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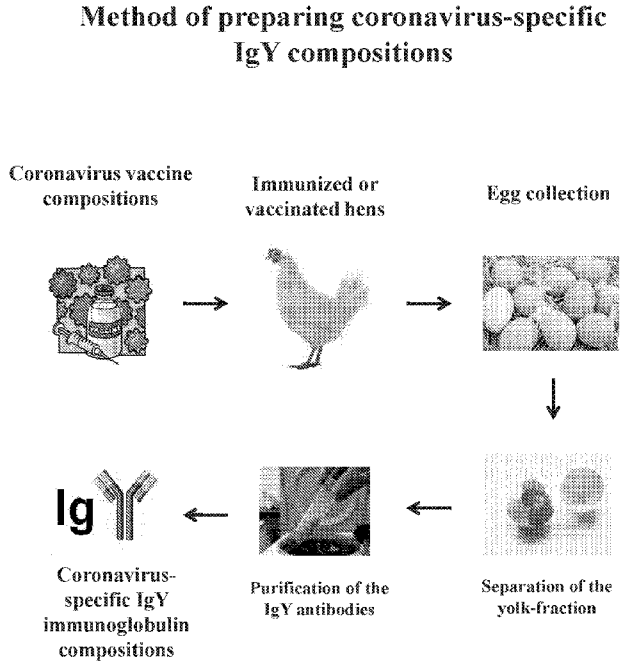

FIG. 1

(57) Abstract: The present disclosure provides a method of preparing compositions or vaccines for immunizing or vaccinating egg-laying fowl such as hens against coronavirus. The eggs can be collected and the coronavirus-specific IgY antibodies can be extracted and purified. The coronavirus-specific IgY immunoglobulin composition can be used to treat individuals exposed to, susceptible to, or infected with coronavirus.

[Continued on next page]

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IGY IMMUNOGLOBULINS TARGETING CORONAVIRUS, METHODS OF PREPARING SAME, AND METHODS OF USING SAME

Technical Field

[0001] The present disclosure generally relates to immunoglobulins from an egg-laying fowl specific to coronavirus. The present disclosure also relates to methods of immunization and methods of treating or preventing coronavirus infections. The immunoglobulins specific for coronavirus disclosed herein provide broad-spectrum immunity to individuals infected with or susceptible to coronavirus.

Cross Reference to Related Applications

[0002] This application claims the benefit of U.S. Provisional Application 63/008,298 filed on April 10, 2020, of which is hereby incorporated by reference in its entirety.

Sequence Listings

[0003] The instant application contains Sequence Listings which have been filed electronically in ASCII format and are hereby incorporated by reference in its entirety. Said ASCII copy, created on April 9, 2021, is named Sequence Listing for 1401870.00011.txt and is 8,192 bytes in size.

Background

[0004] Coronavirus disease 19 (COVID-19) is a highly transmissible and pathogenic viral infection caused by serious acute respiratory coronavirus syndrome 2 (SARS-CoV-2), which originated in Wuhan, China and has spread throughout the world. Genomic analysis has shown that SARS-CoV-2 is phylogenetically linked to severe, acute respiratory syndrome-like (SARS-like) bat viruses, so bats could be the primary reservoir. The intermediate source of origin and transfer to humans is not known but the rapid human transmission to humans has been widely established. No clinically approved antiviral drugs or vaccines for use against COVID-19 are

currently available. However, in clinical trials, few broad-spectrum antiviral drugs were assessed against COVID-19 which resulted in clinical recovery.

[0005] Coronaviruses belong to the family of Coronaviridae family, of the order Nidovirales. The "corona" represents crown-like spikes on the surface of the virus; therefore, it was named as a coronavirus. Coronaviruses are minute in size (65–125 nm in diameter) and contain a single-stranded RNA as a nucleic material with the size ranging from 26 to 32 kbs in length. (Anthony R. Fehr et al., at 1). The subgroups of coronaviruses family are alpha (α), beta (β), gamma (γ) and delta (δ) coronavirus. Most of the coronavirus can cause the infectious diseases in human and vertebrates. The α-CoV and β-CoV mainly infect the respiratory, gastrointestinal, and central nervous system of humans and mammals, while γ-CoV and δ-CoV mainly infect the birds (Stanley Perlman et al., at 1) (Yudong Yin et al., at 132). Usually, several members of the coronavirus cause mild respiratory disease in humans; however, SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV) explored in 2002–2003 and in 2012, respectively, caused fatal severe respiratory diseases (Kathryn V. Holmes et al., at 1949). The SARS-CoV and MERS-CoV belong to the β-CoV. 2019-nCoV also belongs to the β-CoV according to the phylogenetic analysis based on the viral genome (Fan Wu et al., at 267). Although the nucleotide sequence similarity is less than 80% between 2019-nCoV and SARS-CoV (about 79%) or MERS-CoV (about 50%), 2019-nCoV can also cause fetal infection and spread faster than the two other coronaviruses (Peng Zhou et al., at 270).

[0006] Initially, interferons-α nebulization, broad-spectrum antibiotics, and anti-viral drugs were used to reduce the viral load (Chen Seng Ng et al., at 2), (Ben X Wnag et al., at 1), (Manli Wang et al., at 269), however, only remdesivir has shown promising impact against the virus (Maria L. Agostini et al., at 1). Remdesivir only and in combination with chloroquine or interferon beta significantly blocked the SARS-CoV-2 replication and patients were declared as clinically recovered (Timothy P. Sheahan et al., at 10), (Michelle L. Holshue et al, at 931), (Manli Wang et al., at 270). Various other anti-virals are currently being evaluated against infection. Nafamostat, Nitazoxanide, Ribavirin, Penciclovir, Favipiravir, Ritonavir, AAK1, Baricitinib, and Arbidol exhibited moderate results when tested against infection in patients and in-vitro clinical isolates (Timothy P. Sheahan et al., at 10), (Peter Richardson et al., at 30), (Manli Wang et al., at 270). Several combinations, such as combining the antiviral or antibiotics with traditional Chinese

medicines were also evaluated against SARS-CoV-2 induced infection in humans and mice (Timothy P. Sheahan et al., at 3). Recently in Shanghai, doctors isolated the blood plasma from clinically recovered patients of COVID-19 and injected it in the infected patients who showed positive results with rapid recovery (Muhammad Adnam Shereen et al., at 94). In a recent study, it was identified that monoclonal antibody (CR3022) binds with the spike RBD of SARS-CoV-2. This is likely due to the antibody's epitope not overlapping with the divergent ACE2 receptor-binding motif. CR3022 has the potential to be developed as a therapeutic candidate, alone or in combination with other neutralizing antibodies for the prevention and treatment of COVID-19 infection (Muhammad Adnam Shereen et al., at 93).

Summary

[0007] The present inventors developed a method of producing coronavirus-specific IgY immunoglobulins using adenovirus vectors encoding coronavirus antigens and/or compositions comprising coronavirus antigens or coronavirus recombinant proteins. The adenovirus vectors encoding coronavirus antigens and/or compositions coronavirus antigens or recombinant proteins and can be used individually or in combination to immunize or vaccinate egg-laying fowl, such as hens, to generate polyclonal immunoglobulins with broad-spectrum specificity to coronavirus. The IgY immunoglobulins or antibodies can be extracted and purified from the yolk-fraction of the egg. The antibodies produced may be used for the prevention of viral adhesion, viral spread, the treatment of coronavirus infection, or the prevention of coronavirus infection. Antibodies of the IgY isotype from fowl or birds are particularly useful in these applications.

[0008] The coronavirus-specific immunoglobulins can be administered intranasally and/or orally for the treatment of an individual infected with coronavirus. Alternatively, or additionally, the immunoglobulins can be administered to an individual previously exposed or otherwise susceptible to coronavirus infection. The coronavirus-specific immunoglobulins can be in the form of a liquid or a lyophilized powder, reconstituted prior to administration, at a dose effective to reduce, alleviate, or eliminate the symptoms of an infected individual or at a dose effective to prevent infection or reduce the severity of the infection or symptoms for an individual previously exposed or susceptible to coronavirus infection.

[0009] In one embodiment disclosed herein is a method of treating coronavirus infection in an individual having the coronavirus infection, the method comprising administering a first composition to an egg-laying fowl, the first composition comprising one or more adenovirus vector(s) encoding one or more coronavirus antigen(s); and providing, to the individual having the coronavirus infection, a second composition, the second composition comprising a water-soluble fraction of egg yolk from the egg-laying fowl administered the first composition.

[0010] Also disclosed herein, the method of treating coronavirus infection, wherein the second composition is administered to the individual having the coronavirus infection, in an amount that provides a dose of the water-soluble fraction of egg yolk that is about 0.25 mg to about 1.0 mg per kg body weight of the individual having the coronavirus infection.

[0011] In another example embodiment, the method of treating coronavirus infection, wherein the second composition is administered intranasally.

[0012] In one aspect, the method of treating coronavirus infection, wherein the second composition is administered more than once to the individual having the coronavirus infection.

[0013] In one aspect, the method of treating coronavirus infection, wherein the first composition is administered more than once to the egg-laying fowl.

[0014] In another aspect, the method of treating coronavirus infection, wherein the first composition is administered to the egg-laying fowl a second time, at least 14 days after administration of the first composition.

[0015] In one embodiment disclosed herein, the method of treating coronavirus infection, wherein an additional composition is administered to the egg-laying fowl, at least at least 14 days after administration of the first composition.

[0016] In another aspect, the method of treating coronavirus infection, the additional composition comprises live or inactivated coronavirus, adenovirus vector encoding coronavirus antigens, coronavirus recombinant proteins, and/or inactivated Newcastle Disease Virus expressing anchored spike protein.

[0017] One embodiment disclosed herein, a method of preventing, decreasing incidence of, and/or decreasing severity of coronavirus infection in an individual at risk thereof, the method comprising administering a first composition to an egg-laying fowl, the first composition comprising one or more adenovirus vector(s) encoding one or more coronavirus antigen(s); and providing a second composition to the individual, the second composition comprising a water-soluble fraction of egg yolk from the egg-laying fowl who has been administered the first composition.

[0018] In another embodiment disclosed herein, the method of preventing, decreasing incidence of, and/or decreasing severity of coronavirus infection in an individual at risk thereof, wherein the second composition is administered to the individual in an amount that provides a dose of the water-soluble fraction of egg yolk that is about 0.25 mg to about 1.0 mg per kg body weight of the individual.

[0019] In another embodiment disclosed herein, the method of preventing, decreasing incidence of, and/or decreasing severity of coronavirus infection in an individual at risk thereof, wherein the second composition is administered to intranasally.

[0020] In another aspect, the method of preventing, decreasing incidence of, and/or decreasing severity of coronavirus infection in an individual at risk thereof, wherein the second composition is administered more than once to the individual.

[0021] In another embodiment, the method of preventing, decreasing incidence of, and/or decreasing severity of coronavirus infection in an individual at risk thereof, wherein the first composition is administered more than once to the egg-laying fowl.

[0022] In another aspect, the method of preventing, decreasing incidence of, and/or decreasing severity of coronavirus infection in an individual at risk thereof, wherein the first composition is administered to the egg-laying fowl a second time, at least 14 days after administration of the first composition.

[0023] In another aspect, the method of preventing, decreasing incidence of, and/or decreasing severity of coronavirus infection in an individual at risk thereof, wherein an additional

is administered to the egg-laying fowl, at least at least 14 days after administration of the first composition.

[0024] In another embodiment disclosed herein, the method of preventing, decreasing incidence of, and/or decreasing severity of coronavirus infection in an individual at risk thereof, wherein the additional composition comprises live or inactivated coronavirus, adenovirus vector encoding coronavirus antigens, coronavirus recombinant proteins, and/or inactivated Newcastle Disease Virus expressing anchored spike protein.

[0025] It is also understood that the present disclosure contemplates a method of producing an intranasally administrable composition effective to treat, prevent, decrease incidence of, and/or decrease severity of coronavirus infection in an infected individual, the method comprising adding a water-soluble fraction of egg yolk, which has been obtained from an egg-laying fowl administered an one or more adenovirus vector(s) encoding one or more coronavirus antigen(s), to a solution to form the composition.

[0026] Another embodiment disclosed herein, a unit dosage form comprising a therapeutically or prophylactically effective amount of a composition comprising a water-soluble fraction of egg yolk, which has been obtained from an egg-laying fowl administered one or more adenovirus vector(s) encoding one or more coronavirus antigen(s).

[0027] In another aspect, immunoglobulins specific against coronavirus for use in the treatment and/or prevention of coronavirus infection in a subject at risk thereof.

Brief Description of the Drawings

[0028] **FIG. 1** shows an embodiment of a method of making a coronavirus vaccine composition, an embodiment of a method of immunizing or vaccinating an egg-laying fowl, such as a hen, for producing coronavirus-specific IgY immunoglobulins, and an embodiment of a method for collecting and purifying IgY immunoglobulins from eggs laid by the immunized or vaccinated hens.

[0029] **FIG. 2** shows an embodiment of a method of treating individuals exposed to, susceptible to, or infected with coronavirus.

[0030] **FIG. 3** shows the specificity of the coronavirus-specific IgY immunoglobulins from immunized or vaccinated hens, compared to non-vaccinated or control hens (Table Egg IgY).

[0031] **FIG. 4** shows the virus neutralizing activity of the coronavirus-specific IgY immunoglobulins from immunized or vaccinated hens (AdV-S induced IgY), compared to non-vaccinated or control hens (Table Egg IgY and nucleocapsid-specific IgY).

Detailed Description

[0032] Definitions

[0033] Some definitions are provided hereafter. Nevertheless, definitions may be located in the "Embodiments" section below, and the above header "Definitions" does not mean that such disclosures in the "Embodiments" section are not definitions.

[0034] As used herein, "about," "approximately," and "substantially" are understood to refer to numbers in a range of numerals, for example the range of -10% to +10% of the referenced number, preferably -5% to +5% of the referenced number, more preferably -1% to +1% of the referenced number, most preferably -0.1% to +0.1% of the referenced number.

[0035] All numerical ranges herein should be understood to include all integers, whole or fractions, within the range. Moreover, these numerical ranges should be construed as providing support for a claim directed to any number or subset of numbers in that range. For example, a disclosure of from 1 to 10 should be construed as supporting a range of from 1 to 8, from 3 to 7, from 1 to 9, from 3.6 to 4.6, from 3.5 to 9.9, and so forth.

[0036] As used in this disclosure and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a component" or "the component" includes two or more components.

[0037] The words "comprise," "comprises," and "comprising" are to be interpreted inclusively rather than exclusively. Likewise, the terms "include," "including," "containing" and "having" should all be construed to be inclusive, unless such a construction is clearly prohibited from the context. Further in this regard, these terms specify the presence of the stated features but not preclude the presence of additional or further features.

[0038] Nevertheless, the compositions and methods disclosed herein may lack any element that is not specifically disclosed herein. Thus, a disclosure of an embodiment using the term "comprising" is (i) a disclosure of embodiments having the identified components or steps and also additional components or steps, (ii) a disclosure of embodiments "consisting essentially of" the identified components or steps, and (iii) a disclosure of embodiments "consisting of" the identified components or steps. Any embodiment disclosed herein can be combined with any other embodiment disclosed herein.

[0039] The term "and/or" used in the context of "X and/or Y" should be interpreted as "X," or "Y," or "X and Y." Similarly, "at least one of X or Y" should be interpreted as "X," or "Y," or "X and Y."

[0040] Where used herein, the terms "example" and "such as," particularly when followed by a listing of terms, are merely exemplary and illustrative and should not be deemed to be exclusive or comprehensive.

[0041] A "subject" or "individual" is a mammal, preferably a human.

[0042] As used herein, an "effective amount" is an amount that prevents an infection, treats a disease or medical condition in an individual, or, more generally, reduces symptoms, manages progression of the disease, or attenuates the viral infection for a period of time.

[0043] The term "fowl" refers to a wild, domestic, or domesticated egg-laying fowl, such as a chicken or hen, duck, swan, goose, turkey, peacock, guinea hen, ostrich, pigeon, quail, pheasant, dove, or other domestic egg-laying fowl. The terms "fowl," "egg-laying fowl," "chicken," and/or "hen" are used interchangeably herein.

[0044] The term "immunoglobulin" or "antibody" refers to glycoprotein molecules produced by leukocytes and lymphocytes that are involved in the body's immune system and immune response by specifically recognizing and binding to particular antigens and aiding in their neutralization. The terms "immunoglobulins," "antibodies," and "IgY" are used interchangeably herein.

[0045] The term “antigen,” “immunogen,” or “hapten” refers to a substance or structure or small molecule that is or is perceived to be foreign to the body and evokes an immune response alone or after forming a complex with a larger molecule

[0046] The term “passive immunity” or “passive immunization” refers to immunity as a result of the introduction of antibodies into the subject from another person, animal, species, or other external source.

[0047] The terms “active immunity” or “active immunization” refer to immunity as a result of the natural and/or artificial introduction of antigens into the subject.

[0048] The terms “immunize” or “vaccinate” within this disclosure are used interchangeably.

[0049] The term “adjuvant” or “immunologic adjuvant” refers to substances that are often added to vaccines to stimulate the subject’s immune system’s response.

[0050] The terms “treatment” and “treat” include both prophylactic or preventive treatment (that prevent and/or slow the development of a targeted pathologic condition, infection, disorder, or disease) and curative, therapeutic or disease-modifying treatment, including therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition, infection, disorder, or disease. Treatment of subjects at risk of contracting a disease or infection or suspected to have contracted a disease or infection, as well as subjects who are ill or have been diagnosed as suffering from a pathologic condition, infection, disorder, or disease are meant to be included. The terms “treatment” and “treat” do not necessarily imply that a subject is treated until total recovery. The terms “treatment” and “treat” also refer to the maintenance and/or promotion of health in an individual not suffering from a pathologic condition, infection, disorder, or disease but who may be susceptible to the development of a pathologic condition, infection, disorder, or disease. The terms “treatment” and “treat” are also intended to include the potentiation or otherwise enhancement of one or more primary prophylactic or therapeutic measures. As non-limiting examples, a treatment can be performed by a doctor, a healthcare professional, or another human.

[0051] The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for subjects, each unit containing a predetermined quantity of the composition disclosed herein in amount sufficient to produce the desired effect, in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the unit dosage form depend on the particular compounds employed, the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

[0052] The term "sterile" is understood to mean free from any bacteria or other living microorganisms.

[0053] The term "pharmaceutically acceptable" as used herein refers to substances that do not cause substantial adverse allergic or immunological reactions when administered to a subject.

[0054] The terms "food," "food product" and "food composition" mean a product or composition that is intended for ingestion by an animal, and provides at least one nutrient to the animal. Preferred embodiments of a food product include at least one of a protein, a carbohydrate, a lipid, a vitamin, or a mineral. Food products may include macronutrients and/or micronutrients.

[0055] The term "mM", as used herein, refers to a molar concentration unit of an aqueous solution, which is mmol/L. For example, 1.0 mM equals 1.0 mmol/L.

[0056] The terms "substantially no," "essentially free" or "substantially free" as used in reference to a particular component means that any of the component present constitutes no more than about 3.0% by weight, such as no more than about 2.0% by weight, no more than about 1.0% by weight, preferably no more than about 0.5% by weight or, more preferably, no more than about 0.1% by weight.

[0057] All percentages expressed herein are by weight of the total weight of the composition unless expressed otherwise. When reference herein is made to the pH, values correspond to pH measured at about 25°C with standard equipment. "Ambient temperature" or "room temperature" is between about 15°C and about 25°C, and ambient pressure is about 100 kPa.

[0058] The terms "immunize" or "vaccinate" within this disclosure are used interchangeably.

[0059] The term "isolated" means altered or removed from a natural state. For example, a protein naturally present in a living animal is not "isolated," while the same protein partially or completely separated from all or some of the coexisting materials of its natural state is "isolated."

[0060] The terms "peptide," "protein," or "polypeptide" refers to a polymer of amino acid residues covalently linked by peptide bonds. The terms "peptides," "proteins," or "polypeptides," used herein, may also refer to a polymer of amino acids where one or more of the amino acids may be a modified residue, such as an artificial amino acid mimetic or a synthetic amino acid residue. The terms "peptide," "protein," or "polypeptide" are used interchangeably.

[0061] The present disclosure generally relates to an IgY composition comprising purified polyclonal IgY immunoglobulins or antibodies from an egg-laying fowl. The composition may be diluted in sterile buffer, such as saline. The IgY composition can be used to prevent or treat coronavirus-infection and/or to reduce or eliminate the symptoms of coronavirus-infection.

[0062] Some embodiments of the coronavirus-specific IgY immunoglobulin component of the IgY composition are obtained by a method comprising collecting an egg laid by a fowl previously actively vaccinated or immunized against coronavirus and separating the antibody fraction from the yolk-fraction of the egg. The egg-laying fowl is preferably a domesticated fowl. The domesticated fowl is preferably a domesticated chicken. The domesticated chicken may be raised for egg or meat production.

[0063] Some embodiments relate to a method of preparing a first composition or vaccine to be administered to an egg-laying fowl. The first composition or vaccine may comprise live or inactivated coronavirus, adenovirus vectors encoding coronavirus antigens, and/or compositions comprising coronavirus antigens or coronavirus recombinant proteins. The first composition or vaccine can be used to actively vaccinate or immunize an egg-laying fowl against coronavirus. Eggs can be collected from the immunized or vaccinated egg-laying fowl and IgY immunoglobulins or antibodies (i.e., the antibody fraction) then can be extracted from the egg yolk and purified (**FIG. 1**).

[0064] Some embodiments provide a method of preparing a first composition or vaccine, comprising preparing coronavirus antigens or recombinant proteins to be administered to an egg-laying fowl for the purpose of immunizing or vaccinating the fowl to generate coronavirus-specific IgY immunoglobulins or antibodies. Adenovirus vectors can encode coronavirus antigens using the nucleotide sequence of Severe acute respiratory syndrome (SARS) coronavirus 2 spike protein (i.e., the coronavirus spike protein gene; GenBank Accession No. NC_045512.2; SEQ ID NO.: 1). Additionally, or alternatively, the nucleotide sequence of the coronavirus spike protein gene without the transmembrane domain (i.e. extracellular domain of SARS-Cov-2 spike protein), can be codon-optimized for expression in domestic chickens (Genscript). The selected nucleotide sequence can be cloned into the pCI (Promega) between immediate-early enhancer/promoter from Cytomegalovirus (CMV) and a terminator/polyadenylation signal from SV40. Plasmid DNA can be purified using NoEndo JETSTAR Plasmid Kit (Genomed, Germany), or any other method known to a person of ordinary skill in the art. The purified DNA (i.e., plasmid) can be suspended in a suitable buffer (e.g. PBS) at a pH of about 7.4. This purified DNA (i.e., plasmid) can be used to generate coronavirus antigens or coronavirus recombinant proteins or, additionally or alternatively, the purified DNA (i.e., plasmid) can be used as a DNA vaccine to booster or re-immunize egg-laying fowl as described below.

[0065] To generate coronavirus antigens or recombinant proteins, the purified DNA (i.e., plasmid) of approximately 62 μg to approximately 250 μg can be combined with Lipofectin transfection reagent (Life Technologies, USA), as recommended by the manufacturer, or the DNA may be combined with any comparable transfection reagent. The DNA can be subcloned into pFastBacgp67 transfer plasmids (GenScript), or any other comparable method or reagent. The resulting baculovirus-transfer vector can then be transformed into competent DH 10Bac recombinant E. *coli* cells to generate recombinant Bacmid DNA. The recombinant bacmid can be transferred onto Sf9 insect cells (*Spodoptera frugiperda*, for example, without limitation), or other comparable cells, using a transfection reagent, and the expressed coronavirus recombinant proteins or coronavirus antigens can be collected, examined, and purified.

[0066] Another embodiment provides a method of preparing a first composition or vaccine comprising one or more adenovirus vectors encoding coronavirus antigens to be administered to a hen or egg-laying fowl for the purpose of immunizing or vaccinating the hen to generate

coronavirus-specific immunoglobulins. The adenovirus vectors encoding coronavirus antigens can be generated using the nucleotide sequence of the SARS coronavirus 2 spike protein (SEQ ID NO.: 1). The gene can be cloned into the pShuttle/CMV plasmid to allow homologous recombination with a plasmid encoding an adenovirus backbone in BJ5183 E. *coli*. The recombinant adenovirus vector can be generated by transfecting plasmids containing the bioengineered adenovirus genomes encoding the transgenes into 293 cells. The resulting produced vectors (AdV-S) can be purified and titrated according to the AdEasy manual, or a similar method known to a person of ordinary skill in the art. Alternatively the adenovirus vector can be constructed using any commercially available system such as the Ad-HQ system developed by Vector Biolabs.

[0067] Another embodiment provides a method of preparing and storing the first composition or vaccine, the first composition or vaccine comprising live or inactivated coronavirus, adenovirus vectors encoding coronavirus antigens, and/or compositions comprising coronavirus antigens or coronavirus recombinant proteins, in aliquots (e.g., about 1 ml) of saline or a comparable solution at a concentration suitable for vaccinating or immunizing a hen, via intramuscular injection.

[0068] Another aspect of the present disclosure generally relates to the method of preparing a first composition or vaccine, comprising live or inactivated coronavirus, adenovirus vectors encoding coronavirus antigens and/or compositions coronavirus antigens or recombinant proteins, in aliquots emulsified with adjuvant.

[0069] Another embodiment provides a method of administering the first composition or coronavirus vaccine, the first composition or vaccine comprising live or inactivated coronavirus, adenovirus vectors encoding coronavirus antigens, and/or compositions comprising coronavirus antigens or coronavirus recombinant proteins, to an egg-laying fowl. The composition or coronavirus vaccine can be administered intramuscularly into the egg-laying fowl or hen. In some embodiments, at least a portion of the first composition or coronavirus vaccine (most preferably the entirety) is injected into the breast of a hen, for example a first portion in the left breast of the hen and a second portion in the right breast of the hen, optionally in approximately equal amounts such as about 500 mL into the right breast and about 500 mL into the left breast. The hen can be

re-immunized following the initial immunization, for example about 7 days following the initial immunization and/or about 14 days after the initial immunization. The re-immunization composition can be the same composition or vaccine that was initially used to immunize that egg-laying fowl or hen, or the composition or vaccine used for re-immunization can be a different composition comprising live or inactivated coronavirus, adenovirus vectors encoding coronavirus antigens and/or compositions coronavirus antigens or recombinant proteins. Preferrably, re-immunization or repeated immunizations should occur within about 7 days to about 28 days, with respect to the prior immunization. After initial immunization and any re-immunization (e.g., about twenty-seven days after the initial immunization), eggs laid by the immunized hen can be collected for one or more days for purification of coronavirus-specific IgY antibodies. Alternatively, the eggs can be continuously collected during the immunization period.

[0070] Additionally, or alternatively, the re-immunization composition administered to the egg-laying fowl can be inactivated Newcastle Disease Virus expressing anchored spike protein (NDV-S; SEQ ID NO.: 1).

[0071] Some embodiments provide a method of collecting, isolating, and purifying the coronavirus-specific IgY from the eggs laid by the immunized or vaccinated hens. The coronavirus-specific IgY antibodies can be obtained from the egg yolks or water-soluble fraction. One or more egg yolks can be pooled and diluted about 10 times with cooled HCl (e.g. about 3 mM HCl) to give the suspension a pH of about 5 (adjusted with approximately 10% acetic acid). The suspension can be frozen, for example, overnight at about -20°C. After thawing to a predetermined temperature, the mixture can be centrifuged at about 13,000 x g for about 15 minutes at approximately 4°C and the supernatant containing the IgY antibodies can be collected. The IgY antibodies can be further purified by various precipitation methods known to a person of ordinary skill in the art, such as using ammonium sulfate or bio-compatible sodium chloride (*See* Hodek, P. et al., *Optimized Protocol of Chicken Antibody (IgY) Purification Providing Electrophoretically Homogenous Preparations*, 8 Int. J. Electrochem. Sci.113, 113-124 (2013)).

[0072] In some embodiments, IgY purification further comprises collecting the eggs from a hen vaccinated or immunized against coronavirus. The yolk-fraction can be separated and diluted with a solution such as water. Lipids can be separated from water soluble proteins using

a gravitational force. The water soluble fraction can be further concentrated with a ultrafiltration (UF) system. Water soluble proteins and the IgY antibodies may then be separated with ion exchange. The coronavirus-specific IgY antibodies can be further separated using a coarser UF filtration.

[0073] In some embodiments the coronavirus-specific IgY composition is in part comprised by the yolk of the egg, or any IgY antibody-containing fraction thereof. The yolk is the preferable portion of the egg, as the yolk typically contains much higher concentrations of IgY than does the white. However, the egg white may contain concentrations of IgY sufficient for some applications.

[0074] In some embodiments, IgY is concentrated, isolated, or purified from the constituent of the egg. This can be accomplished by a variety of methods, for example, methods known by a person of ordinary skill in the art. *See* Schwarzkopf and Thiele, *Effectivity of Different Methods for the Extraction and Purification of IgY*, ALTEX 13, 35-39 (1996), which is incorporated by reference in its entirety herein. If desired, the titer of IgY antibodies can be determined by immunoassay, for example enzyme-linked immunosorbent assay (ELISA).

[0075] In some embodiments, an antibody composition is made by a method comprising actively vaccinating a hen against coronavirus, collecting eggs from the hen after an immunization period, and separating the antibody fraction from a yolk of the egg. In some embodiments, collecting eggs from the hen can occur continuously after the immunization period.

[0076] Further methods of producing IgY with a specific target are known to those skilled in the art, although these methods are not known to have been previously successfully used to produce antibodies to coronavirus. The antibodies disclosed in this section are suitable for use in any of the methods and compositions described in this disclosure.

[0077] Some embodiments relate to a pharmaceutically acceptable composition of coronavirus-specific IgY immunoglobulin compositions that can be administered to individuals exposed to, susceptible to, or infected with coronavirus.

[0078] One aspect of the present disclosure generally relates to a method of administering an effective amount of coronavirus-specific IgY immunoglobulins at a dose of about 0.25 mg/kg

body weight to about 1 mg/kg body weight. The coronavirus-specific IgY immunoglobulins can be administered more than once to an individual.

[0079] In one embodiment, such antibodies are in the form of compositions, such as but not limited to, pharmaceutical compositions. The compositions disclosed may comprise one or more of such antibodies or immunoglobulin compositions disclosed above, in combination with a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in *Remington: The Science and Practice of Pharmacy* (20th Ed., Lippincott, Williams & Wilkins, Daniel Limmer, editor, which is incorporated by reference in its entirety herein). To form a pharmaceutically acceptable composition suitable for administration, such compositions will contain a therapeutically effective amount of an antibody. The therapeutically effective amount may be an adhesion inhibiting effective amount.

[0080] The pharmaceutical compositions of the disclosure may be used in the treatment and prevention methods of the present disclosure. Such compositions are administered to humans in amounts sufficient to deliver a therapeutically effective amount of the antibody so as to be effective in the treatment and prevention methods disclosed herein. The therapeutically effective amount may vary according to a variety of factors such as, for example, but not limited to, the subject's condition, weight, sex and age. Other factors include the mode and site of administration. The pharmaceutical compositions may be provided to the subject in any method known in the art.

[0081] The compositions of the present disclosure may be administered only one time to the subject or more than one time to the subject. Furthermore, when the compositions are administered to the subject more than once, preferably less than two months. A variety of regimens may be used, such as, for example, but not limited to, one per day, once per week, once per month or once per year or any variation in between. The compositions may also be administered to the subject more than one time per day. The compositions The therapeutically effective amount of the antibodies and appropriate dosing regimens may be identified by routine testing in order to obtain optimal activity, while minimizing any potential side effects. In addition, co-administration or sequential administration of other agents may be desirable.

[0082] The compositions of the present disclosure may be administered systemically, such as by intranasal and/or oral administration (**FIG. 2**). The preferred routes of administration include intranasal and oral. In some embodiments, compositions are administered as an intranasal. In some embodiments, compositions are administered orally.

[0083] Oral administration methods for coronavirus-specific immunoglobulins additionally include, for example, the oral administration of the uncooked yolk or yolk-fraction of the egg, alone or in combination with the white of the egg. Oral administration of the raw yolk or yolk-fraction may be performed for example by eating the yolk-fraction. The yolk-fraction or water-soluble yolk fraction may be administered in combination with other ingredients to make it more palatable or nutritious. Thus the yolk-fraction may be consumed by the subject or individual as a food item; alternatively, the yolk-fraction may be consumed as part of a pharmaceutical composition. It is preferably uncooked or very lightly cooked yolk-fraction as cooking can inactivate the antibody.

[0084] In some embodiments, the water-soluble fraction of the egg yolk can be readily mixed with food of any type or any edible ingredient for oral administration. The compositions can also be formulated to contain or provide a portion of the macronutrient and micronutrient requirements for a subject, and can be provided as a replacement for, or a supplement to, the subject's regular diet. The composition can be provided as, added to, or mixed with a snack, drink, food item, or other supplement to the normal intake of food.

[0085] The compositions of the present disclosure may further comprise additional agents which improve the solubility, half-life, absorption, etc. of the antibody. Furthermore, the compositions of the present disclosure may further comprise agents that attenuate undesirable side effects and/or decrease the toxicity of the antibodies(s). Examples of such agents are described in a variety of texts, such a, but not limited to, *Remington: The Science and Practice of Pharmacy* (20th Ed., Lippincott, Williams & Wilkins, Daniel Limmer, editor, which is incorporated by reference in its entirety herein).

[0086] The compositions of the present disclosure can be administered in a wide variety of dosage forms for administration. For example, the compositions can be administered in forms, such as, but not limited to, injectable solution, lyophilized powder, and/or granules.

[0087] In some embodiments, the pharmaceutical compositions may further comprise a pharmaceutically acceptable carrier. Such carriers include, but are not limited to, vehicles, adjuvants, suspending agents, inert fillers, diluents, excipients, wetting agents, binders, buffering agents, disintegrating agents, and/or carriers. Typically, the pharmaceutically acceptable carrier is chemically inert to the active antibodies and has no detrimental side effects or toxicity under the conditions of use. The pharmaceutically acceptable carriers can include polymers and polymer matrices. The nature of the pharmaceutically acceptable carrier may differ depending on the particular dosage form employed and other characteristics of the composition.

[0088] For instance, for oral administration in solid form, such as but not limited to powders, or granules, the antibody may be combined with an oral, non-toxic pharmaceutically acceptable inert carrier, such as, but not limited to, inert fillers, suitable binders, lubricants, disintegrating agents, and/or accessory agents. Suitable binders include, for example, without limitation, starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include, for example, without limitation, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, and the like. Disintegrators include, for example, without limitation, starch, methyl cellulose, agar, bentonite, xanthum gum and the like.

[0089] Formulations suitable for intranasal or oral administration include aqueous isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the subject, and aqueous suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and/or preservatives. The antibody may be administered in a physiologically acceptable diluent, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose, and/or related sugar solutions, or any other solutions known to a person of ordinary skill in the art.

[0090] Oils which can be used in intranasal or oral formulations include, for example, without limitation, petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in intranasal or oral formulations include polyethylene sorbitan fatty acid esters,

such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol, oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters. Suitable soaps for use in intranasal formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, without limitation, dimethyldialkylammonium halides, and alkylpyridinium halides, (b) anionic detergents such as, for example, without limitation, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, without limitation, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylene polypropylene copolymers, (d) amphoteric detergents such as, for example, without limitation, alkylbeta-aminopropionates, and 2-alkylimidazoline quaternary ammonium salts, and (e) mixtures thereof.

[0091] Suitable preservatives and buffers can be used in such formulations. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17.

[0092] An antibody of the present disclosure may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include, but are not limited to, polyvinyl-pyrrolidone, pyran copolymer, polyhydroxypropylmethacryl-amidephenol, polyhydroxyethylaspartamidephenol, or polyethyl-eneoxidepolylysine substituted with palmitoyl residues. Furthermore, an antibody may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydro-pyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

[0093] The pharmaceutical compositions may be modified to prevent adverse reactions in the subject. Such potential adverse reactions include host recognition, anaphylaxis, localized inflammation and other forms of allergic reaction. Adverse reactions are more common in heterologous antibody treatment than in homologous antibody treatment, although the advantages of IgY antibodies in this respect have been explained. In some embodiments of the pharmaceutical

composition, the antibody is modified to alter the Fc region of the molecule. In further embodiments, the antibody is treated to prevent binding between the Fc region of the antibody and the Fc receptor of a cell.

[0094] In some embodiments, pharmaceutical preparations of the present disclosure can be stored in any pharmaceutically acceptable form, including an aqueous solution, a frozen aqueous solution, a lyophilized powder, or any of the other forms described herein.

[0095] Non-limiting examples of the pharmaceutically acceptable coronavirus-specific IgY immunoglobulin composition preferably further comprises an anti-inflammatory.

[0096] Non-limiting examples of the pharmaceutically acceptable coronavirus-specific IgY immunoglobulin composition preferably further comprise an antigen-binding fragment of an antibody such as a Fab or Fab2 fragment, that may substitute for the antibody. For example, the antigen-binding fragment may be any fragment that includes the antigen-binding region of the original IgY. In some embodiments of the compositions and methods, a modified version of an IgY antibody may substitute for the IgY antibody, so long as the antigen-binding region of the IgY antibody retains its ability to recognize coronavirus.

[0097] Non-limiting examples of the pharmaceutically acceptable coronavirus-specific IgY immunoglobulin composition preferably further comprise a composition of lyophilized powder such as for long-term storage and/or transportation. The lyophilized composition can be reconstituted into a solution, such as saline, to about the original volume before being used for immunization, vaccination, or treatment.

[0098] As can be seen from FIG. 4, IgY antibodies are useful as viral adhesion inhibitors that can neutralize the virus. IgY antibodies from fowl eggs are a cheap and plentiful source of viral adhesion inhibitors. Such antibodies bind to the surface of an antigen-bearing virus (such as coronavirus), thus preventing the initial stages of contact between the virus and a potential host cell. As explained elsewhere in this disclosure, preventing the initial stages of adhesion between a virus and a host cell has numerous applications, including treatment of viral infection and prevention of viral infection.

[0099] In some embodiments of the viral adhesion inhibitors, the inhibitor comprises a constituent of a fowl egg, wherein the fowl egg comprises an adhesion-inhibiting effective amount of IgY specific for coronavirus. The constituent of the fowl egg may be any constituent described as appropriate antibody compositions in this disclosure.

[00100] Some methods provide for preventing viral adhesion to a cell. The first step in the infection of a cell by a virus is contact and adhesion between virus and cell. Although this step is critical to the establishment of infection, methods of preventing infection at this early stage are few. More typically, viral infection is countered using techniques such as active vaccination, which causes the body to produce antibodies that neutralize the virus. If active vaccination is not feasible, most often viral disease is merely treated symptomatically. The methods described here offer an effective means to prevent this early step in the infection process without requiring administration well in advance of the subject's exposure to the pathogen, as is required by active vaccination.

[00101] Antibodies can function to prevent adhesion between virus and cell (i.e., viral adhesion inhibitor) by binding to the virus and interfering with the ability of the virus to bind its target membrane receptor. Immunoglobulins from an egg-laying fowl, such as IgY antibodies, have distinct advantages over mammalian antibodies in this application, particularly when the subject is a mammal. As stated above, the advantages of IgY antibodies include that IgY antibodies as compared to mammalian antibodies are more specific, more stable, and cause fewer unwanted forms of immune response. IgY antibodies can also be easily and cheaply obtained from eggs.

[00102] In some embodiments, the method comprises administering to a subject an amount of coronavirus-specific immunoglobulins effective to inhibit and/or prevent adhesion between the coronavirus and the subject's cells. In some embodiments of the method, the coronavirus-specific immunoglobulins or viral adhesion inhibitors comprises a constituent of a fowl egg, the constituent comprising an adhesion-inhibiting effective amount of IgY-specific for coronavirus. The constituent may be any constituent disclosed herein as an appropriate antibody composition.

[00103] In some embodiments, the coronavirus-specific immunoglobulin composition is a pharmaceutical comprising the contents of a fowl egg, the contents of the fowl egg comprising

an effective amount of IgY-specific for coronavirus. The pharmaceutical may comprise additional components as discussed herein. The pharmaceutical may be administered by any method known in the art or as described herein.

[00104] Some embodiments provide a method for treating an individual exposed to, susceptible to, or infected with coronavirus. The coronavirus-specific IgY immunoglobulin composition(s) may comprise additional components as pharmaceutical components discussed elsewhere in the disclosure. The coronavirus-specific IgY immunoglobulin composition may be administered orally and/or intranasally. Oral administration can prevent or reduce transmission of the virus within the gastrointestinal tract or can eliminate the virus from the gastrointestinal tract.

[00105] Such oral administration methods additionally include the oral administration of the uncooked yolk or yolk-fraction of the egg, alone or in combination with the white of the egg. Oral administration of the raw yolk or yolk-fraction may be performed for example by eating the yolk-fraction. The yolk-fraction may be administered in combination with other ingredients to make it more palatable or nutritious. Thus the yolk-fraction may be consumed by the subject as a food item; alternatively, the yolk-fraction may be consumed as part of a pharmaceutical composition. It is preferably uncooked or very lightly cooked yolk-fraction as cooking can inactivate the antibody.

[00106] Non-limiting examples of the method of treatment include an increased dose of coronavirus-specific IgY immunoglobulin composition administered either intranasally, orally, or in combination, or alternatively, administered at an increased dose and/or frequency.

[00107] Some embodiments provide a method of treating an individual infected with coronavirus, the method comprising administering to the infected individual the coronavirus-specific IgY immunoglobulin composition at a concentration or dose affective to reduce, alleviate, or eliminate symptoms associated with the coronavirus infection.

[00108] Some embodiments provide a method of treating an individual previously exposed to or otherwise susceptible to coronavirus infection, the method comprising administering to the

infected individual the coronavirus-specific IgY immunoglobulin composition at a concentration or dose affective to prevent or reduce the severity of coronavirus infection.

[00109] The coronavirus-specific IgY immunoglobulin composition can be administered intranasally and/or orally. The coronavirus-specific IgY immunoglobulin composition may be administered to the subject more than once.

EXAMPLES

[00110] The following non-limiting examples support the concept of using the pharmaceutically acceptable coronavirus vaccine composition for generation of antibodies to be used for treatment of infected humans or for prevention of infection of humans.

EXAMPLE 1

[00111] Three groups of Chickens (n = 3 per group) were immunized with the coronavirus or adenovirus antigen composition on day 1, day 7, and on day 14. Group 1 received saline as a control (no antigen composition), group 2 received a primary immunization with the coronavirus antigen composition or the adenovirus antigen composition, followed by boosting immunizations, and group 3 received 3 immunizations with the coronavirus antigen composition. Following the second and third immunizations, blood samples were taken to assess antibody titers using antigen-coated ELISA plates. Example 1 demonstrates that the antigen-induced antibody pools have comprehensive specificities to coronavirus and adenovirus after 14 and 28 days.

[00112] EXAMPLE 2

[00113] Thirty weeks old egg-laying hens were immunized intramuscularly with adenovirus vectored vaccine encoding the SARS coronavirus 2 spike protein (SEQ ID NO.: 1), without the transmembrane domain. Two weeks later they were boosted or re-immunized with the same vaccine. Two weeks after the re-immunization the egg-laying hens were further boosted with a DNA vaccine (i.e., plasmid) that contains full length of extracellular domain sequence or SEQ ID NO.: 1 with CMV promoter (DNA-S).

[00114] Eggs laid 5 weeks after first immunization were collected. IgY immunoglobulins from pooled egg yolks were separated and purified using water dilution method followed by precipitation with polyethylenglycol (PEG).

[00115] The specificity of the purified coronavirus-specific immunoglobulins was determined by ELISA compared to non-vaccinated control hens (**FIG. 3**) and virus neutralizing activity was assessed using a virus neutralization assay (**FIG. 4**), compared to non-vaccinated and control groups. As shown in **FIG. 4**, the lowest concentration of the IgY at which the virus neutralization was observed was 0.3 mg/ml.

EXAMPLE 3

[00116] Individuals may be provided a composition comprising coronavirus-specific IgY immunoglobulins. The composition comprising coronavirus-specific IgY immunoglobulins may be administered intranasally and/or orally for the treatment of an individual infected with coronavirus (**FIG. 2**). The composition may be administered only one time to the individual or more than one time to the individual.

Claims

1. A method of treating coronavirus infection in an individual having the coronavirus infection, the method comprising:

administering a first composition to an egg-laying fowl, the first composition comprising one or more adenovirus vector(s) encoding one or more coronavirus antigen(s); and

providing, to the individual having the coronavirus infection, a second composition, the second composition comprising a water-soluble fraction of egg yolk from the egg-laying fowl administered the first composition.

2. The method of Claim 1, wherein the second composition is administered to the individual having the coronavirus infection, in an amount that provides a dose of the water-soluble fraction of egg yolk that is about 0.25 mg to about 1.0 mg per kg body weight of the individual having the coronavirus infection.

3. The method of Claim 1, wherein the second composition is administered intranasally.

4. The method of Claim 1, wherein the second composition is administered more than once to the individual having the coronavirus infection.

5. The method of Claim 1, wherein the first composition is administered more than once to the egg-laying fowl.

6. The method of Claim 5, wherein the first composition is administered to the egg-laying fowl a second time, at least 14 days after a first administration of the first composition.

7. The method of Claim 1, wherein an additional composition is administered to the egg-laying fowl, at least 14 days after a first administration of the first composition.

8. The method of Claim 7, wherein the additional composition comprises live or inactivated coronavirus, adenovirus vector encoding coronavirus antigens, coronavirus recombinant proteins, and/or inactivated Newcastle Disease Virus expressing anchored spike protein.

9. A method of preventing, decreasing incidence of, and/or decreasing severity of coronavirus infection in an individual at risk thereof, the method comprising:

administering a first composition to an egg-laying fowl, the first composition comprising one or more adenovirus vector(s) encoding one or more coronavirus antigen(s); and

providing a second composition to the individual, the second composition comprising a water-soluble fraction of egg yolk from the egg-laying fowl who has been administered the first composition.

10. The method of Claim 9, wherein the second composition is administered to the individual in an amount that provides a dose of the water-soluble fraction of egg yolk that is about 0.25 mg to about 1.0 mg per kg body weight of the individual.

11. The method of Claim 9, wherein the second composition is administered to intranasally.

12. The method of Claim 9, wherein the second composition is administered more than once to the individual.

13. The method of Claim 9, wherein the first composition is administered more than once to the egg-laying fowl.

14. The method of Claim 13, wherein the first composition is administered to the egg-laying fowl a second time, at least 14 days after administration of the first composition.

15. The method of Claim 9, wherein an additional composition is administered to the egg-laying fowl, at least at least 14 days after administration of the first composition.

16. The method of Claim 15, wherein the additional composition comprises live or inactivated coronavirus, adenovirus vector encoding coronavirus antigens, coronavirus recombinant proteins, and/or inactivated Newcastle Disease Virus expressing anchored spike protein.

17. A method of producing an intranasally administrable composition effective to treat, prevent, decrease incidence of, and/or decrease severity of coronavirus infection in an individual infected with the coronavirus, the method comprising adding a water-soluble fraction of egg yolk,

which has been obtained from an egg-laying fowl administered an one or more adenovirus vector(s) encoding one or more coronavirus antigen(s), to a solution to form the composition.

18. A unit dosage form comprising a therapeutically or prophylactically effective amount of a composition comprising a water-soluble fraction of egg yolk, which has been obtained from an egg-laying fowl administered one or more adenovirus vector(s) encoding one or more coronavirus antigen(s).

19. Immunoglobulins specific against coronavirus for use in the treatment and/or prevention of coronavirus infection in a subject at risk thereof.

Method of preparing coronavirus-specific IgY compositions

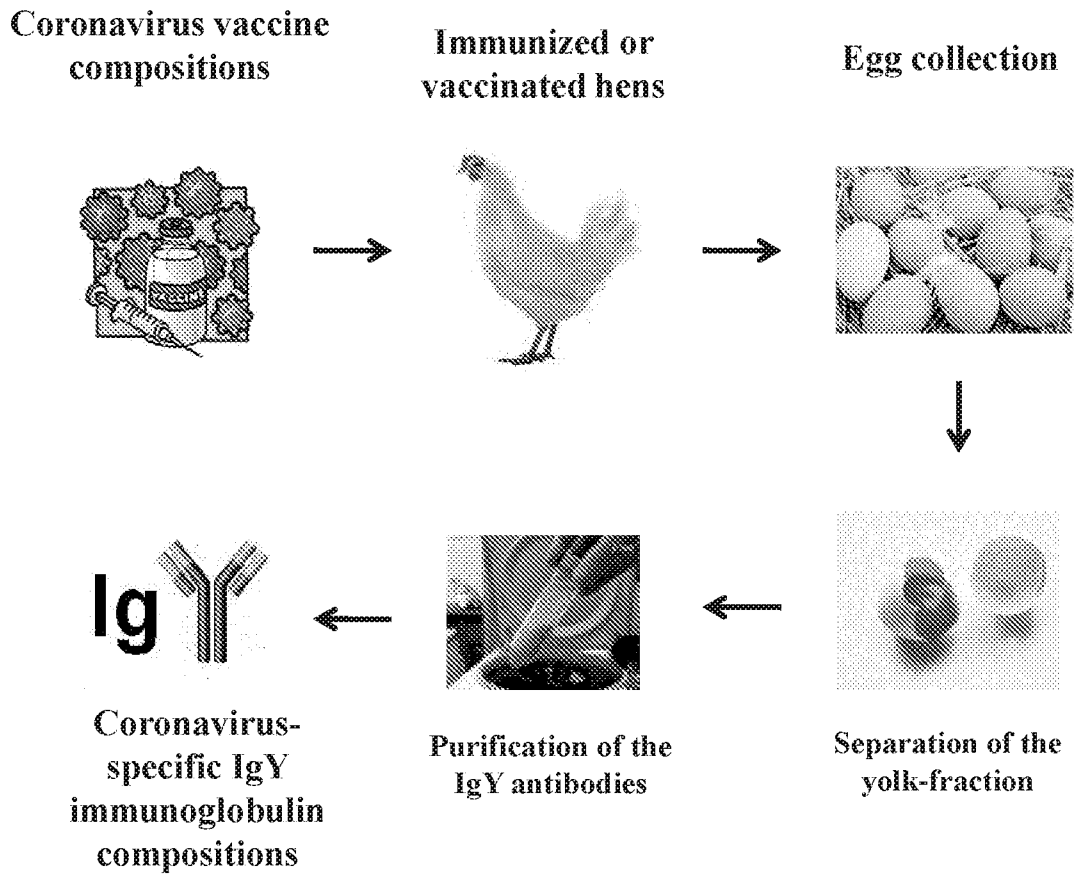

FIG. 1

Methods of treating individuals exposed to, susceptible to, or infected with coronavirus

Coronavirus-specific IgY composition administered intranasally

Coronavirus-specific IgY composition administered orally

FIG. 2

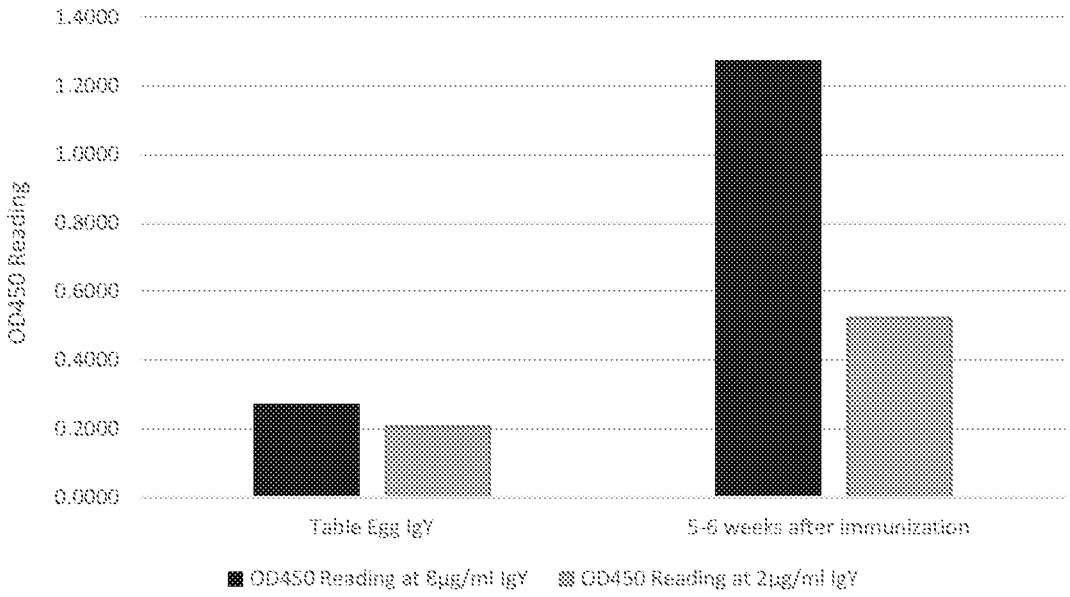
1.4000
1.2000
1.0000
0.8000
0.6000
0.4000
0.2000
0.0000
OD450 Reading
Table Egg IgY
5-6 weeks after immunization
OD450 Reading at 8µg/ml IgY
OD450 Reading at 2µg/ml IgY

FIG. 3

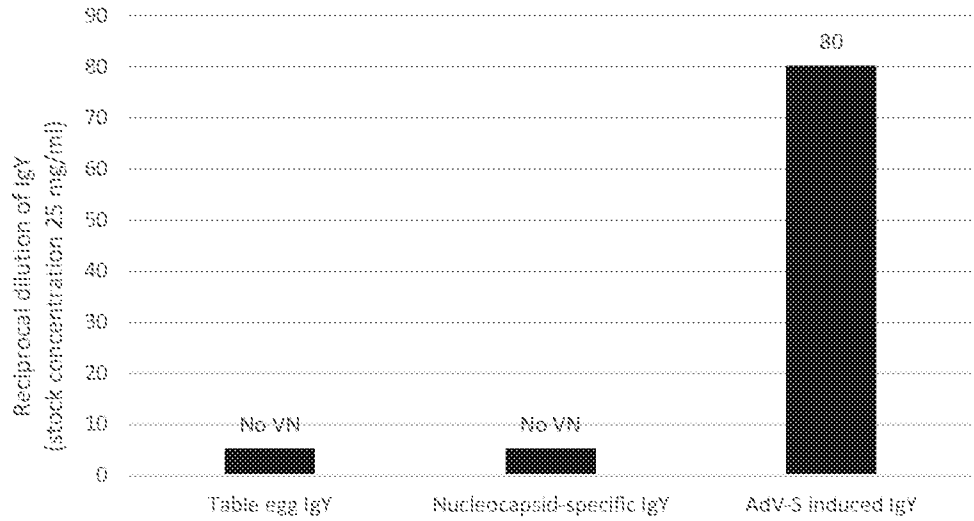
90
80
70
60
50
40
30
20
10
0
Reciprocal dilution of IgY (stock concentration 25 mg/ml)
No VN
No VN
80
Table egg IgY
Nucleocapsid-specific IgY
AdV-5 induced IgY

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2021/052977

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 39/42*** (2006.01), ***A61P 31/14*** (2006.01), ***C07K 14/165*** (2006.01), ***C07K 16/10*** (2006.01), ***C12N 15/50*** (2006.01), ***C12N 15/861*** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Keywords across whole IPC

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel Orbit; Scopus; Canadian Patent Database; Google Scholar; Google; Library Discover Tool; STN
Keywords: coronavirus, corona virus, coronavirus infection, egg, egg yolk, adenovirus, adenovirus vector, corona virus, IgY, immunoglobulin, chicken, fowl, egg-laying fowl

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	FU et al., Journal of Virological Methods, 2006, Vol. 133(1), pp. 112-115, ISSN 0166-0934 (see whole document)	17-19 1-16
X	US9701735 B1 Starzl, T.W., 11 July 2017 (11-07-2017) (see whole document)	17-19
X	WO2006/051091 A1 TER MEULEN et al., 18 May 2006 (18-05-2006) (see whole document)	17-19
X	CN1621417 A WANG XILANG et al., 1 June 2005 (01-06-2005) (see abstract)	17-19

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	
"D" document cited by the applicant in the international application	
"E" earlier application or patent but published on or after the international filing date	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	
	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
	"&" document member of the same patent family

Date of the actual completion of the international search 07 May 2021 (07-05-2021)	Date of mailing of the international search report 23 June 2021 (23-06-2021)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Christiane Hansen (819) 639-7736

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2021/052977

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US20190256579 A1 GRAHAM et al., 2 August 2019 (02-08-2019) (see whole document)	17-19
Y	AFKHAMI et al., Molecular Therapy: Method & Clinical Development, 2016, Vol. 3(C), pp. 1-10, ISSN 2329-0501 (see whole document)	1-16
Y	WO01/88162 A2 DUAN LINGXUN, 22 November 2001 (22-11-2001) (see whole document, especially abstract, page 3, lines 10-20 and page 39, line 14 to page 42, line 7)	1-16
P,Y	CN112279912 A XU QING et al., 29 January 2021 (29-01-2021) (see abstract)	1-16
P, Y	CN112094341 A LI JING et al., 18 December 2020 (18-12-2020) (see abstract)	1-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2021/052977

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

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

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB2021/052977

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US9701735B2	11 July 2017 (11-07-2017)	US2012141458A1	07 June 2012 (07-06-2012)
		AR083973A1	10 April 2013 (10-04-2013)
		AU2011332053A1	02 May 2013 (02-05-2013)
		AU2011332053B2	02 June 2016 (02-06-2016)
		AU2016201651A1	07 April 2016 (07-04-2016)
		AU2016201651B2	22 February 2018 (22-02-2018)
		AU2018203497A1	07 June 2018 (07-06-2018)
		AU2018203497B2	07 May 2020 (07-05-2020)
		BR112013012594A2	16 October 2018 (16-10-2018)
		CA2818827A1	31 May 2012 (31-05-2012)
		CL2013001469A1	07 March 2014 (07-03-2014)
		CN103533958A	22 January 2014 (22-01-2014)
		CN103533958B	08 July 2015 (08-07-2015)
		CN105012950A	04 November 2015 (04-11-2015)
		CO6791606A2	14 November 2013 (14-11-2013)
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