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(54) Title: EPIZOOTIC HEMORRHAGIC DISEASE VACCINE

(57) Abstract: The present disclosure provides for an immunogenic composition against epizootic hemorrhagic disease virus (EHDV). The immunogenic composition has been shown to be efficacious in inducing serum neutralizing antibodies against EHDV and intended to be used to prevent or reduce clinical symptoms associated with EHDV infection in susceptible animals. The disclosure provides for composition and methods that represent an improvement over previous strategies for treating and preventing EHDV.

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EPIZOOTIC HEMORRHAGIC DISEASE VACCINE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to United States Patent Application No. 62/397,265, filed on September 20, 2016, which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Epizootic hemorrhagic disease (EHD) is a vector-borne viral disease of wild and domestic ruminants. EHD virus (EHDV) infection causes severe clinical signs and high mortality in wild ruminants, especially in white-tail deer (WTD), which is the most susceptible species, and can also cause disease in domestic cattle. The disease is economically important for deer and cattle farmers due to the loss of animals and restrictions on trading livestock. Recent concerns about EHDV were caused by the expansion of the affected area and the emergence of serotype 6 in the US. Serotype 6 was isolated from dead WTD in 2006 and was also found to be responsible for morbidity and mortality in cattle.

[0003] Autogenous inactivated vaccines have been used for protection of EHDV infection in North America. There are several steps to make an autogenous vaccine including virus isolation, confirmation of identity, propagation of virus, virus inactivation and proof of virus inactivation, USDA-required safety and purity testing, as well as approval by government authorities. These autogenous vaccines are primarily if not solely used by deer farmers. In Japan, both live modified and inactivated vaccines have been developed to control Ibaraki disease caused by an EHDV-2 strain. Autogenous inactivated vaccines have several limitations: the process needs approximately 12 weeks before delivery to the farm, inability to differentiate between vaccination and infection in the serological test, limitation of protection only to the homologous serotype and vaccine side effects caused by whole inactivated virus/adjuvant.

BRIEF DESCRIPTION OF THE INVENTION

[0004] The present disclosure provides for an immunogenic composition or vaccine against EHD. Further, a method for eliciting an immune response in an animal is provided, where the steps include administration of the immunogenic composition or vaccine disclosed herein to an animal in need thereof. A method for reducing the incidence and/or severity of clinical signs associated with EHD, as well as a method of reducing viremia is also provided as an aspect of the disclosure. Such a method comprises the steps of administration of the immunogenic composition or vaccine disclosed herein to an animal in need thereof.

[0005] In one aspect, the immunogenic composition or vaccine of the present disclosure is preferably a protein subunit or DNA-based. In a preferred embodiment, the protein subunit is selected from VP1, VP2, VP3, VP4, VP5, VP6, VP7, NS1, NS2, NS3a, NS3b, NS4 and any combination thereof as well as the DNA sequences encoding such subunits. In a preferred aspect, the protein subunit is selected from VP2, VP5 and combinations thereof. In some forms, the protein subunits are recombinantly produced.

[0006] The recombinant protein-based vaccine or immunogenic composition utilizes selected immune-dominant proteins as antigens to ensure protection of vaccinates from virus infection. The immunogenic composition of the present disclosure preferably includes at least one VP2 structural protein of EHDV-2 or other EHDV serotype. In preferred forms, VP2 and VP5 structural proteins are used, where the VP2 structural protein can be from more than one EHDV serotype and the VP5 structural protein can be from more than one EHDV serotype. In its most preferred form, structural proteins of multiple EHDV serotypes are included in the immunogenic composition or vaccine. VP2 and VP5 are major structural proteins involved in virus attachment and entry into the host cell. They are also major targets for neutralizing antibodies. The recombinant protein vaccines used in this application revealed specific serological reactivity and neutralizing activity against EHD virus. This disclosure demonstrates that a combination of VP2 and VP5 protein subunits

induces neutralizing antibodies associated with protection against EHDV infection, although the immunogenic composition is not so limited. The examples contained in this disclosure also demonstrated that immunization with the VP2 protein subunit alone stimulates production of EHDV-specific neutralizing antibodies and leads to protection from EHDV infection, although the immunogenic composition is not so limited. The use of recombinant proteins which are safe and protective is an advantage over the previous methods.

[0007] In one aspect, the immunogenic composition of the present disclosure using recombinant protein doesn't use the live EHDV during all processes, so it does not require propagation of infectious virus and virus inactivation steps. The immunogenic composition or vaccine of the present disclosure can be used to prevent EHDV infection and spread in cattle and deer, and is DIVA (differentiating infected from vaccinated animals) compatible, which is an important feature for the control of the disease.

[0008] In another aspect, the immunogenic composition comprises a DNA vector plasmid capable of expressing the protein subunits *in vivo*. The vector or plasmid may also contain an acceptable pharmaceutical or veterinary vehicle. In a preferred aspect, the plasmid or vector comprises a nucleotide sequence of the genome of EHDV, a subunit, a homologue, or fragment thereof. In one aspect, the immunogenic composition or vaccine further comprises a gene coding for an expression product capable of inhibiting or retarding the establishment or development of a virus. Preferred nucleotide sequences include those encoding VP1, VP2, VP3, VP4, VP5, VP6, VP7, NS1, NS2, NS3a, NS3b, NS4 and any combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Figure 1 is Western blot of recombinant VP2 and VP5 proteins of EHDV2 and EHDV6. VP5 recombinant proteins revealed a band of about 60 kDa and VP2 proteins revealed a band of about 115 kDa in a western blot using anti-histidine tag-HRP antibody;

[0010] Fig. 2 is a graph illustrating specific OD values of sera from immunized mice against VP2/EHDV2 protein (*= $p<0.05$);

[0011] Fig. 3 is a graph illustrating specific OD values of sera from immunized mice against VP5/EHDV2 proteins (* = $p<0.05$);

[0012] Fig. 4 is a graph illustrating specific OD values of serum from immunized mice against VP2/EHDV 6 proteins (* = $p<0.05$);

[0013] Fig. 5 is a graph illustrating specific OD values of serum from immunized mice against VP5/EHDV6 protein (* = $p<0.05$);

[0014] Fig. 6 is a graph illustrating Virus Neutralization titers of sera from immunized mice against EHDV 2 virus;

[0015] Fig 7 is a graph illustrating Virus Neutralization titers of sera from immunized mice against EHDV2 virus at 35 dpv;

[0016] Fig. 8 is a graph illustrating Virus Neutralization titers of sera from mice immunized with recombinant proteins against EHDV serotype 6 virus;

[0017] Fig. 9 is a graph illustrating Virus Neutralization titers of sera against EHDV6 virus at 35 dpv;

[0018] Fig. 10 is a graph illustrating specific OD values of serum from immunized cattle against the VP2/EHDV2 protein;

[0019] Fig. 11 is a graph illustrating specific OD values of serum from immunized cattle against the VP5/EHDV2 protein;

[0020] Fig. 12 is a graph illustrating specific OD values of serum from immunized cattle against VP2/EHDV 6 proteins;

[0021] Fig. 13 is a graph illustrating specific OD values of serum from immunized cattle against the VP5/ EHDV 6 protein (*= $p<0.05$); and

[0022] Fig. 14 is a graph illustrating a specific Virus Neutralization titers of serum against EHDV 2 virus at 35 dpv. The individual Virus Neutralization titer presents with mean of respective group with standard deviation.

[0023] Fig. 15 is a graph illustrating a specific Virus Neutralization titers of serum against EHDV 6 virus at 35 dpv. The individual Virus Neutralization titer presents with mean of respective group with standard deviation.

DETAILED DESCRIPTION OF THE INVENTION

[0024] The present disclosure provides for an immunogenic composition or vaccine providing protection against EHDV. The immunogenic composition or vaccine is preferably protein subunit-based or DNA/RNA-based.

[0025] In one aspect, the immunogenic composition or vaccine is a subunit immunogenic composition or vaccine. The subunit is preferably selected from, but not limited to, VP1, VP2, VP3, VP4, VP5, VP6, VP7, NS2, NS3, and any combination thereof. The protein subunits are preferably purified and isolated. The immunogenic composition or vaccine may include 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 subunits, where each subunit may be the same or different. In one aspect, the combination of subunits may be two or more of the same subunits from different strains or serotypes of EHDV. In another aspect, the selected protein subunits could all be from the same strain or serotype of EHDV. Preferred combinations include, but are not limited to, VP2 from one serotype, VP2 of different serotypes, VP5 of one serotype, VP5 of different serotypes, VP2 and VP5 from one serotype or VP2 and VP5 of different serotypes.. As a non-limiting example, the immunogenic composition of the present disclosure may comprise a VP2 from EHDV-1, a VP2 from EHDV-2 and/or the VP2 from EHDV-6. In one aspect, the serotype that the VP2 is derived from determines the serotype of EHDV the vaccine is protective for. The subunits for use in the immunogenic composition or vaccine of the present disclosure are preferably selected from, but not limited to, EHDV-1, EHDV-2, EHDV-6, EHDV-7, or other EHDV serotypes.

[0026] The subunits for purposes of the present disclosure are preferably proteins that are recovered after being produced by a protein expression system. In a preferred aspect, the proteins are produced using a baculovirus expression system; however, the disclosure is not so limited. In a preferred aspect the baculovirus expression system is an insect baculovirus expression system. Other expression systems that may be used include mammalian, bacterial, or yeast or protein expression systems.

[0027] The nucleic acid sequences encoding for the subunits for use with the present disclosure may also be utilized in the form of DNA or RNA in the immunogenic composition or vaccine.

[0028] The EHDV serotype 2 strain for purposes of the present disclosure may be any serotype 2 EHDV strain. Preferably, the EHDV serotype 2 strain is isolated and purified. In one aspect, the protein subunits for use in the immunogenic composition or vaccine is from an EHDV serotype 2 strain having at least 95% sequence homology with the Alberta EHDV serotype 2 strain where values such as at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, and at least 99% sequence homology are envisioned. The proteins utilized in some aspects of the present disclosure are those proteins expressed by sequences having at least 95% sequence homology with proteins of EHDV strain Alberta where values such as at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, and at least 99% sequence homology are envisioned. For purposes of this disclosure the Alberta strain an EHDV2 consisting of 10 segments, GenBank Accession No. AM744997.1 for VP1; GenBank Accession No. AM744998.1 for VP2; GenBank Accession No. AM744999.1 for VP3; GenBank Accession No. AM745000.1 for VP4; GenBank Accession No. AM745001.1 for NS1; GenBank Accession No. AM7745002.1 for VP5; GenBank Accession No. AM745003.1 for VP7; GenBank Accession No. AM745004.1 for NS2; GenBank Accession No. AM745005.1 for VP6; and GenBank Accession No. AM745006.1 for NS3&3a.

[0029] The EHDV serotype 1 strain for purposes of the present disclosure may be any serotype 1 EHDV strain. The EHDV serotype 1 strain is preferably purified and isolated. In one aspect, the protein subunits for use in the immunogenic composition or vaccine are from an EHDV serotype 1 strain having at least 95% sequence homology with the EHDV1/NJ serotype 1 strain where values such as at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, and at least 99% sequence homology are envisioned. The proteins utilized in some aspects of the present disclosure are those proteins expressed by sequences having at least 95% sequence homology with nucleic acid sequences of EHDV1/NJ strain where values such as at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, and at least 99% sequence homology are envisioned. For purposes of this disclosure the EHDV1/NJ strain consists of the following 10 segments: GenBank Accession No. KU140704.1 for VP1; GenBank Accession No. KU140740.1 for VP2; GenBank Accession No. KU140752.1 for VP3; GenBank Accession No. KU140776.1 for VP4; GenBank Accession No. KU140800.1 for NS1; GenBank Accession No. KU1407836.1 for VP5; GenBank Accession No. KU140872.1 for VP7; GenBank Accession No. KU140848.1 for NS2; GenBank Accession No. KU140896.1 for VP6; and GenBank Accession No. KU140920.1 for NS3&3a.

[0030] The EHDV serotype 6 strain for purposes of the present disclosure may be any serotype 6 EHDV strain. The EHDV serotype 6 strain is preferably purified and isolated. In one aspect, the EHDV serotype 6 strain has at least 95% sequence homology with US EHDV6 isolate C/cervidae/Ohio/12-3437-8/2012, where values such as at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, and at least 99% sequence homology are envisioned. The proteins utilized in some aspects of the present disclosure are those proteins expressed by sequences having at least 95% sequence homology with US EHDV6 isolate C/cervidae/Ohio/12-3437-8/2012 where values such as at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, and at least 99% sequence homology are envisioned. For purposes of the

present disclosure, EHDV6 isolate C/cervidae/Ohio/12-3437-8/2012 has 10 segments: GenBank Accession No. KF570133.1 for VP1; GenBank Accession No. KF570134.1 for VP2; GenBank Accession No. KF570135.1 for VP3; GenBank Accession No. KF570136.1 for VP4; GenBank Accession No. KF570137.1 for VP5; GenBank Accession No. KF570138.1 for VP6; GenBank Accession No. KF570139.1 for VP7; GenBank Accession No. KF570140.1 for NS1; GenBank Accession No. KF570141.1 for NS2; and GenBank Accession No. KF570142.1 for NS3&3a.

[0031] In a further aspect, the EHDV strain is preferably a strain having at least 95% sequence homology, at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, or at least 99% sequence homology with VP2 and VP5 genes of the Alberta strain of EHDV2 (Genbank #AM744998.1 for VP2 and AM745002.1 for VP5) or a strain having at least 95% sequence homology, at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, or at least 99% sequence homology with VP2 and VP5 genes the US EHDV6 isolate C/cervidae/Ohio/12-3437-8/2012 (GenBank Accession No. KF570134.1 for VP2 and KF570137.1 for VP5). The proteins utilized in some aspects of the present disclosure are those proteins expressed by sequences having at least 95% sequence homology, at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, or at least 99% sequence homology with the Alberta strain of EHDV2 or a strain having at least 95% sequence homology, at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, or at least 99% sequence homology with the US EHDV6 isolate C/cervidae/Ohio/12-3437-8/2012. In a preferred aspect, the EHDV strain is purified and/or isolated.

[0032] The VP5 subunit for purposes of the present disclosure may be any VP5 from any stain of EHDV. The VP5 subunit protein is preferably isolated and/or purified. In one aspect, the VP5 subunit is the protein expressed by a sequence having at least 80% nucleotide sequence homology to SEQ ID No. 2 or SEQ ID No. 4, where values such as at least 85%, at least 85%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%,

at least 96%, at least 97%, at least 98%, and at least 99% sequence homology are envisioned. In yet another aspect, the VP5 subunit is a protein having an amino acid sequence that has at least 80% sequence homology to SEQ ID No. 6 or SEQ ID No. 7, where values such as at least 85%, at least 85%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99% sequence homology are envisioned.

[0033] The VP5 subunit may come from any one or more of EHDV-1, EHDV-2 and EHDV-6 serotypes, where the immunogenic composition or vaccine of the present disclosure may include one or more VP5 subunits.

[0034] In a further aspect the VP5 subunit for purposes of the present disclosure is the protein expressed by a sequence having at least 95% sequence homology, at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, or at least 99% sequence homology with Gen Bank Accession #AM745002.1 and/or Gen Bank Accession # KF570137.1.

[0035] In yet another aspect, the VP2 subunit for purposes of the present disclosure is the protein expressed by a sequence having at least 95% sequence homology, at least 96% sequence homology, at least 97% sequence homology, at least 98% sequence homology, or at least 99% sequence homology with Gen Bank Accession #AM744998.1 and/or Gen Bank Accession # KF570134.1. The VP2 subunit protein is preferably isolated and/or purified.

[0036] The VP2 subunit for purposes of the present disclosure may be any VP2 from any strain of EHDV. In one aspect, the VP2 subunit is the protein expressed by a sequence having at least 80% sequence homology to SEQ ID No. 1 or SEQ ID No. 3, where values such as at least 85%, at least 85%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99% sequence homology are envisioned. In another aspect, the VP2 subunit is a protein having an amino acid sequence that has at least 80% sequence homology to SEQ ID No. 5 or

SEQ ID No. 7, where values such as at least 85%, at least 85%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99% sequence homology are envisioned. The VP2 subunit may come from any one or more of EHDV-1, EHDV-2 and EHDV-6 serotypes, where the immunogenic composition or vaccine of the present disclosure may include one or more VP2 subunits.

[0037] In one aspect, the immunogenic composition or vaccine of the present disclosure comprises a VP5 subunit which is the protein expressed by a sequence having at least 90% sequence homology with SEQ ID No. 2 or SEQ ID No.4 or having an amino acid sequence that has at least 90% sequence homology with SEQ ID No. 6 or SEQ ID No. 8 and a VP2 subunit which is the protein expressed by a sequence having at least 90% sequence homology with SEQ ID No. 1 or SEQ ID No. 3 or having an amino acid sequence that has at least 90% sequence homology with SEQ ID No. 5 or SEQ ID No. 7.

[0038] In another aspect, the immunogenic composition or vaccine of the present disclosure comprises two or more VP5 subunits expressed by a sequence having at least 90% sequence homology with SEQ ID No. 2 or SEQ ID No.4 or having an amino acid sequence that has at least 90% sequence homology with SEQ ID No. 6 or SEQ ID No. 8.

[0039] In a further aspect, the immunogenic composition or vaccine of the present disclosure comprises two or more VP2 subunits which are expressed by a sequence having at least 90% sequence homology with SEQ ID No. 1 or SEQ ID No. 3 or having an amino acid sequence that has at least 90% sequence homology with SEQ ID No. 5 or SEQ ID No. 7.

[0040] In a further aspect, the immunogenic composition or vaccine of the present disclosure comprises a VP2 subunit. In a preferred aspect, the VP2 subunit is from an EHDV-2 or EHDV-6. In another aspect, the immunogenic composition of the present disclosure comprises a VP2 and VP5 subunit. In a preferred aspect, the VP2 subunit is from an EHDV-2 or EHDV-6, or both. In yet

another aspect, the immunogenic composition or vaccine of the present disclosure comprises a VP5 subunit. In a preferred aspect, the VP5 subunit is from an EHDV-2 or EHDV-6, or both.

[0041] In another aspect, the disclosure provides immunogenic compositions or vaccines comprising a vector or plasmid and an acceptable pharmaceutical or veterinary vehicle, the vector or plasmid comprising a nucleotide sequence of the genome of EHDV, a subunit, a homologue, or fragment thereof. In one aspect, the immunogenic composition or vaccine further comprises a gene coding for an expression product capable of inhibiting or retarding the establishment or development of a virus. Preferred nucleotide sequences have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-4, and combinations thereof.

[0042] The present disclosure also relates to immunogenic compositions or vaccines comprising a cell and an acceptable pharmaceutical or veterinary vehicle, wherein the cell is transformed with a nucleotide sequence of the genome of EDHV, a subunit, a homologue, or a fragment thereof. Preferred nucleotide sequences have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-4, and combinations thereof.

[0043] In one aspect, the present disclosure provides for a vector having the sequence for one or more of a VP2 or VP5 subunit, where the vector expresses the proteins for the subunits in the recipient. Possible combinations, include, but are not limited to, a vector with two or more VP2 sequences, a vector with two or more VP5 sequences, or a vector with one or more VP2 sequences and one or more VP5 sequences, where the VP2 and/or VP5 subunits may come from any one or more of EHDV-1, EHDV-2 and EHDV-6.

[0044] In a further aspect, the immunogenic composition or vaccine may comprise nucleic acid. The nucleic acid component may be inactivated, modified live, or attenuated. In preferred forms, the nucleic acid has at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence homology with at least one of the nucleotide sequences selected from SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, and SEQ ID No. 4. In another aspect, the nucleic acid sequence encodes an amino acid sequence that has at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence homology with at least one of the amino acid sequences selected from SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, and SEQ ID No. 8.

[0045] In one aspect, the immunogenic composition may also comprise additional elements selected from the group consisting of antigens, pharmaceutical carriers, adjuvants, preservatives, stabilizers, or combinations thereof. These additional elements can be combined with any of the protein or nucleic acid immunogenic compositions or vaccines described herein.

[0046] Pharmaceutically acceptable vehicle or carrier is understood as designating a compound or a combination of compounds entering into a pharmaceutical composition or vaccine which does not provoke secondary reactions and which allows, for example, the facilitation of the administration of the active compound, an increase in its duration of life and/or its efficacy in the body, an increase in its solubility in solution or alternatively an improvement in its conservation. These pharmaceutically acceptable vehicles are well known and will be adapted by the person skilled in the art as a function of the nature and of the mode of administration of the chosen active compound.

[0047] As far as the vaccine formulations are concerned, these can comprise adjuvants of the appropriate immunity which are known to the person skilled in the art, such as, for example, Montanide ISA 25, aluminum hydroxide, a representative of the family of muramyl peptides such as one of the peptide derivatives of N-acetyl muramyl, a bacterial lysate, carbomers, or alternatively

Freund's incomplete adjuvant. In a preferred aspect, the adjuvant is Montanide ISA 25.

[0048] The immunogenic composition and vaccine described herein can be administered by the systemic route, in particular by the intravenous route, by the intramuscular, intradermal or subcutaneous route, or by the oral or nasal, or intranasal route. In a more preferred manner, the immunogenic composition or vaccine composition according to the disclosure will be administered by the intramuscular route, through the food, or by nebulization several times, staggered over time. In a further preferred aspect, the immunogenic composition or vaccine composition according to the disclosure will be administered subcutaneously. In preferred forms, such subcutaneous administration occurs twice with a three-week interval.

[0049] Their administration modes, dosages and optimum pharmaceutical forms can be determined according to the criteria generally taken into account in the establishment of a treatment adapted to an animal such as, for example, the age or the weight, the seriousness of its general condition, the tolerance to the treatment and the secondary effects noted. Preferably, the vaccine of the present disclosure is administered in an amount that is protective against EHD.

[0050] The administration of the immunogenic composition or vaccine according to the present disclosure may be administered one time, two times, three times, four times, five times, six times, seven times, eight times, nine times, or at least 10 times. In one aspect, the immunogenic composition or vaccine of the present disclosure is effective after a single dose administration.

[0051] For example, the immunogenic composition or vaccine according to the present disclosure may be administered one time or several times, spread out over time in an amount of about 0.1 to 1000 μg per kilogram weight of the animal, where values and ranges such as, but not limited to, 0.5 to 800 μg per kilogram weight of the animal, 1 to 1000 μg per kilogram weight of the animal, 1 to 500 μg per kilogram weight of the animal, 1 to 300 μg per kilogram weight of the

animal, 1 to 200 µg per kilogram weight of the animal, 1 to 150 µg per kilogram weight of the animal, 1 to 125 µg per kilogram weight of the animal, 1 to 100 µg per kilogram weight of the animal, 5 µg per kilogram weight of the animal, 10 µg per kilogram weight of the animal, 15 µg per kilogram weight of the animal, 20 µg per kilogram weight of the animal, 25 µg per kilogram weight of the animal, 30 µg per kilogram weight of the animal, 35 µg per kilogram weight of the animal, 40 µg per kilogram weight of the animal, 45 µg per kilogram weight of the animal, 50 µg per kilogram weight of the animal, 55 µg per kilogram weight of the animal, 60 µg per kilogram weight of the animal, 65 µg per kilogram weight of the animal, 70 µg per kilogram weight of the animal, 75 µg per kilogram weight of the animal, 80 µg per kilogram weight of the animal, 85 µg per kilogram weight of the animal, 90 µg per kilogram weight of the animal, 95 µg per kilogram weight of the animal, 100 µg per kilogram weight of the animal, 125 µg per kilogram weight of the animal, 150 µg per kilogram weight of the animal, 200 µg per kilogram weight of the animal, 250 µg per kilogram weight of the animal, 300 µg per kilogram weight of the animal, 400 µg per kilogram weight of the animal, 500 µg per kilogram weight of the animal, 600 µg per kilogram weight of the animal, 700 µg per kilogram weight of the animal, 800 µg per kilogram weight of the animal, 900 µg per kilogram weight of the animal and 1000 µg per kilogram weight of the animal are envisioned.

[0052] In some preferred forms, the above amounts are also provided without reference to the weight of the animal.

[0053] In some preferred forms where more than one dose or administration is provided, the time between administrations is from 1 day to 12 weeks, more preferably between 3 days and 11 weeks, still more preferably between 5 days and 10 weeks, even more preferably between 1 week and 9 weeks, still more preferably between 9 days and 8 weeks, even more preferably between 11 days and 7 weeks, still more preferably between 13 days and 6 weeks, even more preferably between 15 days and 5 weeks, still more preferably between 17 days and 4 weeks, and still more preferably about 3 weeks.

[0054] According to the present disclosure, the immunogenic composition or vaccine may include an antigen from at least one further pathogen other than EHDV, including, but not limited to the pathogens responsible for Bluetongue (BT), African Horse Sickness, Crimean-Congo hemorrhagic fever, Foot and mouth disease, lumpy skin disease, peste des petit ruminants, rinderpest virus, schmallenberg virus, sheep pox, goat pox, West Nile virus, BVD, calf diphtheria, calf pneumonia, fog fever, IBR, TB, thrombosis, trypanosomosis, and combinations thereof. Further, the at least one further pathogen may be selected from, but is not limited to, *Burcella* spp., *cryptosporidium*, *Dermatophilus congolensis*, *Escherichia coli*, *Leptospira interrogans*, *Listeria monocytogenes*, pseudocowpox, *Coxiella burneti*, rabies, Trichopyton, Microspora, *Salmonella* spp., *Mycobacterium bovis*, *Mycoplasma Bovis* and combinations thereof.

[0055] A method for reducing the incidence or severity of clinical symptoms of EHD is provided, where the steps of the method include administration of the immunogenic composition or vaccine against EHD to a subject in need thereof.

[0056] The clinical symptoms reduced in incidence or severity include, but are not limited to, loss of appetite, loss of fear of people, weakness, excessive salivation, rapid pulse, rapid respiration rate, fever, lying in bodies of water to reduce body temperature, unconsciousness, blue tongue, head swelling, neck swelling, sloughing or breaking of hooves, lameness, shock, hemorrhages in tissues, including skin and gastrointestinal tract, death, and combinations thereof.

[0057] In one aspect, the method of the present invention reduces the clinical symptoms and/or the severity or incidence of clinical symptoms of EHD in a subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, from about 10% to 50%, from about 10% to 90%, from about 30% to 50%, from about 20% to 60%, from about 30% to 80%, from about 30% to 50%, and from about 50% to 95%, with all values within the recited ranges being envisioned. The reduction of clinical symptoms and/or the reduction of the incidence or severity of clinical symptoms is

when compared to a subject not administered the immunogenic composition or vaccine of the present disclosure.

[0058] A method for preventing clinical symptoms or reducing the incidence or severity of symptoms of subclinical infection of EHDV is provided, where the steps of the method include administration of the immunogenic composition or vaccine against EHD to a subject in need thereof.

[0059] Subclinical infection presents with viremia, virus shedding and mild symptoms included, but not limited to, mild fever and/or loss of appetite. Clinical symptom includes subclinical with more severe fever, oral ulcers, excessive salivation, lameness, coronitis, and combinations thereof.

[0060] In one aspect, the method of the present invention reduces the symptoms of subclinical EHDV infection and/or the severity or incidence of the symptoms of subclinical EHDV infection in a subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, from about 10% to 50%, from about 10% to 90%, from about 30% to 50%, from about 20% to 60%, from about 30% to 80%, from about 30% to 50%, and from about 50% to 95%, with all values within the recited ranges being envisioned. The reduction of clinical symptoms and/or the reduction of the incidence or severity of clinical symptoms is when compared to a subject not administered the immunogenic composition or vaccine of the present disclosure.

[0061] A method for reducing EHD viremia is provided, where the steps of the method include administration of the immunogenic composition or vaccine against EHD to a subject in need thereof.

[0062] The EHD viremia is preferably reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, when compared to an animal not receiving the immunogenic composition or vaccine of the present disclosure.

[0063] The immunogenic composition or vaccine provided in this disclosure may be administered to any subject in need thereof. In one aspect, the subject is selected from, but not limited to, cattle, goats, sheep, giraffes, yaks, deer, antelope, macropods, or any other animal considered a ruminant.

[0064] It must be understood that the present disclosure does not relate to the genomic nucleotide sequences taken in their natural environment, that is to say, in the natural state. It concerns sequences for which it has been possible to isolate, purify or partially purify, starting from separation methods such as, for example, ion-exchange chromatography, by exclusion based on molecular size, or by affinity, or alternatively fractionation techniques based on solubility in different solvents, or starting from methods of genetic engineering such as amplification, cloning and subcloning, it being possible for the sequences of the disclosure to be carried by vectors.

[0065] Definitions

[0066] “Homologous nucleotide sequence” or “having sequence homology” in the sense of the present disclosure is understood as meaning a nucleotide or amino acid sequence having at least a percentage identity with the nucleotide bases of a or amino acid sequence according to the disclosure of at least 80%, where ranges and values, including but not limited to, from 80% to 85%, 85% to 96%, 80% or 95%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, 99.9%, and higher are envisioned for the present disclosure, where this percentage being purely statistical and it being possible to distribute the differences between the two sequences at random and over the whole of their length.

[0067] An “immunogenic composition” as used herein, means a EHDV composition which elicits an “immunological response” in the host of a cellular and/or antibody-mediated immune response to the EHDV sequence, whether the sequence is killed/inactivated, modified live, a subunit of the nucleotide sequence or peptide expressed by that sequence, or in a vector. Preferably, this immunogenic

composition is capable of reducing the incidence or severity of clinical and subclinical signs of infection and/or conferring protective immunity against EHDV infection or subclinical infection and the clinical symptoms associated therewith.

[0068] “Sequence Identity” as it is known in the art refers to a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, namely a reference sequence and a given sequence to be compared with the reference sequence. Sequence identity is determined by comparing the given sequence to the reference sequence after the sequences have been optimally aligned to produce the highest degree of sequence similarity, as determined by the match between strings of such sequences. Upon such alignment, sequence identity is ascertained on a position-by-position basis, e.g., the sequences are “identical” at a particular position if at that position, the nucleotides or amino acid residues are identical. The total number of such position identities is then divided by the total number of nucleotides or residues in the reference sequence to give % sequence identity. Sequence identity can be readily calculated by known methods, including but not limited to, those described in Computational Molecular Biology, Lesk, A. N., ed., Oxford University Press, New York (1988), Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York (1993); Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H. G., eds., Humana Press, New Jersey (1994); Sequence Analysis in Molecular Biology, von Heinge, G., Academic Press (1987); Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York (1991); and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988), the teachings of which are incorporated herein by reference. Preferred methods to determine the sequence identity are designed to give the largest match between the sequences tested. Methods to determine sequence identity are codified in publicly available computer programs which determine sequence identity between given sequences. Examples of such programs include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research, 12(1):387 (1984)), BLASTP, BLASTN and FASTA (Altschul, S. F. et al., J. Molec. Biol., 215:403-410 (1990)). The BLASTX program is

publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al., NCBI NLM NIH Bethesda, MD 20894, Altschul, S. F. et al., J. Molec. Biol., 215:403-410 (1990), the teachings of which are incorporated herein by reference). These programs optimally align sequences using default gap weights in order to produce the highest level of sequence identity between the given and reference sequences. As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 85%, preferably 90%, even more preferably 95% "sequence identity" to a reference nucleotide sequence, it is intended that the nucleotide sequence of the given polynucleotide is identical to the reference sequence except that the given polynucleotide sequence may include up to 15, preferably up to 10, even more preferably up to 5 point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, in a polynucleotide having a nucleotide sequence having at least 85%, preferably 90%, even more preferably 95% identity relative to the reference nucleotide sequence, up to 15%, preferably 10%, even more preferably 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 15%, preferably 10%, even more preferably 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. Analogously, by a polypeptide having a given amino acid sequence having at least, for example, 85%, preferably 90%, even more preferably 95% sequence identity to a reference amino acid sequence, it is intended that the given amino acid sequence of the polypeptide is identical to the reference sequence except that the given polypeptide sequence may include up to 15, preferably up to 10, even more preferably up to 5 amino acid alterations per each 100 amino acids of the reference amino acid sequence. In other words, to obtain a given polypeptide sequence having at least 85%, preferably 90%, even more preferably 95% sequence identity with a reference amino acid sequence, up to 15%, preferably up to 10%, even more preferably up to 5% of the amino acid

residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 15%, preferably up to 10%, even more preferably up to 5% of the total number of amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or the carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in the one or more contiguous groups within the reference sequence. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. However, conservative substitutions are not included as a match when determining sequence identity.

[0069] The definition of sequence identity given above is the definition that would be used by one of skill in the art. The definition by itself does not need the help of any algorithm, said algorithms being helpful only to achieve the optimal alignments of sequences, rather than the calculation of sequence identity.

[0070] From the definition given above, it follows that there is a well-defined and only one value for the sequence identity between two compared sequences which value corresponds to the value obtained for the best or optimal alignment.

[0071] The BLAST N or BLAST P “BLAST 2 sequence”, software which is available in html at the web site ncbi.nlm.nih.gov/gorf/b12, is habitually used by the skilled man for comparing and determining the identity between two sequences and calculates the statistical significance.

[0072] A “conservative substitution” refers to the substitution of an amino acid residue or nucleotide with another amino acid residue or nucleotide having similar characteristics or properties including size, hydrophobicity, etc., such that the overall functionality does not change significantly.

[0073] “Isolated or purified” means altered “by the hand of man” from its natural state, i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or polypeptide naturally present in a living organism is not “isolated or purified,” but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is “isolated or purified”, as the term is employed herein.

[0074] “Modified or modified live” nucleotide sequence will be understood as meaning any nucleotide sequence obtained by mutagenesis according to techniques well known to the person skilled in the art, and containing modifications with respect to the normal sequences according to the disclosure, for example mutations in the regulatory and/or promoter sequences of polypeptide expression, especially leading to a modification of the rate of expression of said polypeptide or to a modulation of the replicative cycle.

[0075] “Nucleotide, polynucleotide, nucleic acid or nucleic acid sequence” will be understood according to the present disclosure as meaning both a double-stranded or single-stranded RNA or DNA in the monomeric and dimeric (so-called in tandem) forms and the transcription products of said DNAs.

[0076] All ranges provided herein include each and every value in the range as well as all sub-ranges there-in-between as if each such value or sub-range was disclosed. Further, all aspects and embodiments of the disclosure comprise, consist essentially of, or consist of any aspect or embodiment, or combination of aspects and embodiments disclosed herein.

[0077] Examples

[0078] This written description uses examples to disclose the invention, including the best mode, and also to enable any person skilled in the art to practice the invention, including making and using any devices or systems and performing any incorporated methods. The patentable scope of the invention is defined by the claims, and may include other examples that occur to those skilled in

the art. Such other examples are intended to be within the scope of the claims if they have structural elements that do not differ from the literal language of the claims, or if they include equivalent structural elements with insubstantial differences from the literal languages of the claims.

[0079] Example 1

[0080] This example illustrates the efficacy of the immunogenic composition or vaccine of the present disclosure.

[0081] The expression of VP2 and VP5 proteins of EHDV in baculovirus. The recombinant VP2 and VP5 proteins of EHDV2 and EHDV6 were produced using the baculovirus expression system (BaculoDirect vector, Invitrogen, US). The expression of each VP2 and VP5 protein was confirmed by western blot using anti-histidine tag antibodies and polyclonal serum against EHDV2. The expressed proteins revealed a band of 60kDa and VP5 and 115kDa for VP2 (Fig.1).

[0082] Immunogenicity studies of recombinant EHDV vaccines in mice.

[0083] 1) Experimental groups

[0084] Nine groups of mice with 5 animals per group were immunized with either VP2 or VP5 or combination of VP2& VP5 proteins of EHDV2 or EHDV6. EHDV2 and EHDV 6 inactivated virus vaccines were used as a positive control for each serotype, as detailed in Table 1. Vaccines (10 - 20 µg of purified protein mixed with adjuvant Montanide ISA25, Seppic, in 200 ul) were administered subcutaneously twice at an interval of 3 weeks. Mice were bled via saphenous vein on 0 and 21 day post vaccination (dpv) and at the end of study on 35 dpv.

[0085] **Table 1.** Experimental groups of mouse study #1

Group	Vaccine candidate	Doses
M1	rVP2/EHDV 2 (VP2/2)	200 µl (20 ug purified protein)
M2	rVP5/EHDV 2 (VP5/2)	

M3	rVP2/EHDV2 + rVP5 /EHDV 2	(with adjuvant; ISA25)
M4	EHDV 2 inactivated virus	10 ⁵ TCID ₅₀ with adjuvant
M5	rVP2/EHDV 6 (VP2/6)	200 µl (20 ug purified protein) (with adjuvant; ISA25)
M6	rVP5/EHDV 6 (VP5/6)	
M7	rVP2/EHDV6 + rVP5/EHDV 6	
M8	EHDV 6 inactivated virus	10 ⁵ TCID ₅₀ with adjuvant
M9	Mock	200 µl of PBS with adjuvant

[0086] Evaluation of immunogenicity (vaccine efficacy)

[0087] ELISA test

[0088] ELISA test was used to evaluate the humoral immune responses in serum from immunized mice. Pooled serum samples collected on 0 and 21 dpv and individual serum samples collected on 35 dpv were tested. Briefly, Maxisorp 96-well plates were coated with respective recombinant protein (100 ng/well). The negative control wells were coated with baculovirus-infected insect cell lysate. Serum samples were diluted 1/100 and allowed to react with respective antigens in 96 well plate for 1 hour at room temperature. Anti-mouse IgG conjugated with HRP was used as secondary antibody. Then plates were incubated with TMB substrate for 15 minutes and after addition of the stop solution (0.16 M sulfuric acid) OD values were measured in the plate reader at 450 nm.

[0089] Protein specific signal was calculated as shown: *Specific OD value = (OD of sample – OD of negative serum) in target protein coated well – (OD of sample – OD of negative serum) in proteins from baculovirus infected cell lysate - coated well*

[0090] Mice immunized with different recombinant proteins elicited EHDV-specific antibodies against target proteins as shown in Figs 2, 3, 4 and 5. Individual data of ELISA testing of samples are shown in Tables 2 and 3.

[0091] **Table 2.** Specific OD value of each recombinant protein in ELISA test.

Specific OD value of recombinant protein										
α -VP2-EHDV2						α -VP5-EHDV2				
DPV [†]	M1 VP2/2	M3 VP2/2+V P5/2	M7 VP2/6+V P5/6	M4 Inact. EHDV2	M9 Mock	M2 VP5/2	M3 VP2/2+V P5/2	M7 VP2/6+V P5/6	M4 Inact. EHDV2	M9 Mock
0 D	-0.048	0.013	0.005	0.076	0	-0.038	0.055	0.028	0.057	0
21 D	1.370	0.776	0.378	0.058	0	0.328	0.683	0.551	0.029	0
35 D	1.542	1.720	1.176	0.458	0	0.932	1.466	1.438	0.441	0
35D SD*	0.158	0.129	0.357	0.365	0	0.470	0.267	0.244	0.428	0
α -VP2-EHDV6						α -VP5-EHDV6				
DPV	M5 VP2/6	M7 VP2/6+V P5/6	M3 VP2/2+V P5/2	M8 Inact. EHDV6	M9 Mock	M6 VP5/6	M7 VP2/6+V P5/6	M3 VP2/2+V P5/2	M8 Inact. EHDV6	M9 Mock
0 D	0.009	-0.182	-0.086	0.006	0	-0.001	0.002	-0.025	0.030	0
21 D	2.204	2.124	0.094	0.017	0	0.083	0.233	0.294	0.068	0
35 D	2.577	2.432	0.516	0.269	0	0.892	0.904	0.759	0.244	0
35D SD	0.05	0.102	0.213	0.131	0	0.230	0.170	0.163	0.069	0

[0092] DPV[†] = day post vaccination; SD* = standard deviation.

[0093] **Table 3.** The specific OD values of serum from individual animals at 35 dpv

Specific OD.value of serum from immunized mouse against respective protein					
Group	mouse #	Anti-VP2/EHDV2	Anti- VP5/EHDV2	Anti-VP2/EHDV6	Anti-VP5/EHDV6
M1: VP2/2	1-1	1.675	NT*	NT	NT
	1-2	1.443	NT	NT	NT
	1-3	1.313	NT	NT	NT
	1-4	1.624	NT	NT	NT
	1-5	1.656	NT	NT	NT
M2: VP5/2	2-1	NT	1.370	NT	NT
	2-2	NT	1.404	NT	NT
	2-3	NT	0.876	NT	NT
	2-4	NT	0.751	NT	NT
	2-5	NT	0.861	NT	NT
M3: VP2/2+VP5/2	3-1	1.599	1.367	0.482	0.727
	3-2	1.737	1.571	0.697	0.820
	3-3	1.599	1.084	0.217	0.496
	3-4	1.911	1.807	0.745	0.920
	3-5	1.755	1.501	0.438	0.834
M4: Inactivated EHDV2	4-1	0.022	0.557	NT	NT
	4-2	0.535	0.517	NT	NT
	4-3	0.735	0.736	NT	NT
	4-4	0.859	0.948	NT	NT

	4-5	0.141	0.026	NT	NT
M5 : VP2/6	5-1	NT	NT	2.508	NT
	5-2	NT	NT	2.556	NT
	5-3	NT	NT	2.621	NT
	5-4	NT	NT	2.622	NT
	5-5	NT	NT		NT
M6 : VP5/6	6-1	NT	NT	NT	0.781
	6-2	NT	NT	NT	1.135
	6-3	NT	NT	NT	0.907
	6-4	NT	NT	NT	1.073
	6-5	NT	NT	NT	0.565
M7: VP2/6+VP5/6	7-1	1.599	1.695	2.525	1.064
	7-2	0.868	1.382	2.426	0.942
	7-3	1.529	1.666	2.370	1.018
	7-4	0.981	1.111	2.299	0.631
	7-5	0.904	1.337	2.539	0.868
M8 : Inactivated EHDV 6	8-1	NT	NT	0.117	0.283
	8-2	NT	NT	0.325	0.254
	8-3	NT	NT	0.374	0.318
	8-4	NT	NT	0.390	0.231
	8-5	NT	NT	0.242	0.136
M9 : Mock	9-1	0	0	0	0
	9-2	0	0	0	0
	9-3	0	0	0	0
	9-4	0	0	0	0
	9-5	0	0	0	0

[0094] There was a strong seroconversion against homologous protein in vaccinates of all recombinant vaccine administered groups detected as early as 21 dpv after single immunization. ELISA signal was increased on 35 dpv after booster vaccination. In addition, sera from mice immunized with VP2+VP5 of EHDV2 (Group M3) revealed specific OD signal against VP2 of EHDV 6 and VP5 of EHDV6 (Figs. 2 and 3). Sera from mice immunized with VP2 + VP5 of EHDV6 (Group M7) also showed cross reactivity against VP2 of EHDV2 and VP5 of EHDV2 (Figs. 4 and 5). This indicates serological cross-reactivity between the VP2 and VP5 proteins of EHDV2 and EHDV6.

[0095] Virus Neutralization test (VN test)

[0096] The virus neutralization test was done based on OIE and USDA protocols. EHDV serotype 2 (strain 600544 or EHDV2/Alberta Strain) and EHDV serotype 6 (2006 US isolate were C/cervidae/Ohio/12-3437-8/2012) used for

neutralization test. In brief, sera from immunized mice were initially diluted 10 fold and then 2 fold dilutions from 1/10 to 1/3200 were made in 96-well culture plate. Virus with 100 TCID₅₀/50ul was added into each well and the plates were incubated for 1 hour at 37°C. Then Vero cells were added (2×10^4 cell per well) and incubated for 5 days at 37°C with 5% CO₂. Wells were scored for the degree of CPE. The serum titer was calculated and recorded as the highest serum dilution capable of preventing virus replication (CPE) in more than 50% of the infected wells.

[0097] Virus neutralization assays against EHDV2 showed neutralizing activity in serum from mice immunized with VP2 of EHDV2 (Group M1) and with combination of VP2 and VP5 proteins of EHDV2 (Group M3) on 35 dpv. Sera from mice, immunized with VP5 alone (Group M2), with combination of VP2+VP5 of EHDV6 (Group M7) and inactivated EHDV2 virus (Group M4) did not show any anti-EHDV2 neutralizing activity (Fig. 6 & 7, Table 4, 5). No significant neutralizing titers were detected on 21 dpv after single immunization. In contrast to ELISA results, there was no cross-neutralizing activity between EHDV2 and EHDV6 serotypes.

[0098] **Table 4.** VN titer of serum against EHDV serotype 2 virus

DPV	VN titer (mean) against EHDV serotype 2 virus					
	M1 VP2/2	M2 VP5/2	M3 VP2/2 +VP5/2	M7 VP2/6 +VP5/6	M4 I-EHDV2	M9 Mock
0 D	0	0	0	0	0	0
21 D	0	0	0	0	0	0
35 D	53.33	0	64.6	0	0	0
(Mean±SD)	±19.3		±27			

[0099] **Table 5.** VN titer of serum from each individual mouse at 35

dpv

	VN titer of individual serum sample against EHDV2 virus				
	#1	#2	#3	#4	#5
M1 : VP2/2	40	66.67	40	40	80
M2: VP5/2	0	0	0	0	0

M3 : VP2/2+VP5/2	80	66.67	80	80	16.67
M7 : VP2/6+VP5/6	0	0	0	0	0
M4 : I-EHDV2	0	0	0	0	0
M9 : Mock	0	0	0	0	0

[0100] Virus neutralization assays against EHDV6 showed neutralizing activity in serum from mice immunized with VP2 of EHDV6 (Group M5) and with a combination of VP2 and VP5 proteins of EHDV6 (Group M7) at 35 dpv. Some virus neutralizing activity in serum from mice immunized with VP2 of EHDV6 (Group M5) was already observed on day 21 post vaccination. Sera from mice, immunized with VP5 of EHDV6 (Group M6), with combination of VP2+VP5 of EHDV2 (M3) and inactivated EHDV6 virus (M8) did not show any anti-EHDV6 virus neutralizing activity (Fig. 8 & 9, Table 6, 7). Similarly to the EHDV2 VN assay, there was no cross-neutralizing activity between EHDV2 and EHDV6 serotypes.

[0101] **Table 6.** VN titer of serum against EHDV serotype 6 virus

DPV	VN titer against EHDV serotype 6 virus					
	M5 VP2/6	M6 VP5/6	M7 VP2/6 +VP5/6	M3 VP2/2 +VP5/2	M8 I-EHDV6	M9 Mock
0 D	0	0	0	0	0	0
21 D	80	0	0	0	0	0
35 D	1800	0	2000	0	0	0
(Mean±SD)	±1006		±1200			

[0102] **Table 7.** VN titer of serum from individual mouse at 35 dpv

	VN titer of individual serum against EHDV2 virus				
	#1	#2	#3	#4	#5
M5 : VP2/6	1600	3200	1600	800	
M6: VP5/6	0	0	0	0	0
M7 : VP2/6+VP5/6	3200	400	1600	1600	3200
M3 : VP2/2+VP5/2	0	0	0	0	0
M8 : I-EHDV6	0	0	0	0	0
M9 : Mock	0	0	0	0	0

[0103] Immunogenicity studies of recombinant EHDV vaccines in cattle

[0104] Experimental groups

[0105] Five groups of cattle with 3 animals per group were immunized with either VP2 or a combination of VP2 & VP5 proteins of EHDV2 or EHDV6. PBS with adjuvant was used for mock-control, as detailed in Table 8. The purified protein (150µg) mixed with Montanide ISA25 adjuvant (Seppic, France) in 2 mL per dose, was administered subcutaneously twice at an interval of 3 weeks. Blood was collected via the jugular vein on days 0, 21 and 35 post vaccination (dpv). The collected blood was centrifuged at 2,000 rpm for 10 minutes to collect the serum. Serum samples for VN test were mixed with equal volume of PBS and incubated at 56°C for 30 minutes to inactivate the complement. All serum samples were stored at -70°C until use.

[0106] **Table 8.** The experimental groups of cattle study

Group	Vaccine candidate	Dose	Animals
C1	rVP2/EHDV 2	2 ml (150 µg purified protein with ISA25)	3
C2	rVP2/EHDV 2 + rVP5/EHDV 2		3
C3	rVP2-EHDV 6		3
C4	rVP2/EHDV 6 + rVP5-EHDV6		3
C5	Non vaccine control	2 ml PBS with ISA25	3

[0107] Evaluation of immunogenicity (vaccine efficacy)

[0108] ELISA test. ELISA tests to evaluate the humoral immune responses in sera from immunized cattle were done using individual EHDV recombinant proteins as coating antigens. Briefly, a Maxisorp 96 well plate was coated with respective recombinant protein (100 ng/well). The negative control wells were coated with baculovirus-infected insect cell lysate. Sera from immunized cattle at 1:200 dilution was added into the well and reacted with respective antigens to analyze the level of specific antibodies. Anti-Bovine IgG conjugated with HRP was

used as secondary antibody. The plate was then stained with TMB substrate for 15 minutes and following treatment with 0.16M sulfuric acid to stop the reaction. After staining the OD value was measured at 450nm.

[0109] Protein specific titer was calculated as shown below:

[0110] *Specific OD value (S.OD value) = (OD of sample – OD of negative serum) in target protein coated well – (OD of sample – OD of negative serum) in protein from baculovirus infected cell - coated well*

[0111] Cattle immunized with different recombinant proteins elicited specific antibodies against each of the proteins, as shown in Figures 10, 11, 12 and 13. Individual data of ELISA testing of samples are shown in Tables 9 and 10. There was a strong seroconversion against homologous protein in vaccinates of all recombinant vaccine administered groups detected as early as on 21 dpv after single immunization. ELISA signal was increased on 35 dpv after booster vaccination. In addition, sera from cattle immunized with VP2/EHDV2 (Group C1) and combination of VP2/EHDV2 +VP5/EHDV2 (Group C2) revealed cross-reactivity against VP2/EHDV6 and VP5/EHDV6. Similarly, sera from cattle administered VP2/EHDV6 (Group C3) and combination of VP2/EHDV6 + VP5/EHDV6 (Group C4) had cross reactivity with recombinant proteins of EHDV2. Similar cross-reactivity between EHDV2 and EHDV6 serotypes was observed in the mice study described above.

[0112] **Table 9.** Mean of specific OD value of experimental groups against target proteins

Specific OD value of recombinant protein								
vaccine	α -VP2-EHDV2					α -VP5-EHDV2		
	C1: VP2/2	C2: VP2/2+ VP5/2	C3: VP2/6	C4: VP2/6 + VP5/6	C5: Mock	C2: VP2+ VP5/2	C4: VP2/6 + VP5/6	Mock
0 D	0.043	0.003	0.006	0.072	0.007	0.001	0.021	0.001
21 D	0.660	0.475	0.768	0.965	0.128	1.015	1.372	0.004
35 D	2.439	2.364	1.736	1.898	0.122	2.172 $\pm$ 0.41	2.423 $\pm$ 0.34	0.165 $\pm$ 0.02
(Mean $\pm$ SD)	$\pm$ 0.26	$\pm$ 0.39	$\pm$ 0.42	$\pm$ 0.2	$\pm$ 1.05			
α -VP2-EHDV6						α -VP5-EHDV6		

vaccine	VP2/2	VP2/2+ VP5/2	VP2/6	VP2/6 + VP5/6	Mock	VP2+ VP5/2	VP2/6 + VP5/6	Mock
0 D	0.077	0.040	0.000	0.049	0.021	0.013	0.053	0.031
21 D	1.258	1.554	1.585	1.964	0.242	0.264	0.625	0.220
35 D	2.095	2.598	2.925	3.185	0.197			
(Mean±SD)	±0.68	±0.35	±0.31	±0.05	±0.06	2.091±0.14	2.440±0.12	0.141±0.09

[0113] **Table 10.** Specific OD values of serum samples from individual animals

Specific OD values of serum from immunized cattle against target recombinant protein							
Group	# cattle	α -VP2/EHDV 2			α -VP5/EHDV 2		
		0 dpv	21 dpv	35 dpv	0 dpv	21 dpv	35 dpv
C1: VP2/2	677	-0.007	0.177	2.276	N	N	N
	709	0.104	0.458	2.305	N	N	N
	743	0.033	1.344	2.735	N	N	N
C2: VP2/2+VP5/2	712	-0.025	0.552	2.124	0.001	0.750	2.104
	794	0.0272	0.552	2.158	0.002	0.663	1.797
	800	0.007	0.321	2.810	0.001	1.632	2.614
C3: VP2/6	714	0.006	0.428	1.625	N	N	N
	792	0.768	0.888	2.199	N	N	N
	855	1.736	0.987	1.384	N	N	N
C4: VP2/6+VP5/6	742	0.051	1.391	2.039	0.032	2.101	2.731
	745	-0.011	0.75	1.986	-0.030	1.108	2.479
	779	0.175	0.755	1.670	0.063	0.908	2.060
Mock	707	0.008	0.191	0.147	0.001	0.164	0.152
	825	0.006	0.072	0.098	0.001	-0.029	0.178
	851	0.007	0.121	0.122	0.002	-0.122	0.165
Group	# cattle	α -VP2/EHDV 6			α -VP5/EHDV 6		
		0 dpv	21 dpv	35 dpv	0 dpv	21 dpv	35 dpv
C1: VP2/2	677	-0.098	0.506	1.58115	N	N	N
	709	0.153	1.044	1.83535	N	N	N
	743	0.177	2.224	2.86745	N	N	N
C2: VP2/2+VP5/2	712	-0.066	1.189	2.4633	-0.003	0.510	1.980
	794	0.177	1.353	2.3407	0.019	0.391	2.041
	800	0.009	2.121	2.9907	0.022	0.712	2.251
C3: VP2/6	714	0.032	1.062	2.812	N	N	N
	792	0.003	1.726	3.280	N	N	N
	855	-0.024	1.967	2.682	N	N	N
C4: VP2/6+VP5/6	742	0.119	2.387	3.215	0.068	0.763	2.379
	745	-0.021	1.633	3.210	-0.034	0.415	2.368
	779	0.968	1.872	3.129	0.126	0.698	2.574
Mock	707	0.085	0.267	0.236	0.068	0.260	0.207
	825	-0.043	0.207	0.158	-0.018	0.121	0.075
	851	-0.051	0.252	0.197	0.043	0.280	0.141

[0114] Virus Neutralization test (VN test)

[0115] The virus neutralization test was done as described above in the VN section for the mice experiment. Virus neutralization assays against EHDV2 showed high neutralizing titers in serum from cattle immunized with VP2/EHDV2 protein (Group C1) and VP2/EHDV2+ VP5/EHDV2 protein (Group C2) at 35 dpv. (Fig. 14 and Tables 11, 12). Some low serum neutralization titers against EHDV2 were detectable on 21 dpv after one dose immunization. Sera from cattle, immunized with VP2 of EHDV6 (Group C3) or with combination of VP2+VP5 of EHDV6 (Group C4) did not show any anti-EHDV2 neutralizing activity. Similarly to the mice studies, there was no cross-neutralization reactivity between EHDV2 and EHDV6 serotypes.

[0116] **Table 11.** VN titer of serum against EHDV2 virus

VN titer against EHDV serotype 2 virus (Mean±SD)					
DPV	C1: VP2/2	C2: VP2/2+VP5/2	C3: VP2/6	C4: VP2/6+VP5/6	C5: Mock
0	0	0	0	0	0
21	10 ±16.0	0	0	0	0
35	706.7 ± 384	720.0 ± 2114.2 04.2± 7.0	4.2±7.0	0	0

[0117] **Table 12.** VN titer of individual animal against EHDV 2 virus

Group	# cattle	VN titer against EHDV 2		
		0 dpv	21 dpv	35 dpv
C1: VP2	677	0	0	1120
	709	0	30	360
	743	0	0	640
C2: VP2/2+VP5/2	712	0	0	800
	794	0	0	480
	800	0	0	880
C3: VP2/6	714	0	0	0
	792	0	0	12.5
	855	0	0	0
C4: VP2/6+VP5/6	742	0	0	0
	745	0	0	0

	779	0	0	0
Mock	707	0	0	0
	825	0	0	0
	851	0	0	0

[0118] Virus neutralization assays against EHDV6 showed high neutralizing titers in serum from cattle immunized with VP2/EHDV6 protein (Group C3) and VP2+VP5/EHDV6 proteins (Group C4) at 35 dpv. Sera from cattle, immunized with VP2 of EHDV2 (Group C1) or with a combination of VP2+VP5 of EHDV2 (Group C2) did not show any anti-EHDV6 neutralizing activity (Fig. 15 and Tables 13, 14). Some low serum neutralization titers against EHDV6 were detectable on 21 dpv after one dose immunization. Similarly to the mice studies, there was no cross-neutralization reactivity between EHDV2 and EHDV6 serotypes.

[0119] **Table 13.** Summary VN titers of serum against EHDV6 serotype (Mean $\pm$ SD)

VN titer against EHDV serotype 2 virus (Mean $\pm$ SD)					
DPV	C1: VP2/2	C2: VP2/2+VP5/2	C3: VP2/6	C4: VP2/6+VP5/6	C5: Mock
0 D	0	0	0	0	0
21 D	0	0	5.8 $\pm$ 8.0	20 $\pm$ 11.4	0
35 D	5.8 $\pm$ 5.2	0	226.7 $\pm$ 83	400 $\pm$ 138	0

[0120] **Table 14.** VN titers in serum of individual animals against EHDV6 serotype.

Group	# cattle	VN titer against EHDV 2		
		0 dpv	21 dpv	35 dpv
C1: 2-VP2	677	0	0	0
	709	0	30	10
	743	0	0	7.5
C2: VP2/2+VP5/2	712	0	0	0
	794	0	0	0

	800	0	0	0
C3: VP2/6	714	0	0	200
	792	0	2.5	160
	855	0	15	320
C4: VP2/6+VP5/6	742	0	7.5	480
	745	0	22.5	480
	779	0	30	240
Mock	707	0	0	0
	825	0	0	0
	851	0	0	0

[0121] **Results and Conclusions**

[0122] One aspect of this investigation was to develop a subunit EHD vaccine to protect ruminants from EHDV infection. The recombinant proteins expressed in baculovirus system were used as vaccine antigens. The immunogenicity of expressed EHDV antigens was evaluated in mice and cattle. The VP2 and VP5 genes of US isolates of EHDV serotypes 1, 2 and 6 were expressed to produce recombinant proteins. The specificity of the proteins was confirmed by western blot using polyclonal anti-EHDV serum and anti-histidine antibody. Expressed EHDV serotype 2 and EHDV serotype 6 proteins were tested for immunogenicity in mice and cattle. In the mouse study, 9 groups of five six-week-old mice (CD1) were immunized with 20µg of either VP2 or VP5 recombinant protein individually or a combination of the two proteins. In the cattle study, five groups of cattle with 3 animals per group were immunized with 150µg of either VP2 or a combination of VP2 and VP5 recombinant proteins derived from EHDV serotypes 2 and 6. The vaccine candidates were administrated subcutaneously twice at an interval of 3 weeks. The EHDV specific immune responses in serum from immunized mice and cattle were evaluated using an indirect ELISA and virus neutralization tests. The ELISA results revealed that EHDV-specific antibodies were produced against each of the immunogens. Virus neutralization assays showed the presence of neutralizing antibodies in sera from mice and cattle immunized with VP2 alone, and in sera of mice and cattle immunized with a combination of both, VP2 and VP5 recombinant proteins of both EHDV serotypes (2 & 6).

[0123] The results indicate that the recombinant protein VP2 alone and a combination of recombinant proteins VP2/VP5 can elicit neutralizing anti-EHDV antibody responses thus providing protection against EHDV infection; these data support the efficacy of the EHDV vaccine for white tail deer (WTD) and cattle based on baculovirus-expressed EHDV VP2 and VP5 proteins.

WHAT IS CLAIMED IS:

1. An immunogenic composition comprising a VP2 subunit and a VP5 subunit from EHDV and at least one component selected from the group consisting of antigens, pharmaceutical carriers, adjuvants, preservatives, stabilizers, or combinations thereof.

2. The immunogenic composition of claim 1, wherein the VP2 subunit and VP5 subunit are proteins.

3. The immunogenic composition of claim 2, wherein the proteins are expressed using a baculovirus expression system.

4. The immunogenic composition of claim 1, wherein the at least one component is an adjuvant.

5. The immunogenic composition of claim 4, wherein the adjuvant is Montanide ISA 25.

6. The immunogenic composition of claim 1, wherein the VP2 subunit is the protein expressed by a sequence having at least 95% sequence homology with SEQ ID No. 1 or SEQ ID No. 3 and the VP5 subunit is the protein expressed by a sequence having at least 95% sequence homology with SEQ ID No. 2 or SEQ ID No. 4.

7. The immunogenic composition of claim 1, wherein the VP2 subunit has an amino acid sequence that has at least 95% sequence homology with SEQ ID No. 5 or SEQ ID No. 7 or the VP5 subunit has an amino acid sequence that has at least 95% sequence homology with SEQ ID No. 6 or SEQ ID No. 8.

8. An immunogenic composition comprising at least two VP2 subunits from different serotypes of EHDV and at least one component selected from the group consisting of antigens, pharmaceutical carriers, adjuvants, preservatives, stabilizers, or combinations thereof.

9. The immunogenic composition of claim 8, wherein the VP2 subunits are proteins.

10. The immunogenic composition of claim 9, wherein the proteins are expressed using a baculovirus expression system.

11. The immunogenic composition of claim 8, wherein the at least one component is an adjuvant

12. The immunogenic composition of claim 11, wherein the adjuvant is Montanide ISA 25.

13. The immunogenic composition of claim 8, wherein the at least two VP2 subunits are the protein expressed by a sequence having at least 95% sequence homology with SEQ ID No. 1 or SEQ ID No. 3.

14. The immunogenic composition of claim 8, wherein the at least two VP2 subunits have an amino acid sequence that has at least 95% sequence homology with SEQ ID No. 5 or SEQ ID No. 7.

15. An immunogenic composition comprising at least two VP5 subunits from different serotypes of EHDV and at least one component selected from the group consisting of antigens, pharmaceutical carriers, adjuvants, preservatives, stabilizers, or combinations thereof.

16. The immunogenic composition of claim 15, wherein the VP5 subunits are proteins.

17. The immunogenic composition of claim 16, wherein the proteins are expressed using a baculovirus expression system.

18. The immunogenic composition of claim 15, wherein the at least one component is an adjuvant.

19. The immunogenic composition of claim 18, wherein the adjuvant is Montanide ISA 25.

20. The immunogenic composition of claim 15, wherein the at least two VP5 subunits are the protein expressed by a sequence having at least 95% sequence homology with SEQ ID No. 2 or SEQ ID No. 4.

21. The immunogenic composition of claim 15, wherein the at least two VP5 subunits have an amino acid sequence that has at least 95% sequence homology with SEQ ID No. 6 or SEQ ID No. 8.

22. A method of reducing the incidence or severity of at least one clinical symptom of EHD, comprising the step of administering an immunogenic composition comprising a VP2 subunit and a VP5 subunit from EHDV and at least one component selected from the group consisting of antigens, pharmaceutical carriers, adjuvants, preservatives, stabilizers, or combinations thereof.

23. The method of claim 22, wherein the clinical symptoms of EHD are selected from the group consisting of loss of appetite, loss of fear of people, weakness, excessive salivation, rapid pulse, rapid respiration rate, fever, lying in bodies of water to reduce body temperature, unconsciousness, blue tongue, head swelling, neck swelling, sloughing or breaking of hooves, lameness, shock, death, and combinations thereof.

24. The method of claim 22, wherein the VP2 subunit and VP5 subunit are proteins.

25. The method of claim 24, wherein the proteins are produced using a baculovirus expression system.

26. The method of claim 22, wherein the at least one component is an adjuvant.

27. The method of claim 26, wherein the adjuvant is Montanide ISA 25.

28. The method of claim 22, wherein the VP2 subunit is the protein expressed by a sequence having at least 95% sequence homology with SEQ ID No. 1 or SEQ ID No. 3 and the VP5 subunit is the protein expressed by a sequence having at least 95% sequence homology with SEQ ID No. 2 or SEQ ID No. 4.

29. The method of claim 22, wherein the VP2 subunit has an amino acid sequence that has at least 95% sequence homology with SEQ ID No. 5 or SEQ ID No. 7 and the VP5 subunit has an amino acid sequence that has at least 95% sequence homology with SEQ ID No. 6 or SEQ ID No. 8.

30. A method of reducing the incidence or severity of at least one clinical symptom associated with subclinical EHD, comprising the step of administering an immunogenic composition comprising a VP2 subunit and a VP5 subunit from EHDV and at least one component selected from the group consisting of antigens, pharmaceutical carriers, adjuvants, preservatives, stabilizers, or combinations thereof.

31. The method of claim 30, wherein the symptoms of subclinical EHD are selected from the group consisting of viremia, fever, oral ulcers, excessive salivation, lameness, coronitis, and combinations thereof.

32. The method of claim 30, wherein the VP2 subunit and VP5 subunit are proteins.

33. The method of claim 30, wherein the proteins are produced using a baculovirus expression system.

34. The method of claim 30, wherein the at least one component is an adjuvant.

35. The method of claim 34, wherein the adjuvant is Montanide ISA 25.

36. The method of claim 30, wherein the VP2 subunit is the protein expressed by a sequence having at least 95% sequence homology with SEQ ID No. 1 or SEQ ID No. 3 and the VP5 subunit is the protein expressed by a sequence having at least 95% sequence homology with SEQ ID No. 2 or SEQ ID No. 4.

37. The method of claim 30, wherein the VP2 subunit has an amino acid sequence that has at least 95% sequence homology with SEQ ID No. 5 or SEQ ID No. 7 and the VP5 subunit has an amino acid sequence that has at least 95% sequence homology with SEQ ID No. 6 or SEQ ID No. 8.

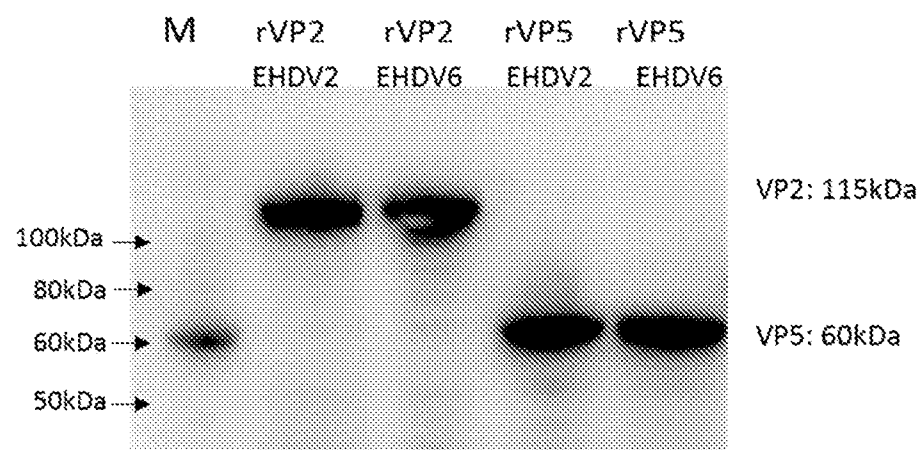


Fig. 1

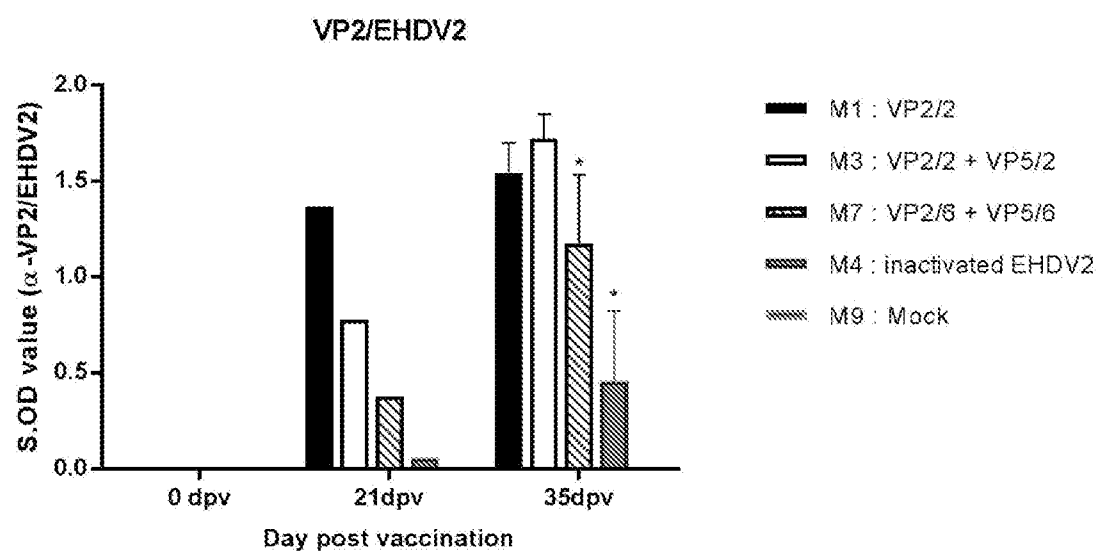


Fig. 2

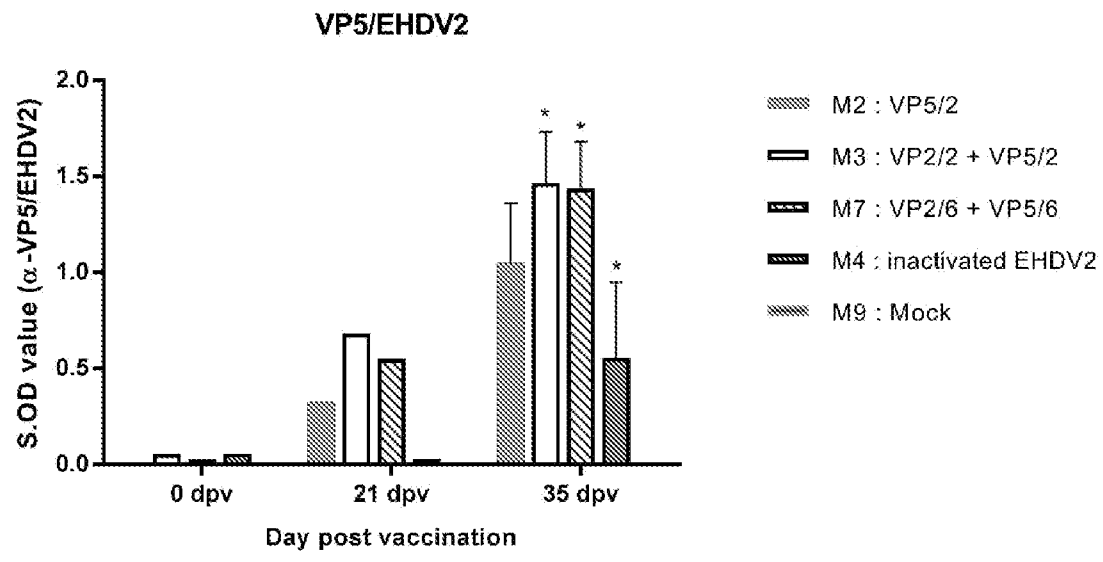


Fig. 3

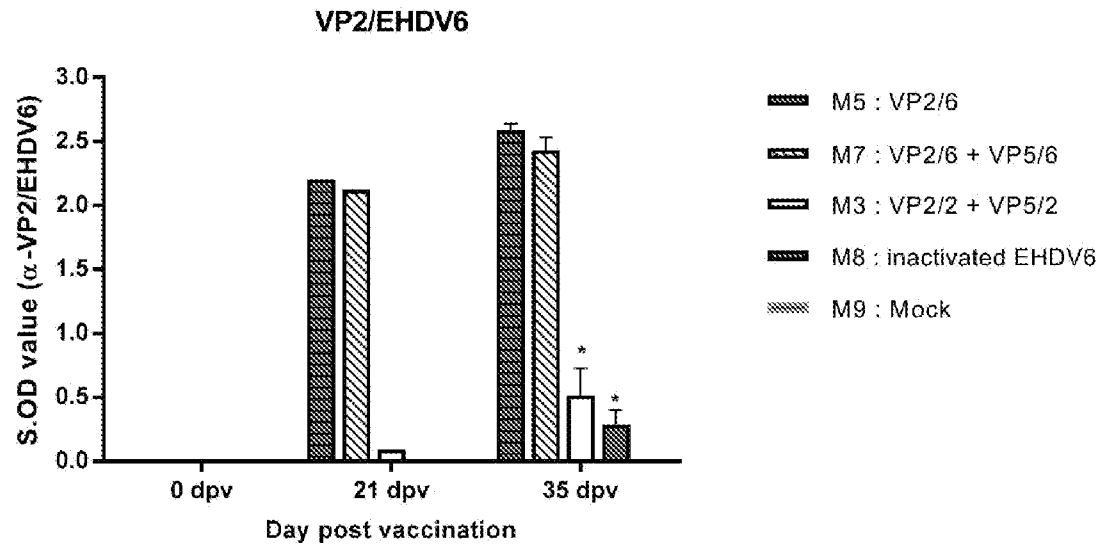


Fig. 4

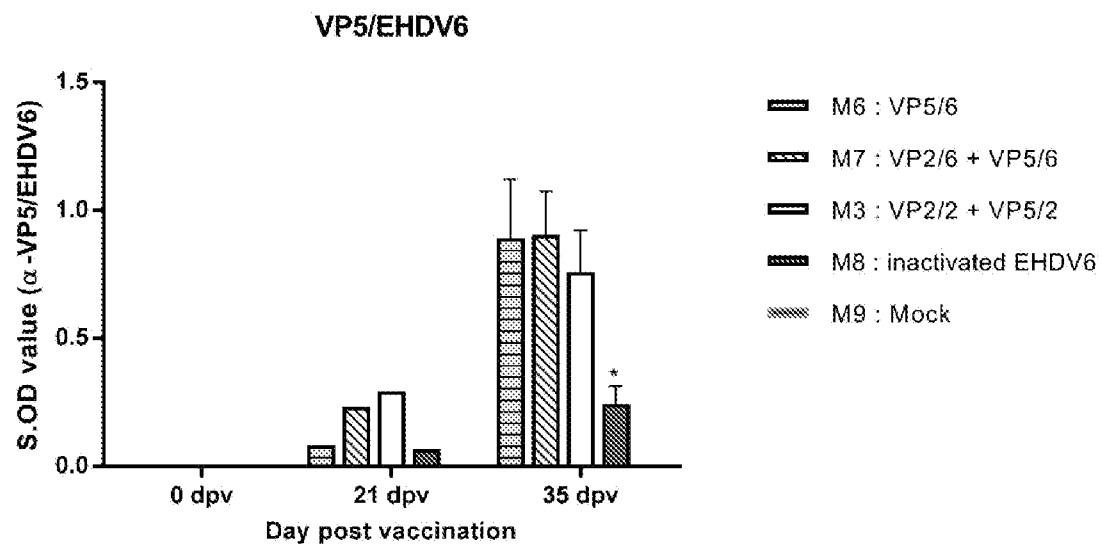


Fig. 5

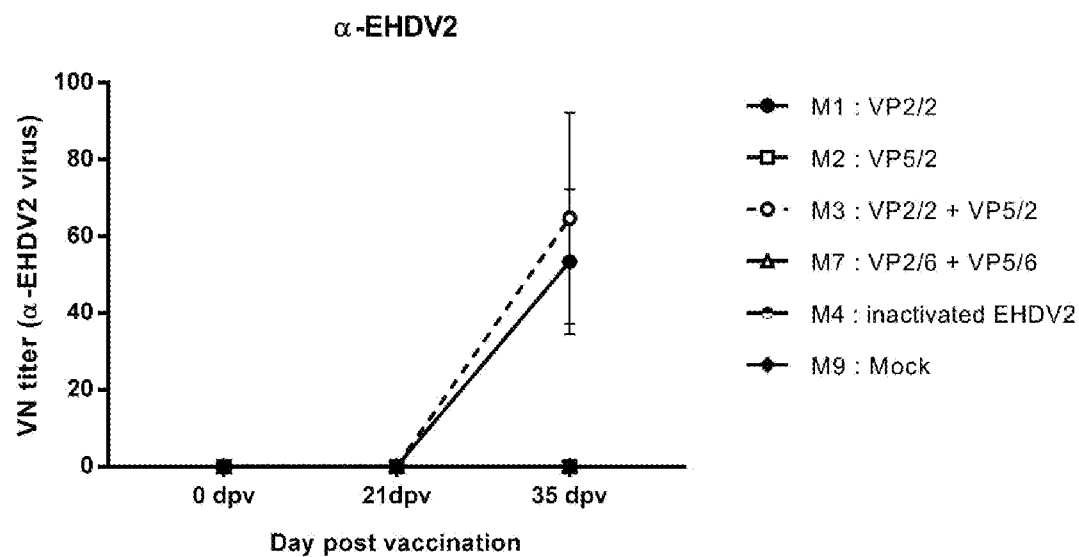


Fig. 6

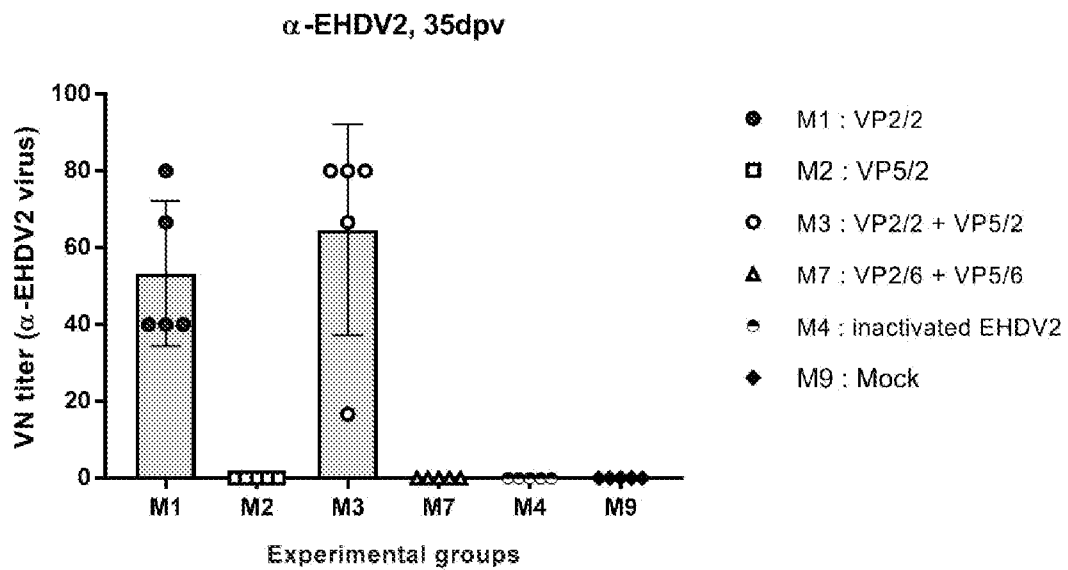


Fig. 7

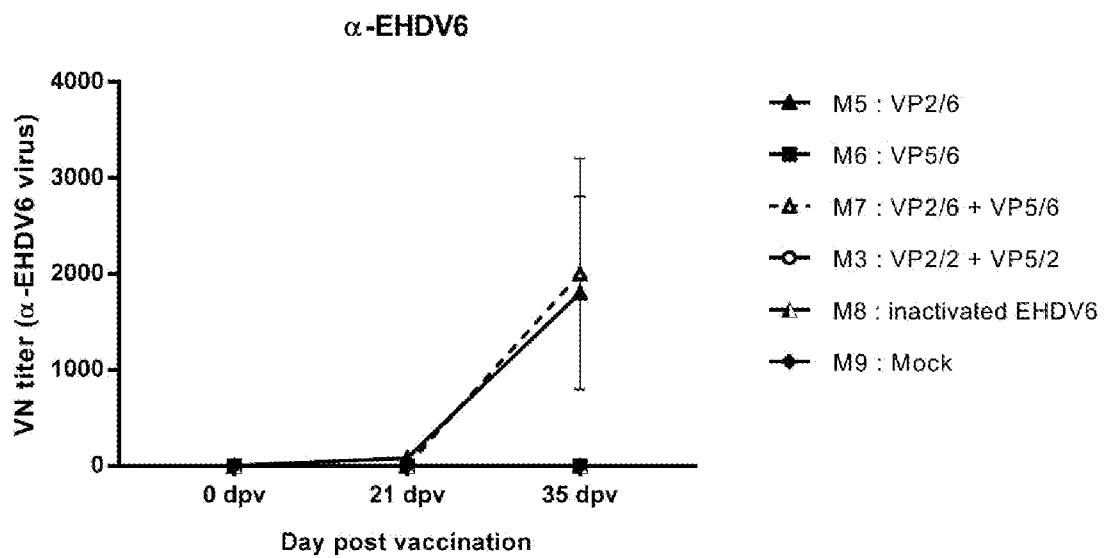


Fig. 8

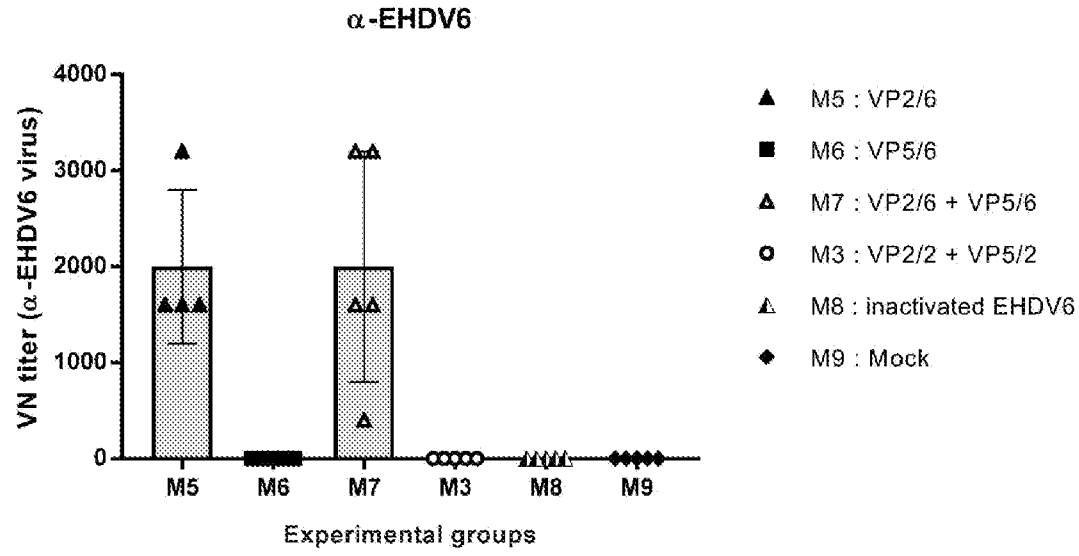


Fig. 9

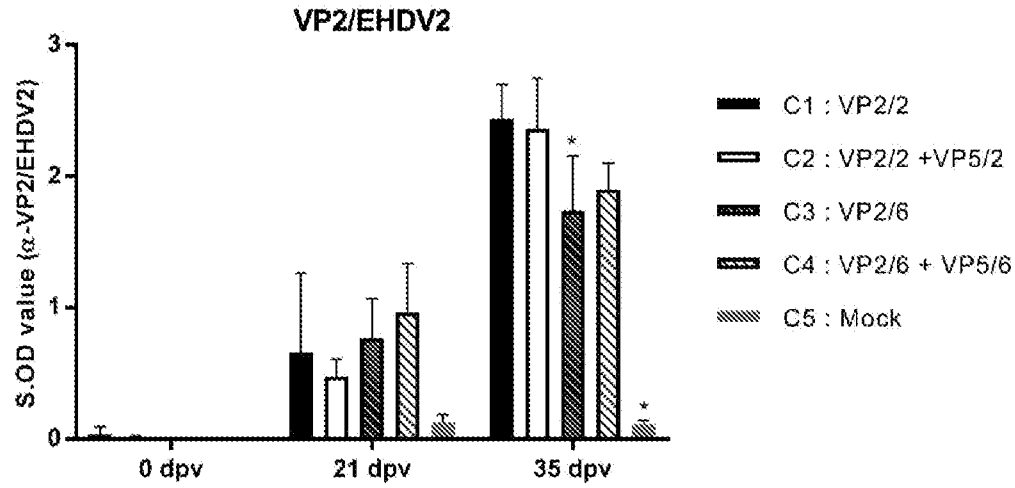


Fig. 10

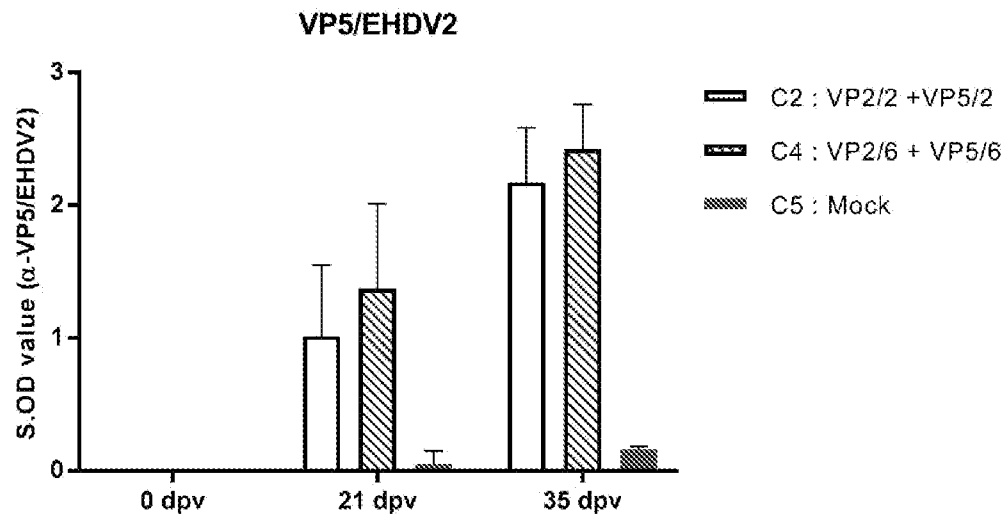


Fig. 11

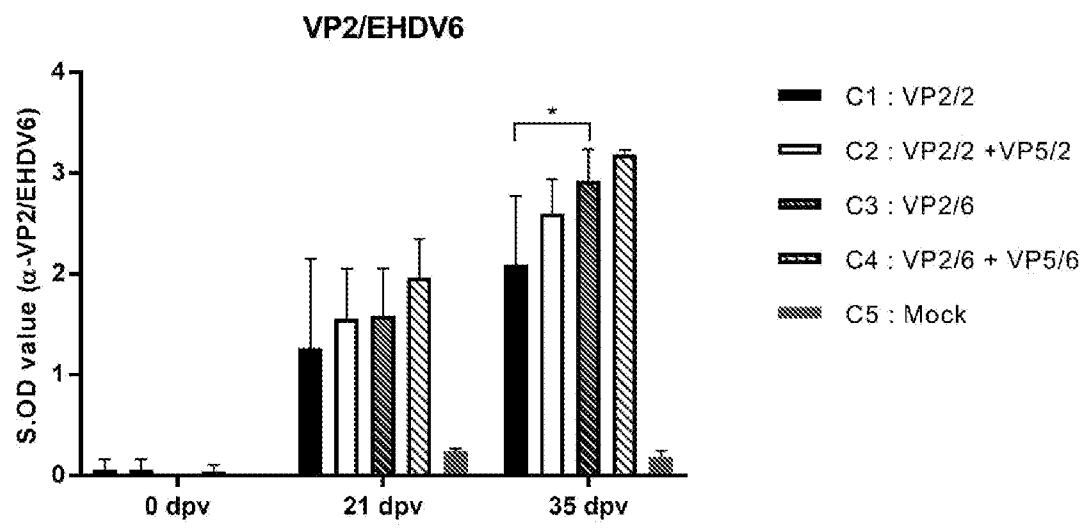


Fig. 12

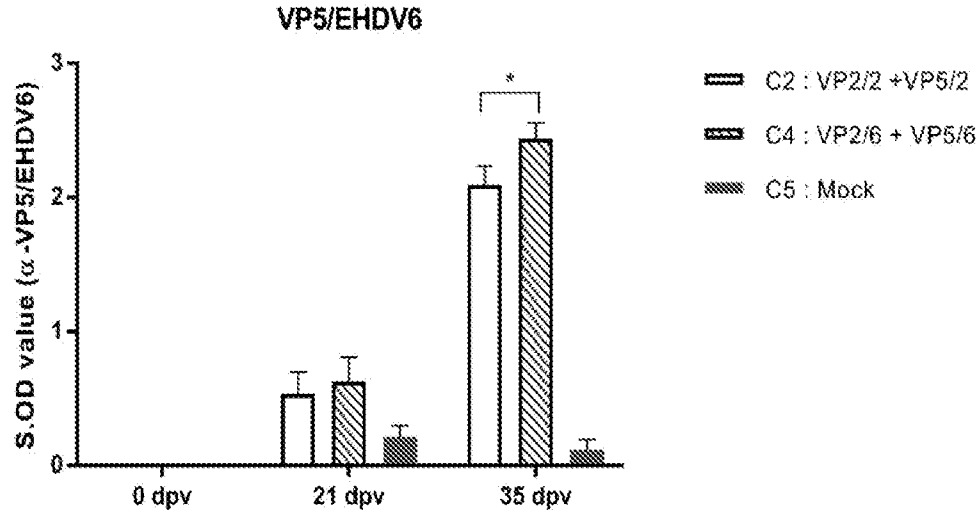


Fig. 13

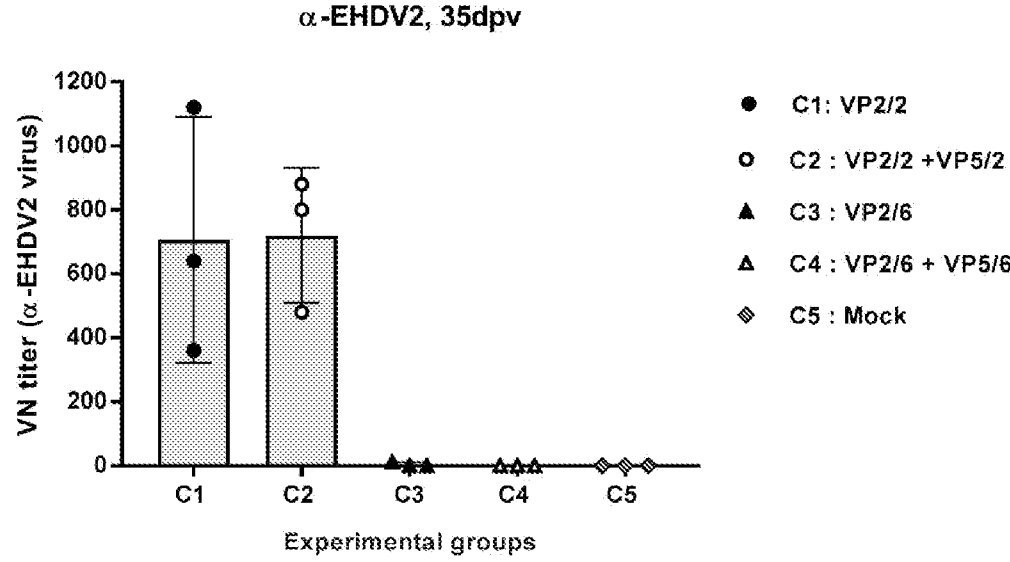


Fig. 14

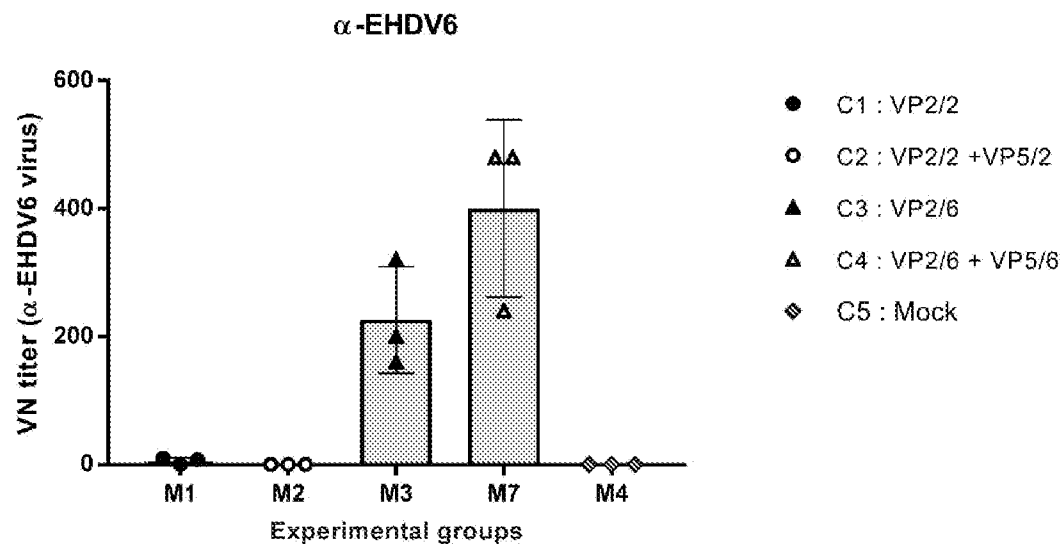


Fig. 15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/52084

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/866; C07K 14/14; A61K 39/12, 39/15 (2017.01)

CPC - C12N 2720/12111, 2720/12134, 15/866, 2710/14143; A61K 39/15; C07K 14/14

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Alshaikhahmed et al. Generation of virus-like particles for emerging epizootic haemorrhagic disease virus: Towards the development of safe vaccine candidates. Vaccine 17 February 2016	1-4
----	Vol 34 No 8 Pages 1103-1108. Especially abstract, pg 1104 col 1 para 5, pg 1104 col 2 para 5.	-----
Y		5-7
Y	US 2001/0033845 A1 (Ganne). 25 October 2001 (25.10.2001). Especially para [0007], [0051], claim 1.	5
Y	GenBank Accession number AM744998.1. Epizootic hemorrhagic disease virus (serotype 2 / strain Alberta) segment 2. (online) 07 March 2015 [retrieved 18 November 2017]. Available on the internet: <URL: https://www.ncbi.nlm.nih.gov/nucore/AM744998 >. Especially pg 1-2	6, 7
Y	GenBank Accession number AM745002.1 Epizootic hemorrhagic disease virus (serotype 2 / strain Alberta) segment 6. (online) 07 March 2015 [retrieved 18 November 2017]. Available on the internet: <URL: https://www.ncbi.nlm.nih.gov/nucore/AM745002 >. Especially pg 1-2.	6, 7

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

21 November 2017

Date of mailing of the international search report

01 FEB 2018

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/52084

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

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

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

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

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

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

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

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

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

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

3. Additional comments:

GenCore ver 6.4.1 SEQ ID NOs: 1, 2, 5, 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/52084

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

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

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

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

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

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

This International Searching Authority found multiple inventions in this international application, as follows:
-----Go to Extra Sheet for continuation-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-21 limited to SEQ ID NOs: 1, 2, 6, 7 (claims 1-7)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/52084

Continuation of Box III: Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-21, drawn to an immunogenic composition comprising viral protein subunits from EHDV [Epizootic hemorrhagic disease virus].

The immunogenic composition will be searched to the extent to encompass a VP2 subunit and a VP5 subunit; wherein the VP2 subunit comprises the first named nucleic acid sequence SEQ ID NO: 1 (claim 6) and amino acid sequence SEQ ID NO: 5 (claim 7), and wherein the VP5 unit comprises the first named nucleic acid SEQ ID NO: 2 (claim 6) and amino acid sequence SEQ ID NO: 6 (claim 7). It is believed that claims 1-7 read on this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 1, 2, 5, 6. Additional viral protein subunits will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected VP2 or VP5 nucleic acid and/or protein sequences. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: an immunogenic composition comprising two VP2 subunits comprising nucleic acid and amino acid sequences SEQ ID NOs: 3 and 7 (claims 8-14).

Group II: Claims 22-37, drawn to a method of reducing the incidence or severity of EHD.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group I+ has the special technical feature of an immunogenic composition, not required by Group II.

Group II has the special technical feature of a method of treating a disease, not required by Group I+.

No technical features are shared between the VP2 nucleotide and amino acid sequences of Group I+ and, accordingly, these groups lack unity a priori.

No technical features are shared between the VP5 nucleotide and amino acid sequences of Group I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of Claims 1, 8 and 15. These shared technical features are previously disclosed by the publication titled "Generation of virus-like particles for emerging epizootic haemorrhagic disease virus: Towards the development of safe vaccine candidates" by Alshaikhahmed et al. (hereinafter "Alshaikhahmed") [published 17 February 2016; Vaccine Vol 34 No 8 Pages 1103-1108], in view of the publication titled "Genetic and phylogenetic analysis of the outer-coat proteins VP2 and VP5 of epizootic haemorrhagic disease virus (EHDV): comparison of genetic and serological data to characterise the EHDV serogroup" by Anthony et al. (hereinafter "Anthony") [published November 2009 in Virus Res Vol 145 No 2 Pages 200-210]

Alshaikhahmed teaches an immunogenic composition comprising a VP2 subunit and a VP5 subunit from EHDV (abstract; "Here we expressed four major capsid proteins (VP2, VP3, VP5 and VP7) of EHDV-1 using baculovirus multiple gene expression systems and demonstrated that three-layered VLPs were assembled mimicking the authentic EHDV particles but lacking the viral genomic RNA segments and the transcriptase complex (TC). Antibodies generated with VLPs not only neutralized EHDV-1 infection in cell culture but also showed cross neutralizing reactivity against two other serotypes, EHDV-2 and EHDV-6. For proof of concept, we demonstrated that EHDV-2 VLPs could be generated rapidly by expressing the EHDV-2 variable outer capsid proteins (VP2, VP5) together with EHDV-1 VP3 and VP7, the two inner capsid proteins, which are highly conserved among the 7 serotypes").

Alshaikhahmed teaches an immunogenic composition comprising adjuvant (pg 1104 col 2 para 5; "Animals were immunized first with 500ug of antigen in 10 sites followed by boosting twice with 250ug antigen at 14 and 28 respectively in the presence of Incomplete Freund's adjuvant").

The inventions of Group I+ and II further share the technical feature of an immunogenic composition comprising a VP2 subunit and a VP5 subunit from EHDV, which is addressed by Alshaikhahmed, as above.

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As to claim 8, concerning an immunogenic composition comprising at least two VP2 subunits from different serotypes of EHDV and at least one component selected from the group consisting of adjuvants, Alshaikhahmed teaches an immunogenic composition comprising a VP2 subunit of serotype 1 (abstract) and an adjuvant (pg 1104 col 2 para 5), as indicated above. Alshaikhahmed does not teach that the immunogenic composition comprising at least two VP2 subunit from different serotypes. However, Anthony teaches the amino acid and nucleotide sequences of VP2 subunits from serotype 1, serotype 2, and serotype 6 for example (pg 203 Table 3; "Summary of percentage sequence identities for segment 2/VP2 and segment 6/VP5 for eastern viruses, western viruses and all strains respectively. Nucleotide (NT) and amino acid (AA) identities are both presented"; pg 202 Table 2; Genetic information of genome segment 2. Grey shading indicates western viruses. No shading indicates eastern viruses. Indicated: GenBank Accessions: AM744978, AM744988, AM745038.1 [serotypes 1, 2, 6 respectively]). An artisan of ordinary skill in the art would have known how to obtain the nucleotide sequences for VP2 capsids of at least 2 serotypes provided by Anthony and use them in the baculovirus expression systems taught by Alshaikhahmed to generate an immunogenic composition comprising at least two VP2 subunits to provide wider application of the vaccine, without undue effort and a high probability of success.

As to claim 15, concerning an immunogenic composition comprising at least two VP5 subunits from different serotypes of EHDV and at least one component selected from the group consisting of adjuvants, Alshaikhahmed teaches an immunogenic composition comprising a VP5 subunit of serotype 1 (abstract) and an adjuvant (pg 1104 col 2 para 5), as indicated above. Alshaikhahmed does not teach that the immunogenic composition comprising at least two VP5 subunits. However, Anthony teaches the amino acid and nucleotide sequences of VP2 subunits from serotype 1, serotype 2, and serotype 6 for example (Pg 203 Table 3; Page 202 Table 2). An artisan of ordinary skill in the art would have known how to obtain the nucleotide sequences for VP5 capsids of at least 2 serotypes provided by Anthony and use them in the baculovirus expression systems taught by Alshaikhahmed to generate an immunogenic composition comprising at least two VP5 subunits, to provide wider application of the vaccine, without undue effort and a high probability of success.

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I+ and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.