was higher in MLV vaccinated birds (FIG. **4**B), albeit non-significant. These results highlight the ability of the heterologous pQAC/MVA-N vaccine strategy to elicit robust IBV-specific immune responses.

[0088] Heterologous Vaccine is More Effective than the Homologous Vaccine Strategy.

[0089] Twenty-one days post initial vaccination (21 dpv) and seven days post final boost, immunized birds were challenged with a virulent strain of IBV Arkansas DPI Serotype via the intranasal route to evaluate vaccine efficacy. Immunization with homologous 2×MVA-N did not confer any protection against the challenge; no reduction in clinical severity was observed (FIG. **5**A). In contrast, immunization with heterologous pQAC/MVA-N and MLV resulted in a significant reduction in clinical severity with the birds asymptomatic when compared to unvaccinated PBS group birds (FIG. **5**A). Viral RNA in lachrymal fluid and tracheal swabs were evaluated using qRT-PCR. Only the best performing experimental vaccine group as determined by viral shedding in lachrymal fluid along with the control groups was taken for quantifying viral shedding in the tracheal swabs. A significant reduction in viral load was observed both in the lachrymal fluid and swabs of pQAC/MVA-N vaccinated birds in comparison to the unvaccinated and 2×MVA-N vaccinated birds (FIG. **5**B). More importantly, reduction in viral load in tracheal swabs was comparable to levels seen in commercial MLV vaccinated birds (FIG. **5**C). In contrast, no reduction in viral load was observed in 2×MVA-N vaccinated birds, which correlated well with clinical severity scoring (FIGS. **5**A and **5**B). Vaccination with the heterologous pQAC/MVA-N confers protection against IBV challenge significantly higher than the homologous 2×MVA-N (FIG. **5**B). This protection might be attributed to the induction of CD8+ and TCRγδ+ memory T-cell responses (FIG. **4**).

[0090] Impact of MPLA Addition on IBV Vaccine Protection.

[0091] MPLA is a potent mucosal adjuvant and TLR 4 ligand that stimulates expression of inflammatory-related genes, important of viral control in poultry. We hypothesized that inclusion of MPLA in addition to Quil-A and Chitosan would further improve protection observed with pQAC/MVA-N vaccination. To investigate this, we immunized SPF birds with a triple adjuvant system (MPLA+QAC) loaded with pCAG-N plasmid at day-1 followed by MVA-N immunization (pmQAC/MVA-N) at day-14 similar to the pQAC/MVA-N group in the previous trial. Reduction in clinical severity and viral burden in tracheal swabs was observed comparable to the MLV group (FIGS. **6**A-B). Protective efficacy of pmQAC/MVA-N was very similar to and not significantly different from pQAC/MVA-N (FIGS. **6**A-B). Our results indicate that addition of MPLA does not improve vaccine performance. Overall, these results highlight the ability of the heterologous vaccine strategy to elicit potent IBV specific T-cell responses and protect vaccinated birds against virulent IBV challenge.

[0092] Discussion:

[0093] Many experimental viral vectored vaccines primarily based on Newcastle Disease Virus (NDV) have been developed against IBV [31, 44, 45]. Recombinant NDV encoding for IBV Spike protects against homologous challenge and resulted in a reduction of clinical severity and viral shedding[31, 45]. Recombinant MVA based vaccines have

been developed for use in chickens against Infectious Bursal Disease Virus (IBDV) and Influenza[46-48]. The heterologous vaccine strategy involving a DNA prime followed by a viral vector booster dose has been evaluated against multiple human and animal viruses with modest success[37, 38, 49-51]. Intranasally administered vaccines are highly favorable for mass vaccinations in the field. Unfortunately, mucosal surfaces are vaccine hostile leading to poor immunogen uptake and bioavailability, rapid degradation and weak immune responses[27]. In a previous study, we demonstrated the ability of a nano adjuvant system, QAC to facilitate the intranasal delivery of DNA immunogens leading to a protection against IBV in poultry and SARS-CoV-2 in transgenic mice [30, 37, 38]. In this study we evaluated the efficacy of an intranasally delivered heterologous QAC complexed DNA prime-MVA boost vaccine strategy. To our knowledge, the use of heterologous and MVA based vaccine strategies against IBV infection in chickens have not been extensively studied.

[0094] DNA viral vectors like MVA can accommodate and stably express multiple foreign immunogens, making them ideal candidates for vaccine use. In our hands, although the recombinant MVA-N had similar replication rates in cell-culture when compared to the parental MVA-GFP, the titers were 100-fold lower, albeit non-significant. This could mean that constitutive expression of IBV N-6×His protein potentially weakened the MVA vector replication in permissive CEF cells. The safety and efficacy of MVA-based vaccines in chicken hosts have been well documented[52-54]. Experimental MVA-hemagglutinin based influenza vaccines protects chickens against both lethal high- and low-pathogenicity avian influenza[52, 53]. Furthermore, the safety and replication of MVA in chicken embryos have been extensively characterized with no embryonic death observed even after in ovo inoculation[54]. We have previously shown that QAC based DNA vaccines are well tolerated by chicken hosts when administered via the IN and in ovo routes. Similarly, we observed that chickens intranasally administered MVA-N and pQAC/MVA-N did not show any signs of respiratory distress, in appetence or depression pre-challenge.

[0095] Very few studies have investigated the efficacy of MVA based vaccines in poultry. Ocular administration of MVA based flu vaccine protects birds against avian influenza challenge[47]. Mixing and matching viral vector and nucleic acid SARS-CoV-2—vaccines also boost the immunogenicity of homologous vaccines[55, 56]. In our hands, the heterologous DNA prime followed by MVA boost was more immunogenic and protective than the homologous MVA vaccination. Reduction in clinical severity and viral burden both in lachrymal fluid and tracheal swabs were observed to levels comparable with MLV vaccination. The protection is most likely due to the induction of local lymphocyte responses by the pQAC-N priming followed by the expansion of T-cells facilitated by the MVA-N boost. We observed a similar phenomenon with our QAC-based COVID-19 vaccines in mice, where the heterologous DNA/MVA vaccine was more immunogenic than the homologous vaccine strategy[37, 38].

[0096] In a previous study we showed that 2 doses of pQAC-N vaccine protected vaccinated SPF and commercial birds against IBV challenge comparable to protection observed with MLV[30]. A robust T-cell immune response without a complementing humoral response was induced post vaccination with 2×pQAC-N. We hypothesized that boosting with MVA viral vector instead of DNA vaccine would further expand CD4+ T-cells leading to an induction of complementing humoral responses. We observed that immunization with MVA-N, both in the homologous and heterologous group did not lead to significant induction of both IgY and IgA as assayed using IBV specific binding ELISA. Instead, low level IBV-specific IgA and IgY was observed in the experimental vaccine groups at 3 dpc, indicating presence of an anamnestic response with pQAC-N based vaccines. In contrast, significant induction of humoral responses was observed with commercial MLV vaccine. Irrespective of the vaccine platform used, homologous MVA and heterologous DNA/MVA used in this study and homologous DNA used in the previous study, significant induction of N specific humoral responses are not observed [30]. The absence of humoral responses could be a consequence of using the N immunogen exclusively and not the vaccine platform itself. The N protein here will be intracellularly expressed in cells that take up the vaccine and not secreted. Moreover, it is unlikely that antibodies generated against N will be neutralizing given the intra-virion nature of the protein. With mouse hepatitis virus (MHV), a CoV infecting mice, N specific antibodies fail to neutralize MHV in cell culture[57].

[0097] Previously, sequential immunization approach of DNA prime-viral vector boost has led to the initial induction of cell-mediated immune (CMI) responses followed by MVA boost which expands induced CD8+ T-cells and Th1 T-cells[58]. We have previously shown that the potency of unadjuvanted plasmid DNA vaccine was enhanced by QAC nanoparticle formulation leading to induction of robust CD8+ and TCRγδ+ T-cells, potentially a hallmark of the QAC adjuvant system[30]. Similarly, lung cells harvested from pQAC/MVA-N immunized chickens responded well to IBV-N antigen recall stimulation. Furthermore, higher stimulation of TCRγδ+ and CD8+ T-cells was observed in pQAC/MVA-N immunized chickens, albeit non-significant. Although no significance was observed in T-cell specific responses, statistically higher proliferation was observed with total lung cells. This could mean that there are other lymphocytes (non TCRγδ+, CD8+ or CD4+ T-cells) in the lungs responding to IBV antigen that were not specifically evaluated in this study. We believe that an MVA boost after DNA prime further expanded the lung lymphocytes elicited by the initial DNA vaccination leading to protection. These results are in accordance with our previous data where a similar heterologous DNA/MVA vaccine elicited better local type-1 and type-17 T-cell responses in mice not observed with the homologous vaccine strategy[38]. Further studies are still warranted to evaluate the exact mechanism of action for the pQAC/MVA-N vaccine.

[0098] To further improve on the efficacy of the pQAC/MVA-N vaccine we added MPLA to our QAC vaccine formulation. MPLA is a synthetic low toxic form of LPS can engage with TLR4 (toll-like receptor) leading to an enhanced Th1 response[59]. MPLA is the only licensed TLR agonist approved for human use and is currently used as part of AS04 adjuvant in hepatitis B and human papillomavirus vaccines[60, 61]. Engagement of TLRs by agonists like lipopolysaccharides (LPS), Poly I:C and CpG dinucleotides leads to a cascade of intracellular signaling leading to induction of factors and cytokines which enhance immunity [62]. The new tri-adjuvant system based heterologous vac-

cine dubbed pmQAC/MVA-N with MPLA did not significantly improve protection observed with pQAC/MVA-N when administered intranasally.

[0099] Results presented here highlight the utility of a nano-adjuvant complexed DNA prime/viral vector boost vaccine strategy against IBV in chickens which reduces clinical severity and viral load in trachea and lachrymal fluid. The heterologous vaccine strategy outperformed the homologous MVA/MVA immunization and resulted in the induction of local-IBV specific T-cells in the lungs. Moreover, the protection observed with the heterologous vaccine strategy was very comparable with the commercial MLV vaccine's efficacy.

[0100] In general, CD8+ T-cells are important for early protection against IBV infection but CD4+ T-cells and systemic humoral responses are needed for sterilizing long term immunity[63]. We did not observe IBV specific antibody responses with the heterologous vaccine. The use of additional adjuvants and a secreted IBV S protein as an additional immunogen to the pQAC/MVA-N formulation could help in generating a complementing humoral immune response [64]. 2-dose vaccine regimens like the heterologous vaccine strategy described here might also have poor field applicability. Single dose vaccines administered at day-1 are preferred for poultry considering the need for early protection against IBV and the short lifespan of broilers in the poultry industry. Many experimental MVA based vaccines for use in humans are currently undergoing clinical trials. Therefore, use of MVA in poultry might confer people coming in contact with vaccinated birds with pre-existing immunity against the viral vector limiting the efficacy of subsequent human MVA based vaccines. That being said, the utility of this heterologous vaccine platform can be extended for use against other respiratory coronaviruses which necessitate robust local immune responses for protection. As highlighted with the ongoing COVID-19 pandemic, mix and match heterologous vaccines can not only improve immunogenicity, but also help in mitigating global vaccine supply chain shortages.

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cgctctctcg aggttttgtt tcaaggcccc ggccatcatc atcaccatca tcatcattcc  3420
gcttggagcc accctcaatt tgaaaagggt ggtggctctg gtggcggcgg ctcaggcggt  3480
tcagcctggt cccatcccca atttgaaaag taa                               3513

SEQ ID NO: 8           moltype = DNA  length = 1257
FEATURE                Location/Qualifiers
source                 1..1257
                       mol_type = other DNA
                       organism = Infectious Bronchitis Virus
SEQUENCE: 8
atggcaagcg gtaaagcaac tggaaagaca gacgccccag cgccagtcat caaactagga  60
ggaccaaagc cacctaaagt tggttcttct ggaaatgcat cttggtttca agcaataaaa  120
gccaagaagc taaattcaca tccacctaag tttgaaggta gcggtgttcc tgataatgaa  180
aatcttaaaa caagtcagca acatggatac tggaggcgcc aagccaggtt taagccagtt  240
aaaggcggaa gaaaaccagt cccagatgct tggtacttct attatactgg aacaggacca  300
gccgctgacc tgaattgggg tgatagccaa gatggtatag tgtgggttgc tgcaaagggt  360
gctgatgtta aatctagatc tcaccagggt acaagggacc ctgacaagtt tgaccaatat  420
ccactacgat tctcggacgg aggacctgat ggtaatttcc gttgggactt cattcctctg  480
aatcgtggta ggagtggaag atcaacagca gcttcatcag cagcatctag tagagcaccg  540
tcgcgtgacg gctcgcgtgg tcgtagaagt ggttctgaag atgatcttat tgctcgtgca  600
gcaaagataa tccaggatca gcagaagaag ggttctcgca ttactaaggt taaggctgat  660
gaaatggctc accgccggta ttgcaagcgc actattccac ctggttataa ggttgatcaa  720
gtctttggcc cccgtactaa aggtaaggag ggaaattttg gtgatgacaa gatgaatgag  780
gaaggtatta aggatgggcg tgttacagca atgctcaacc tagtccctag cagccatgct  840
tgtctttttg gaagtagagt gacgcccaaa ctacaaccag atgggctgca cttgaaattt  900
gaatttacta ctgtggtccc acgtgatgat ccgcagtttg ataattatgt taaaatttgt  960
gatcagtgtg ttgatggtgt aggaacacgt ccaaaagatg atgaaccgag accaaagtca  1020
cgctcaagtt caagacctgc tacaagaaca agttctccgg cgccaagaca acaacgccca  1080
aagaaggaga aaaagccaaa gaagcaggat gatgaagtag ataaagcatt gacctcaaat  1140
gaggagagga acaatgcaca gctggaattt gatgaggaac ccaaggtgat taactggggg  1200
gatgcagctc taggagagaa tgaacttgga ggaggtcatc atcaccatca ccactaa     1257

SEQ ID NO: 9           moltype = DNA  length = 1257
FEATURE                Location/Qualifiers
source                 1..1257
                       mol_type = other DNA
                       organism = Infectious Bronchitis Virus
SEQUENCE: 9
atggcaagcg gtaaggcaac tggaaagaca gatgccccag ctccagtcat caaactagga  60
ggaccaaagc cacctaaagt tggttcttct ggaaatgtat cttggtttca agcaataaaa  120
gccaagaagt taaattcacc tccgcctaag tttgaaggta gcggtgttcc tgataatgaa  180
aatctaaaac caagtcagca gcatggatat tggagacgcc aagctaggtt taagccaggt  240
aaaggtggaa gaaaaccagt cccagatgct tggtattttt actatactgg aacaggacca  300
gccgctaacc tgaattgggg tgatagccaa gatggtatag tgtgggttgc tggtaagggt  360
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gctgatacta aatttagatc taatcagggt actcgtgact ctgacaagtt tgaccaatat  420
ccgctacggt tttcagacgg aggacctgat ggtaatttcc gttgggattt cattcctctg  480
aatcgtggca ggagtgggag atcaacagca gcttcatcag cagcatctag tagagcacca  540
tcacgtgaag tttcgcgtgg tcgcaggagt ggttctgaag atgatcttat tgctcgtgca  600
gcaaggataa ttcaggatca gcagaagaag ggttctcgca ttacaaaggc taaggctgat  660
gaaatggctc accgccggta ttgcaagcgc actattccac ctaattataa ggttgatcaa  720
gtgtttggtc cccgtactaa aggtaaggag ggaaattttg gtgatgacaa gatgaatgag  780
gaaggtatta aggatgggcg cgttacagca atgctcaacc tagttcctag cagccatgct  840
tgtctttttcg gaagtagagt gacgcccaga cttcaaccag atgggctgca cttgaaattt  900
gaatttacta ctgtggtccc acgtgatgat ccgcagtttg ataattatgt aaaaatttgt  960
gatcagtgtg ttgatggtgt aggaacacgt ccaacagatg atgaaccaag accaaagtca 1020
cgctcaagtt caagacctgc aacaagagga aattctccag cgccaagaca gcagcgccct 1080
aagaaggaga aaaagccaaa gaagcaggat gatgaagtgg ataaagcatt gacctcagat 1140
gaggagagga acaatgcaca gctggaattt gatgatgaac ccaaggtaat taactggggg 1200
gattcagccc taggagagaa tgaacttgga ggaggtcatc atcaccatca ccactaa     1257

SEQ ID NO: 10          moltype = AA  length = 412
FEATURE                Location/Qualifiers
source                 1..412
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 10
MASGKATGKT DAPAPVIKLG GPKPPKVGSS GNVSWFQAIK AKKLNSPPPK FEGSGVPDNE  60
NLKPSQQHGY WRRQARFKPG KGGRKPVPDA WYFYYTGTGP AANLNWGDSQ DGIVWVAGKG  120
ADTKFRSNQG TRDSDKFDQY PLRFSDGGPD GNFRWDFIPL NRGRSGRSTA ASSAASSRAP  180
SREVSRGRRS GSEDDLIARA ARIIQDQQKK GSRITKAKAD EMAHRRYCKR TIPPNYKVDQ  240
VFGPRTKGKE GNFGDDKMNE EGIKDGRVTA MLNLVPSSHA CLFGSRVTPR LQPDGLHLKF  300
EFTTVVPRDD PQFDNYVKIC DQCVDGVGTR PTDDEPRPKS RSSSRPATRG NSPAPRQQRP  360
KKEKKPKKQD DEVDKALTSD EERNNAQLEF DDEPKVINWG DSALGENELG GG          412

SEQ ID NO: 11          moltype = AA  length = 1165
FEATURE                Location/Qualifiers
source                 1..1165
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 11
MLVTPLLLVT LLCALCSAVL YDSSSYVYYY QSAFRPPNGW HLQGGAYAVV NISSEFNNAG  60
SSSGCTVGII HGGRVVNASS IAMTAPSSGM AWSSSQFCTA HCNFSDTTVF VTHCYKHGGC  120
PITGMLQQNL IRVSAMKNGQ LFYNLTVSVA KYPTFRSFQC VNNLTSVYLN GDLVYTSNET  180
IDVTSAGVYF KAGGPITYKV MREVKALAYF VNGTAQDVIL CDGSPRGLLA CQYNTGNFSD  240
GFYPFTNSSL VKQKFIVYRE NSVNTTCTLH NFIFHNETGA NPNPSGVQNI QTYQTKTAQS  300
GYYNFNFSFL SSFVYKESNF MYGSYHPSCN FRLETINNGL WFNSLSVSIA YGPLQGGCKQ  360
SVFKGRATCC YAYSYGGPSL CKGVYSGELD HNFECGLLVY VTKSGGSRIQ TATEPPVITQ  420
NNYNNITLNT CVDYNIYGRT GQGFITNVTD SAVSYNYLAD AGLAILDTSG SIDIFVVQGE  480
YGLNYYKVNP CEDVNQQFVV SGGKLVGILT SRNETGSQLL ENQFYIKITN GTGGGVPSIT  540
ENVANCPYVS YGKFCIKPDG SIATIVPKQL EQFVAPLFNV TENVLIPNSF NLTVTDEYIQ  600
TRMDKVQINC LQYVCGSSLD CRKLFQQYGP VCDNILSVVN SVGQKEDMEL LNFYSSTKPA  660
GFNTPVLSNV STGEFNISLL LTTPSSRRKR SLIEDLLFTS VESVGLPTND AYKNCTAGPL  720
GFFKDLACAR EYNGLLVLPP IITAEMQALY TSSLVASMAF GGITAAGAIP FATQLQARIN  780
HLGITQSLLL KNQEKIAASF NKAIGHMQEG FRSTSLALQQ IQDVVSKQSA ILTETMASLN  840
KNFGAISSVI QEIYQQFDAI QANAQVDRLI TGRLSSLSVL ASAKQAEYIR VSQQRELATQ  900
KINECVKSQS IRYSFCGNGR HVLTIPQNAP NGIVFIHFSY TPDSFVNVTA IVGFCVKPAN  960
ASQYAIVPAN GRGIFIQVNG SYYITARDMY MPRAITAGDV VTLTSCQANY VSVNKTVITT 1020
FVDNDDFDFN DELSKWWNDT KHELPDFDKF NYTVPILDID SEIDRIQGVI QGLNDSLIDL 1080
EKLSILKTYI KWPGSGYIPE APRDGQAYVR KDGEWVLLST FLGRSLEVLF QGPGSAWSHP 1140
QFEKGGGSGG GGSGGSAWSH PQFEK                                       1165

SEQ ID NO: 12          moltype = AA  length = 1172
FEATURE                Location/Qualifiers
source                 1..1172
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 12
MLVKSLFLVT ILFALCSANL YDNESFVYYY QSAFRPGHGW HLYGGAYAVV NVSSENNNAG  60
TAPSCTAGAI GYSKNLSAAS VAMTAPLSGM SWSANSFCTA HCNFTSYIVF VTHCYKSGSN  120
SCPLTGLIPS GYIRIAAMKH GSAMPGHLFY NLTVSVTKYP KFRSLQCVNN YTSVYLNGDL  180
VFTSNYTEDV VAAGVHFKSG GPITYKVMRE VKALAYFVNG TAHDVILCDD TPRGLLACQY  240
NTGNFSDGFY PFTNTSIVKD KFIVYRESSV NTTLTLTNFT FSNESGAPPN TGGVDSFILY  300
QTQTAQSGYY NFNFSFLSSF VYRESYYMYG SYHPRCSFRP ETLNNGLWFN SLSVSLTYGP  360
IQGGCKQSVF NGKATCCYAY SYGGPRACKG VYRGELTQHF ECGLLVYVTK SDGSRIQTAT  420
QPPVLTQNFY NNINLGKCVD YNIYGRIGQG LITNVTDLAV SYNYLSDAGL AILDTSGAID  480
IFVVQGEYGP NYYKVNPCED VNQQFVVSGG KLVGILTSRN ETGSQLLENQ FYIKITNGTG  540
GGVPSVTENV TNCPYVSYGK FCIKPDGSIS VIVPKELDQF VAPLLNVTEY VLIPNSFNLT  600
VTDEYIQTRM DKIQINCLQY VCGNSLACRK LFQQYGPVCD NILSVVNSVG QKEDMELLNF  660
YSSTKPARFN TPVFSNLSTG EFNISLLLTP PSSPRRRSFI EDLLFTSVES VGLPTDDAYK  720
MRTAGPLGFL KDLACAREYN GLLVLPPIIT AEMQTLYTSS LVASMAFGGI TAAGAIPFAT  780
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QLQARINHLG ITQSLLLKNQ EKIAASFNKA IGHMQEGFRS TSLALQQIQD VVNKQSAILT  840
ETMLALNKNF GAISSVIQDI YQQLDSIQAD AQVDRLITGR LSSLSVLASA KQSEYIRVSQ  900
QRELATQKIN ECVKSQSIRY SFCGNGRHVL TIPQNAPNGI VFIHFTYTPE SFINVTAVVG  960
FCVSPANASQ YAIVPANGRG IFIQVNGSYY ITARDMYMPR DITAGDIVTL TSCQANYVSV  1020
NKTVITTFVD NDDFDFDDEL SKWWNETKHE LPDFDKFNYT VPILDIDSEI DRIQGVIQGL  1080
NDSLIDLETL SILKTYIKWP GSGYIPEAPR DGQAYVRKDG EWVLLSTFLG RSLEVLFQGP  1140
GSAWSHPQFE KGGGSGGGGS GGSAWSHPQF EK                                1172

SEQ ID NO: 13          moltype = AA  length = 1175
FEATURE                Location/Qualifiers
source                 1..1175
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 13
MLVKSPFIVT LLCALCSASL YDNGSYVYYY QSAFRPSIGW HLHGGAYAVV NVTQEYNNAG  60
SASECTAGAI VWSKNFSAAS VAMTAPHSGM SWSVKQFCTA HCNFTNFVVF VTHCFKDGLN  120
TCPLTGRIDQ GYIRIAAMKN TGTGPRDLFY NFTVSVTKYP SFKSLQCVNN QTSVYLNGDL  180
VFTSNETVDV SGAGVHFKAG GPITYKVMRE VKALAYFVNG TAQDVILCDS SPRGLLACQY  240
NTGNFSDGFY PFTNSSVVKE KFIVYSENSV NTTLVLHNFT FYNESDAPPN SQQSSAGVGG  300
LTTYQTQTAQ SGYYNFNFSF LSSFVYKESN FMYGSYHPQC NFRPENINNG LWFNSLSVSI  360
TYGPLQGGCK QSVFSHRATC CYAYSYNGPH ICKGVYSGQL HNNFECGLLV YITKTDGSRI  420
QTATTPPVRT QHFYNNITLH KCVEYNIYGR VGQGFITNVT DSVAGYNYLQ DGGLAILDTS  480
GAIDIFAVQG GYGLNFYKVN PCEDVNQQFV VSGGNLVGIL TSRNETDSQP LENQFFVKLI  540
NGTGGGVPSI SENVTSCSFV SYGKFCIKPD GSISTIVPKE MEQFVAPLLN VTEHVLIPDS  600
FNLTVTDEYI QTRMDKVQIN CLQYVCGNSF ECRQLFQQYG PVCDNILSVV NSVGQKEDME  660
LLSFYSSTKP AGYNTPVFNI STGDFNISLL LPPSSAPSGR SFIEDLLFTS VESVGLPTDE  720
AYKKCTAGPL GFLKDLACAR EYNGLLVLPP IITAEMQTLY TSSLVASMAL GGITAAGAIP  780
FATQLQARIN HLGITQTVLL KNQEKIAASF NKAIGHMQEG FKSTSLALQQ IQDVVNKQSA  840
ILTETMASLN KNFGAISSVI QEIYQQLDAI QANAQVDRLI TGRLSSLSVL ASSKQAEYLR  900
VSQQRELATQ KINECVKSQS TRYSFCGNGR HVLTIPQNAP NGIVFIHFTY TPESFVNVTA  960
IVGFCINPAN ASQYAIVPAN GRGIFIQVNG TYYITARDMF MPRDITAGDV VTLTSCQANY  1020
VSVNKTVITT FVESDDFDFD DELSKWWNET KHEFPDFDQF NYTIPVLNIT YDIDKIEEVI  1080
KGLNDSLIDL ETLSILKTYI KWPGSGYIPE APRDGQAYVR KDGEWVLLST FLGRSLEVLF  1140
QGPGSAWSHP QFEKGGGSGG GGSGGSAWSH PQFEK                             1175

SEQ ID NO: 14          moltype = AA  length = 1169
FEATURE                Location/Qualifiers
source                 1..1169
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 14
MLVKSLFTVI PLFALCSATL YDSGSYVYYY QSAFRPPNGW QLHGGAYAVV NVSTETGSAN  60
RCTAGAISFS KNFSAASVAM TAPANGMTWS DAQFCTAHCN FTNIVVFVTH CFKNRPNYCS  120
LTGLIPQNYI RIAAMKSNGT GPSDLFYNLT VPVTKYPKFR SLQCVNNQTS VYLNGDLVFT  180
SNETVDISGA GVHFAAGGPI TYKVMREVKA LAYFVNGTAQ DVILCDGTPR GLLACQYNTG  240
NFSDGFYPFT NSSLVKERFI VYRENSVNTT LVLHNVTFFN ETSAPNGGDL NANFQIYQTV  300
TAQSGYYNFN FSFLSGFVYK ESDFMYGSYH PNCNFRPENI NNGLWFNSLS ISLAYGPLQG  360
GCKQSVFNRR ATCCYAYSYN GPHACKGVYR GQLTQLFECG LLVYITKSDG SRIQTATKAL  420
VVTTNFYNNI TLDRCVEYNI YGRVGQGFIT NVTDSTADYN YLADGGLAIL DTSGAIDIFV  480
VQGVYGLNFY KVNPCEDVNQ QFVVSGGKLV GILTSRNETD SQFLENQFYI KLTNETHGGG  540
VPVSENVTSC PYVSYGKFCI KPDGSISTIV PEELKQFVSP LLNVTEYVLI PDSFNLTVTD  600
EYIQTRMDKV QINCLQYVCG NSFECRNLFQ QYGPVCDNIL SVVNSVGQKE DMELLTFYSS  660
TKPAGYNTPV FNNISTGDFN ISLLLTPPST PSGRSFIEDL LFTSVESVGL PTDEAYKKCT  720
AGPLGFLKDL ACAREYNGLL VLPPIITAEM QTLYTSSLVA SMALGGITAA GAIPFATQLQ  780
ARINHLGITQ TILLKNQEKI AASFNKAIGH MQEGFKSTSL ALQQIQDVVN KQSAILTETM  840
ASLNKNFGAI SSVIQEIYQQ LDSIQANAQV DRIITGRLSS LSVLASSKQA EYLRVSQQRE  900
LATQKINECV KSQSTRYSFC GNGRHVLTIP QNAPNGIVFI HFTYTPESFV NVTAIVGFCV  960
NPANASQYAI VPANGRGIFI QVNGSYYITA RDMYMPRDIT AGDIVTLTSC QANYVSVNKT  1020
VITTLVDNDD FDFHDELSKW WNETKHELPD FDQFNYTIPV LNITYDIDKI EEVIKGLNDS  1080
LIDLETLSIL KTYIKWPGSG YIPEAPRDGQ AYVRKDGEWV LLSTFLGRSL EVLFQGPGSA  1140
WSHPQFEKGG GSGGGGSGGS AWSHPQFEK                                    1169

SEQ ID NO: 15          moltype = AA  length = 1172
FEATURE                Location/Qualifiers
source                 1..1172
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 15
MSVLLPLLVT LLCALCSAVL YDINSYVYYY QSAFRPSNGW HLYGGAYAVV NVSNENNNAG  60
SASTCTAGAI GYSKNFSAAS IAMTAPPSGM AWSTAAFCTA HCNFTNIVVF VTHCYKSGSG  120
SCPLTGFIQS GYIRISAMKK ECSGPSCLFY NLTESVSKYP TFRSLQCVNN YTSVYLNGDL  180
VFTSNYTQDV VAAGVHFKSG GPITYKVMRE VKALAYFVNG TAQDVILCDD TPRGLLACQY  240
NTGNFSDGFY PFTNTSIVKD KFIVYRESSV NTTLTLTNFT FSNESGAPPN TGGVNSFILY  300
QTQTAQSGYY NFNFSFLSGF VYEESNYMYG SYHPLCSFRP ENINNGLWFN SLSVSITYGP  360
LQGGCKQSFF QGRATCCYAY SYNGPRACKG VYSGELTQSF ECGLLVYITK SDGSRIQTAT  420
KAPVVTTNFY NNITLDKCVE YNIYGRVGQG FITNVTDSAF GYNYLQDGGL AILDTSGAID  480
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IFVVKGVYGL NYYKVNPCED VNQQFVVSGG TLVGVLTSRN ETGSQFLENQ FYIKLTNGTH  540
GGGVPVNENV TSCPYVSYGK FCIKPDGSTS VIVPKELEQF VTPLLNATEY VPIPDSFNLT  600
VTDEYIQTRM DKVQINCLQY VCGNSFECRN LFQQYGPVCD NILSIVNSVS QKEDMELLTF  660
YSSTKPFGFN TPILSNLSTG DFNISLLLTP PSSTTGRSFI EDLLFTSVES VGLPTDDAYK  720
KCTAGPLGFL KDLACAREYN GLLVLPPIIT AEMQTMYTSS LVASMALGGI TAAGAIPFAT  780
QLQARINHLG ITQAVLLKNQ EKIAASFNKA IGQMQEGFRS TSLALQQIQD VVNKQSAILT  840
ETMASLNKNF GAISSVIQDI YQQLDVIQAD AQVDRLITGR LSSLSVLASA KQSEHIIASQ  900
QRELATQKIN ECVKSQSTRY SFCGNGRHVL TIPQNAPNGI VFIHFTYTPE SFVNVTAIVG  960
FCVKPANASQ YAIVPANGRG IFIQFNGSYY ITARDMYMPR NITAGDIVTL TSCQSNYVSV  1020
NKTVITTFVD NDDFDFDDEL SKWWNDTKHE LPDFDEFNYT APILDIDSEI DRIQGVIQGL  1080
NDSLIDLETL SILKTYIKWP GSGYIPEAPR DGQAYVRKDG EWVLLSTFLG RSLEVLFQGP  1140
GSAWSHPQFE KGGGSGGGGS GGSAWSHPQF EK                                 1172

SEQ ID NO: 16          moltype = AA  length = 1162
FEATURE                Location/Qualifiers
source                 1..1162
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 16
MLVTPLLLVT LLFALCSAAL YDNSSYVYYY QSAFRPPNGW HLHGGAYAVV NTSIESNNLR  60
ECIVGIIGGD RVVNASSIAM TAPQPGMDWS SRQFCTAHCN FSDITVFVTH CYKHNGCPIT  120
GSIPQHSIRV SAMKKGRLFY NLTVSVNKYP TFKSFQCVNN FTSVYLNGDL VYTSNETTDV  180
TSAGVYFNAG GPITYKVMRE VKALAYFVNG TAQDVILCDG SPRGLLSCQY NTGNFSDGFY  240
PFTNSSLVKQ KFIVYRENSI NTTLKLHNFT FHNETGANPN LSGVQNIQTY QTQTAQSGYY  300
NFNFSFLSGF VYKESNFMYG SYHPSCNFRP ETINNGLWFN SLSVSIAYGP LQGGCKQSVF  360
SGRATCCYAY SYGGPSLCKG VYLGELKSDF ECGLLVYVTK SDGSRIQTAT EPPVITQHNY  420
NNITLNTCVD YNIYGRTGQG FITNVTDSAV SYNYLADAGM AILDTSGSID IFVVQGEYGL  480
TYYKVNPCED VNQQFVVSGG KLVGILTSRN ETGSQLLENQ FYIKITNGTG GGVPSITANV  540
TNCPYVSYGK FCIKPDGSVS AIVPKELEQF VAPLLNVTEN VLIPNSFNLT VTDEYIQTRM  600
DKIQINCMQY VCGNSLDCRK LFQQYGPVCD NILSVVNSVG QKEDMELLNF YSSTKPSGFN  660
TPVFSNLSTG DFNISLLLTP PSSTTGRSFI EDLLFTSVES VGLPTDEAYK KCTAGPLGFL  720
KDLACAREYN GLLVLPPIIT AEMQTLYTSS LVASMAFGGI TAAGAIPFAT QLQARINHLG  780
ITQSLLQKNQ EKIAASFNKA IAVVQEGFRS TSLALQQVQD VVNKQSAILT ETMASLNKNF  840
GAISSVIQDI YQQLDAIQAN AQVDRLITGR LSSLSVLASA KQAEYIRVSQ QRELATQKIN  900
ECVKSQSIRY SFCGNGRHVL TIPQNAPNGI VFIHFTYTPE SFVNVTAIVG FCVKPANASQ  960
YAIVPANGRG IFIQVNGSYY ITARDMYMPR DITAGDIVTL TSCQANYVSV NKTVITTFVD  1020
NDDFDFDDEL SKWWNDTKHE LPDFDEFNYT VPILDIGSEI DRIQGVIQGL NDSLIDLETL  1080
SILKTYIKWP GSGYIPEAPR DGQAYVRKDG EWVLLSTFLG RSLEVLFQGP GSAWSHPQFE  1140
KGGGSGGGGS GGSAWSHPQF EK                                            1162

SEQ ID NO: 17          moltype = AA  length = 1162
FEATURE                Location/Qualifiers
source                 1..1162
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 17
MLGKSLFIVT LLLALCEGGL VGVNYTYYYQ SRYRPPNGWH MQGGAYKVVN KTTISYTSQE  60
CTIGVIRGGV TINQSAIAFT SATGRVGVKK GVCTVYCNYT SFYVFVTHCG GTGHNCIVNT  120
KKLGVLVFGV KNYNDQFIYN ITLNAAGPYA NFKAWQCLSN YTSVFLNGNL LYTSNYTEDV  180
KAAGVYAKQV NGLERRVMRD TPVMAYFVNG TVQDVILCDD SPKGRLACQY NTGNFSDGLY  240
PVYEEPVASN FTFVPLHTSS TSYGVLHNFT FNNVTGVAPN QEHIARFNIS TISEGYVNFK  300
FNFLNSFTYV ESDFDRGSYY GKPGSRCNFG LESINRGLSF NSLTVSIGYG PISGGCKQSV  360
WKNEATCCFA YKYNGGSRNC KGLYTFDRDV NYECVLLVFI SKPDGSRIRT ATSPPVYSNN  420
NVNINLGLCV DYNVYGITGR GLITNITESV HPGYLDHGGL VLLDATGSID TFVLHSDKLT  480
SYYKVNPCSD INEQYVVSGG NLVGKLTSNN QTVAQQLGDM FYVKFSTSGG GGVPATSENV  540
TSCPYVTYGK FCIKPDGDIS NIVPEEVKDY TSLLLNRTDY VLIPNSFNLT VTDEFIQTQF  600
QKIQINCIQY VCGSSIQCKQ LFQQYGSVCG NILSIVNGIA LQDNAEMLHF YSSTKPRGFD  660
TNSFVNFTAG EFNISLVLPK NGQPTGRCLI EDLLFDKVES LGLPGDSAYQ KCTSGPLGFV  720
KDLVCAQNYN GLLVLPPIIT AEMQTLYTSS LVVSMAFGGI TAARAIPFAT QIQARINHLG  780
ITQTVLQKNQ EKIAASFNKA MKHMQDGFSA TSLALQQVQD VVNEQGAILQ QTMHSLNKNF  840
GAISHVIQDI YKQLDALEAN AQVDRIITGR LSSLSVLASA KQLEYTKVSQ QRELAKEKIN  900
ECVKSQSNRH GFCGEGMHIM SIPQNAPNGI VFLHFTYTPE TYANVTAVVG FCVKPGNGTE  960
YGLVPVVGRG IFIEVNGTYY ITGRDMYSPR AITAGDVVKL TPCQANYQSI NRTVITTFVD  1020
EDDFDFDHEL SKWWNETSRD FPNLDEFNYT IPVLNISNEI DKIQEVIQGL NDSIIDLETL  1080
SILKTYIKWP GSGYIPEAPR DGQAYVRKDG EWVLLSTFLG RSLEVLFQGP GSAWSHPQFE  1140
KGGGSGGGGS GGSAWSHPQF EK                                            1162

SEQ ID NO: 18          moltype = AA  length = 412
FEATURE                Location/Qualifiers
source                 1..412
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 18
MASGKATGKT DAPAPVIKLG GPKPPKVGSS GNASWFQAIK AKKLNSHPPK FEGSGVPDNE  60
NLKTSQQHGY WRRQARFKPV KGGRKPVPDA WYFYYTGTGP AADLNWGDSQ DGIVWVAAKG  120
ADVKSRSHQG TRDPDKFDQY PLRFSDGGPD GNFRWDFIPL NRGRSGRSTA ASSAASSRAP  180
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SRDGSRGRRS GSEDDLIARA AKIIQDQQKK GSRITKVKAD EMAHRRYCKR TIPPGYKVDQ  240
VFGPRTKGKE GNFGDDKMNE EGIKDGRVTA MLNLVPSSHA CLFGSRVTPK LQPDGLHLKF  300
EFTTVVPRDD PQFDNYVKIC DQCVDGVGTR PKDDEPRPKS RSSSRPATRT SSPAPRQQRP  360
KKEKKPKKQD DEVDKALTSN EERNNAQLEF DEEPKVINWG DAALGENELG GG          412

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FEATURE                Location/Qualifiers
source                 1..22
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 19
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SEQ ID NO: 20          moltype = DNA  length = 21
FEATURE                Location/Qualifiers
source                 1..21
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 20
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SEQ ID NO: 21          moltype = AA  length = 1169
FEATURE                Location/Qualifiers
source                 1..1169
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
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TAPSCTAGAI GYSKNLSAAS VAMTAPLSGM SWSANSFCTA HCNFTSYIVF VTHCYKSGSN  120
SCPLTGLIPS GYIRIAAMKH GSAMPGHLFY NLTVSVTKYP KFRSLQCVNN YTSVYLNGDL  180
VFTSNYTEDV VAAGVHFKSG GPITYKVMRE VKALAYFVNG TAHDVILCDD TPRGLLACQY  240
NTGNFSDGFY PFTNTSIVKD KFIVYRESSV NTTLTLTNFT FSNESGAPPN TGGVDSFILY  300
QTQTAQSGYY NFNFSFLSSF VYRESYYMYG SYHPRCSFRP ETLNNGLWFN SLSVSLTYGP  360
IQGGCKQSVF NGKATCCYAY SYGGPRACKG VYRGELTQHF ECGLLVYVTK SDGSRIQTAT  420
QPPVLTQNFY NNINLGKCVD YNIYGRIGQG LITNVTDLAV SYNYLSDAGL AILDTSGAID  480
IFVVQGEYGP NYYKVNPCED VNQQFVVSGG KLVGILTSRN ETGSQLLENQ FYIKITNGTR  540
RSRRSVTENV TNCPYVSYGK FCIKPDGSIS VIVPKELDQF VAPLLNVTEY VLIPNSFNLT  600
VTDEYIQTRM DKIQINCLQY VCGNSLACRK LFQQYGPVCD NILSVVNSVG QKEDMELLNF  660
YSSTKPARFN TPVFSNLSTG EFNISLLLTP PSSPRRRSFI EDLLFTSVES VGLPTDDAYK  720
MRTAGPLGFL KDLACAREYN GLLVLPPIIT AEMQTLYTSS LVASMAFGGI TAAGAIPFAT  780
QLQARINHLG ITQSLLLKNQ EKIAASFNKA IGHMQEGFRS TSLALQQIQD VVNKQSAILT  840
ETMLALNKNF GAISSVIQDI YQQLDSIQAD AQVDRLITGR LSSLSVLASA KQSEYIRVSQ  900
QRELATQKIN ECVKSQSIRY SFCGNGRHVL TIPQNAPNGI VFIHFTYTPE SFINVTAVVG  960
FCVSPANASQ YAIVPANGRG IFIQVNGSYY ITARDMYMPR DITAGDIVTL TSCQANYVSV  1020
NKTVITTFVD NDDFDFDDEL SKWWNETKHE LPDFDKFNYT VPILDIDSEI DRIQGVIQGL  1080
NDSLIDLETL SILKTYIKWP WYVWLAIAFA TIIFILILGW LFFMTGCCGC CCGCFGIIPL  1140
MSKCGKKSSY YTTFDNDVVT EQYRPKKSV                                    1169

SEQ ID NO: 22          moltype = AA  length = 3510
FEATURE                Location/Qualifiers
source                 1..3510
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 22
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CACCTCTACG GAGGCGCCTA TGCAGTGGTA AATGTGTCTA GTGAAAACAA TAACGCCGGG  180
ACCGCGCCGT CCTGCACAGC AGGGGCCATT GGCTATTCCA AAAACCTCAG TGCTGCCTCC  240
GTAGCCATGA CAGCTCCCCT CTCTGGGATG TCCTGGAGTG CCAATTCATT CTGCACCGCT  300
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AGTTGCCCCC TCACAGGGTT GATACCTTCT GGATATATCC GAATTGCAGC AATGAAGCAC  420
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AAATTCCGTT CCCTGCAGTG TGTGAACAAC TACACTTCTG TTTACTTGAA CGGAGACCTA  540
GTCTTCACTA GCAATTATAC CGAAGATGTG GTCGCTGCTG GGGTGCACTT CAAATCAGGG  600
GGCCCAATTA CGTACAAGGT GATGAGAGAA GTGAAGGCAC TAGCCTATTT CGTAAACGGT  660
ACTGCACACG ATGTCATCCT ATGTGACGAC ACACCTCGCG GATTGTTAGC CTGCCAGTAT  720
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AAATTTATTG TTTACCGGGA GAGTTCTGTC AACACAACAC TAACACTCAC AAATTTTACA  840
TTCTCTAATG AATCAGGAGC GCCACCAAAT ACGGGTGGAG TGGATTCTTT CATACTCTAT  900
CAGACACAAA CCGCTCAATC AGGGTATTAC AACTTTAATT TCAGCTTTCT CAGTTCATTT  960
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ATTCAGGGCG GCTGCAAACA GTCTGTCTTC AACGGAAAAG CTACCTGCTG CTATGCTTAC  1140
TCGTATGGAG GGCCCAGAGC TTGCAAGGGA GTGTATCGAG GAGAACTCAC ACAGCACTTT  1200
GAGTGCGGCC TGTTGGTTTA CGTCACTAAA TCCGACGGCA GTAGGATTCA GACAGCAACA  1260
CAGCCACCAG TGTTAACCCA GAACTTTTAT AATAATATAA ACCTAGGGAA ATGTGTTGAC  1320
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TACAACATTT ATGGAAGAAT CGGGCAGGGG CTGATCACCA ATGTCACAGA TCTTGCTGTT  1380
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GTGAACCAGC AGTTTGTTGT TAGCGGGGGC AAGCTTGTTG GCATACTCAC CAGCAGAAAT  1560
GAAACTGGAT CCCAGTTACT TGAAAATCAG TTCTACATCA AAATCACTAA CGGTACACGA  1620
AGGAGCCGAA GGTCAGTCAC GGAGAACGTT ACTAACTGTC CTTATGTCAG TTACGGTAAG  1680
TTCTGCATTA AGCCAGATGG AAGCATTAGC GTGATCGTCC CCAAGGAACT GGATCAGTTT  1740
GTAGCTCCAT TGCTTAATGT TACAGAATAT GTGCTTATTC CAAACTCCTT CAACCTGACT  1800
GTGACTGATG AATACATTCA GACCAGGATG GACAAGATAC AAATAAACTG CCTGCAATAT  1860
GTTTGTGGGA ATAGTCTAGC ATGCAGGAAA CTGTTTCAGC AGTATGGCCC CGTGTGTGAT  1920
AATATCCTTA GTGTCGTTAA TAGTGTTGGA CAGAAGGAGG ATATGGAGCT GCTCAATTTC  1980
TACAGCAGTA CTAAACCAGC CCGGTTCAAT ACTCCTGTGT TTTCCAACCT CTCTACAGGA  2040
GAATTTAATA TCTCTCTGCT GTTAACCCCG CCCTCCAGCC CTCGGCGGCG GTCATTTATC  2100
GAAGACCTCC TTTTCACCTC AGTGGAGTCA GTAGGTCTGC CCACGGATGA TGCATACAAG  2160
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CTGGTGGCAT CAATGGCGTT CGGAGGAATA ACCGCCGCCG GAGCAATTCC CTTCGCCACA  2340
CAACTGCAGG CCAGAATCAA CCATCTGGGG ATCACTCAGA GCTTATTGCT GAAGAACCAG  2400
GAGAAGATCG CTGCATCGTT CAACAAAGCG ATAGGCCACA TGCAGGAAGG TTTCCGCTCC  2460
ACCTCTTTGG CTTTGCAACA GATACAGGAC GTAGTCAATA AGCAGTCTGC AATCTTGACG  2520
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TTAAGCAGCC TATCTGTTTT GGCCAGCGCA AAACAATCTG AATATATCCG GGTCTCCCAA  2700
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TCGTTCTGTG GGAACGGCAG GCATGTGCTG ACTATCCCCC AGAATGCTCC TAACGGTATC  2820
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GACATCACAG CTGGCGACAT AGTAACACTT ACTAGCTGTC AAGCGAACTA CGTGAGTGTC  3060
AATAAAACTG TTATCACGAC CTTCGTGGAC AATGATGATT TTGACTTCGA CGATGAGCTT  3120
TCTAAATGGT GGAATGAGAC TAAGCATGAG CTGCCCGACT TTGATAAGTT TAACTACACA  3180
GTACCCATTC TCGATATTGA TAGTGAGATC GATCGTATTC AGGGTGTTAT CCAAGGACTT  3240
AACGACAGCC TGATAGACCT GGAGACGCTG AGCATTCTGA AGACATACAT AAAGTGGCCA  3300
TGGTACGTCT GGCTGGCCAT TGCCTTTGCG ACAATAATTT TTATTTTGAT TCTGGGCTGG  3360
CTCTTCTTCA TGACTGGTTG CTGTGGCTGT TGTTGCGGGT GCTTCGGTAT CATCCCTCTG  3420
ATGAGCAAGT GTGGCAAAAA GTCAAGCTAT TATACTACCT TTGATAACGA TGTGGTAACA  3480
GAGCAGTACC GCCCGAAAAA GTCCGTGTGA                                   3510

SEQ ID NO: 23          moltype = AA  length = 1172
FEATURE                Location/Qualifiers
source                 1..1172
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
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SASECTAGAI VWSKNFSAAS VAMTAPHSGM SWSVKQFCTA HCNFTNFVVF VTHCFKDGLN  120
TCPLTGRIDQ GYIRIAAMKN TGTGPRDLFY NFTVSVTKYP SFKSLQCVNN QTSVYLNGDL  180
VFTSNETVDV SGAGVHFKAG GPITYKVMRE VKALAYFVNG TAQDVILCDS SPRGLLACQY  240
NTGNFSDGFY PFTNSSVVKE KFIVYSENSV NTTLVLHNFT FYNESDAPPN SQQSSAGVGG  300
LTTYQTQTAQ SGYYNFNFSF LSSFVYKESN FMYGSYHPQC NFRPENINNG LWFNSLSVSI  360
TYGPLQGGCK QSVFSHRATC CYAYSYNGPH ICKGVYSGQL HNNFECGLLV YITKTDGSRI  420
QTATTPPVRT QHFYNNITLH KCVEYNIYGR VGQGFITNVT DSVAGYNYLQ DGGLAILDTS  480
GAIDIFAVQG GYGLNFYKVN PCEDVNQQFV VSGGNLVGIL TSRNETDSQP LENQFFVKLI  540
NGTRRSRRSI SENVTSCSFV SYGKFCIKPD GSISTIVPKE MEQFVAPLLN VTEHVLIPDS  600
FNLTVTDEYI QTRMDKVQIN CLQYVCGNSF ECRQLFQQYG PVCDNILSVV NSVGQKEDME  660
LLSFYSSTKP AGYNTPVFNI STGDFNISLL LPPSSAPSGR SFIEDLLFTS VESVGLPTDE  720
AYKKCTAGPL GFLKDLACAR EYNGLLVLPP IITAEMQTLY TSSLVASMAL GGITAAGAIP  780
FATQLQARIN HLGITQTVLL KNQEKIAASF NKAIGHMQEG FKSTSLALQQ IQDVVNKQSA  840
ILTETMASLN KNFGAISSVI QEIYQQLDAI QANAQVDRLI TGRLSSLSVL ASSKQAEYLR  900
VSQQRELATQ KINECVKSQS TRYSFCGNGR HVLTIPQNAP NGIVFIHFTY TPESFVNVTA  960
IVGFCINPAN ASQYAIVPAN GRGIFIQVNG TYYITARDMF MPRDITAGDV VTLTSCQANY  1020
VSVNKTVITT FVESDDFDFD DELSKWWNET KHEFPDFDQF NYTIPVLNIT YDIDKIEEVI  1080
KGLNDSLIDL ETLSILKTYI KWPWYVWLAI FFAIIIFILV LGWIFFMTGC CGCCCGCFGI  1140
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SEQ ID NO: 24          moltype = DNA  length = 3519
FEATURE                Location/Qualifiers
source                 1..3519
                       mol_type = other DNA
                       organism = Infectious Bronchitis Virus
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ttaggctgga tcttctttat gaccggatgc tgtggctgtt gctgtgggtg ctttgggatt 3420
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gtggtgacag agcagtatcg acctaagaag agtgtttga                        3519
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SEQ ID NO: 25          moltype = AA  length = 1166
FEATURE                Location/Qualifiers
source                 1..1166
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 25
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LTGLIPQNYI RIAAMKSNGT GPSDLFYNLT VPVTKYPKFR SLQCVNNQTS VYLNGDLVFT  180
SNETVDISGA GVHFAAGGPI TYKVMREVKA LAYFVNGTAQ DVILCDGTPR GLLACQYNTG  240
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TAQSGYYNFN FSFLSGFVYK ESDFMYGSYH PNCNFRPENI NNGLWFNSLS ISLAYGPLQG  360
GCKQSVFNRR ATCCYAYSYN GPHACKGVYR GQLTQLFECG LLVYITKSDG SRIQTATKAL  420
VVTTNFYNNI TLDRCVEYNI YGRVGQGFIT NVTDSTADYN YLADGGLAIL DTSGAIDIFV  480
VQGVYGLNFY KVNPCEDVNQ QFVVSGGKLV GILTSRNETD SQFLENQFYI KLTNETHRSR  540
RSVSENVTSC PYVSYGKFCI KPDGSISTIV PEELKQFVSP LLNVTEYVLI PDSFNLTVTD  600
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TKPAGYNTPV FNNISTGDFN ISLLLTPPST PSGRSFIEDL LFTSVESVGL PTDEAYKKCT  720
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ARINHLGITQ TILLKNQEKI AASFNKAIGH MQEGFKSTSL ALQQIQDVVN KQSAILTETM  840
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VITTLVDNDD FDFHDELSKW WNETKHELPD FDQFNYTIPV LNITYDIDKI EEVIKGLNDS  1080
LIDLETLSIL KTYIKWPWYV WLAIFFAIII FILILGWVFF MTGCCGCCCG CFGIIPLMSK  1140
CGKKSSYYTT FDNDVVTEQY RPKKSV                                       1166

SEQ ID NO: 26          moltype = DNA  length = 3501
FEATURE                Location/Qualifiers
source                 1..3501
                       mol_type = other DNA
                       organism = Infectious Bronchitis Virus
SEQUENCE: 26
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cagctgcatg gaggcgccta tgcagtggtg aatgtctcaa ccgagacagg ttccgccaac  180
aggtgtactg cgggagccat aagtttcagc aaaaattttt ccgccgcgag cgtagctatg  240
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gtatatcggg agaactcagt caacaccacg cttgttctgc acaacgttac attctttaat  840
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acagcgcaaa gtggatatta taacttcaac ttttctttcc tgtctggatt cgtttataaa  960
gaaagcgatt ttatgtatgg atcctaccat ccaaactgca acttccgccc tgaaaatatt  1020
aataatgggc tgtggtttaa cagtctgagc atcagcctgg cttatggtcc cctgcaaggc  1080
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SEQ ID NO: 27          moltype = AA  length = 1169
FEATURE                Location/Qualifiers
source                 1..1169
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organism = Infectious Bronchitis Virus

SEQUENCE: 27

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SEQ ID NO: 28 moltype = DNA length = 3510

FEATURE Location/Qualifiers

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mol_type = other DNA

organism = Infectious Bronchitis Virus

SEQUENCE: 28

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cacttgtatg ggggagccta tgcagtggtt aatgtctcca atgaaaataa caatgccggc  180
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SEQ ID NO: 29          moltype = AA  length = 1159
FEATURE                Location/Qualifiers
source                 1..1159
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
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SEQ ID NO: 30          moltype = AA  length = 3480
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source                 1..3480
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                       organism = Infectious Bronchitis Virus
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source                 1..3480
                       mol_type = other DNA
                       organism = Infectious Bronchitis Virus
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tctttttacg tttttgtgac tcactgcggg ggtactggtc acaactgcat tgttaacact  360
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attactctga atgccgctgg accctatgcc aactttaaag cctggcagtg tctgagtaac  480
tatacgagtg ttttcttaaa tggaaacctc ttatatacat cgaattacac agaagatgtt  540
aaggcagcgg gggtatatgc aaagcaggtc aacggacttg agagaagagt gatgagggac  600
accccagtca tggcctactt tgtaaatggt accgtgcagg atgtcatctt gtgtgatgat  660
tctccaaaag ggaggctagc ctgccagtat aatacaggga actttagcga cggactatat  720
cctgtatatg aggaaccagt ggcttcgaac ttcaccttcg tccctctcca cacatcttct  780
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tgctgtggtt gcttcggtat cataccactc atgtcaaaat gtggaaagaa aagctcctat 3420
tacaccacct ttgacaacga tgtagtaact gaacagtaca gacctaagaa gtcagtgtga 3480

SEQ ID NO: 33 moltype = AA length = 1162
FEATURE Location/Qualifiers
source 1..1162
mol_type = protein
organism = Infectious Bronchitis Virus
SEQUENCE: 33
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PITGMLQKNF LRVSAMKNGQ LFYNLTVSVA KYPTFKSFQC VNNLTSVYLN GDLVYTSNET 180
TDVTSAGVYF KAGGPITYKV MREVKALAYF VNGTAQDVIL CDGSPRGLLA CQYNTGNFSD 240
GFYPFINSSL VKQKFIVYRE NSVNTTFTLH NFTFHNETGA NPNPSGVQNI QTYQTQTAQS 300
GYYNFNFSFL SSFVYKESNF MYGSYHPSCN FRLETINNGL WFNSLSVSIA YGPLQGGCKQ 360
SVFSGRATCC YAYSYGGPSL CKGVYSGELD LNFECGLLVY VTKSGGSRIQ TATEPPVITR 420
HNYNNITLNT CVDYNIYGRT GQGFITNVTD SAVSYNYLAD AGLAILDTSG SIDIFVVQGE 480
YGLTYYKVNP CEDVNQQFVV SGGKLVGILT SRNETGSQLL ENQFYIKITN GTRRFRRSIT 540
ENVANCPYVS YGKFCIKPDG SIATIVPKQL EQFVAPLLNV TENVLIPNSF NLTVTDEYIQ 600
TRMDKVQINC LQYVCGNSLD CRDLFQQYGP VCDNILSVVN SIGQKEDMEL LNFYSSTKPA 660
GFNTPFLSNV STGEFNISLL LTTPSSPRRR SFIEDLLFTS VESVGLPTDD AYKNCTAGPL 720
GFLKDLACAR EYNGLLVLPP IITAEMQTLY TSSLVASMAF GGITAAGAIP FATQLQARIN 780
HLGITQSLLL KNQEKIAASF NKAIGRMQEG FRSTSLALQQ IQDVVNKQSA ILTETMASLN 840
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KINECVKSQS IRYSFCGNGR HVLTIPQNAP NGIVFIHFSY TPDSFVNVTA IVGFCVKPAN 960
ASQYAIVPAN GRGIFIQVNG SYYITARDMY MPRAITAGDI VTLTSCQANY VSVNKTVITT 1020
FVDNDDFDFN DELSKWWNDT KHELPDFDKF NYTVPILDID SEIDRIQGVI QGLNDSLIDL 1080
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SEQ ID NO: 34 moltype = DNA length = 3489
FEATURE Location/Qualifiers
source 1..3489
mol_type = other DNA

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organism = Infectious Bronchitis Virus

SEQUENCE: 34

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tgcgacgggt cacctcgtgg cttgttggcc tgccagtaca atacaggaaa ttttagcgat  720
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tgtgggtgct gctgcggatg ttttggaatc atgcccctga tgtccaaatg cggaaagaag 3420
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tctgtttga                                                        3489
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SEQ ID NO: 35 moltype = AA length = 409

FEATURE Location/Qualifiers

source 1..409

mol_type = protein

organism = Infectious Bronchitis Virus

SEQUENCE: 35

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ADTKFRSNQG TRDSDKFDQY PLRFSDGGPD GNFRWDFIPL NRGRSGRSTA ASSAASSRAP  180
SREVSRGRRS GSEDDLIARA ARIIQDQQKK GSRITKAKAD EMAHRRYCKR TIPPNYKVDQ  240
VFGPRTKGKE GNFGDDKMNE EGIKDGRVTA MLNLVPSSHA CLFGSRVTPR LQPDGLHLKF  300
EFTTVVPRDD PQFDNYVKIC DQCVDGVGTR PTDDEPRPKS RSSSRPATRG NSPAPRQQRP  360
KKEKKPKKQD DEVDKALTSD EERNNAQLEF DDEPKVINWG DSALGENEL              409
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SEQ ID NO: 36 moltype = DNA length = 1230
FEATURE Location/Qualifiers
source 1..1230
mol_type = other DNA
organism = Infectious Bronchitis Virus
SEQUENCE: 36
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gccaagaagc tgaattctcc acccccaag tttgaaggtt ccggggtccc tgataacgag 180
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SEQ ID NO: 37 moltype = AA length = 409
FEATURE Location/Qualifiers
source 1..409
mol_type = protein
organism = Infectious Bronchitis Virus
SEQUENCE: 37
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NLKTSQQHGY WRRQARFKPV KGGRKPVPDA WYFYYTGTGP AADLNWGDSQ DGIVWVAAKG 120
ADVKSRSHQG TRDPDKFDQY PLRFSDGGPD GNFRWDFIPL NRGRSGRSTA ASSAASSRAP 180
SRDGSRGRRS GSEDDLIARA AKIIQDQQKK GSRITKVKAD EMAHRRYCKR TIPPGYKVDQ 240
VFGPRTKGKE GNFGDDKMNE EGIKDGRVTA MLNLVPSSHA CLFGSRVTPK LQPDGLHLKF 300
EFTTVVPRDD PQFDNYVKIC DQCVDGVGTR PKDDEPRPKS RSSSRPATRT SSPAPRQQRP 360
KKEKKPKKQD DEVDKALTSN EERNNAQLEF DEEPKVINWG DAALGENEL 409

SEQ ID NO: 38 moltype = DNA length = 1230
FEATURE Location/Qualifiers
source 1..1230
mol_type = other DNA
organism = Infectious Bronchitis Virus
SEQUENCE: 38
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gatgccgctt taggagagaa cgagctgtga 1230

SEQ ID NO: 39 moltype = AA length = 1173
FEATURE Location/Qualifiers
source 1..1173
mol_type = protein
organism = Infectious Bronchitis Virus
SEQUENCE: 39

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PITGMLQQNL IRVSAMKNGQ LFYNLTVSVA KYPTFRSFQC VNNLTSVYLN GDLVYTSNET  180
IDVTSAGVYF KAGGPITYKV MREVKALAYF VNGTAQDVIL CDGSPRGLLA CQYNTGNFSD  240
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YGLNYYKVNP CEDVNQQFVV SGGKLVGILT SRNETGSQLL ENQFYIKITN GTGGGVPSIT  540
ENVANCPYVS YGKFCIKPDG SIATIVPKQL EQFVAPLFNV TENVLIPNSF NLTVTDEYIQ  600
TRMDKVQINC LQYVCGSSLD CRKLFQQYGP VCDNILSVVN SVGQKEDMEL LNFYSSTKPA  660
GFNTPVLSNV STGEFNISLL LTTPSSRRKR SLIEDLLFTS VESVGLPTND AYKNCTAGPL  720
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FVDNDDFDFN DELSKWWNDT KHELPDFDKF NYTVPILDID SEIDRIQGVI QGLNDSLIDL  1080
EKLSILKTYI KWPGSGYIPE APRDGQAYVR KDGEWVLLST FLGRSLEVLF QGPGHHHHHH  1140
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SEQ ID NO: 40          moltype = AA  length = 1180
FEATURE                Location/Qualifiers
source                 1..1180
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 40
MLVKSLFLVT ILFALCSANL YDNESFVYYY QSAFRPGHGW HLYGGAYAVV NVSSENNNAG  60
TAPSCTAGAI GYSKNLSAAS VAMTAPLSGM SWSANSFCTA HCNFTSYIVF VTHCYKSGSN  120
SCPLTGLIPS GYIRIAAMKH GSAMPGHLFY NLTVSVTKYP KFRSLQCVNN YTSVYLNGDL  180
VFTSNYTEDV VAAGVHFKSG GPITYKVMRE VKALAYFVNG TAHDVILCDD TPRGLLACQY  240
NTGNFSDGFY PFTNTSIVKD KFIVYRESSV NTTLTLTNFT FSNESGAPPN TGGVDSFILY  300
QTQTAQSGYY NFNFSFLSSF VYRESYYMYG SYHPRCSFRP ETLNNGLWFN SLSVSLTYGP  360
IQGGCKQSVF NGKATCCYAY SYGGPRACKG VYRGELTQHF ECGLLVYVTK SDGSRIQTAT  420
QPPVLTQNFY NNINLGKCVD YNIYGRIGQG LITNVTDLAV SYNYLSDAGL AILDTSGAID  480
IFVVQGEYGP NYYKVNPCED VNQQFVVSGG KLVGILTSRN ETGSQLLENQ FYIKITNGTG  540
GGVPSVTENV TNCPYVSYGK FCIKPDGSIS VIVPKELDQF VAPLLNVTEY VLIPNSFNLT  600
VTDEYIQTRM DKIQINCLQY VCGNSLACRK LFQQYGPVCD NILSVVNSVG QKEDMELLNF  660
YSSTKPARFN TPVFSNLSTG EFNISLLLTP PSSPRRRSFI EDLLFTSVES VGLPTDDAYK  720
MRTAGPLGFL KDLACAREYN GLLVLPPIIT AEMQTLYTSS LVASMAFGGI TAAGAIPFAT  780
QLQARINHLG ITQSLLLKNQ EKIAASFNKA IGHMQEGFRS TSLALQQIQD VVNKQSAILT  840
ETMLALNKNF GAISSVIQDI YQQLDSIQAD AQVDRLITGR LSSLSVLASA KQSEYIRVSQ  900
QRELATQKIN ECVKSQSIRY SFCGNGRHVL TIPQNAPNGI VFIHFTYTPE SFINVTAVVG  960
FCVSPANASQ YAIVPANGRG IFIQVNGSYY ITARDMYMPR DITAGDIVTL TSCQANYVSV  1020
NKTVITTFVD NDDFDFDDEL SKWWNETKHE LPDFDKFNYT VPILDIDSEI DRIQGVIQGL  1080
NDSLIDLETL SILKTYIKWP GSGYIPEAPR DGQAYVRKDG EWVLLSTFLG RSLEVLFQGP  1140
GHHHHHHHHS AWSHPQFEKG GGSGGGGSGG SAWSHPQFEK                        1180

SEQ ID NO: 41          moltype = AA  length = 1183
FEATURE                Location/Qualifiers
source                 1..1183
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 41
MLVKSPFIVT LLCALCSASL YDNGSYVYYY QSAFRPSIGW HLHGGAYAVV NVTQEYNNAG  60
SASECTAGAI VWSKNFSAAS VAMTAPHSGM SWSVKQFCTA HCNFTNFVVF VTHCFKDGLN  120
TCPLTGRIDQ GYIRIAAMKN TGTGPRDLFY NFTVSVTKYP SFKSLQCVNN QTSVYLNGDL  180
VFTSNETVDV SGAGVHFKAG GPITYKVMRE VKALAYFVNG TAQDVILCDS SPRGLLACQY  240
NTGNFSDGFY PFTNSSVVKE KFIVYSENSV NTTLVLHNFT FYNESDAPPN SQQSSAGVGG  300
LTTYQTQTAQ SGYYNFNFSF LSSFVYKESN FMYGSYHPQC NFRPENINNG LWFNSLSVSI  360
TYGPLQGGCK QSVFSHRATC CYAYSYNGPH ICKGVYSGQL HNNFECGLLV YITKTDGSRI  420
QTATTPPVRT QHFYNNITLH KCVEYNIYGR VGQGFITNVT DSVAGYNYLQ DGGLAILDTS  480
GAIDIFAVQG GYGLNFYKVN PCEDVNQQFV VSGGNLVGIL TSRNETDSQP LENQFFVKLI  540
NGTGGGVPSI SENVTSCSFV SYGKFCIKPD GSISTIVPKE MEQFVAPLLN VTEHVLIPDS  600
FNLTVTDEYI QTRMDKVQIN CLQYVCGNSF ECRQLFQQYG PVCDNILSVV NSVGQKEDME  660
LLSFYSSTKP AGYNTPVFNI STGDFNISLL LPPSSAPSGR SFIEDLLFTS VESVGLPTDE  720
AYKKCTAGPL GFLKDLACAR EYNGLLVLPP IITAEMQTLY TSSLVASMAL GGITAAGAIP  780
FATQLQARIN HLGITQTVLL KNQEKIAASF NKAIGHMQEG FKSTSLALQQ IQDVVNKQSA  840
ILTETMASLN KNFGAISSVI QEIYQQLDAI QANAQVDRLI TGRLSSLSVL ASSKQAEYLR  900
VSQQRELATQ KINECVKSQS TRYSFCGNGR HVLTIPQNAP NGIVFIHFTY TPESFVNVTA  960
IVGFCINPAN ASQYAIVPAN GRGIFIQVNG TYYITARDMF MPRDITAGDV VTLTSCQANY  1020
VSVNKTVITT FVESDDFDFD DELSKWWNET KHEFPDFDQF NYTIPVLNIT YDIDKIEEVI  1080
KGLNDSLIDL ETLSILKTYI KWPGSGYIPE APRDGQAYVR KDGEWVLLST FLGRSLEVLF  1140
QGPGHHHHHH HHSAWSHPQF EKGGGSGGGG SGGSAWSHPQ FEK                    1183

SEQ ID NO: 42          moltype = AA  length = 1177
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FEATURE                 Location/Qualifiers
source                  1..1177
                        mol_type = protein
                        organism = Infectious Bronchitis Virus
SEQUENCE: 42
MLVKSLFTVI PLFALCSATL YDSGSYVYYY QSAFRPPNGW QLHGGAYAVV NVSTETGSAN  60
RCTAGAISFS KNFSAASVAM TAPANGMTWS DAQFCTAHCN FTNIVVFVTH CFKNRPNYCS  120
LTGLIPQNYI RIAAMKSNGT GPSDLFYNLT VPVTKYPKFR SLQCVNNQTS VYLNGDLVFT  180
SNETVDISGA GVHFAAGGPI TYKVMREVKA LAYFVNGTAQ DVILCDGTPR GLLACQYNTG  240
NFSDGFYPFT NSSLVKERFI VYRENSYVNT LVLHNVTFFN ETSAPNGGDL NANFQIYQTV  300
TAQSGYYNFN FSFLSGFVYK ESDFMYGSYH PNCNFRPENI NNGLWFNSLS ISLAYGPLQG  360
GCKQSVFNRR ATCCYAYSYN GPHACKGVYR GQLTQLFECG LLVYITKSDG SRIQTATKAL  420
VVTTNFYNNI TLDRCVEYNI YGRVGQGFIT NVTDSTADYN YLADGGLAIL DTSGAIDIFV  480
VQGVYGLNFY KVNPCEDVNQ QFVVSGGKLV GILTSRNETD SQFLENQFYI KLTNETHGGG  540
VPVSENVTSC PYVSYGKFCI KPDGSISTIV PEELKQFVSP LLNVTEYVLI PDSFNLTVTD  600
EYIQTRMDKV QINCLQYVCG NSFECRNLFQ QYGPVCDNIL SVVNSVGQKE DMELLTFYSS  660
TKPAGYNTPV FNNISTGDFN ISLLLTPPST PSGRSFIEDL LFTSVESVGL PTDEAYKKCT  720
AGPLGFLKDL ACAREYNGLL VLPPIITAEM QTLYTSSLVA SMALGGITAA GAIPFATQLQ  780
ARINHLGITQ TILLKNQEKI AASFNKAIGH MQEGFKSTSL ALQQIQDVVN KQSAILTETM  840
ASLNKNFGAI SSVIQEIYQQ LDSIQANAQV DRIITGRLSS LSVLASSKQA EYLRVSQQRE  900
LATQKINECV KSQSTRYSFC GNGRHVLTIP QNAPNGIVFI HFTYTPESFV NVTAIVGFCV  960
NPANASQYAI VPANGRGIFI QVNGSYYITA RDMYMPRDIT AGDIVTLTSC QANYVSVNKT  1020
VITTLVDNDD FDFHDELSKW WNETKHELPD FDQFNYTIPV LNITYDIDKI EEVIKGLNDS  1080
LIDLETLSIL KTYIKWPGSG YIPEAPRDGQ AYVRKDGEWV LLSTFLGRSL EVLFQGPGSA  1140
WSHPQFEKGG GSGGGGSGGH HHHHHHHSAW SHPQFEK                           1177

SEQ ID NO: 43           moltype = AA  length = 1180
FEATURE                 Location/Qualifiers
source                  1..1180
                        mol_type = protein
                        organism = Infectious Bronchitis Virus
SEQUENCE: 43
MSVLLPLLVT LLCALCSAVL YDINSYVYYY QSAFRPSNGW HLYGGAYAVV NVSNENNNAG  60
SASTCTAGAI GYSKNFSAAS IAMTAPPSGM AWSTAAFCTA HCNFTNIVVF VTHCYKSGSG  120
SCPLTGFIQS GYIRISAMKK ECSGPSCLFY NLTESVSKYP TFRSLQCVNN YTSVYLNGDL  180
VFTSNYTQDV VAAGVHFKSG GPITYKVMRE VKALAYFVNG TAQDVILCDD TPRGLLACQY  240
NTGNFSDGFY PFTNTSIVKD KPIVYRESSV NTTLTLTNFT FSNESGAPPN TGGVNSFILY  300
QTQTAQSGYY NFNFSFLSGF VYEESNYMYG SYHPLCSFRP ENINNGLWFN SLSVSITYGP  360
LQGGCKQSFF QGRATCCYAY SYNGPRACKG VYSGELTQSF ECGLLVYITK SDGSRIQTAT  420
KAPVVTTNFY NNITLDKCVE YNIYGRVGQG FITNVTDSAF GYNYLQDGGL AILDTSGAID  480
IFVVKGVYGL NYYKVNPCED VNQQFVVSGG TLVGVLTSRN ETGSQFLENQ FYIKLTNGTH  540
GGGVPVNENV TSCPYVSYGK FCIKPDGSTS VIVPKELEQF VTPLLNATEY VPIPDSFNLT  600
VTDEYIQTRM DKVQINCLQY VCGNSFECRN LFQQYGPVCD NILSIVNSVS QKEDMELLTF  660
YSSTKPFGFN TPILSNLSTG DFNISLLLTP PSSTTGRSFI EDLLFTSVES VGLPTDDAYK  720
KCTAGPLGFL KDLACAREYN GLLVLPPIIT AEMQTMYTSS LVASMALGGI TAAGAIPFAT  780
QLQARINHLG ITQAVLLKNQ EKIAASFNKA IGQMQEGFRS TSLALQQIQD VVNKQSAILT  840
ETMASLNKNF GAISSVIQDI YQQLDVIQAD AQVDRLITGR LSSLSVLASA KQSEHIIASQ  900
QRELATQKIN ECVKSQSTRY SFCGNGRHVL TIPQNAPNGI VFIHFTYTPE SFVNVTAIVG  960
FCVKPANASQ YAIVPANGRG IFIQFNGSYY ITARDMYMPR NITAGDIVTL TSCQSNYVSV  1020
NKTVITTFVD NDDFDFDDEL SKWWNDTKHE LPDFDEFNYT APILDIDSEI DRIQGVIQGL  1080
NDSLIDLETL SILKTYIKWP GSGYIPEAPR DGQAYVRKDG EWVLLSTFLG RSLEVLFQGP  1140
GHHHHHHHHS AWSHPQFEKG GGSGGGGSGG SAWSHPQFEK                        1180

SEQ ID NO: 44           moltype = AA  length = 1170
FEATURE                 Location/Qualifiers
source                  1..1170
                        mol_type = protein
                        organism = Infectious Bronchitis Virus
SEQUENCE: 44
MLVTPLLLVT LLFALCSAAL YDNSSYVYYY QSAFRPPNGW HLHGGAYAVV NTSIESNNLR  60
ECIVGIIGGD RVVNASSIAM TAPQPGMDWS SRQFCTAHCN FSDITVFVTH CYKHNGCPIT  120
GSIPQHSIRV SAMKKGRLFY NLTVSVNKYP TFKSFQCVNN FTSVYLNGDL VYTSNETTDV  180
TSAGVYFNAG GPITYKVMRE VKALAYFVNG TAQDVILCDG SPRGLLSCQY NTGNFSDGFY  240
PFTNSSLVKQ KFIVYRENSI NTTLKLHNFT FHNETGANPN LSGVQNIQTY QTQTAQSGYY  300
NFNFSFLSGF VYKESNFMYG SYHPSCNFRP ETINNGLWFN SLSVSIAYGP LQGGCKQSVF  360
SGRATCCYAY SYGGPSLCKG VYLGELKSDF ECGLLVYVTK SDGSRIQTAT EPPVITQHNY  420
NNITLNTCVD YNIYGRTGQG FITNVTDSAV SYNYLADAGM AILDTSGSID IFVVQGEYGL  480
TYYKVNPCED VNQQFVVSGG KLVGILTSRN ETGSQLLENQ FYIKITNGTG GGVPSITANV  540
TNCPYVSYGK FCIKPDGSVS AIVPKELEQF VAPLLNVTEN VLIPNSFNLT VTDEYIQTRM  600
DKIQINCMQY VCGNSLDCRK LFQQYGPVCD NILSVVNSVG QKEDMELLNF YSSTKPSGFN  660
TPVFSNLSTG DFNISLLLTP PSSTTGRSFI EDLLFTSVES VGLPTDEAYK KCTAGPLGFL  720
KDLACAREYN GLLVLPPIIT AEMQTLYTSS LVASMAFGGI TAAGAIPFAT QLQARINHLG  780
ITQSLLQKNQ EKIAASFNKA IAVVQEGFRS TSLALQQVQD VVNKQSAILT ETMASLNKNF  840
GAISSVIQDI YQQLDAIQAN AQVDRLITGR LSSLSVLASA KQAEYIRVSQ QRELATQKIN  900
ECVKSQSIRY SFCGNGRHVL TIPQNAPNGI VFIHFTYTPE SFVNVTAIVG FCVKPANASQ  960
YAIVPANGRG IFIQVNGSYY ITARDMYMPR DITAGDIVTL TSCQANYVSV NKTVITTFVD  1020
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NDDFDFDDEL SKWWNDTKHE LPDFDEFNYT VPILDIGSEI DRIQGVIQGL NDSLIDLETL  1080
SILKTYIKWP GSGYIPEAPR DGQAYVRKDG EWVLLSTFLG RSLEVLFQGP GHHHHHHHHS  1140
AWSHPQFEKG GGSGGGGSGG SAWSHPQFEK                                   1170

SEQ ID NO: 45          moltype = AA  length = 1170
FEATURE                Location/Qualifiers
source                 1..1170
                       mol_type = protein
                       organism = Infectious Bronchitis Virus
SEQUENCE: 45
MLGKSLFIVT LLLALCEGGL VGVNYTYYYQ SRYRPPNGWH MQGGAYKVVN KTTISYTSQE  60
CTIGVIRGGV TINQSAIAFT SATGRVGVKK GVCTVYCNYT SFYVFVTHCG GTGHNCIVNT  120
KKLGVLVFGV KNYNDQFIYN ITLNAAGPYA NFKAWQCLSN YTSVFLNGNL LYTSNYTEDV  180
KAAGVYAKQV NGLERRVMRD TPVMAYFVNG TVQDVILCDD SPKGRLACQY NTGNFSDGLY  240
PVYEEPVASN FTFVPLHTSS TSYGVLHNFT FNNVTGVAPN QEHIARFNIS TISEGYVNFK  300
FNFLNSFTYV ESDFDRGSYY GKPGSRCNFG LESINRGLSF NSLTVSIGYG PISGGCKQSV  360
WKNEATCCFA YKYNGGSRNC KGLYTFDRDV NYECVLLVFI SKPDGSRIRT ATSPPVYSNN  420
NVNINLGLCV DYNVYGITGR GLITNITESV HPGYLDHGGL VLLDATGSID TFVLHSDKLT  480
SYYKVNPCSD INEQYVVSGG NLVGKLTSNN QTVAQQLGDM FYVKFSTSGG GGVPATSENV  540
TSCPYVTYGK FCIKPDGDIS NIVPEEVKDY TSLLLNRTDY VLIPNSFNLT VTDEFIQTQF  600
QKIQINCIQY VCGSSIQCKQ LFQQYGSVCG NILSIVNGIA LQDNAEMLHF YSSTKPRGFD  660
TNSFVNFTAG EFNISLVLPK NGQPTGRCLI EDLLFDKVES LGLPGDSAYQ KCTSGPLGFV  720
KDLVCAQNYN GLLVLPPIIT AEMQTLYTSS LVVSMAFGGI TAARAIPFAT QIQARINHLG  780
ITQTVLQKNQ EKIAASFNKA MKHMQDGFSA TSLALQQVQD VVNEQGAILQ QTMHSLNKNF  840
GAISHVIQDI YKQLDALEAN AQVDRIITGR LSSLSVLASA KQLEYTKVSQ QRELAKEKIN  900
ECVKSQSNRH GFCGEGMHIM SIPQNAPNGI VFLHFTYTPE TYANVTAVVG FCVKPGNGTE  960
YGLVPVVGRG IFIEVNGTYY ITGRDMYSPR AITAGDVVKL TPCQANYQSI NRTVITTFVD  1020
EDDFDFDHEL SKWWNETSRD FPNLDEFNYT IPVLNISNEI DKIQEVIQGL NDSIIDLETL  1080
SILKTYIKWP GSGYIPEAPR DGQAYVRKDG EWVLLSTFLG RSLEVLFQGP GHHHHHHHHS  1140
AWSHPQFEKG GGSGGGGSGG SAWSHPQFEK                                   1170

SEQ ID NO: 46          moltype = DNA  length = 22
FEATURE                Location/Qualifiers
source                 1..22
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 46
atgctcaacc tagtccctag ca                                           22

SEQ ID NO: 47          moltype = DNA  length = 21
FEATURE                Location/Qualifiers
source                 1..21
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 47
tcaaactgcg gatcatcacg t                                            21
```

What is claimed:

1. A vaccine composition comprising a polynucleotide that encodes an infectious bronchitis virus (IBV) spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein.

2. The vaccine composition of claim **1**, further comprising an adjuvant.

3. The vaccine composition of claim **2**, wherein the adjuvant comprises disaggregated spherical nanostructures comprising Quil-A and chitosan, and wherein the Quil-A and chitosan are present at a ratio between 1:15 and 1:100.

4. The vaccine composition of claim **3**, wherein the chitosan is functionalized by treatment with 5-formyl-2-furan sulfonic acid and sodium borohydride, such that the chitosan surface is negatively charged.

5. The vaccine composition of claim **2**, wherein the spherical nanostructures are between about 5 nm and about 100 nm in diameter in the absence of a payload molecule.

6. The vaccine composition of claim **1**, wherein the S protein comprises one or more of the group consisting of SEQ ID NOs:11-17, 21, 23, 25, 27, 29, 31, and 33.

7. The vaccine composition of claim **1**, wherein the N protein comprises one or more of the group consisting of SEQ ID NOs:10, 18, 35, and 37.

8. A vaccine composition comprising an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein.

9. The vaccine composition of claim **8**, wherein the vaccine composition comprises a viral vector and the viral vector comprises a polynucleotide encoding the S protein, the N protein or both the S protein and the N protein.

10. The vaccine composition of claim **9**, wherein the viral vector is selected from an adeno-associated virus or a poxvirus.

11. The vaccine composition of claim **8**, wherein the S protein comprises one or more of the group consisting of SEQ ID NOs:11-17, 21, 23, 25, 27, 29, 31, and 33.

12. The vaccine composition of claim **8**, wherein the N protein comprises one or more of the group consisting of SEQ ID NOs:10, 18, 35, and 37.

13. The vaccine composition of claim **8**, further comprising an adjuvant.

14. The vaccine composition of claim **13**, wherein the adjuvant comprises disaggregated spherical nanostructures

comprising Quil-A and chitosan, and wherein the Quil-A and chitosan are present at a ratio between 1:15 and 1:100.

15. The vaccine composition of claim **14**, wherein the chitosan is functionalized by treatment with 5-formyl-2-furan sulfonic acid and sodium borohydride, such that the chitosan surface is negatively charged.

16. A method of inducing an immune response against infectious bronchitis virus (IBV) in a subject, the method comprising: administering the vaccine composition of claim **1** in an amount effective to induce the immune response against at least one IBV antigen in the subject.

17. The method of claim **16**, wherein the administration is by a route selected from the group consisting of intranasal, intramuscular, aerosol via inhalation, oral and in ovo.

18. The method of claim **16**, wherein the subject is an avian subject.

19. A method of claim **16**, further comprising administering a second vaccine composition comprising a protein, nucleic acid or viral vectored vaccine composition comprising a polynucleotide encoding the IBV S protein, the IBV N protein, or both or a polypeptide of the IBV S protein, the IBV N protein or both,

wherein administration of the vaccine composition of claim **1** and the second vaccine composition induces the immune response against at least one IBV antigen in the subject, and wherein administration of the second vaccine composition occurs at least two weeks after administration of the vaccine composition of claim **1**.

20. The method of claim **19**, wherein the second vaccine composition comprises an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein.

21. The method of claim **19**, wherein both the first vaccine composition and the second vaccine composition are administered by route selected from intranasal, intramuscular, oral, intranasal, and in ovo and the first vaccine composition and the second vaccine composition may be administered via the same or different routes.

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