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(54) Titre : NOUVELLES SOUCHES DE ROTAVIRUS HUMAIN ET NOUVEAUX VACCINS

(54) Title: NEW HUMAN ROTAVIRUS STRAINS AND VACCINES

(57) Abrégé/Abstract:

A vaccine composition and method of vaccination are provided useful for immunizing a subject against a rotavirus. The vaccines include rotavirus strains CDC-9 and CDC-66, fragments thereof, homologues thereof, or combinations thereof. Inventive vaccines may include a fragment of CDC-9, CDC-66, homologues thereof, or combinations thereof. Methods of inducing an immunological response are provided by administering an inventive vaccine.

ABSTRACT

A vaccine composition and method of vaccination are provided useful for immunizing a subject against a rotavirus. The vaccines include rotavirus strains CDC-9 and CDC-66, fragments thereof, homologues thereof, or combinations thereof. Inventive vaccines
5 may include a fragment of CDC-9, CDC-66, homologues thereof, or combinations thereof. Methods of inducing an immunological response are provided by administering an inventive vaccine.

NEW HUMAN ROTAVIRUS STRAINS AND VACCINES**GOVERNMENT SPONSORSHIP**

[0001] This invention was made by the Centers for Disease Control and Prevention, an agency of the United States Government.

5 **CROSS REFERENCE TO RELATED APPLICATIONS**

[0002] This application is a division of application 2763359 filed May 12, 2010.

FIELD OF THE INVENTION

[0003] The present invention relates generally to virus vaccine strains as well as vaccine compositions and methods relating thereto. More specifically, the present invention relates to
10 human rotavirus A vaccine strains, vaccine compositions and methods of use to induce an immunological response against rotavirus A in a subject.

BACKGROUND OF THE INVENTION

[0004] Of the various enteric pathogenic viruses causing severe diarrhea in children, rotavirus is the most common causing an average of 611,000 deaths per year. Virtually all
15 children are infected by rotavirus by age 5. The virus is believed to be highly contagious and has been described as a "democratic" virus since the infection affects no particular socioeconomic or geographic group disproportionately. While the majority of children having access to adequate supportive and palliative medical care survive infection with no significant long-term consequences, the number of deaths associated with severe diarrhea, vomiting,
20 dehydration and shock is unacceptable and requires preventative intervention if possible.

[0005] Rotavirus A is an icosahedral virus in the family Reoviridae with a distinct hub-and-spoke morphology. Particular rotaviruses are classified by group, subgroup and serotype according to properties characteristic of the viral capsid proteins. Rotavirus particles contain 3 protein layers surrounding the viral genome which consists of 11 segments of double- stranded
25 RNA, each segment encoding a protein. The viral proteins include structural proteins called VPs and nonstructural proteins designated NSPs. A number of the structural proteins are particularly

important in eliciting an immune response in a host since these proteins are present on the outermost surface of the viral particles. In particular the proteins VP7 and VP4 both figure prominently in host immune response and therefore have also played a central role in development of rotavirus vaccines.

5 [0006] Variants of VP7 and VP4 structural proteins characterize distinct rotavirus A serotypes. In particular, variants of human VP7 are identified as “G” serotypes including at least serotypes G1, G2, G3, G4, as well as the less common G5, G6, G8, G9, G10, G11, G12, G13 and G14. Variants of the VP4 structural protein are identified as “P” serotypes including P1A, P1B, P2A, P3, P4, P5, P6 and P8. Because intact rotaviruses are characterized by both a VP7
10 protein and a VP4 protein, individual virus serotypes are named according to the identity of the variant of these two proteins contained in the particular virus. For example, a common rotavirus A contains both G1 and P[8] variants of VP7 and VP4, respectively. The G1, P[8] serotype of rotavirus A is one of the most common forms of the virus which cause disease worldwide. The G1 serotype of rotavirus A is the most common serotype associated with human disease
15 worldwide. A number of vaccines have been developed which use rotavirus A G1 strains with the goal of developing immunity in a host against rotavirus A G1 strains as well as rotavirus A strains having other serotypes. However, this approach has been limited by important differences between the G1 and G2 serotypes. In particular, rotavirus A G2 strains are derived from a different lineage than most other rotavirus strains. This is demonstrated by nucleic acid
20 hybridization experiments showing that labeled transcripts of the 11 gene segments of G2 strains, also known as the DS-1 genogroup, do not hybridize with corresponding nucleic acids from the strains known as the Wa genogroup of rotavirus A which includes G1, G3, G4, and G9. The lack of hybridization of these homologous genes indicates that differences in the encoded proteins, such as the outer capsid proteins VP4 and VP7 and inner capsid protein VP6, are
25 substantial. These genetic differences support the observations that individuals infected or immunized using a G1 strain are less likely to show cross protection against G2 strains than other strains of the Wa genogroup.

[0007] In addition, to the common G1 and G2 rotavirus A strains, a diversity of human rotavirus types is increasingly recognized as contributing to acute severe diarrhea disease
30 worldwide. This diversity underscores the need for robust vaccines capable of generating immunity against several strains. Recently, the United States Food and Drug Administration suspended the use of the ROTARIX vaccine citing contaminants in its preparation. Thus, the

number of available vaccines for rotavirus is declining at a time when infections remain a serious worldwide problem. Another vaccine, RotaTeq®, appears safe and effective in preventing diarrhea among children in middle and high income countries and are currently licensed and recommended for use in infants throughout the world. However, the efficacy of this vaccine is reduced in low income countries of Africa and Asia.

[0008] Thus, there is a continuing need for vaccines against human rotavirus A of both common and less common types.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. 1A is a xerographic image of an electron micrograph showing intact isolated rotavirus A virions identified as strain CDC-9, having serotype P[8], G1;

[0010] FIG. 1B is a xerographic image of an electron micrograph showing intact isolated rotavirus A virions identified as strain CDC-66, having serotype P[4], G2;

[0011] FIG. 2 shows a xerographic reproduction of an image of a polyacrylamide gel illustrating RNA profiles of rotavirus A strain CDC-9 isolated from a stool sample (S) and Vero cells (V) and showing typical long (CDC-9) RNA electropherotypes for the rotavirus strain;

[0012] FIG. 3 shows a xerographic reproduction of an image of a polyacrylamide gel illustrating RNA profiles of the rotavirus A strain CDC-66 isolated from a stool sample and Vero cells and showing typical short RNA electropherotypes for this rotavirus strain;

[0013] FIG. 4A shows bands of CsCl purified rotavirus particles of rotavirus A strain CDC-9;

[0014] FIG. 4B shows identified structural viral proteins of rotavirus A strain CDC-9 analyzed by SDS-PAGE in comparison to molecular weight markers;

[0015] FIG. 5A is a bar graph showing total antibody titers in response to thermally inactivated rotavirus in control and vaccinated mice;

[0016] FIG. 5B is a bar graph showing neutralizing antibody titers in response to thermally inactivated rotavirus in control and vaccinated mice;

[0017] FIG. 6 is a bar graph showing total serum antibody responses to thermally inactivated rotavirus formulated with $\text{Al}(\text{OH})_3$ in control and vaccinated mice.

[0018] FIG. 7A shows virus shedding in fecal samples of piglets vaccinated with no antigen and with 750 micrograms of aluminum phosphate in 4 animals;

[0019] FIG. 7B shows virus shedding in fecal samples from piglets immunized with antigen and no adjuvant;

[0020] FIG. 7C shows virus shedding in fecal samples of piglets immunized with antigen and adjuvant;

[0021] FIG. 7D shows virus shedding measured in fecal samples of piglets immunized with buffer only;

5 [0022] FIG. 8A is a bar graph showing rotavirus specific IgG antibody response in sera of piglets vaccinated with no antigen and with 600 micrograms of aluminum phosphate (solid bars) or piglets vaccinated with 50 micrograms of antigen and with 600 micrograms of aluminum phosphate (hatched bars);

[0023] FIG. 8B is a bar graph showing neutralizing antibody response in sera of piglets
10 vaccinated with no antigen and with 600 micrograms of aluminum phosphate or piglets vaccinated with 50 micrograms of antigen and with 600 micrograms of aluminum phosphate;

[0024] FIG. 9A shows virus shedding in fecal samples of piglets vaccinated with no antigen and with 600 micrograms of aluminum phosphate; and

[0025] FIG. 9B shows virus shedding in fecal samples of piglets vaccinated with 50
15 micrograms of antigen and with 600 micrograms of aluminum phosphate.

SUMMARY OF THE INVENTION

[0026] A vaccine composition is provided including one or more isolated rotavirus strains illustratively strain CDC-9 or CDC-66 in combination with a pharmaceutically acceptable carrier. An inventive vaccine optionally includes an adjuvant.

20 [0027] The CDC-9 or CDC-66 strains in an inventive vaccine are optionally live attenuated rotavirus or inactivated rotavirus.

[0028] It is appreciated that an inventive vaccine optionally includes at least two isolated rotavirus strains. The at least two isolated rotavirus strains each independently have a G group serotype of G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13 or G14. Optionally, the
25 at least two isolated rotavirus strains each independently have a P group serotype of P1A, P1B, P2A, P3, P4, P5, P6, P8, P11, or P12.

[0029] An inventive vaccine is optionally administered parenterally or orally.

[0030] An isolated rotavirus strain is also provided that is illustratively CDC-9 or CDC-66 strain.

30 [0031] An inventive vaccine is provided that includes a pharmaceutically acceptable carrier admixed with an isolated rotavirus strain characterized as having a G1 group serotype and an isolated rotavirus strain characterized as having a G2 group serotype. The G1 or G2 group

serotype strains optionally each independently have a P group serotype of P1A, P1B, P2A, P3, P4, P5, P6, P8, P11 or P12. In some embodiments the human rotavirus strain characterized as having a G1 group serotype is CDC-9, or the human rotavirus strain characterized as having a G2 group serotype is CDC-66.

5 [0032] A method of inducing an immunological response to a rotavirus in a subject is provided including administering a vaccine composition including a pharmaceutically acceptable carrier admixed with an isolated human rotavirus strain of CDC-9 or CDC-66.

[0033] A method of inducing an immunological response to a rotavirus in a subject is provided including administering a vaccine composition including a pharmaceutically acceptable
10 carrier admixed with an isolated human rotavirus strain characterized as having a G1 group serotype and an isolated human rotavirus strain characterized as having a G2 group serotype.

[0034] Also provided is a vaccine including a pharmaceutically acceptable carrier admixed with a portion of an isolated human rotavirus. The isolated human rotavirus portion is a peptide or polypeptide including an amino acid sequence of SEQ ID No. 2; SEQ ID No. 5; SEQ ID No.
15 8; SEQ ID No. 11; SEQ ID No. 14; SEQ ID No. 17; SEQ ID No. 20; SEQ ID No. 23; SEQ ID No. 26; SEQ ID No. 29; SEQ ID No. 32; SEQ ID No. 3; SEQ ID No. 6; SEQ ID No. 9; SEQ ID No. 12; SEQ ID No. 15; is SEQ ID No. 18; SEQ ID No. 21; SEQ ID No. 24; SEQ ID No. 27; SEQ ID No. 30; SEQ ID No. 33; SEQ ID No. 71; SEQ ID No. 77; SEQ ID No. 83; SEQ ID No. 89; SEQ ID No. 95; SEQ ID No. 101; SEQ ID No. 107; SEQ ID No. 113; SEQ ID No. 119; SEQ
20 ID No. 125; SEQ ID No. 131; SEQ ID No. 72; SEQ ID No. 78; SEQ ID No. 84; SEQ ID No. 90; SEQ ID No. 96; SEQ ID No. 102; SEQ ID No. 108; SEQ ID No. 114; SEQ ID No. 120; SEQ ID No. 126; SEQ ID No. 132; a homolog thereof or a fragment thereof.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0035] Novel isolated human rotavirus A strains, vaccines including human rotavirus A
25 strains, vaccines including a human rotavirus A polypeptide and/or an immunogenic fragment thereof, anti-rotavirus A antibodies and methods for vaccinating humans against rotavirus A disease are provided according to embodiments of the present invention.

[0036] Scientific and technical terms used herein are intended to have the meanings commonly understood by those of ordinary skill in the art. Such terms are found defined and
30 used in context in various standard references illustratively including J. Sambrook and D.W. Russell, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press; 3rd Ed., 2001; F.M. Ausubel, Ed., Short Protocols in Molecular Biology, Current Protocols; 5th Ed.,

2002; B. Alberts et al., *Molecular Biology of the Cell*, 4th Ed., Garland, 2002; D.L. Nelson and M.M. Cox, *Lehninger Principles of Biochemistry*, 4th Ed., W.H. Freeman & Company, 2004; Wild, D., *The Immunoassay Handbook*, 3rd Ed., Elsevier Science, 2005; Gosling, J. P., *Immunoassays: A Practical Approach*, Practical Approach Series, Oxford University Press, 2005; Antibody Engineering, Kontermann, R. and Dübel, S. (Eds.), Springer, 2001; Harlow, E. and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1988; Ausubel, F. et al., (Eds.), *Short Protocols in Molecular Biology*, Wiley, 2002; J. D. Pound (Ed.) *Immunochemical Protocols*, Methods in Molecular Biology, Humana Press; 2nd ed., 1998; B.K.C. Lo (Ed.), *Antibody Engineering: Methods and Protocols*, Methods in Molecular Biology, Humana Press, 2003; and Kohler, G. and Milstein, C., *Nature*, 256:495-497 (1975); the contents of each of which are incorporated herein by reference.

Human Rotaviruses

[0037] Novel human rotavirus A strains of the present invention are illustratively identified as CDC-9 and CDC-66, fragments thereof or homologues thereof.

[0038] The CDC-9 rotavirus A strain was isolated from a fecal specimen of a 5-month boy in Providence, Rhode Island. Human rotavirus strain CDC-9 was characterized by RT-PCR using G and P type-specific primers. RT-PCR analysis indicates that isolated strain CDC-9 is a strain having a genotype P[8], G1. Particular characteristics of CDC-9, its identification, isolation, and passages in Vero cells are described by Esona, MD, et al., *Human Vaccines*, 2010; 6:1-7, the entire contents of which are incorporated herein by reference.

[0039] Following isolation from the fecal sample, isolated rotavirus strain CDC-9 was adapted to grow in MA104 cells that were prepared and frozen before 1980 and have complete documentation. The CDC-9 strain was then adapted to grow in Vero cells qualified for vaccine production. CDC-9 was purified by performing 3 rounds of limiting dilution and after amplification in Vero cells, was further purified by performing 3 rounds of plaque assays. The isolated strain CDC-9 was passaged 7 and 38 times in MA104 and Vero cells, respectively (total 45 passages). The adaptation and all passages are done using standard operation procedures and certified raw materials and reagents and under the Good Laboratory Practice Guidelines. Unlike other reference or laboratory strains, the isolated strain CDC-9 has complete passage history and documentation.

[0040] The titer of passaged human rotavirus strain CDC-9 is about 10^7 ffu/ml.

[0041] Isolated human rotavirus strain CDC-9 was studied by electron microscopy using CDC-9 virions isolated from the medium of infected Vero cell cultures. FIG. 1A shows an electron micrograph of isolated CDC-9 virions. The micrograph shows the virions to have the morphology typical of human rotavirus A virions.

5 [0042] Isolated human rotavirus strain CDC-9 was further examined using polyacrylamide gel electrophoresis of RNA isolated from the strain. As shown in FIG. 2, CDC-9 has a typical long RNA electropherotype and the RNA profiles of both the original isolate from the stool and the Vero passaged rotavirus are identical. Also shown in FIG. 2 is a standard for comparison including an RNA profile of a Wa genogroup human rotavirus.

10 [0043] Isolated human rotavirus strain CDC-9 in stool and Vero cells (passage 27) was analyzed by sequence analysis of entire genome.

[0044] CDC9 amino acid sequences of proteins encoded by nucleic acids isolated from a stool sample: CDC9 NSP1 aa – stool is SEQ ID No. 2; CDC9 NSP 2 aa – stool is SEQ ID No. 5; CDC9 NSP 3 aa – stool is SEQ ID No. 8; CDC9 NSP 4 aa – stool is SEQ ID No. 11; CDC9 NSP
15 5 aa – stool is SEQ ID No. 14; CDC9 VP1 aa – stool is SEQ ID No. 17; CDC9 VP 2 aa – stool is SEQ ID No. 20; CDC9 VP 3 aa – stool is SEQ ID No. 23; CDC9 VP 4 aa – stool is SEQ ID No. 26; CDC9 VP 6 aa – stool is SEQ ID No. 29; and CDC9 VP 7 aa – stool is SEQ ID No. 32.

[0045] CDC9 nucleotide sequences of nucleic acids isolated from a stool sample: CDC9 NSP1 nt – stool is SEQ ID No. 35; CDC9 NSP 2 nt – stool is SEQ ID No. 38; CDC9 NSP 3 nt –
20 stool is SEQ ID No. 41; CDC9 NSP 4 nt – stool is SEQ ID No. 44; CDC9 NSP 5 nt – stool is SEQ ID No. 47; CDC9 VP1 nt – stool is SEQ ID No. 50; CDC9 VP 2 nt – stool is SEQ ID No. 53; CDC9 VP 3 nt – stool is SEQ ID No. 56; CDC9 VP 4 nt – stool is SEQ ID No. 59; CDC9 VP 6 nt – stool is SEQ ID No. 62; and CDC9 VP 7 nt – stool is SEQ ID No. 65.

[0046] CDC9 amino acid sequences of proteins encoded by nucleic acids isolated from
25 CDC9 rotavirus at passage 27 isolated from Vero cells: CDC9 NSP1 aa – Vero is SEQ ID No. 3; CDC9 NSP 2 aa – Vero is SEQ ID No. 6; CDC9 NSP 3 aa – Vero is SEQ ID No. 9; CDC9 NSP 4 aa – Vero is SEQ ID No. 12; CDC9 NSP 5 aa – Vero is SEQ ID No. 15; CDC9 VP1 aa – Vero is SEQ ID No. 18; CDC9 VP 2 aa – Vero is SEQ ID No. 21; CDC9 VP 3 aa – Vero is SEQ ID No. 24; CDC9 VP 4 aa – Vero is SEQ ID No. 27; CDC9 VP 6 aa – Vero is SEQ ID No. 30; and
30 CDC9 VP 7 aa – Vero is SEQ ID No. 33.

[0047] CDC9 nucleotide sequences of nucleic acids isolated from CDC9 rotavirus at passage 27 isolated from Vero cells: CDC9 NSP1 nt – Vero is SEQ ID No. 36; CDC9 NSP 2 nt –

Vero is SEQ ID No. 39; CDC9 NSP 3 nt – Vero is SEQ ID No. 42; CDC9 NSP 4 nt – Vero is SEQ ID No. 45; CDC9 NSP 5 nt – Vero is SEQ ID No. 48; CDC9 VP1 nt – Vero is SEQ ID No. 51; CDC9 VP 2 nt – Vero is SEQ ID No. 54; CDC9 VP 3 nt – Vero is SEQ ID No. 57; CDC9 VP 4 nt – Vero is SEQ ID No. 60; CDC9 VP 6 nt – Vero is SEQ ID No. 63; and CDC9 VP 7 nt –

5 Vero is SEQ ID No. 66.

[0048] Nucleotide and amino acid sequences of entire genome from CDC-9 rotavirus isolated from stool and infected culture were compared with amino acid and nucleotide sequences of entire genome from reference KU or other G1P8 strains of rotavirus A, as shown herein.

10 **[0049]** Additionally, as shown in Table 2, CDC-9 genes (except for segment 3) share high sequence identity with the corresponding genes of the prototype P[8],G1 human KU strain.

[0050] Table 2. Percentages of nucleotide (NT) and deduced amino acid (AA) identity of rotavirus vaccine strain CDC-9 gene segments compared with cognate gene sequences of prototype rotavirus strain KU.

Gene	CDC-9	
	% NT	% AA
VP1	88	96
VP2	95	98
VP3	77	80
VP4	91	94
VP6	91	98
VP7	93	96
NSP1	83	81
NSP2	90	94
NSP3	93	95
NSP4	93	94
NSP5	93	94

15 **[0051]** In addition, changes in nt and aa sequences of entire genome of CDC-9 strain from stool to passage 27 in Vero cells have been documented, as shown in Table 3.

Table 3 Changes of nt and aa in genes of CDC-9 from stool to passage 27 in Vero cells

Gene segment	# of nt changes	nt position	# of aa changes	aa position
NSP1	1	396 A→G	1	122 Q→R
NSP2	0		0	
NSP3	0		0	
NSP4	0	0	0	
NSP5	1	155C→T	1	45 A→I
VP1	0		0	
VP2	0		0	
VP3 (DS-1)	0		0	
VP4	6	161G→A, 1001C→T, 1101G→A, 1162G→C, 1171A→C, 2025T→C	5	51G→D, 331S→F, 364M→I, 385D→H, 388I→L
VP6	1	325C→T	1	101A→V
VP7	1	678G→A	0	
Total:	10		8	

[0052] Isolated rotavirus CDC-9 in Vero cells is a reassortant that has all (except segment 3) genes from a KU-like strain. CDC-9 has a segment 3 derived from a DS-1 like strain as CDC-9 VP3 shares a high identity with the cognate gene of DS-1 strain. This reassortment might have occurred during natural infection or when G1P8 and G2P4 rotaviruses in the fecal specimen were adapted and passaged in cell culture. Rotavirus VP3 has been described to possess guanylyltransferase and may be involved in viral replication and morphogenesis.

[0053] The CDC-66 rotavirus A strain was isolated from a fecal specimen of an 11-month girl in Providence, Rhode Island. Human rotavirus strain CDC-66 was characterized by RT-PCR using G and P type-specific primers. RT-PCR analysis indicates that isolated strain CDC-66 is a strain having a serotype P[4], G2.

[0054] Following isolation from the fecal sample, isolated rotavirus strain CDC-66 was adapted to grow in MA104 cells that were prepared and frozen before 1980 and have complete passage history and documentation. The CDC-66 strain was then adapted to grow in Vero cells qualified for vaccine production. CDC-66 was purified by performing 3 rounds of limiting dilution and after amplification in Vero cells, was further purified by performing 3 rounds of plaque assays. The isolated strain CDC-66 was passaged 5 and 40 times in MA104 and Vero

cells, respectively (total 45 passages). The adaptation and all passages are done using standard operation procedures and certified raw materials and reagents and under the Good Laboratory Practice Guidelines. Unlike other reference and laboratory strains, the isolated strain CDC-66 has complete passage history and documentation.

5 [0055] The titer of passaged human rotavirus strain CDC-66 is about 10^7 pfu/ml.

[0056] Isolated human rotavirus strain CDC-66 was studied by electron microscopy using CDC-66 virions isolated from the medium of infected Vero cell cultures. FIG. 1B shows an electron micrograph of isolated CDC-66 virions. The micrograph shows the virions to have the morphology typical of human rotavirus virions.

10 [0057] Isolated human rotavirus strain CDC-66 was further examined using polyacrylamide gel electrophoresis of RNA isolated from the strain. As shown in FIG. 3, CDC-66 has a typical short RNA electropherotype and the RNA profiles of both the original isolate from the stool and the Vero passaged rotavirus are identical. Also shown in FIG. 3 are standards for comparison including a DNA molecular weight marker III (Roche) in the far left lane and an RNA profile of
15 rhesus rotavirus, RRV.

[0058] Isolated human rotavirus strain CDC-66 in stool and Vero cells (passage 27) was analyzed by sequence analysis of entire genome.

[0059] CDC66 amino acid sequences of proteins encoded by nucleic acids isolated from a stool sample: CDC66 NSP1 aa – stool is SEQ ID No. 71; CDC66 NSP 2 aa – stool is SEQ ID
20 No. 77; CDC66 NSP 3 aa – stool is SEQ ID No. 83; CDC66 NSP 4 aa – stool is SEQ ID No. 89; CDC66 NSP 5 aa – stool is SEQ ID No. 95; CDC66 VP1 aa – stool is SEQ ID No. 101; CDC66 VP 2 aa – stool is SEQ ID No. 107; CDC66 VP 3 aa – stool is SEQ ID No. 113; CDC66 VP 4 aa – stool is SEQ ID No. 119; CDC66 VP 6 aa – stool is SEQ ID No. 125; and CDC66 VP 7 aa – stool is SEQ ID No. 131.

25 [0060] CDC-66 nucleotide sequences of proteins encoded by nucleic acids isolated from a stool sample: CDC66 NSP1 nt – stool is SEQ ID No. 68; CDC66 NSP 2 nt – stool is SEQ ID No. 74; CDC66 NSP 3 nt – stool is SEQ ID No. 80; CDC66 NSP 4 nt – stool is SEQ ID No. 86; CDC66 NSP 5 nt – stool is SEQ ID No. 92; CDC66 VP1 nt – stool is SEQ ID No. 98; CDC66 VP 2 nt – stool is SEQ ID No. 104; CDC66 VP 3 nt – stool is SEQ ID No. 110; CDC66 VP 4 nt
30 – stool is SEQ ID No. 116; CDC66 VP 6 nt – stool is SEQ ID No. 122; and CDC66 VP 7 nt – stool is SEQ ID No. 128.

[0061] CDC-66 amino acid sequences of proteins encoded by nucleic acids isolated at passage 27 isolated from Vero cells: CDC66 NSP1 aa – Vero is SEQ ID No. 72; CDC66 NSP 2 aa – Vero is SEQ ID No. 78; CDC66 NSP 3 aa – Vero is SEQ ID No. 84; CDC66 NSP 4 aa – Vero is SEQ ID No. 90; CDC66 NSP 5 aa – Vero is SEQ ID No. 96; CDC66 VP1 aa – Vero is SEQ ID No. 102; CDC66 VP 2 aa – Vero is SEQ ID No. 108; CDC66 VP 3 aa – Vero is SEQ ID No. 114; CDC66 VP 4 aa – Vero is SEQ ID No. 120; CDC66 VP 6 aa – Vero is SEQ ID No. 126; and CDC66 VP 7 aa – Vero is SEQ ID No. 132.

[0062] CDC-66 nucleotide sequences of proteins encoded by nucleic acids isolated at passage 27 isolated from Vero cells: CDC66 NSP1 nt – Vero is SEQ ID No. 69; CDC66 NSP 2 nt – Vero is SEQ ID No. 75; CDC66 NSP 3 nt – Vero is SEQ ID No. 81; CDC66 NSP 4 nt – Vero is SEQ ID No. 87; CDC66 NSP 5 nt – Vero is SEQ ID No. 93; CDC66 VP1 nt – Vero is SEQ ID No. 99; CDC66 VP 2 nt – Vero is SEQ ID No. 105; CDC66 VP 3 nt – Vero is SEQ ID No. 111; CDC66 VP 4 nt – Vero is SEQ ID No. 117; CDC66 VP 6 nt – Vero is SEQ ID No. 123; and CDC66 VP 7 nt – Vero is SEQ ID No. 129.

[0063] Entire amino acid and nucleotide sequences from CDC-66 rotavirus isolated from stool and Vero cells are compared with entire amino acid and nucleotide sequences of a strain of rotavirus A considered to be the closest related known rotavirus A, human rotavirus DS-1 strain or another closely related strain, as shown herein.

[0064] As shown in Table 4, CDC-66 genes share high sequence identity with the corresponding genes of the prototype P[4],G2 strain DS-1.

[0065] Table 4. Percentages of nucleotide (NT) and deduced amino acid (AA) identity of rotavirus vaccine strain CDC-66 genome compared with genome sequence of prototype rotavirus strain DS-1.

Table 4.

Gene	nt %	aa %
VP1	90.85	97.24
VP2	94.11	98.86
VP3	92.9	95.69
VP4	94.02	96.52
VP6	87.98	98.74
VP7	93.88	96.32
NSP1	93.12	93.21
NSP2	86.89	93.99
NSP3	95.31	97.12
NSP4	94.76	95.43
NSP5	92.57	96.5

[0066] Changes in nt and aa sequences of entire genome of CDC-66 strain from stool to the passage in Vero cells have been documented, as shown in Table 5.

5 Table 5. Changes of nt and aa in genes of CDC-66 from stool to passage 27 in Vero cells

Gene	nt change	aa change
NSP1	none	none
NSP2	470 C→T	142 H → Y
NSP3	none	none
NSP4	none	none
NSP5	126 T→C, 199 A→G	60 I→V
VP1	440 A→G	141 N→S
VP2	none	none
VP3	760 T→C, 1143 T→C, 1882 A→G	365 L→S
VP4	770C→A, 1109T→C, 1162G→A, 1184A→G	254T→K, 367V→A, 385D→N, 392E→G
VP6	none	none
VP7	none	none

[0067] Vaccines

[0068] Vaccines and methods for their use to induce active immunity and protection against
 10 rotavirus induced illness in a subject are provided according to the present invention.

[0069] In particular embodiments, vaccine compositions for enhancing immunological protection against a rotavirus-mediated disease in a subject are provided according to the present invention which includes a human rotavirus strain admixed with a pharmaceutically acceptable carrier.

5 [0070] The term “vaccine composition” is used herein to refers to a composition including a biological agent capable of inducing an immune response in a subject inoculated with the vaccine composition. In particular embodiments, the biological agent is a live attenuated and/or inactive rotavirus. In further embodiments, the biological agent is an antigenic portion of a rotavirus.

10 [0071] In particular embodiments, a human rotavirus strain included in a vaccine composition of the present invention is CDC-9 or CDC-66. Combinations of these human rotavirus strains are optionally included in vaccine compositions of the present invention. Additionally, a human rotavirus strain other than CDC-9 or CDC-66 is optionally included in a vaccine composition of the present invention.

15 [0072] In particular embodiments of a vaccine composition according to the present invention, at least two rotavirus strains are included. The two or more rotavirus strains each independently have a G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13 or G14 G serotype. Thus, for example, at least one of CDC-9 or CDC-66 is present in a vaccine composition of the present invention along with at least a second human rotavirus strain which
20 has a G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13 or G14 G serotype.

[0073] Each of the at least two rotavirus strains included in a vaccine composition has a P serotype which is P1A, P1B, P2A, P3, P4, P5, P6, P8, P11, or P12 in particular embodiments.

[0074] A vaccine composition for enhancing immunological protection against a rotavirus-mediated disease in a subject includes a first human rotavirus strain characterized as having a G1
25 serotype and a second human rotavirus strain characterized as having a G2 serotype in particular embodiments of a vaccine composition of the present invention. Each of the two rotavirus strains independently has a P group serotype which is P1A, P1B, P2A, P3, P4, P5, P6, P8, P11 or P12.

[0075] In some embodiments, the human rotavirus strain having a G1 serotype is CDC-9.

30 [0076] In some embodiments, the human rotavirus strain having a G2 serotype is CDC-66.

[0077] Combinations of human rotavirus strains in particular embodiments of a vaccine composition of the present invention include CDC-9 and CDC-66.

[0078] A human rotavirus strain included in a vaccine composition according to the present invention is a live attenuated rotavirus or an inactivated rotavirus. The choice of live attenuated rotavirus or inactivated rotavirus depends on factors such as route of vaccine composition administration.

5 [0079] In a particular embodiment, a vaccine composition including a human rotavirus A strain CDC-9, and/or CDC-66, one or more rotavirus A CDC-9, and/or CDC-66 polypeptides and/or an immunogenic fragment of one or more rotavirus A CDC-9, and/or CDC-66 polypeptides, stimulates generation of neutralizing antibodies to a rotavirus A CDC-9, and/or CDC-66 strain.

10 [0080] Vaccine compositions are provided according to embodiments of the present invention which include one or more rotavirus A polypeptides and/or an immunogenic fragment of one or more rotavirus A polypeptides. In particular embodiments of an inventive vaccine composition, a CDC-9, and/or CDC-66 polypeptide, a homolog thereof, and/or an immunogenic fragment thereof is included.

15 [0081] The term “homolog” refers to a polypeptide characterized by amino acid sequence homology to a reference CDC-9 or CDC-66 rotavirus A polypeptide.

CDC-9 Sequences

[0082] Accordingly, the present invention provides a virus including an NSP1 having SEQ
20 ID NO: 2 or a homolog having an amino acid sequence that is greater than 80%, is greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 2. Further, the
25 present invention provides a virus including an NSP1 having SEQ ID NO: 3 or a homolog having an amino acid sequence that is greater than 80%, is greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%,
30 greater than 98% or greater than 99% identical to SEQ ID NO: 3.

[0083] The present invention provides a virus including an NSP2 having SEQ ID NO: 5 or a homolog having an amino acid sequence that is greater than 80%, is greater than 81%, greater

than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 5.

5 **[0084]** The present invention provides a virus including an NSP3 having SEQ ID NO: 8 or a homolog having an amino acid sequence that is greater than 80%, is greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%,
10 greater than 98% or greater than 99% identical to SEQ ID NO: 8.

[0085] The present invention provides a virus including an NSP4 having SEQ ID NO: 11 or a homolog having an amino acid sequence that is greater than 80%, is greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%,
15 greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 11.

[0086] The present invention provides a virus including an NSP5 having SEQ ID NO: 14 or a homolog having an amino acid sequence that is greater than 80%, is greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%,
20 greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 14. Further, the present invention provides a virus including an NSP5 having SEQ ID NO: 15 or a homolog having an amino acid sequence that is greater than 80%, is greater than 81%, greater than 82%, greater than 83%,
25 greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 15.

[0087] Accordingly, the present invention provides a virus including a VP1 having SEQ ID
30 NO: 17 or a homolog having an amino acid sequence that is greater than 80%, is greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%,

greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 17.

[0088] The present invention provides a virus including a VP2 having SEQ ID NO: 20 or a homolog having an amino acid sequence that is greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 20.

[0089] The present invention provides a virus including a VP3 having SEQ ID NO: 23 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 23.

[0090] The present invention provides a virus including a VP4 having SEQ ID NO: 26 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 26. Further, the present invention provides a virus including an VP4 having SEQ ID NO: 27 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 27.

[0091] The present invention provides a virus including a VP6 having SEQ ID NO: 29 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 29. Further, the present invention

provides a virus including an VP6 having SEQ ID NO: 30 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 30.

[0092] The present invention provides a virus including a VP7 having SEQ ID NO: 32 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 32.

CDC-66 Sequences

[0093] The present invention provides a virus including an NSP1 having SEQ ID NO: 71 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 71.

[0094] The present invention provides a virus including an NSP2 having SEQ ID NO: 77 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 77. Further, the present invention provides a virus including an NSP2 having SEQ ID NO: 78 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 78.

[0095] The present invention provides a virus including an NSP3 having SEQ ID NO: 83 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 83.

[0096] The present invention provides a virus including an NSP4 having SEQ ID NO: 89 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 89.

[0097] The present invention provides a virus including an NSP5 having SEQ ID NO: 95 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 95. Further, the present invention provides a virus including an NSP5 having SEQ ID NO: 96 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 96.

[0098] Accordingly, the present invention provides a virus including a VP1 having SEQ ID NO: 101 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 101. Further, the present invention provides a virus including an VP1 having SEQ ID NO: 102 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%,

greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 102.

5 [00099] The present invention provides a virus including a VP2 having SEQ ID NO: 107 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%,
10 greater than 98% or greater than 99% identical to SEQ ID NO: 107.

[00100] The present invention provides a virus including a VP3 having SEQ ID NO: 113 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%,
15 greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 113. Further, the present invention provides a virus including an VP3 having SEQ ID NO: 114 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%,
20 greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 114.

[00101] The present invention provides a virus including a VP4 having SEQ ID NO: 119 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 119. Further, the present invention provides a virus including an VP4 having SEQ ID NO: 120 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%,
30

greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 120.

[00102] The present invention provides a virus including a VP6 having SEQ ID NO: 125 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 125.

[00103] The present invention provides a virus including a VP7 having SEQ ID NO: 131 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 131.

[00104] The present invention provides an isolated or purified NSP1, NSP2, NSP3, NSP4, NSP5, VP1, VP2, VP3, VP4, VP6, or VP7. The term “purified” or “isolated” as used herein, is intended to refer to a composition, isolatable from other components, wherein the composition is purified to any degree relative to its naturally-obtainable state, i.e., in this case, relative to its purity within a cell, relative to its purity within a virion, or relative to its purity within an infective organism. An isolated composition, therefore, also refers to a protein, peptide, nucleic acid, or oligonucleotide, substantially free from the environment in which it may naturally occur.

[00105] It is recognized that numerous variants, analogues, or homologues are within the scope of the present invention including amino acid substitutions, alterations, modifications, or other amino acid changes that increase, decrease, or do not alter the function or immunogenic propensity of the inventive immunogen or vaccine. It is further appreciated that the inventive sequences are optionally modified by the addition of one or more amino acids, sugars, nucleotides, pendent groups, fluorophores, lumiphores, radioactive molecules, lipids, fatty acids, derivatives thereof, or other groups known in the art. Illustratively, an inventive immunogen is conjugated to a protein. Optionally, an inventive immunogen is conjugated to a protein that promotes the immunogenicity of an immunogen, illustratively, keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), or modifications thereof, as well as BLUE CARRIER immunogenic protein from Thermo Scientific, Rockford, IL. Other sources of natural or

artificial immunogenic protein conjugates are known in the art. Optionally, an inventive immunogen is conjugated to an antibody. Optionally, an inventive immunogen is conjugated to other regions of G-protein that may or may not also contain epitopes.

[00106] In some embodiments, the NSP1 has SEQ ID NO: 2 or SEQ ID No. 3, or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 2 or to SEQ ID No. 3. In a further embodiment, the NSP1 has SEQ ID NO: 71 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 71.

[00107] The present invention provides an isolated or purified NSP2. In one embodiment, the NSP2 has SEQ ID NO: 5 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 5. In a further embodiment, the NSP2 has SEQ ID NO: 77 or SEQ ID NO: 78 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 77 or SEQ ID NO: 78.

[00108] The present invention provides an isolated or purified NSP3. In one embodiment, the NSP3 has SEQ ID NO: 8 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%,

greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 8. In a further embodiment, the NSP3 has SEQ ID NO: 83 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 83.

[00109] The present invention provides an isolated or purified NSP4. In one embodiment, the NSP4 has SEQ ID NO: 11 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 11. In a further embodiment, the NSP4 has SEQ ID NO: 89 or a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 89

[00110] The present invention provides an isolated or purified NSP5. In one embodiment, the NSP5 has SEQ ID NO: 14 or SEQ ID NO: 15 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 14 or SEQ ID NO: 15. In a further embodiment, the NSP5 has SEQ ID NO: 95 or SEQ ID NO: 96 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 95 or SEQ ID NO: 96.

[00111] The present invention provides an isolated or purified VP1. In one embodiment, the VP1 has SEQ ID NO: 17 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 17. In a further embodiment, the VP1 has SEQ ID NO: 101 or SEQ ID NO.102 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 101 or SEQ ID NO.102.

[00112] The present invention provides an isolated or purified VP2. In one embodiment, the VP2 has SEQ ID NO: 20 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 20. In a further embodiment, the VP2 has SEQ ID NO: 107 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 107.

[00113] The present invention provides an isolated or purified VP3. In one embodiment, the VP3 has SEQ ID NO: 23 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 23. In a further embodiment, the VP3 has SEQ ID NO: 113 or SEQ ID NO. 114 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than

82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 113 or SEQ ID NO. 114.

5 **[00114]** The present invention provides an isolated or purified VP4. In one embodiment, the VP4 has SEQ ID NO: 26 or SEQ ID NO. 27 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%,
10 greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 26 or SEQ ID NO. 27. In a further embodiment, the VP4 has SEQ ID NO: 119 or SEQ ID NO. 120 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%,
15 greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 119 or SEQ ID NO. 120.

[00115] The present invention provides an isolated or purified VP6. In one embodiment, the VP6 has SEQ ID NO: 29 or SEQ ID NO. 30 or is a homolog having an amino acid sequence that
20 is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 29 or SEQ ID NO. 30. In a further embodiment, the VP6 has SEQ ID
25 NO: 125 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 125.

30 **[00116]** The present invention provides an isolated or purified VP7. In one embodiment, the VP7 has SEQ ID NO: 32 or is a homolog having an amino acid sequence that is greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%,

greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 32. In a further embodiment, the VP7 has SEQ ID NO: 131 or is a homolog having an amino acid sequence that is greater than 97%, greater than 98% or greater than 99% identical to SEQ ID NO: 131.

[00117] An isolated or purified nucleic acid encoding an above-described protein or fragment thereof is provided according to embodiments of the present invention. Optionally, the isolated or purified nucleic acid encoding an above-described protein or fragment thereof is included in a vector.

[00118] A nucleic acid encoding NSP1 includes the nucleotide sequence of SEQ ID NO: 35; SEQ ID NO: 36; SEQ ID NO: 68 or a fragment thereof encoding at least nine contiguous amino acids. A nucleic acid encoding NSP2 includes the nucleotide sequence of SEQ ID NO: 38; SEQ ID NO: 74; SEQ ID NO: 75; or a fragment thereof encoding at least nine contiguous amino acids. A nucleic acid encoding NSP3 includes the nucleotide sequence of SEQ ID NO: 41; SEQ ID NO: 80; or a fragment thereof encoding at least nine contiguous amino acids. A nucleic acid encoding NSP4 includes the nucleotide sequence of SEQ ID NO: 44; SEQ ID NO: 86; or a fragment thereof encoding at least nine contiguous amino acids. A nucleic acid encoding NSP5 includes the nucleotide sequence of SEQ ID NO: 47; SEQ ID NO: 48; SEQ ID NO: 92; SEQ ID NO: 93; or a fragment thereof encoding at least nine contiguous amino acids. A nucleic acid encoding VP1 includes the nucleotide sequence of SEQ ID NO: 50; SEQ ID NO: 98; SEQ ID NO: 99; or a fragment thereof encoding at least nine contiguous amino acids. A nucleic acid encoding VP2 includes the nucleotide sequence of SEQ ID NO: 53; SEQ ID NO: 104; or a fragment thereof encoding at least nine contiguous amino acids. A nucleic acid encoding VP3 includes the nucleotide sequence of SEQ ID NO: 23; SEQ ID NO: 110; SEQ ID NO: 111; or a fragment thereof encoding at least nine contiguous amino acids. A nucleic acid encoding VP4 includes the nucleotide sequence of SEQ ID NO: 59; SEQ ID NO: 60; SEQ ID NO: 116; SEQ ID NO: 117; or a fragment thereof encoding at least nine contiguous amino acids. A nucleic acid encoding VP6 includes the nucleotide sequence of SEQ ID NO: 62; SEQ ID NO: 63; SEQ ID NO: 122; or a fragment thereof encoding at least nine contiguous amino acids. A nucleic acid encoding VP7 includes the nucleotide sequence of SEQ ID NO: 65; SEQ ID NO: 128; SEQ ID NO: 129; or a fragment thereof encoding at least nine contiguous amino acids.

[00119] One of skill in the art will appreciate that, due to the degeneracy of the genetic code, a particular polypeptide or fragment thereof can be encoded by more than one nucleic acid sequence.

[00120] Mutations can be introduced using standard molecular biology techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. One of skill in the art will recognize that one or more amino acid mutations can be introduced without altering the functional properties of rotavirus polypeptides. For example, one or more amino acid substitutions, additions, or deletions can be made without altering the functional properties of rotavirus polypeptides. It is also appreciated that several mutations optionally increase, decrease, or do not change the immunogenicity of an inventive polypeptide.

[00121] Conservative amino acid substitutions can be made in rotavirus polypeptides to produce homologs. Conservative amino acid substitutions are art recognized substitutions of one amino acid for another amino acid having similar characteristics. For example, each amino acid may be described as having one or more of the following characteristics: electropositive, electronegative, aliphatic, aromatic, polar, hydrophobic and hydrophilic. A conservative substitution is a substitution of one amino acid having a specified structural or functional characteristic for another amino acid having the same characteristic. Acidic amino acids include aspartate, glutamate; basic amino acids include histidine, lysine, arginine; aliphatic amino acids include isoleucine, leucine and valine; aromatic amino acids include phenylalanine, glycine, tyrosine and tryptophan; polar amino acids include aspartate, glutamate, histidine, lysine, asparagine, glutamine, arginine, serine, threonine and tyrosine; and hydrophobic amino acids include alanine, cysteine, phenylalanine, glycine, isoleucine, leucine, methionine, proline, valine and tryptophan; and conservative substitutions include substitution among amino acids within each group. Amino acids may also be described in terms of relative size, alanine, cysteine, aspartate, glycine, asparagine, proline, threonine, serine, valine, all typically considered to be small.

[00122] In making such changes, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art. It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. Those indices are: isoleucine

(+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

5 **[00123]** It is believed that the relative hydropathic character of the amino acid determines the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, such as enzymes, substrates, receptors, antibodies, antigens, and the like. It is known in the art that an amino acid can be substituted by another amino acid having a similar hydropathic index and still obtain a functionally equivalent polypeptide. In such
10 changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

[00124] Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly, where the biological functional equivalent polypeptide or peptide thereby created is
15 intended for use in immunological embodiments. The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 $\pm$ 1); glutamate (+3.0 $\pm$ 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5 $\pm$ 1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is
20 understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

25 **[00125]** As outlined above, amino acid substitutions are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include (original residue: exemplary substitution): (Ala: Gly, Ser), (Arg: Lys), (Asn: Gln, His), (Asp:
30 Glu, Cys, Ser), (Gln: Asn), (Glu: Asp), (Gly: Ala), (His: Asn, Gln), (Ile: Leu, Val), (Leu: Ile, Val), (Lys: Arg), (Met: Leu, Tyr), (Ser: Thr), (Thr: Ser), (Tyr: Trp, Phe), and (Val: Ile, Leu). Embodiments of this disclosure thus contemplate functional or biological equivalents

of a polypeptide as set forth above. In particular, embodiments of the polypeptides can include variants having about 50%, 60%, 70%, 80%, 90%, and 95% sequence identity to the polypeptide of interest.

5 [00126] Rotavirus particles, nucleic acids, polypeptides and fragments thereof can be produced in recombinant host cells using well-known conventional techniques. Any nucleic acid construct, which is effective in producing the encoded protein or fragment thereof in a host cell, can be used to produce rotavirus particles, rotavirus polypeptides or fragments thereof.

[00127] One of ordinary skill in the art recognizes many ways to make the inventive CDC-9 or CDC-66 viruses for use in an inventive vaccine composition or in inventive processes. 10 Illustratively, it is common practice for one to isolate a putative rotavirus from a stool or other biological sample optionally including passaging in cell culture such as in Vero cells similar to the methods illustrated in Esona, MD, et al., *Human Vaccines*, 2010; 6:1-7; the contents of which are incorporated herein by reference. One of skill in the art regularly isolates virus strains and characterizes the genome sequence by techniques well known in the art. It is common practice 15 for one of skill in the art to sequence a viral genome and compare the output sequence to a model sequence such as the sequences of CDC-9 or CDC-66 disclosed herein to determine whether the isolated virus has the required genetic sequence to be CDC-9, CDC-66, or homologues thereof.

[00128] One of skill in the art also knows methods of modifying a model rotavirus such as KU or DS-1 to make CDC-9 or CDC-66 viruses. One such method uses the reverse genetics 20 approach of Komoto, S., et al., *PNAS USA*, 2006; 103:4646-4651, the contents of which are incorporated herein by reference. Briefly, each of the genes of strain KU can be isolated and amplified by taking culture fluid from infected MA104 cells, extracting the viral dsRNA and synthesizing cDNA using with avian myeloblastosis virus reverse transcriptase (Seikagaku Kogyo, Tokyo, Japan) using a starting primer. It is well within the level of skill in the art to 25 design primers for synthesizing cDNA. Numerous free and commercially available programs for primer synthesis are known to those of skill in the art. Illustratively, primers for the KU VP4 gene are described in Komoto, S., et al., *PNAS USA*, 2006; 103:4646-4651. Modification of the sequences of KU or any other strain to those of CDC-9 or CDC-66 are illustratively accomplished by establishing the sequence modifications such as by sequence alignments. Once 30 the nucleotide substitutions are elucidated, modifications of the cDNA can be achieved by using the QUICKCHANGE XL site-directed mutagenesis kit available from Agilent Technologies, Santa Clara, CA. Modified gene sequences to conform to that of CDC-9, CDC-66, or

homologues thereof are optionally inserted into a cell line such as COS-7 cells along with a helper virus such as KU used to serve as a base for gene insertion into new viruses. The modified viruses are subsequently isolated by known techniques. An optional iterative process is used whereby each individual gene of CDC-9 or CDC-66 is substituted for the gene of the helper virus step wise whereby the isolated substituted virus strain from the substitution of the first gene is used as a helper virus for substitution of the second gene and so on until CDC-9 or CDC-66 is created from a source strain.

[00129] Illustrative examples of helper viruses or model rotaviruses can be found at GenBank Accession Nos: (a) VP3 strains: RV161-00 (DQ490547), RV176-00 (DQ490553), DRC88 (DQ005112), DRC86 (DQ005123), TB-Chen (AY787654), DS-1 (AY277914), AU-1 (DQ490537), T152 (DQ146701), Hosokawa (DQ870491), Hoshi (AY277915), Wa (AY267335), L26 (AY277918), KU (AB022767), Dhaka25-02 (DQ146651), Dhaka12-03 (DQ146662), B4633-03 (DQ146640), PO-13 (AB009631).

[00130] (b) VP7 (G1) strains: SK417 (EU839907), DH415 (EU839906), MMC82 (EU839913), Dhaka18-02 (AY631051), MMC56 (EU839911), Matlab159-02 (AY631055), ISO-4 (AY098670), Thai-2104 (DQ512982), CMH042/04 (EF199713), 417 (D16328), T73 (AF450291), TE1 (D17721), K18 (D16319), Chi-87 (DQ512998), J-4825 (DQ512989), 88H249 (AB081795), 421 (D16326), RV-4 (M64666), AU007 (AB081799), HOU8697 (U88717), Mvd9607 (AF480295), 80 (D16325), DC03 (AF183859), KU (AB222788), K2 (D16323), K8 (D16344), Egy-8 (U26374), Brz-5 (U26367), Wa (K02033), D (AB118022), C95 (L24165), T449 (M92651), DS-1 (AB118023).

[00131] (c) VP4 P[8] strains: ITO (AB008280), D (EF672570), Wa (L34161), Hoshi (AB008295), Odelia (AB008296), VA70 (AJ540229), CH32 (AB008274), MO (AB008278), KU (AB222784), Wi61 (EF672619), F45 (U30716) P (EF672598), AI-39 (AB008283), 90-544 (AB008304), B4633-03 (DQ146641), Dhaka25-02 (DQ146652), SK438 (EU839955), DH402 (EU839958), DH415 (EU839955), DS-1 (AB118025).

[00132] (d) NSP4 strains: Dhaka16-03 (DQ492678), 1099 (AJ236759), Dhaka12-03 (DQ146669), Dhaka25-02 (DQ146658), KU (AB022772), Wa (AF093199), RMC321 (AF541921), OSU (D88831), AU-1 (D89873), CMH120/04 (DQ923799), B4106 (AY740732), C-11 (AF144793), DRC86 (DQ005116), DRC88 (DQ005105), DS-1 (AF174305), TB-Chen (AY787650), Ch-1 (AB065287).

[00133] Each of the files and sequences at the aforementioned accession numbers are incorporated herein by reference.

[00134] Other methods, primers, isolation techniques, sequencing techniques, and characterization techniques are known to those of skill in the art and are similarly operable
5 herein. Illustratively, one can reconstitute CDC-9 or CDC-66 viruses de novo from isolated genes such as by assembly of virus particles with captured genes illustratively by the techniques of or modifications of Gonzalez, SA, and Affranchino, JL, J. Gen. Virol., 1995; 76:2357-2360, the contents of which are incorporated herein by reference.

[00135] Expression constructs and methods for their generation and use to express a desired
10 protein are known in the art, as described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2001; Ausubel, F. et al., (Eds.), Short Protocols in Molecular Biology, Wiley, 2002; and S. J. Higgins and B. D. Hames (Eds.), Protein Expression: A Practical Approach, Oxford University Press, USA, 1999.

[00136] Illustratively, a nucleic acid molecule encoding one or more rotavirus polypeptides
15 and/or rotavirus polypeptide fragments is operably linked to regulatory sequences that control transcriptional expression in an expression vector. The expression vector is introduced into a host cell and the produced rotavirus particles, one or more rotavirus polypeptides and/or rotavirus polypeptide fragments can then be isolated. Illustrative constructs include operably linking a rotavirus nucleic acid molecule into plasmid pT7 by first amplifying the nucleic acid
20 molecule using primers including a T7 promoter sequence and ligating the amplified nucleic acids into the T7 expression vector pX8dT as described by Schnell, MJ, et al., EMBO J, 1994; 13:4195-4203, the contents of which are incorporated herein by reference.

[00137] Non-limiting examples of regulatory sequences that control transcriptional
25 expression in an expression vector illustratively include a promoter, an enhancer, a splicing signal, a transcription start site, a transcription termination signal, a polyadenylation signal, an internal ribosome entry site (IRES) and combinations of these or other regulatory sequences.

[00138] Expression vectors include, but are not limited to, viral vectors and bacterial vectors
30 used to express a desired protein. Non-limiting examples of expression vectors include bacterial plasmids, bacteriophage, adenovirus, adeno-associated virus, herpes virus, vaccinia virus and lentivirus.

[00139] A host cell for expression of polypeptides and fragments thereof can be prokaryotic or eukaryotic, such as bacterial, plant, insect, fungus, yeast, and mammalian cells.

[00140] An expression vector is introduced into a host cell using well-known techniques such as infection or transfection, including calcium phosphate transfection, liposome-mediated transfection, electroporation and sonoporation.

[00141] In addition to recombinant methodology, chemical synthetic techniques can be used to produce polypeptides and fragments thereof. For example, solid phase synthesis, solution phase synthesis, partial solid phase synthesis or fragment condensation can be used.

[00142] The term “isolated” as used herein refers to a substance that has been separated from other cellular components associated with the substance in nature or when recombinantly produced not intended to be associated with the substance and that may interfere with use of the substance in therapeutic, prophylactic, diagnostic, research or other uses. Generally, an isolated substance described herein is at least about 80% pure, at least about 90% pure, at least about 95% pure, or greater than about 99% pure. Purification is achieved using well-known standard methodology such as fractionation and/or chromatography, such as ammonium sulfate precipitation and elution chromatography such as size exclusion chromatography, displacement chromatography, ion exchange chromatography and bioaffinity chromatography. Exemplary purification methodology is described in S. Doonan, Protein Purification Protocols Humana Press, 1996.

[00143] Percent identity is determined by comparison of amino acid or nucleic acid sequences, including a reference rotavirus A amino acid or nucleic acid sequence and a putative homologue amino acid or nucleic acid sequence. Algorithms used for determination of percent identity illustratively include the algorithms of S. Karlin and S. Altshul, PNAS, 90:5873-5877, 1993; T. Smith and M. Waterman, Adv. Appl. Math. 2:482-489, 1981, S. Needleman and C. Wunsch, J. Mol. Biol., 48:443-453, 1970, W. Pearson and D. Lipman, PNAS, 85:2444-2448, 1988 and others incorporated into computerized implementations such as, but not limited to, GAP, BESTFIT, FASTA, TFASTA; and BLAST, for example incorporated in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI and publicly available from the National Center for Biotechnology Information.

[00144] Rotavirus A CDC-9, and/or CDC-66 polypeptides, a homolog thereof, and/or an immunogenic fragment thereof may be prepared by any of various methods, including isolation of virus particles from sources such as cultured cells or patient samples, isolation of one or more polypeptides and/or one or more polypeptide fragments from viral particles, recombinant production of viral polypeptides, fragments and/or viral particles, including intact and virus-like

particles, and/or by chemical synthetic techniques. Methods of isolation of virus particles, virus polypeptides and/or one or more virus polypeptide fragments, recombinant production of virus polypeptides, virus polypeptide fragments and/or virus particles are described in detail herein, in references cited herein, and are known to those of skill in the art.

5 [00145] An antigen may be made more immunogenic if desired by linkage to a carrier molecule such bovine serum albumin or keyhole limpet hemocyanin and/or by use of an adjuvant. Carrier linkage may be accomplished by any of various techniques, illustratively including, but not limited to, conjugation and expression of a fusion protein.

[00146] Recombinantly expressed polypeptides or peptides may be tagged to allow for easier
10 isolation. For instance, such polypeptides and peptides are optionally tagged illustratively, Fc-tagged, 6xHIS-tagged, FLAG-tagged, or by other tag suitable for isolation of a tagged polypeptide.

Vaccine formulation

15 [00147] In particular embodiments of the invention, a rotavirus A strain for inclusion in a vaccine composition of the present invention is prepared by standard methods typically used for preparation of live or inactivated rotavirus. For example, generally a compatible cell type is inoculated with a rotavirus strain and the cells are maintained under conditions which allow for viral replication and production of infectious particles.

20 [00148] A particular example of a cell type which permits rotavirus infection, replication and particle production is a mammalian cell line such as a Vero cell line.

[00149] Rotavirus particles are harvested, typically from cell culture supernatant for inclusion in a vaccine composition. The rotavirus particles may be isolated from the cell culture supernatant, for example by filtration and/or centrifugation. The isolated rotavirus particles are
25 optionally lyophilized, such as for later resuspension in a pharmaceutically acceptable carrier.

[00150] The term “pharmaceutically acceptable carrier” refers to a carrier which is substantially non-toxic to a subject and substantially inert to the rotavirus included in a vaccine composition. A pharmaceutically acceptable carrier is a solid, liquid or gel in form and is typically sterile and pyrogen free.

30 [00151] A vaccine composition of the present invention may be in any form suitable for administration to a subject.

[00152] A vaccine composition is administered by any suitable route of administration including oral and parenteral such as intravenous, intradermal, intramuscular, mucosal, nasal, or subcutaneous routes of administration.

[00153] For example, a vaccine composition for parenteral administration may be formulated as an injectable liquid including a rotavirus and a pharmaceutically acceptable carrier. Examples of suitable aqueous and nonaqueous carriers include water, ethanol, polyols such as propylene glycol, polyethylene glycol, glycerol, and the like, suitable mixtures thereof; vegetable oils such as olive oil; and injectable organic esters such as ethyloleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of a desirable particle size in the case of dispersions, and/or by the use of a surfactant, such as sodium lauryl sulfate. A stabilizer is optionally included such as, for example, sucrose, EDTA, EGTA, and an antioxidant.

[00154] A solid dosage form for administration or for suspension in a liquid prior to administration illustratively includes capsules, tablets, powders, and granules. In such solid dosage forms, a rotavirus is admixed with at least one carrier illustratively including a buffer such as, for example, sodium citrate or an alkali metal phosphate illustratively including sodium phosphates, potassium phosphates and calcium phosphates; a filler such as, for example, starch, lactose, sucrose, glucose, mannitol, and silicic acid; a binder such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia; a humectant such as, for example, glycerol; a disintegrating agent such as, for example, agar-agar, calcium carbonate, plant starches such as potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate; a solution retarder such as, for example, paraffin; an absorption accelerator such as, for example, a quaternary ammonium compound; a wetting agent such as, for example, cetyl alcohol, glycerol monostearate, and a glycol; an adsorbent such as, for example, kaolin and bentonite; a lubricant such as, for example, talc, calcium stearate, magnesium stearate, a solid polyethylene glycol or sodium lauryl sulfate; a preservative such as an antibacterial agent and an antifungal agent, including for example, sorbic acid, gentamycin and phenol; and a stabilizer such as, for example, sucrose, EDTA, EGTA, and an antioxidant.

[00155] Solid dosage forms optionally include a coating such as an enteric coating. The enteric coating is typically a polymeric material. Preferred enteric coating materials have the characteristics of being bioerodible, gradually hydrolyzable and/or gradually water-soluble polymers. The amount of coating material applied to a solid dosage generally dictates the time

interval between ingestion and drug release. A coating is applied having a thickness such that the entire coating does not dissolve in the gastrointestinal fluids at pH below 3 associated with stomach acids, yet dissolves above pH 3 in the small intestine environment. It is expected that any anionic polymer exhibiting a pH-dependent solubility profile is readily used as an enteric coating in the practice of the present invention to achieve delivery of the active agent to the lower gastrointestinal tract. The selection of the specific enteric coating material depends on properties such as resistance to disintegration in the stomach; impermeability to gastric fluids and active agent diffusion while in the stomach; ability to dissipate at the target intestine site; physical and chemical stability during storage; non-toxicity; and ease of application.

5 [00156] Suitable enteric coating materials illustratively include cellulosic polymers such as hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, methyl cellulose, ethyl cellulose, cellulose acetate, cellulose acetate phthalate, cellulose acetate trimellitate, hydroxypropylmethyl cellulose phthalate, hydroxypropylmethyl cellulose succinate and carboxymethylcellulose sodium; acrylic acid polymers and copolymers, preferably formed from acrylic acid, methacrylic acid, methyl acrylate, ammonium methylacrylate, ethyl acrylate, methyl methacrylate and/or ethyl; vinyl polymers and copolymers such as polyvinyl pyrrolidone, polyvinyl acetate, polyvinylacetate phthalate, vinylacetate crotonic acid copolymer, and ethylene-vinyl acetate copolymers; shellac; and combinations thereof. A particular enteric coating material includes acrylic acid polymers and copolymers described for example U.S. Patent No. 6,136,345.

20 [00157] The enteric coating optionally contains a plasticizer to prevent the formation of pores and cracks that allow the penetration of the gastric fluids into the solid dosage form. Suitable plasticizers illustratively include, triethyl citrate (Citroflex 2), triacetin (glyceryl triacetate), acetyl triethyl citrate (Citroflex A2), Carbowax 400 (polyethylene glycol 400), diethyl phthalate, tributyl citrate, acetylated monoglycerides, glycerol, fatty acid esters, propylene glycol, and dibutyl phthalate. In particular, a coating composed of an anionic carboxylic acrylic polymer typically contains approximately 10% to 25% by weight of a plasticizer, particularly dibutyl phthalate, polyethylene glycol, triethyl citrate and triacetin. The coating can also contain other coating excipients such as detackifiers, antifoaming agents, lubricants (e.g., magnesium stearate), and stabilizers (e.g. hydroxypropylcellulose, acids or bases) to solubilize or disperse the coating material, and to improve coating performance and the coated product.

30

[00158] Liquid dosage forms for oral administration include rotavirus and a pharmaceutically acceptable carrier formulated as an emulsion, solution, suspension, syrup, or elixir. A liquid dosage form of a vaccine composition of the present invention may include a wetting agent, an emulsifying agent, a suspending agent, a sweetener, a flavoring, or a perfuming agent.

5 [00159] Detailed information concerning customary ingredients, equipment and processes for preparing dosage forms is found in *Pharmaceutical Dosage Forms: Tablets*, eds. H. A. Lieberman et al., New York: Marcel Dekker, Inc., 1989; and in L.V. Allen, Jr. et al., *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems*, 8th Ed., Philadelphia, PA: Lippincott, Williams & Wilkins, 2004, throughout and in chapter 16; A. R. Gennaro, *Remington: The Science and Practice of Pharmacy*, Lippincott Williams & Wilkins, 21st ed., 2005, particularly chapter 89; and J. G. Hardman et al., *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, McGraw-Hill Professional, 10th ed., 2001.

[00160] An adjuvant is optionally included in a virus composition according to embodiments of the present invention. Adjuvants are known in the art and illustratively include Freund's
15 adjuvant, aluminum hydroxide, aluminum phosphate, aluminum oxide, saponin, dextrans such as DEAE-dextran, vegetable oils such as peanut oil, olive oil, and/or vitamin E acetate, mineral oil, bacterial lipopolysaccharides, peptidoglycans, and proteoglycans.

[00161] The term "subject" is used herein to refer to a human. Non-human animals, illustratively including other primates, cows, horses, sheep, goats, pigs, dogs, cats, birds, poultry,
20 and rodents, are also referred to by the term subject in particular embodiments of the present invention.

[00162] The isolated rotavirus is treated to inactivate or attenuate the rotavirus. Thus, in particular embodiments a vaccine for human rotavirus includes a live attenuated human rotavirus or an inactivated human rotavirus.

25 [00163] The term "live attenuated rotavirus" refers to a rotavirus having the ability to infect an appropriate host or host cell and replicate and the term is used to distinguish an "inactivated" rotavirus. The term "live attenuated rotavirus" refers to a rotavirus characterized by substantially diminished virulence compared to wild type human rotavirus. The term "virulence" is used to describe the degree of pathogenicity of a rotavirus to a host cell or a host organism. Virulence is
30 determined using any of various assays recognized in the art. For example, virulence may be assessed by exposing cultured host cells to an attenuated rotavirus and determining the number of cells which display a pathogenic response and/or the severity of pathogenic response elicited.

Diminished virulence is present where an attenuated rotavirus has decreased capability to cause one or more pathogenic effects in a host cell and/or host organism.

5 **[00164]** The term “inactivated” rotavirus is used herein to refer to rotavirus that has been killed and which is therefore capable of neither replication nor infection of a host cell or host organism.

[00165] Inactivation is achieved by any of various techniques illustratively including inactivation using one or more chemical agents, thermal inactivation and/or UV light inactivation.

10 **[00166]** Chemical agents used to inactivate a rotavirus are known in the art and include such agents as ethyleneimines such as binary ethyleneimine; cross-linking aldehydes such as formaldehyde and glutaraldehyde; proteases illustratively including pronase, trypsin and/or chymotrypsin; and detergents such as octylphenol ethoxylates and alkyl trimethylammonium salts. Rotavirus may be inactivated by treatment with a base, for example by incubation of the rotavirus at a pH above pH 10.0.

15 **[00167]** Thermal inactivation may be achieved by heating at temperatures above 50° centigrade, for example.

[00168] Inactivation is assessed by techniques standard in the art, illustratively including sampling virus at various times during an inactivation procedure and observing cytopathic effects or infectivity of a sample on suitable cells, such as Vero cells.

20 **[00169]** It is appreciated that, in addition to live attenuated and inactivated rotavirus, an antigenic portion of a rotavirus is optionally included in a vaccine composition of the present invention. Thus, for example, a rotavirus-derived protein or peptide capable of inducing an immunological response in a subject is considered within the scope of the present invention. In particular, an antigenic portion of a human rotavirus strain identified as CDC-9 or CDC-66 is
25 optionally included in a vaccine composition of the present invention.

[00170] Methods of inducing an immunological response against a rotavirus-mediated disease in a subject are provided according to embodiments of the present invention which include administering a therapeutic amount of a vaccine composition including at least one human rotavirus strain.

30 **[00171]** The phrase “therapeutically effective amount” is used herein to refer to an amount effective to induce an immunological response sufficient to prevent or ameliorate signs or symptoms of a rotavirus-mediated disease. Induction of an immunological response in a subject

can be determined by any of various techniques known in the art, illustratively including detection of anti-rotavirus antibodies, measurement of anti-rotavirus antibody titer and/or lymphocyte proliferation assay. Illustrative methods for detection of anti-rotavirus antibodies are illustrated by Tsunemitsu, H, et al., *J. Clin. Microbiol.*, 1992; 30:2129-2134, the contents of which are incorporated herein by reference. Signs and symptoms of rotavirus-mediated disease may be monitored to detect induction of an immunological response to administration of a vaccine composition of the present invention in a subject. An immunological response is illustratively a reduction of clinical signs and symptoms of rotavirus-mediated disease such as reduction of the amount of virus shed in feces, reduction of the number of days on which virus is shed in feces, reduction in the number of days the subject has diarrhea, reduction in mortality, reduction in morbidity, reduction in weight loss or weight gain. An immunological response is illustratively, development of anti-rotavirus antibodies, activation of T-cells, B-cells, or other immune cells following administration of an inventive composition, or other immune responses known in the art.

[00172] In a particular embodiment, a method of inducing an immunological response against a rotavirus-mediated disease in a subject includes administering 10^4 to 10^8 ffu of live attenuated vaccine or 1 to 25 micrograms of inactivated virus in a typical vaccine composition.

[00173] In some embodiments, a method of inducing an immunological response against a rotavirus-mediated disease in a subject includes administering a therapeutically effective amount of a vaccine composition including a human rotavirus strain CDC-9 and/or CDC-66, polypeptide fragments thereof, homologues thereof, or combinations thereof.

[00174] In a further embodiment, a method of inducing an immunological response against a rotavirus-mediated disease in a subject includes administering a therapeutically effective amount of a vaccine composition including a G1 group serotype and a second human rotavirus strain characterized as having a G2 group serotype.

[00175] Administration of a vaccine composition according to a method of the present invention includes administration of one or more doses of a vaccine composition to a subject at one time in particular embodiments. Alternatively, two or more doses of a vaccine composition are administered at time intervals of days, weeks, or years. A suitable schedule for administration of vaccine composition doses depends on several factors including age and health status of the subject, type of vaccine composition used and route of administration, for example.

One of skill in the art is able to readily determine a dose and schedule of administration to be administered to a particular subject.

[00176] Embodiments of inventive compositions and methods are illustrated in the following examples. These examples are provided for illustrative purposes and are not considered
5 limitations on the scope of inventive compositions and methods.

EXAMPLES

Example 1 - Adaptation and passaging:

[00177] One ml of a 10% virus suspension in DMEM is supplemented with neomycin in a
10 1.7 ml sterile low bind tube, mixed well and then centrifuged for 10 min at 3,000 rpm in an Eppendorf microcentrifuge. The supernatant is transferred to a new tube and centrifuged for 10 min at 10,000 rpm (8,000xg). The clarified supernatant is sterilized by passing through a 0.45 micron pore filter. The supernatant is tested by EIA (Rotacalone; Meridian Biosciences) and if OD value is > 1.0, store it at 4 °C before use for infection. Stool extraction and Rotacalone testing
15 can be done the day before infection.

[00178] The culture medium is removed from cell monolayers in individual roller tubes. Each roller tube is washed with 2 ml of maintenance medium, then 2 ml maintenance medium is added to each tube and incubated at 37 °C in a rolling apparatus until virus inoculum is ready.

[00179] An aliquot of 0.5 ml of supernatant is transferred to a sterile tube and 1 microliter of
20 CaCl₂ stock (300grams per liter) is added to make a final concentration of 800 micrograms per milliliter. The tube is incubated at room temperature for 30 min before adding 3 microliters of porcine trypsin stock (2.5 milligrams per milliliter) - final concentration of 15 micrograms per milliliter. The mixture is incubated for 60 min at 37°C. The same volume of MEM is treated in the same way as a mock inoculum. Separate pipette tips are used for pipetting virus
25 suspension and trypsin solutions. All pipetting of virus should be done within the biological safety cabinet.

[00180] Medium is removed from each roller tube and 0.2 to 0.3 milliliter of trypsinized virus suspension or mock inoculums is added to each roller tube using separate sterile pipette. The caps are tightened and the tubes incubated at 37°C on a roller tube apparatus located in an
30 incubator. After 2 hrs incubation, inoculum is removed using a 1 ml pipette and washed gently with 2ml maintenance medium.

[00181] Two milliliters of maintenance medium containing various concentrations (10, 20 or 30 micrograms per ml depending on strain) of trypsin is added into each tube and incubated for 2 hours at 37°C on a roller tube apparatus located in an incubator.

5 [00182] The cells are observed daily for cytopathic effect (CPE), harvested at day 4 and stored at -70°C. The cells are subjected to freeze-thaw two times before the next passage.

[00183] The freeze-thawed cell lysates are treated with CaCl₂ and trypsin as described above and subsequent passages are performed as above. The cells are subjected to freeze-thaw at least once and assayed for rotavirus antigen by Rotaclone kit or virus titer is determined by FFA
10 assays.

Example 2 - Production and Purification of Rotavirus Strains

[00184] Production of rotavirus is accomplished by use of large scale production roller bottles. Briefly described, Vero cells are cultured in Dulbecco's Modified Eagle Medium
15 supplemented with 5% fetal bovine serum (Invitrogen Corp., Grand Island, NY) and 50 micrograms/milliliter of neomycin (Sigma Corp., St. Louis, MO). Confluent monolayers of Vero cells in roller bottles are infected with a particular rotavirus strain at a multiplicity of infection of 0.1.

[00185] Rotavirus obtained by large scale production is purified according to standard
20 operating procedures. Briefly described, rotavirus is harvested from infected cultures of Vero cells at four days post-infection. Triple-layered rotavirus particles are purified from supernatants by centrifugation through 40% sucrose cushions in TNC buffer for 2 hours at 106,750 x g using an SW32Ti rotor and then through isopycnic centrifugations in CsCl gradients for 17 hours at 111,160 g using an SW40Ti rotor. Rotavirus particles may also be purified using sucrose
25 gradients. TNC buffer is 10mM Tris, pH 8.0, 140 mM NaCl, and 10mM CaCl₂. Purified rotavirus particles are resuspended in diluent buffer which is Hanks Balanced Salt Solution with CaCl₂ and MgCl₂, obtained from Invitrogen Corp., Grand Island, NY, supplemented with 10% sorbitol (Sigma Corp., St. Louis, MO). The resuspended isolated rotavirus is stored at -70°C until it is inactivated and administered to a subject in this example.

30 [00186] Purified rotavirus is analyzed for purity and identity by any of various techniques, illustratively including SDS-PAGE followed by Coomassie blue staining, western blot using a

rotavirus-specific antibody and/or electron microscopy. In addition, purity and identity of purified rotavirus strains is accomplished by identification of particular structural viral proteins.

[00187] FIG. 4A shows CsCl purified rotavirus of strain CDC-9. FIG. 4B shows identified structural viral proteins of double- and triple-layered CDC-9 analyzed by SDS-PAGE in comparison to molecular weight markers.

Example 3 - Immunogenicity of inactivated rotavirus (IRV)

[00188] Immunogenicity of rotavirus strains is tested in a mouse model. Purified killed rotavirus particles are administered intramuscularly to mice without an adjuvant. Animals are immunized with amounts of killed rotavirus protein in the range between 2 and 20 micrograms.

[00189] Immunogenicity is assayed by measuring immunoglobulin titers including IgM, IgA and IgG in blood samples obtained at various times after administration. Neutralizing antibody titers are measured by microneutralization assay as described in detail in Jiang, B., *Vaccine*, 17:1005-1013, 1999, the contents of which are incorporated herein by reference. Briefly described, mouse sera are serially diluted two-fold in duplicate wells and incubated with trypsin-inactivated RRV rotavirus. Activated rotavirus or similarly treated serum-free MEM medium is incubated in the absence of mouse serum and serve as positive and negative controls, respectively. MA104 cells in MEM medium supplemented with a final concentration of 10 micrograms/milliliter trypsin and 0.5% chick serum, obtained from Invitrogen Corp., Grand Island, NY, are added to each well. After incubation at 37°C for 18 hours, cells are fixed with formalin. Rotavirus antigens in MA104 cells are detected by incubating cells with rabbit anti-RRV hyperimmune serum, HRP-labeled anti-rabbit IgG, and then tetramethyl benzidine. Neutralizing antibody titer in a serum is defined as the reciprocal of the highest dilution giving a 70% reduction in absorbance value compared to that in the virus control.

[00190] Antibody titers in mice injected with killed purified rotavirus particles are compared with antibody titers in control mice. Antibody titers in control mice are typically less than 100. FIGs. 5A and 5B illustrate total antibody and neutralizing antibody titers in control and vaccinated mice. Total serum antibody (FIG. 5A) and neutralizing antibody (FIG. 5B) responses to thermally inactivated rotavirus in mice. Mice are vaccinated I.M. twice with killed YK-1 and rotavirus-specific total (IgA, IgG, and IgM) and neutralizing antibodies are determined by EIA as described. For total antibody, each serum specimen is tested at an initial dilution of 1:100. Pre-bleed serum specimens have no detectable antibody at this dilution. A value of 20 is used for

determining geometric mean titers and illustration. Neutralizing antibody is tested at an initial dilution of 1:20. Antibody titers are expressed as the geometric means for each group (n = 7 or 6). Error bars represent 1 standard error.

5 **Example 4 - Adjuvant**

[00191] In a further example, $\text{Al}(\text{OH})_3$ is added as an adjuvant to rotavirus particles in a vaccine administered to mice. Animals are immunized intramuscularly once with 2 micrograms or 0.2 micrograms of killed purified rotavirus particles in the presence or absence of 600 micrograms $\text{Al}(\text{OH})_3$. $\text{Al}(\text{OH})_3$ dramatically enhances total antibody titers in mice at both
10 concentrations of rotavirus administered. No antibody titers (less than 100 dilutions) are detected in control mice immunized with 600 micrograms of $\text{Al}(\text{OH})_3$.

[00192] FIG. 6 is a bar graph showing total serum antibody responses to thermally inactivated rotavirus formulated with $\text{Al}(\text{OH})_3$ in control and vaccinated mice. Mice are vaccinated I.M. once with killed YK-1 in the absence or presence of $\text{Al}(\text{OH})_3$ and rotavirus-specific total (IgA, IgG, and IgM) antibodies are determined by EIA as described. For total
15 antibody, each serum specimen is tested at an initial dilution of 1:100. Pre-bleed serum specimens have no detectable antibody at this dilution. A value of 20 is used for determining geometric mean titers and illustration. Antibody titers are expressed as the geometric means for each group (n = 6). Error bars represent 1 standard error.

20

Example 5 – Gnotobiotic piglet model

[00193] A gnotobiotic piglet model of rotavirus disease is used. This piglet model allows testing under defined conditions avoiding problems of environment exposure of animals and using disease as the outcome variable. This model also allows testing of an inactivated rotavirus
25 vaccine having a G1 serotype against a homotypic Wa challenge. Gnotobiotic piglets are the best current animal model for infection and disease with human rotavirus strains. (See Saif LJ, et al., *Archives of Virology*, 1996;12:S153-61; and Iosef C, et al., *Vaccine*, 2002;20:1741-53 ; the contents of each of which are incorporated herein by reference.)

[00194] Thirteen infant gnotobiotic piglets are selected and randomly assigned to 4 groups as
30 indicated in Table 6.

Table 6. Piglet Study Design

Group Name	Number of Piglets in Group	CDC-9 Antigen (micrograms)	Aluminum phosphate (micrograms)
AA	4	0	750
BB	4	75	0
CC	3	75	750
DD	2	0 (buffer)	0 (buffer)

[00195] Each group of animals indicated in Table 6 is kept in separate isolators. Animals in groups BB and CC are vaccinated intramuscularly 3 times with an inactivated rotavirus vaccine without or with an adjuvant, respectively. The vaccine formulation in this example includes 75 micrograms of killed purified CDC-9 rotavirus in diluent mixed with 750 micrograms of aluminum phosphate. Animals in groups AA and DD are vaccinated with 750 micrograms of aluminum phosphate and buffer, respectively, in the same manner. Antigen adsorption is determined by the Bradford method which showed that about 50% of the antigen was bound to aluminum phosphate. Both bound and unbound antigen was injected in these immunizations.

10 [00196] As shown in Table 6, piglets are immunized with a vaccine formulation including no antigen and 750 micrograms of aluminum phosphate; 75 micrograms of antigen and no aluminum phosphate; 75 micrograms of antigen and 750 micrograms of aluminum phosphate; or no antigen and no aluminum phosphate, that is buffer alone. Each vaccination is carried out by injecting 0.5 milliliters of the vaccine formulation into muscles of the hind legs of the piglets.

15 After 3 doses of the vaccine formulation administered at intervals of 10-12 days, piglets are orally challenged with virulent Wa rotavirus. Prior to virus challenge, each piglet is inoculated with 3 milliliters of sodium bicarbonate to neutralize acids in the stomach. Fecal specimens are collected from the challenged piglets daily for 10 days. Blood samples are collected throughout the experiment at intervals of 7-14 days. FIG. 7A shows virus shedding in fecal samples of piglets vaccinated with no antigen and with 750 micrograms of aluminum phosphate in 4 animals. FIG. 7B shows virus shedding in fecal samples from piglets immunized with antigen and no adjuvant. FIG. 7C shows virus shedding in fecal samples of piglets immunized with antigen and adjuvant. FIG. 7D shows virus shedding measured in fecal samples of piglets immunized with buffer only. These illustrate that piglets that are mock vaccinated with

20 aluminum phosphate only or diluent buffer only all shed rotavirus up to 5 days and at high titer. By contrast, piglets that are vaccinated with inactivated rotavirus without aluminum phosphate

25

are partially protected, as evidenced by a shortened 1-day shedding or a delayed and reduced shedding. Of the 3 piglets that are vaccinated with inactivated rotavirus and aluminum phosphate, 2 are completely protected and 1 has only a short, 1-day, reduced shedding. Thus, these results show effectiveness of vaccine formulation according to embodiments of the present invention.

Example 6 – Gnotobiotic piglet model – II

[00197] To repeat the experiment above, eleven infant gnotobiotic piglets are selected and randomly assigned to 2 groups as indicated in Table 7.

Table 7. Piglet Study Design

Group Name	Number of Piglets in Group	CDC-9 Antigen (micrograms)	aluminum phosphate (micrograms)
GG	5	0	600
HH	6	50	600

[00198] As shown in Table 7, and as described by Wang, Y, et al., Inactivated rotavirus vaccine induces protective immunity in gnotobiotic piglets, in press, the contents of which are incorporated herein by reference, piglets are immunized with a vaccine formulation including no antigen and 600 micrograms of aluminum phosphate or 50 micrograms of antigen and 600 micrograms of aluminum phosphate. Each vaccination is carried out by injecting 0.5 milliliters of the vaccine formulation into muscles of the hind legs of the piglets. After 3 doses of the vaccine formulation administered at intervals of 10-12 days, piglets are orally challenged with virulent Wa rotavirus. Prior to virus challenge, each piglet is inoculated with 3 milliliters of sodium bicarbonate to neutralize acids in the stomach. Fecal specimens are collected from the challenged piglets daily for 10 days. Blood samples are collected throughout the experiment at intervals of 7-14 days.

[00199] FIG. 8A shows the level of rotavirus specific IgG antibody response in sera of piglets vaccinated with no antigen and with 600 micrograms of aluminum phosphate (solid bars) or piglets vaccinated with 50 micrograms of antigen and with 600 micrograms of aluminum phosphate (hatched bars). FIG. 8B shows neutralizing antibody response in sera of piglets vaccinated with no antigen and with 600 micrograms of aluminum phosphate (solid bars) or piglets vaccinated with 50 micrograms of antigen and with 600 micrograms of aluminum phosphate (hatched bars). Piglets vaccinated with antigen develop significant levels of serum IgG. Oral challenge with rotavirus further enhances the serum IgG levels. The levels of neutralizing activity are significantly higher in piglets vaccinated with 50 micrograms of antigen and with 600 micrograms of aluminum phosphate than mock immunized animals.

[00200] FIG. 9A shows virus shedding in fecal samples of piglets vaccinated with 50 micrograms of antigen and with 600 micrograms of aluminum phosphate. FIG. 9B shows virus shedding in fecal samples of piglets vaccinated with no antigen and with 600 micrograms of aluminum phosphate. These figures illustrate that piglets that are mock vaccinated with

aluminum phosphate only or diluent buffer only all shed rotavirus up to 5 days and at high titer. By contrast, piglets that are vaccinated with inactivated rotavirus and aluminum phosphate are protected from shedding.

[00201] These results show effectiveness of vaccine formulation according to embodiments of the present invention and confirm the results in first piglet experiment. These results clearly demonstrate that IRV formulated with alum is highly immunogenic and protective against infection in piglets and consequently establish proof of concept for inactivated rotavirus vaccine.

Example 7 – Gnotobiotic piglet model – III- Immunization with CDC-66:

[00202] To repeat the experiment above using CDC-66 rotavirus, eleven infant gnotobiotic piglets are selected and randomly assigned to 2 groups as indicated in Table 8.

Table 8. Piglet Study Design

Group Name	Number of Piglets in Group	CDC-66 Antigen (micrograms)	aluminum phosphate (micrograms)
GG	5	0	600
HH	6	50	600

[00203] As shown in Table 7, piglets are immunized with a vaccine formulation including no antigen and 600 micrograms of aluminum phosphate or 50 micrograms of antigen and 600 micrograms of aluminum phosphate (AlPO_4). Each vaccination is carried out by injecting 0.5 milliliters of the vaccine formulation into muscles of the hind legs of the piglets. After 3 doses of the vaccine formulation administered at intervals of 10-12 days, piglets are orally challenged with virulent DS-1 rotavirus. Prior to virus challenge, each piglet is inoculated with 3 milliliters of sodium bicarbonate to neutralize acids in the stomach. Fecal specimens are collected from the challenged piglets daily for 10 days. Blood samples are collected throughout the experiment at intervals of 7-14 days.

[00204] Piglets vaccinated with antigen develop significant levels of serum IgG. Oral challenge with rotavirus further enhances the serum IgG levels. The levels of neutralizing activity are significantly higher in piglets vaccinated with 50 micrograms of CDC-66 antigen and with 600 micrograms of aluminum phosphate than mock immunized animals.

[00205] Piglets that are mock vaccinated with aluminum phosphate only or diluent buffer only all shed rotavirus up to 5 days and at high titer. By contrast, piglets that are vaccinated with inactivated rotavirus CDC-66 and aluminum phosphate are protected from shedding.

5 [00206] These results show effectiveness of vaccine formulation according to embodiments of the present invention and confirm the results in first piglet experiment. These results clearly demonstrate that IRV formulated with alum is highly immunogenic and protective against infection in piglets and consequently establish proof of concept for inactivated rotavirus vaccine.

[00207] Any patents or publications mentioned in this specification are incorporated herein by reference to the same extent as if each individual publication is specifically and individually
10 indicated to be incorporated by reference.

[00208] The compositions and methods described herein are presently representative of preferred embodiments, exemplary, and not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art. Such changes and other
15 uses can be made without departing from the scope of the invention as set forth in the claims.

CLAIMS:

1. A vaccine composition for inducing an immunological response against a rotavirus in a subject, comprising a pharmaceutically acceptable carrier admixed with an isolated rotavirus strain CDC-66.
- 5 2. The vaccine composition of claim 1, further comprising an adjuvant.
3. The vaccine composition of claim 1 or claim 2, wherein the isolated human rotavirus strain is a live attenuated rotavirus.
4. The vaccine composition of claim 1 or claim 2, wherein the isolated human rotavirus strain is an inactivated rotavirus.
- 10 5. The vaccine composition of any one of claims 1-4, comprising at least two isolated rotavirus strains.
6. The vaccine composition of claim 5 wherein the at least two isolated rotavirus strains each independently have a G group serotype of G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13 or G14.
- 15 7. The vaccine composition of any one of claims 1-6, formulated for parenteral administration to a subject.
8. The vaccine composition of any one of claims 1-6, formulated for oral administration to a subject.
9. The vaccine composition of any one of claims 1-8, further comprising an
20 isolated rotavirus strain characterized as having a G1 group serotype.
10. The vaccine composition of claim 9, wherein the human rotavirus strain characterized as having a G1 group serotype has a P group serotype of P1A, P1B, P2A, P3, P4, P5, P6, P8, P11 or P12.
11. The vaccine composition of any one of claims 1-10, wherein rotavirus
25 CDC-66 strain comprises a NSP1, NSP2, NSP3, NSP4, NSP5, VP1, VP2, VP3, VP4, VP5, VP6 and VP7 protein, and wherein:

- the NSP1 protein has greater than 95% identity to SEQ ID NO: 71;
- the NSP2 protein has greater than 95% identity to SEQ ID NO: 77 or SEQ ID NO: 78;
- the NSP3 protein has greater than 95% identity to SEQ ID NO: 83;
- 5 the NSP4 protein has greater than 95% identity to SEQ ID NO: 89;
- the NSP5 protein has greater than 95% identity to SEQ ID NO: 95 or SEQ ID NO: 96;
- the VP1 protein has greater than 95% identity to SEQ ID NO: 101 or SEQ ID NO: 102;
- 10 the VP2 protein has greater than 95% identity to SEQ ID NO: 107;
- the VP3 protein has greater than 95% identity to SEQ ID NO: 113 or SEQ ID NO: 114;
- the VP4 protein has greater than 95% identity to SEQ ID NO: 119 or SEQ ID NO: 120;
- 15 the VP6 protein has greater than 95% identity to SEQ ID NO: 125; and
- the VP7 protein has greater than 95% identity to SEQ ID NO: 131.
12. The vaccine composition of claim 11, wherein:
- the NSP1 protein comprises SEQ ID NO: 71;
- the NSP2 protein comprises SEQ ID NO: 77 or SEQ ID NO: 78;
- 20 the NSP3 protein comprises SEQ ID NO: 83;
- the NSP4 protein comprises SEQ ID NO: 89;
- the NSP5 protein comprises SEQ ID NO: 95 or SEQ ID NO: 96;
- the VP1 protein comprises SEQ ID NO: 101 or SEQ ID NO: 102;

the VP2 protein comprises SEQ ID NO: 107;

the VP3 protein comprises SEQ ID NO: 113 or SEQ ID NO: 114;

the VP4 protein comprises SEQ ID NO: 119 or SEQ ID NO: 120;

the VP6 protein comprises SEQ ID NO: 125; and

5 the VP7 protein comprises SEQ ID NO: 131.

13. The vaccine composition of any one of claims 1-12, for use in inducing an immunological response to a rotavirus in a subject.

14. An isolated rotavirus, wherein the rotavirus is strain CDC-66.

1/7

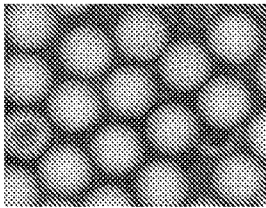


Figure 1A

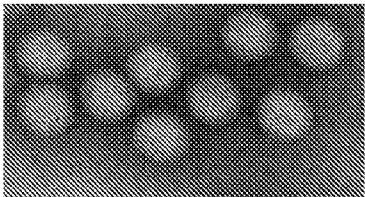


Figure 1B

Wa	CDC-9
Stool	Vero

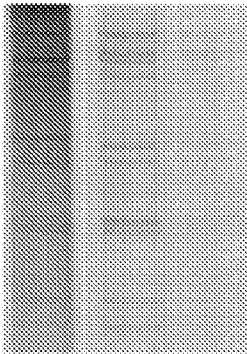


Figure 2

MM	RRV	CDC-66
Stool	Vero	

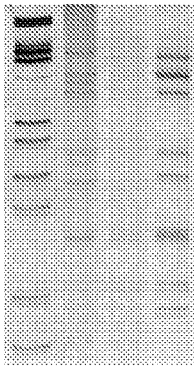


Figure 3

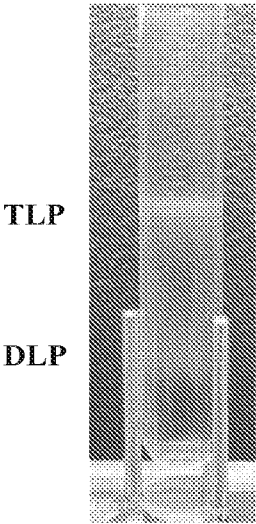


Figure 4A

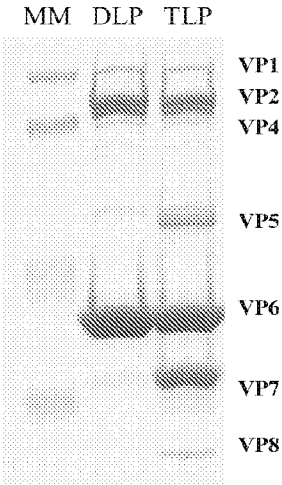


Figure 4B

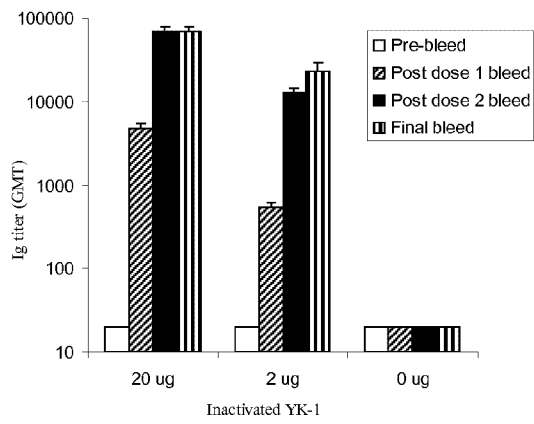


Figure 5A

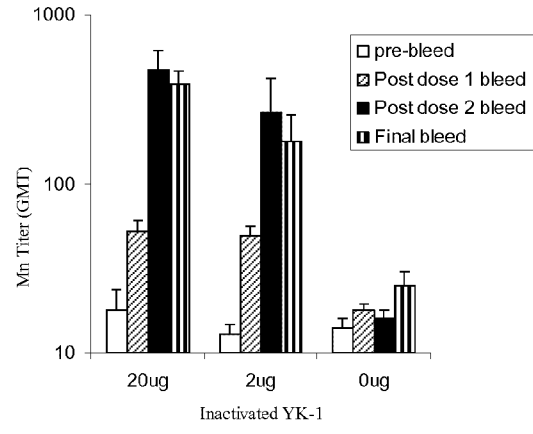


Figure 5B

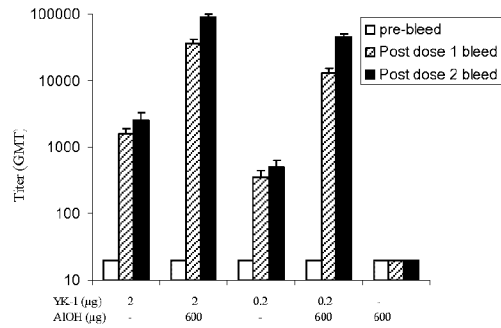


Figure 6

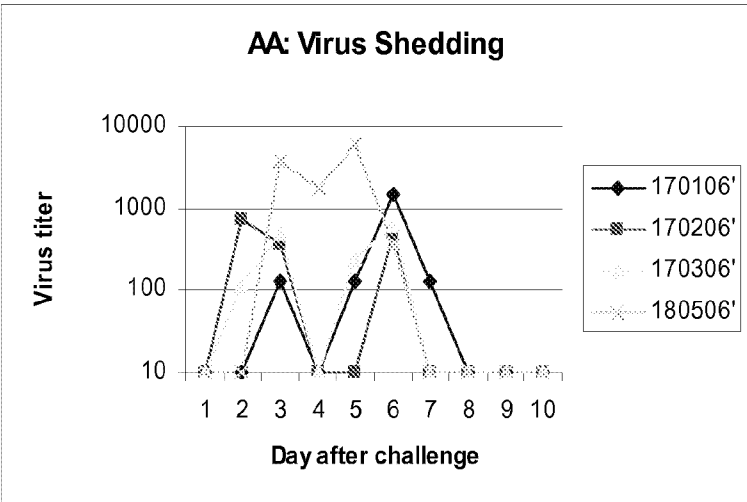


Figure 7A

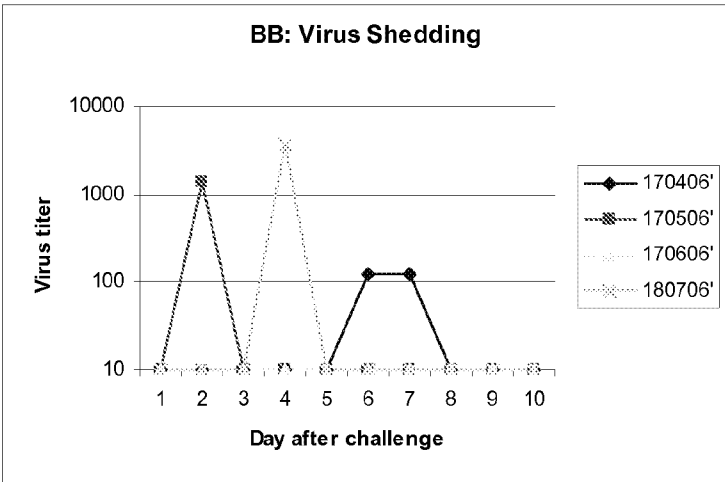


Figure 7B

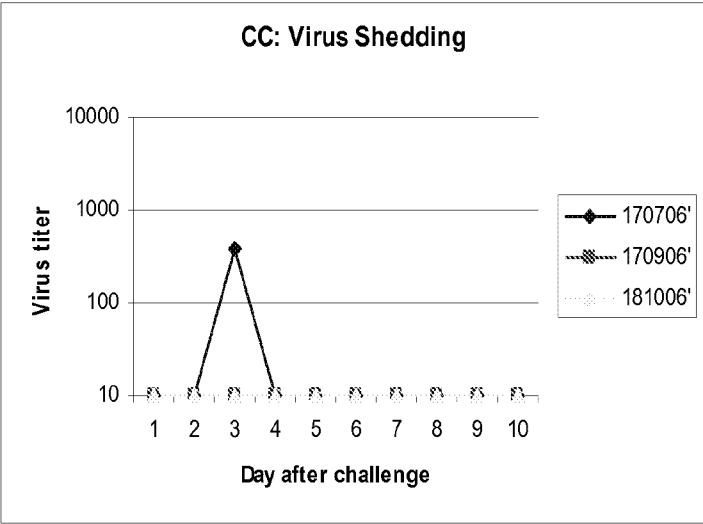


Figure 7C

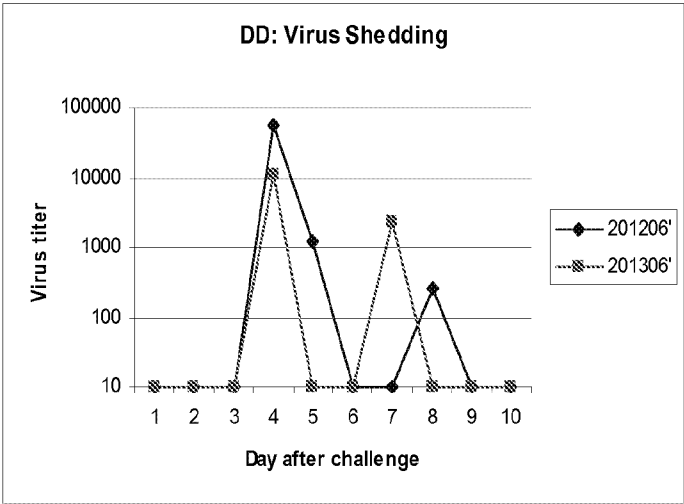


Figure 7D

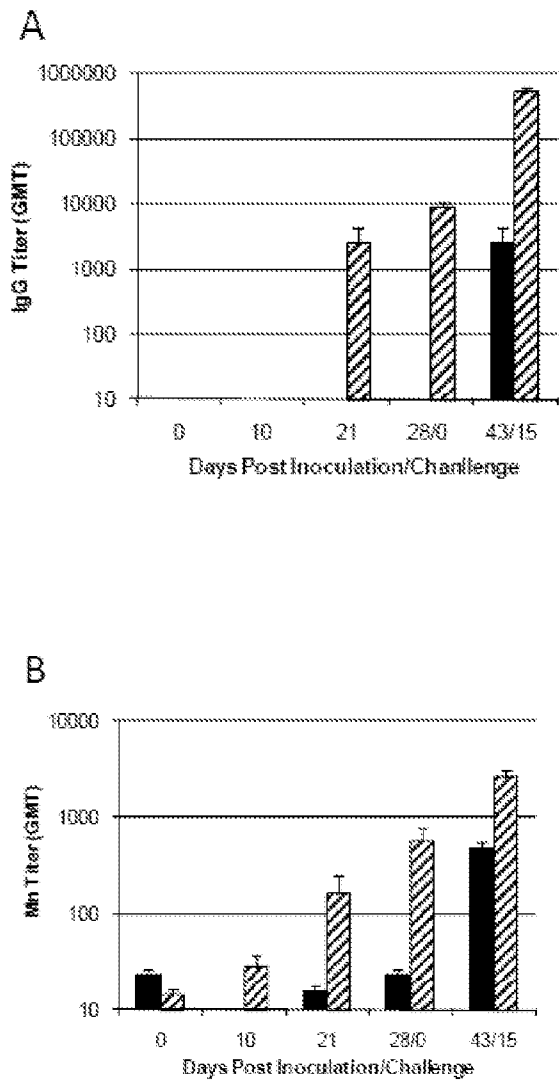


FIG. 8

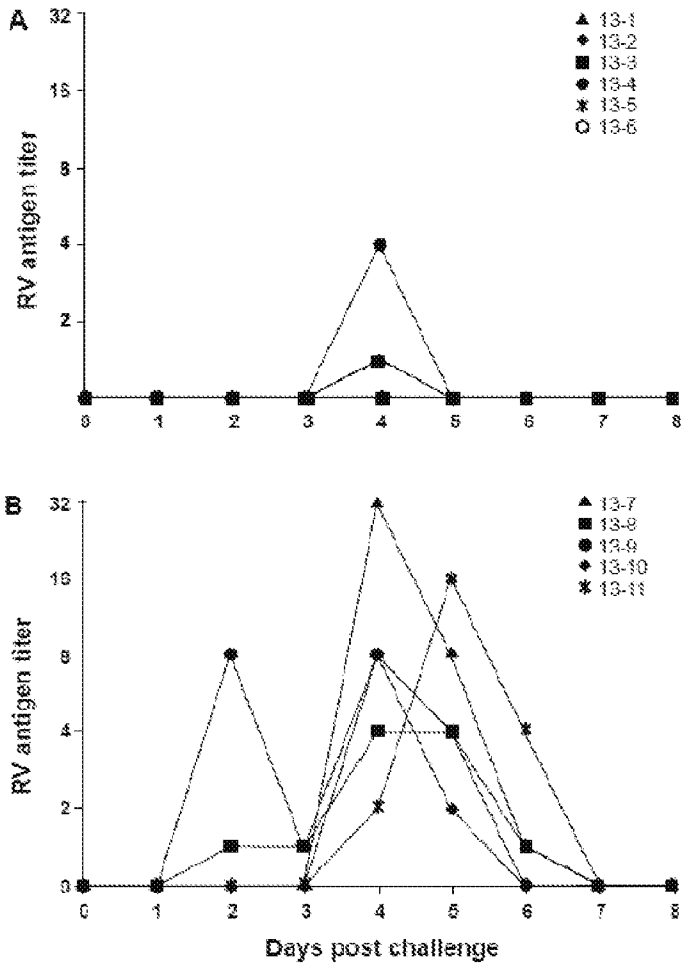


FIG. 9