Title: NEWCASTLE DISEASE VIRUS VECTORED HERPESVIRUS VACCINES

Abstract: The present invention encompasses recombinant Newcastle Disease Virus - Herpesvirus vaccines or compositions. The invention encompasses recombinant NDV vectors encoding and expressing herpesvirus pathogen, antigens, proteins, epitopes or immunogens. Such vaccines or compositions can be used to protect animals against disease.

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NEWCASTLE DISEASE VIRUS VECTORED HERPESVIRUS VACCINES

CROSS-REFERENCE TO RELATED APPLICATIONS


FIELD OF THE INVENTION

[0002] The present invention encompasses NDV-vectored herpesvirus vaccines or compositions.

BACKGROUND OF THE INVENTION


[0004] NDV belongs to the Paramyxovirinae family and the Avulavirus genus. NDV replicates in respiratory and gastrointestinal tracts, in the oviduct, and for some isolates, in the nerve system. The transmission is aerogenic and by oral and fecal routes. NDV causes a highly contagious and fatal disease affecting all species of birds, and can infect some mammalian species. The disease can vary from clinically unapparent to highly virulent forms, depending on the virus strain and the host species. The continuous spectrum of virulence displayed by NDV strains enabled the grouping of them into three different pathotypes: lentogenic, mesogenic, and velogenic (Alexander, D. J., Diseases of Poultry, Iowa State Uni. Press, Ames IA, 541-569, 1997). Lentogenic strains do not usually cause disease in adult chickens and are widely used as live vaccines in poultry industries in the United States and other countries. Viruses of intermediate virulence are termed mesogenic, while viruses that cause high mortality are termed velogenic. The disease has a worldwide distribution and remains a constant major threat to commercial poultry production.

[0005] The NDV genome is a non-segmented negative strand of RNA of approximately 15kb. The genomic RNA contains six genes that encode the following proteins in the order of: the nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F),
haemagglutinin-neuramimidase (HN) and large polymerase protein (L). Two additional proteins, V and W, of unknown function are produced by RNA editing during P gene transcription (Steward et al., 1993, Journal of General Virology 74:2539-2547).

[0006] The development of methods to recover non-segmented negative RNA viruses entirely from cloned cDNA, established in recent years, opened up the possibility of genetically manipulating this virus group, including NDV (Conzelmann, K.K., Ann. Rev. Genet. 32, 123-162, 1998; Roberts and Rose, Virology 247, 1-6, 1998). This unique molecular genetic methodology, termed "reverse genetics", provides a means not only to investigate the functions of various virus-encoded genes (Palese et al, PNAS 93, 11354-11358, 1996; Nagai, Y., Rev. Med. Virol. 9, 83-99, 1999) but also to allow the use of these viruses to express heterologous genes (Bukreyev et al, J. Virol. 70, 6634-6641, 1996; Mebatsion et al, PNAS 93, 7310-7314, 1996; Schnell et al, PNAS 93, 11359-11365, 1996; Hasan et al, J. Gen. Virol. 78, 2813-2820, 1997; He et al, Virology 237, 249-260, 1997; Sakai et al., FEBS Lett. 45, 221-226, 1999). This provides a new method of generating improved vaccines and vaccine vectors. Recently, NDV was used as a vector for expression of avian influenza antigens (US2010/0255029, Merial Limited).

[0007] The Herpesvirus glycoprotein D (gD) is essential for FHV-1 (Feline Herpesvirus -1) entry and is involved in interaction with host cell (binding to receptors). The gD protein has haemagglutination activities on feline red blood cells (Maeda et al, Virology 202, 1034-8, 1994; Maeda et al, Virus Res. 46, 75-80, 1996). The Herpesvirus glycoprotein B (gB) is essential for FHV entry and is involved in fusion process (Spatz and Maes, Virology 197, 125-36, 1993; Maeda et al, Virus Res 39, 55-61, 1995). Both glycoproteins can induce neutralizing antibodies (Horimoto et al., Arch Virol 111, 127-32, 1990).

[0008] Considering the susceptibility of animals, including humans, to herpesvirus, a means of preventing herpesvirus infection and protecting animals is essential. Accordingly, there is a need for an effective vaccine against herpesvirus.

[0009] Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

30 SUMMARY OF THE INVENTION

[0010] The present invention relates to an NDV-vectored vaccine or composition that comprises one or more engineered, recombinant NDV vectors that harbor and express certain herpesvirus antigens, such as a feline herpesvirus antigen, and optionally a pharmaceutically
or veterinarily acceptable carrier, adjuvant, excipient, or vehicle. The NDV may be the AVINEW® NDV strain, a modified live vaccine commercialized by Merial Limited.

[0011] The herpesvirus antigen may be a glycoprotein. The herpesvirus antigen may be a glycoprotein B (gB) or glycoprotein D (gD) antigen from a feline herpesvirus.

[0012] The invention also relates to a method of vaccinating an animal comprising administering to the animal an effective amount of one or more vaccines or compositions which may comprise an effective amount of a recombinant NDV vector and optionally a pharmaceutically or veterinarily acceptable carrier, adjuvant, excipient, or vehicle. The administering may be by in ovo, oro-nasal, eye drop, spray, drinking water or parenteral (subcutaneous, intramuscular, transdermal, intradermal) administration.

[0013] The invention further relates to administration of the vaccine or composition using prime-boost protocol. The invention further encompasses a kit for performing a method of eliciting or inducing an immune response that may comprise any one of the recombinant herpesvirus immunological compositions or vaccines, or inactivated immunological compositions or vaccines, and instructions for performing the method.

[0014] Accordingly, it is an object of the invention to not encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product.

[0015] These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0016] The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may be best understood in conjunction with the accompanying drawings, in which:

[0017] Figure 1 is a table showing the SEQ ID NO assigned to the DNA and protein sequences.
Figure 2A depicts a genetic map of the full length NDV genome; Figure 2B depicts a map illustrating the genetic map of two engineered NDV vectors with herpesvirus gB or gD insertion into two representative intergenic insertion sites on the full length NDV genome; Figure 2C is an example of flow diagram of the NDV reverse genetics system.

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Figure 3 depicts the generation of NDV transcription plasmid containing feline herpesvirus (FHV) gB gene (pFR14 plasmid) or gD gene (pFR16 plasmid).

Figure 4 depicts the maps of pFR14 and pFR16 plasmids.

Figure 5 shows the average rectal temperature of cats after the challenge. Group A is NDV-HV by ON, group B is NDV-HV by SC, group C is positive control (vaccine containing attenuated feline Herpesvirus F2 strain, Merial Limited), group D is negative control (no vaccination).

Figure 6 shows the average bodyweight of cats after the challenge. Group A is NDV-HV by ON, group B is NDV-HV by SC, group C is positive control (vaccine containing attenuated feline Herpesvirus F2 strain, Merial Limited), group D is negative control (no vaccination).

Figure 7 shows the data collected on clinical signs of the cats after challenge. Group A is NDV-HV by ON, group B is NDV-HV by SC, group C is positive control (vaccine containing attenuated feline Herpesvirus F2 strain, Merial Limited), group D is negative control (no vaccination).

Figure 8 shows the statistical analysis of the clinical signs of the cats after the challenge. Group A is NDV-HV by ON, group B is NDV-HV by SC, group C is positive control (vaccine containing attenuated feline Herpesvirus F2 strain, Merial Limited), group D is negative control (no vaccination).

Figure 9 depicts the viral shedding of the cats after the challenge. Group A is NDV-HV by ON, group B is NDV-HV by SC, group C is positive control (vaccine containing attenuated feline Herpesvirus F2 strain, Merial Limited), group D is negative control (no vaccination).

Figure 10 is the statistical analysis of the viral shedding of the cats after the challenge. Group A is NDV-HV by ON, group B is NDV-HV by SC, group C is positive control (vaccine containing attenuated feline Herpesvirus F2 strain, Merial Limited), group D is negative control.

Figure 11 shows the evolution of the mean FHV Ab (anti-gB) titer per group. Group A is NDV-HV by ON, group B is NDV-HV by SC, group C is positive control (vaccine
containing attenuated feline Herpesvirus F2 strain, Merial Limited), group D is negative control (no vaccination).

[0028] Figure 12 shows the gB protein sequence alignment and sequence identity percentage.

[0029] Figure 13 shows the gD protein sequence alignment and sequence identity percentage.

**DETAILED DESCRIPTION**

[0030] It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0031] Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a", "an", and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise.

[0032] In the present invention, AVINEW® strain is used as the NDV vector (US2010/0255029).

[0033] The present invention relates to a vaccine or composition that may comprise an effective amount of one or more engineered NDV vectors, and optionally a pharmaceutically or veterinarily acceptable carrier, adjuvant, excipient, or vehicle.

[0034] The present invention encompasses an engineered NDV vector expressing a herpesvirus protein, polypeptide, antigen, epitope or immunogen that elicits an immunogenic response in an animal. The herpesvirus protein, polypeptide, antigen, epitope or immunogen may be a feline herpesvirus protein, polypeptide, antigen, epitope or immunogen.

[0035] As used herein, the term "herpesvirus polypeptide, antigen, epitope or immunogen" refers to any polypeptide, antigen, epitope or immunogen of a herpesvirus. The herpesvirus may be a feline herpesvirus, canine herpesvirus, phocid herpesvirus. The herpesvirus polypeptide may be herpesvirus glycoprotein, including but not limited to herpesvirus gB or gD protein.
By "animal" is intended mammals, human, birds, and the like. The animal may be selected from the group consisting of equine (e.g., horse), canine (e.g., dogs, wolves, foxes, coyotes, jackals), feline (e.g., lions, tigers, domestic cats, wild cats, other big cats, and other feline including cheetahs and lynx), ovine (e.g., sheep), bovine (e.g., cattle, cow, buffalo), swine (pig), avian (e.g., chicken, duck, goose, turkey, quail, pheasant, parrot, finches, hawk, crow, ostrich, emu and cassowary), primate (e.g., prosimian, tarsier, monkey, gibbon, ape), and fish. The term "animal" also includes an individual animal in all stages of development, including embryonic and fetal stages.

In one embodiment, the herpesvirus immunological composition or vaccine comprises one or more engineered NDV vectors, and optionally a pharmaceutical or veterinary acceptable excipient, adjuvant, carrier or vehicle. The engineered NDV vector may be an NDV expression vector comprising a polynucleotide encoding a herpesvirus protein, polypeptide, antigen, epitope or immunogen. The herpesvirus protein, polypeptide, antigen, epitope or immunogen may be a glycoprotein, or any fragment thereof. The herpesvirus protein, polypeptide, antigen, epitope or immunogen may be a gB or gD protein, or any fragment thereof.

As used herein, the term "antigen" or "immunogen" means a substance that induces a specific immune response in a host animal. The antigen may comprise a whole organism, killed, attenuated or live; a subunit or portion of an organism; a recombinant vector containing an insert expressing an epitope, polypeptide, peptide, protein, or fragment thereof with immunogenic properties; a piece or fragment of nucleic acid capable of inducing an immune response upon presentation to a host animal; a protein, a polypeptide, a peptide, an epitope, a hapten, or any combination thereof. Alternately, the immunogen or antigen may comprise a toxin or antitoxin.

The term "immunogenic protein or peptide" as used herein also includes peptides and polypeptides that are immunologically active in the sense that once administered to the host, it is able to evoke an immune response of the humoral and/or cellular type directed against the protein. Preferably the protein fragment is such that it has substantially the same immunological activity as the total protein. Thus, a protein fragment according to the invention comprises or consists essentially of or consists of at least one epitope or antigenic determinant. The term epitope, also known as antigenic determinant, is the part of a macromolecule recognized by the immune system and able to induce an immune reaction of the humoral type (B cells) and/or cellular type (T cells).
The term "immunogenic protein or peptide" further contemplates deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein. In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic—aspartate and glutamate; (2) basic—lysine, arginine, histidine; (3) non-polar—alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar—glycine, asparagine, glutamine, cysteine, serine threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. It is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, or vice versa; an aspartate with a glutamate or vice versa; a threonine with a serine or vice versa; or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the reference molecule but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of the protein are, therefore, within the definition of the reference polypeptide.

The term epitope is the part of a macromolecule recognized by the immune system and able to induce an immune reaction of the humoral type (B cells) and/or cellular type (T cells). The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site". Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen.

An "immunological response" to a composition or vaccine is the development in the host of a cellular and/or antibody-mediated immune response to a composition or vaccine of interest. Usually, an "immunological response" includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, and/or cytotoxic T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or protective immunological response such that resistance to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host, a quicker recovery time and/or a lowered viral titer in the infected host.
The term "immunogenic" protein or polypeptide as used herein also refers to an amino acid sequence which elicits an immunological response as described above. An "immunogenic" protein or polypeptide, as used herein, includes the full-length sequence of the protein, analogs thereof, or immunogenic fragments thereof. By "immunogenic fragment" is meant a fragment of a protein which includes one or more epitopes and thus elicits the immunological response described above. Such fragments can be identified using any number of epitope mapping techniques, well known in the art. See, e.g., Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66 (Glenn E. Morris, Ed., 1996). For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Such techniques are known in the art and described in, e.g., U.S. Pat. No. 4,708,871; Geysen et al, 1984; Geysen et al, 1986. Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols, supra.

Synthetic antigens are also included within the definition, for example, polyepitopes, flanking epitopes, and other recombinant or synthetically derived antigens. Immunogenic fragments, for purposes of the present invention, will usually include at least about 3 amino acids, about 5 amino acids, about 10-15 amino acids, about 15-25 amino acids or more amino acids, of the molecule. There is no critical upper limit to the length of the fragment, which could comprise nearly the full-length of the protein sequence, or even a fusion protein comprising at least one epitope of the protein.

Accordingly, a minimum structure of a polynucleotide expressing an epitope is that it comprises or consists essentially of or consists of nucleotides to encode an epitope or antigenic determinant of herpesvirus protein or polypeptide. A polynucleotide encoding a fragment of the total protein or polypeptide comprises or consists essentially of or consists of a minimum of 15 nucleotides, advantageously about 30-45 nucleotides, and preferably about 45-75, at least 57, 87 or 150 consecutive or contiguous nucleotides of the sequence encoding the total protein or polypeptide. Epitope determination procedures, such as, generating overlapping peptide libraries (Hemmer et al, 1998), Pepsan (Geysen et al, 1984; Geysen et al, 1985; Van der Zee R. et al, 1989; Geysen, 1990; Multipin.RTM. Peptide Synthesis Kits
de Chiron) and algorithms (De Groot et al., 1999), can be used in the practice of the invention, without undue experimentation.

A "polynucleotide" is a polymeric form of nucleotides of any length that contains deoxyribonucleotides, ribonucleotides, and analogs in any combination. Polynucleotides may have three-dimensional structure, and may perform any function, known or unknown. The term "polynucleotide" includes double-, single-, and triple-stranded helical molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide encompasses both the double stranded form and each of two complementary forms known or predicted to make up the double stranded form of either the DNA, RNA or hybrid molecule.

The term "codon optimization" refers to the process of optimally configuring the nucleic acid sequence encoding a protein, polypeptide, antigen, epitope, domain or fragment for expression/translation in a selected host. In general, gene expression levels depend on many factors, such as promoter sequences and regulatory elements. One of the most important factors is the adaptation of the codon usage of the transcript gene to the typical codon usage of the host (Lithwich, G. and Margalit, H., Genome Res. 13, 2665-2673, 2003). Therefore, highly expressed genes in prokaryotic genomes under translational selection have a pronounced codon usage bias. This is because they use a small subset of codons that are recognized by the most abundant tRNA species (Ikemura, T., J. Mol. Biol. 151, 389-409, 1981). The force that modulates this codon adaptation is called translational selection and its strength is important in fast-growing bacteria (Rocha, E.P., Genome Res. 14, 2279-2286, 2004; Sharp, P.M. et al, Nucleic Acids Res. 33, 1141-1 153). If a gene contains codons that are rarely used by the host, its expression level will not be maximal. This may be one of the limitations of heterologous protein expression (Gustafsson, C. et al, Trends Biotechnol. 22, 346-353, 2004) and the development of DNA vaccines (Ivory, C. and Chadee, K., Genet. Vaccines Ther. 2, 17, 2004). A high number of synthetic genes have been re-designed to increase their expression level. The Synthetic Gene Database (SGDB) (Wu, G. et al, Nucleic Acids Res. 35, D76-D79, 2007) contains information from more than 200 published experiments on synthetic genes. In the design process of a nucleic acid sequence that will be inserted into a new host to express a certain protein in optimal amounts, codon usage optimization is usually one of the first steps (Gustafsson, C., Trends Biotechnol. 22, 346-353, 2004). Codon usage optimization basically involves altering the rare codons in the target gene so that they more closely reflect the codon usage of the host without modifying the
amino acid sequence of the encoded protein (Gustafsson, C., Trends Biotechnol. 22, 346-353, 2004). The information usually used for the optimization process is therefore the DNA or protein sequence to be optimized and a codon usage table (reference set) of the host.

[0048] There are several public web servers and stand-alone applications that allow some kind of codon optimization by anyone skilled in the art. 'GeneDesign' (Richardson, S.M. et al, Genome Res. 16, 550-556, 2006), 'Synthetic Gene Designer' (Wu, G. et al, Protein Expr. Purif. 47, 441-445, 2006) and 'Gene Designer' (Villalobos, A. et al, BMC Bioinformatics 7, 285, 2006) are packages that provide a platform for synthetic gene design, including a codon optimization step. With regard to the methods for codon usage optimization available in each server or program, the first programs developed used only the ‘one amino acid-one codon’ approach. More recent programs and servers now include further methods to create some codon usage variability. This variability reflects the codon usage variability of natural highly expressed genes and enables additional criteria to be introduced (such as the avoidance of restriction sites) in the optimization process. Most applications and web servers described herein provide three methods of codon optimization: a complete optimization of all codons, an optimization based on the relative codon usage frequencies of the reference set that uses a Monte Carlo approach and a novel approaches designed to maximize the optimization with the minimum changes between the query and optimized sequences.

[0049] In one embodiment, the nucleic acid sequence encoding the recombinant protein, antigen, peptide, polypeptide, fragment, domain, or epitope is codon optimized for expression in animal. In another embodiment, the codon optimized sequences encode feline herpesvirus proteins, antigens, peptides, polypeptides, fragments, domains, or epitopes for animal expression. In yet another embodiment, the codon optimized sequences encode herpesvirus gB or gD proteins, antigens, peptides, polypeptides, fragments, domains, or epitopes for animal expression.

[0050] The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, siRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, uracil, other sugars and linking groups such as fluororibose and thiolate, and nucleotide branches. The sequence of nucleotides may be further modified after polymerization, such as
by conjugation, with a labeling component. Other types of modifications included in this
definition are caps, substitution of one or more of the naturally occurring nucleotides with an
analog, and introduction of means for attaching the polynucleotide to proteins, metal ions,
labeling components, other polynucleotides or solid support. The polynucleotides can be
obtained by chemical synthesis or derived from a microorganism.

[0051] The term "gene" is used broadly to refer to any segment of polynucleotide associated
with a biological function. Thus, genes include introns and exons as in genomic sequence, or
just the coding sequences as in cDNAs and/or the regulatory sequences required for their
expression. For example, gene also refers to a nucleic acid fragment that expresses mRNA or
functional RNA, or encodes a specific protein, and which includes regulatory sequences.

[0052] The invention further comprises a complementary strand to a polynucleotide encoding
a herpesvirus protein, antigen, epitope or immunogen. The complementary strand can be
polymeric and of any length, and can contain deoxyribonucleotides, ribonucleotides, and
analogs in any combination thereof.

[0053] The terms "protein", "peptide", "polypeptide" and "polypeptide fragment" are used
interchangeably herein to refer to polymers of amino acid residues of any length. The
polymer can be linear or branched, it may comprise modified amino acids or amino acid
analogues, and it may be interrupted by chemical moieties other than amino acids. The terms
also encompass an amino acid polymer that has been modified naturally or by intervention;
for example disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation,
or any other manipulation or modification, such as conjugation with a labeling or bioactive
component.

[0054] An "isolated" polynucleotide or polypeptide is one that is substantially free of the
materials with which it is associated in its native environment. By substantially free, is meant
at least 50%, at least 70%, at least 80%, at least 90%, or at least 95% free of these materials.

[0055] Hybridization reactions can be performed under conditions of different stringency.
Conditions that increase stringency of a hybridization reaction are well known. See for
eexample, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook et al.,
1989). Examples of relevant conditions include (in order of increasing stringency):
incubation temperatures of 25°C, 37°C, 50°C, and 68°C; buffer concentrations of 10 x SSC, 6
x SSC, 1 x SSC, 0.1 x SSC (where SSC is 0.15 M NaCl and 15 mM citrate buffer) and their
equivalent using other buffer systems; formamide concentrations of 0%, 25%, 50%, and
75%; incubation times from 5 minutes to 24 hours; 1, 2 or more washing steps; wash
incubation times of 1, 2, or 15 minutes; and wash solutions of 6 x SSC, 1 x SSC, 0.1 x SSC, or deionized water.

[0056] The invention further encompasses polynucleotides encoding functionally equivalent variants and derivatives of the herpesvirus polypeptides and functionally equivalent fragments thereof that may enhance, decrease or not significantly affect inherent properties of the polypeptides encoded thereby. These functionally equivalent variants, derivatives, and fragments display the ability to retain the activity. For instance, changes in a DNA sequence that do not change the encoded amino acid sequence, as well as those that result in conservative substitutions of amino acid residues, one or a few amino acid deletions or additions, and substitution of amino acid residues by amino acid analogs are those which will not significantly affect properties of the encoded polypeptide. In one embodiment, the variants have at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, 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% homology or identity to the herpesvirus polynucleotide or polypeptide of interest.

[0057] In one aspect, the present invention provides herpesvirus polypeptides, particularly herpesvirus gB polypeptides. In another aspect, the present invention provides a polypeptide having a sequence as set forth in SEQ ID NO: 1, 7, 8, 9, 11, 13, or 15, and variant or fragment thereof.

[0058] In another aspect, the present invention provides a polypeptide having at least 70%>, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to herpesvirus gB polypeptide of the invention, particularly to the polypeptide having a sequence as set forth in SEQ ID NO: 1, 7, 8, 9, 11, 13, or 15.

[0059] In yet another aspect, the present invention provides fragments and variants of the herpesvirus gB polypeptides identified above (SEQ ID NO: 1, 7, 8, 9, 11, 13, or 15) which may readily be prepared by one of skill in the art using well-known molecular biology techniques.

[0060] Variants are homologous polypeptides having an amino acid sequence at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the antigenic polypeptides of the invention, particularly to the amino acid sequence as set forth in SEQ ID NO: 1, 7, 8, 9, 11, 13, or 15.
An immunogenic fragment of a herpesvirus gB polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids, at least 21 amino acids, at least 23 amino acids, at least 25 amino acids, or at least 30 amino acids of the herpesvirus gB polypeptide having a sequence as set forth in SEQ ID NO: 1, 7, 8, 9, 11, 13, or 15, or variants thereof. In another embodiment, a fragment of the herpesvirus gB polypeptide includes a specific antigenic epitope found on a full-length herpesvirus gB polypeptide.

In another aspect, the present invention provides a polynucleotide encoding a herpesvirus gB polypeptide, such as a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 1, 7, 8, 9, 11, 13, or 15. In yet another aspect, the present invention provides a polynucleotide encoding a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 1, 7, 8, 9, 11, 13, or 15, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. The polynucleotide encoding the herpesvirus gB polypeptide may be codon-optimized for expression in a specific animal species.

In another aspect, the present invention provides a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 2, 3, 10, 12, 14, or 16, or a variant thereof. In yet another aspect, the present invention provides a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO: 2, 3, 10, 12, 14, or 16, or a variant thereof.

In one aspect, the present invention provides herpesviruses polypeptides, particularly herpesvirus gD polypeptides. In another aspect, the present invention provides a polypeptide having a sequence as set forth in SEQ ID NO: 4, 17, 19, 21, or 23, and variant or fragment thereof.

In another aspect, the present invention provides a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a herpesvirus gD polypeptide of the invention, particularly to the polypeptides having a sequence as set forth in SEQ ID NO: 4, 17, 19, 21, or 23.
In yet another aspect, the present invention provides fragments and variants of the herpesvirus gD polypeptides identified above (SEQ ID NO: 4, 17, 19, 21, or 23) which may readily be prepared by one of skill in the art using well-known molecular biology techniques.

Variants are homologous polypeptides having an amino acid sequence at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the antigenic polypeptides of the invention, particularly to the amino acid sequence as set forth in SEQ ID NO: 4, 17, 19, 21, or 23.

An immunogenic fragment of a herpesvirus gD polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids, at least 21 amino acids, at least 23 amino acids, at least 25 amino acids, or at least 30 amino acids of the herpesvirus gD polypeptide having a sequence as set forth in SEQ ID NO: 4, 17, 19, 21, or 23, or variants thereof. In another embodiment, a fragment of a herpesvirus gD polypeptide includes a specific antigenic epitope found on a full-length herpesvirus gD polypeptide.

In another aspect, the present invention provides a polynucleotide encoding a herpesvirus gD polypeptide, such as a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 4, 17, 19, 21, or 23. In yet another aspect, the present invention provides a polynucleotide encoding a polypeptide having at least 70%>, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 4, 17, 19, 21, or 23, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. The polynucleotide encoding the herpesvirus gD polypeptide may be codon-optimized for expression in a specific animal species.

In another aspect, the present invention provides a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 5, 6, 18, 20, 22, or 24, or a variant thereof. In yet another aspect, the present invention provides a polynucleotide having at least 70%>, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to one of a polynucleotide having a sequence as set forth in SEQ ID NO: 5, 6, 18, 20, 22, or 24, or a variant thereof.

In general, comparison of amino acid sequences is accomplished by aligning an amino acid sequence of a polypeptide of a known structure with the amino acid sequence of a polypeptide of unknown structure. Amino acids in the sequences are then compared and groups of amino acids that are homologous are grouped together. This method detects
conserved regions of the polypeptides and accounts for amino acid insertions and deletions. Homology between amino acid sequences can be determined by using commercially available algorithms (see also the description of homology above). In addition to those otherwise mentioned herein, mention is made of the programs BLAST, gapped BLAST, BLASTN, BLASTP, and PSI-BLAST, provided by the National Center for Biotechnology Information. These programs are widely used in the art for this purpose and can align homologous regions of two amino acid sequences.

Alternatively or additionally, the term "homology" or "identity", for instance, with respect to a nucleotide or amino acid sequence, can indicate a quantitative measure of homology between two sequences. The percent sequence identity can be calculated as \( \frac{(N_{\text{ref}} - N_{\text{dif}})}{N_{\text{ref}}} \times 100 \), wherein \( N_{\text{dif}} \) is the total number of non-identical residues in the two sequences when aligned and wherein \( N_{\text{ref}} \) is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC (\( N_{\text{ref}} = 8; N_{\text{dif}} = 2 \)).

Alternatively or additionally, "homology" or "identity" with respect to sequences can refer to the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur et al., 1983), for instance, using a window size of 20 nucleotides, a word length of 4 nucleotides, and a gap penalty of 4, and computer-assisted analysis and interpretation of the sequence data including alignment can be conveniently performed using commercially available programs (e.g., Vector NTI Software™, Invitrogen Inc. CA, USA). When RNA sequences are said to be similar, or have a degree of sequence identity or homology with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence. Thus, RNA sequences are within the scope of the invention and can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences. And, without undue experimentation, the skilled artisan can consult with many other programs or references for determining percent homology.

The invention further encompasses the herpesvirus polynucleotides contained in a vector molecule or an expression vector and operably linked to a promoter element and optionally to an enhancer.

A "vector" refers to a recombinant DNA or RNA plasmid, bacteriophage, or virus that comprises a heterologous polynucleotide to be delivered to a target cell, either in vitro or in...
vivo. The heterologous polynucleotide may comprise a sequence of interest for purposes of prevention or therapy, and may optionally be in the form of an expression cassette. As used herein, a vector needs not be capable of replication in the ultimate target cell or subject. The term "vector" includes vectors for cloning as well as viral vectors.

[0076] The term "engineered" or "recombinant" means a polynucleotide of semisynthetic, or synthetic origin that either does not occur in nature or is linked to another polynucleotide in an arrangement not found in nature.

[0077] "Heterologous" means derived from a genetically distinct entity from the rest of the entity to which it is being compared. For example, a polynucleotide may be incorporated by genetic engineering techniques into a plasmid or vector derived from a different source, and is thus a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence other than the native sequence is a heterologous promoter.

[0078] The polynucleotides of the invention may comprise additional sequences, such as additional encoding sequences within the same transcription unit, controlling elements such as promoters, ribosome binding sites, 5'UTR, 3'UTR, transcription terminators, polyadenylation sites, additional transcription units under control of the same or a different promoter, sequences that permit cloning, expression, homologous recombination, and transformation of a host cell, and any such construct as may be desirable to provide embodiments of this invention.

[0079] Elements for the expression of a herpesvirus polypeptide, antigen, epitope or immunogen are advantageously present in an inventive vector. In minimum manner, this comprises, consists essentially of, or consists of an initiation codon (ATG), a stop codon and a promoter, and optionally also a polyadenylation sequence for certain vectors such as plasmid and certain viral vectors. When the polynucleotide encodes a polypeptide fragment, e.g. a herpesvirus peptide, advantageously, in the vector, an ATG is placed at 5' of the reading frame and a stop codon is placed at 3'. Other elements for controlling expression may be present, such as enhancer sequences, stabilizing sequences, such as intron and or untranslated 5' or 3' sequences and signal sequences permitting the secretion of the protein.

[0080] Methods for making and/or administering a vector or recombinants or plasmid for expression of gene products of the invention either in vivo or in vitro can be any desired method, e.g., a method which is by or analogous to the methods disclosed in documents cited in: U.S. Patent Nos. 4,603,12; 4,769,330; 4,394,448; 4,722,848; 4,745,051; 4,769,331;
4,945,050; 5,494,807; 5,514,375; 5,744,140; 5,744,141; 5,756,103; 5,762,938; 5,766,599;
5,990,091; 5,174,993; 5,505,941; ... plasmid, as well as linear forms of the plasmid, are
intended to be within the scope of the invention.

[0081] The present invention also relates to a composition or vaccine comprising vectors,
such as expression vectors. The composition or vaccine can comprise, consist essentially of,
or consist of one or more vectors, e.g., expression vectors, such as in vivo expression vectors,
comprising, consisting essentially or consisting of (or expressing) one or more of herpesvirus
polypeptides, antigens, epitopes or immunogens. The vector contains and expresses a
polynucleotide that comprises, consists essentially of, or consists of a polynucleotide coding
for (or expressing) a herpesvirus antigen, epitope or immunogen, in a pharmaceutically or
veterinarily acceptable carrier, adjuvant, excipient or vehicle.

[0082] According to another embodiment, the vector or vectors in the composition or vaccine
comprise, or consist essentially of, or consist of polynucleotide(s) encoding one or more
proteins or fragment(s) thereof a herpesvirus polypeptide, antigen, epitope or immunogen.
The inventive composition or vaccine comprises, consists essentially of, or consists of, one or
more vectors comprising, consisting essentially of, or consisting of, and advantageously also
expressing, in vivo under appropriate conditions or suitable conditions or in a suitable host
cell, polynucleotides from different herpesvirus isolates encoding the same proteins and/or
for different proteins. The invention is also directed at mixtures of vectors that contain,
consist essentially of, or consist of coding for, and express, different herpesvirus proteins,
polypeptides, antigens, epitopes or immunogens, e.g., a herpesvirus polypeptide, antigen,
epitope or immunogen from different species such as, but not limited to, feline, humans,
canine, equine, bovine (e.g., cattle), swine, or avian.

[0083] The term plasmid covers any DNA transcription unit comprising a polynucleotide
according to the invention and the elements necessary for its in vivo expression in a cell or
cells of the desired host or target; and, in this regard, it is noted that a supercoiled plasmid
and all of its topoisomers, open-circular plasmid, as well as linear forms of the plasmid, are
intended to be within the scope of the invention.
Each plasmid comprises or contains or consists essentially of, in addition to the heterologous polynucleotide encoding a recombinant protein, antigen, epitope or immunogen, optionally fused with a polynucleotide encoding a heterologous peptide sequence, variant, analog or fragment, operably linked to a promoter or under the control of a promoter or dependent upon a promoter. In general, it is advantageous to employ a strong promoter that is functional in eukaryotic cells. The preferred strong promoter is the immediate early cytomegalovirus promoter (CMV-IE) of human or murine origin, or optionally having another origin such as the rat or guinea pig. The CMV-IE promoter can comprise the actual promoter segment, which may or may not be associated with the enhancer segment.


In more general terms, the promoter is either of a viral or a cellular origin. A strong viral promoter other than CMV-IE that may be usefully employed in the practice of the invention is the early/late promoter of the SV40 virus or the LTR promoter of the Rous sarcoma virus. A strong cellular promoter that may be usefully employed in the practice of the invention is the promoter of a gene of the cytoskeleton, such as e.g. the desmin promoter (Kwissa et al., 2000), or the actin promoter (Miyazaki et al., 1989).

Functional sub-fragments of these promoters, i.e., portions of these promoters that maintain an adequate promoting activity, are included within the present invention, e.g., truncated CMV-IE promoters according to PCT Application No. WO98/00166 or U.S. Patent No. 6,156,567. A promoter in the practice of the invention consequently includes derivatives and sub-fragments of a full-length promoter that maintain an adequate promoting activity and hence function as a promoter, preferably promoting activity substantially similar to that of the actual or full-length promoter from which the derivative or sub-fragment is derived, e.g., akin to the activity of the truncated CMV-IE promoters of U.S. Patent No. 6,156,567 to the activity of full-length CMV-IE promoters. Thus, a CMV-IE promoter in the practice of the invention can comprise or consist essentially of or consist of the promoter portion of the full-length promoter and/or the enhancer portion of the full-length promoter, as well as derivatives and sub-fragments.

Preferably, the plasmids comprise or consist essentially of other expression control elements. It is particularly advantageous to incorporate stabilizing sequence(s), e.g., intron
sequence(s), preferably the first intron of the hCMV-IE (PCT Application No. WO89/01036), the intron II of the rabbit β-globin gene (van Ooyen et al., 1979).

[0088] As to the polyadenylation signal (polyA) for the plasmids and viral vectors other than poxviruses, use can more be made of the poly(A) signal of the bovine growth hormone (bGH) gene (see U.S. Patent No. 5,122,458), or the poly(A) signal of the rabbit β-globin gene or the poly(A) signal of the SV40 virus.

[0089] According to another embodiment of the invention, the expression vectors are expression vectors used for the in vitro expression of proteins in an appropriate cell system. The expressed proteins can be harvested in or from the culture supernatant after, or not after secretion (if there is no secretion a cell lysis typically occurs or is performed), optionally concentrated by concentration methods such as ultrafiltration and/or purified by purification means, such as affinity, ion exchange or gel filtration-type chromatography methods.

[0090] A "host cell" denotes a prokaryotic or eukaryotic cell that has been genetically altered, or is capable of being genetically altered by administration of an exogenous polynucleotide, such as a recombinant plasmid or vector. When referring to genetically altered cells, the term refers both to the originally altered cell and to the progeny thereof. Host cells include, but are not limited to, baby hamster kidney (BHK) cells, colon carcinoma (Caco-2) cells, COS7 cells, MCF-7 cells, MCF-IOA cells, Madin-Darby canine kidney (MDCK) lines, mink lung (MvlLu) cells, MRC-5 cells, U937 cells, Chinese hamster ovary (CHO) cells, monkey Vero cells (cell line with the origin of the kidney of an African green monkey), quail (Quail muscle cell line QM7), chicken cell line DF1, and VERO cells. Polynucleotides comprising a desired sequence can be inserted into a suitable cloning or expression vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification. Polynucleotides can be introduced into host cells by any means known in the art. The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including direct uptake, endocytosis, transfection, f-mating, electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is infectious, for instance, a retroviral vector). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[0091] In one embodiment of the present invention, the vector is a Newcastle Disease Virus (NDV) vector as described in US 2010/0255029 (incorporated herein by reference in its
entireties). Newcastle disease virus designated as avian paramyxovirus 1 (APMV1, family Paramyxoviridae, subfamily Paramyxovirinae, genus Avulavirus) is an avian pathogen whose naturally occurring strains exhibit a wide range of disease severity. NDV is particularly advantageous as a vaccine vector for veterinary use because the vector itself serves as a needed poultry vaccine. NDV strain pathotypes are asymptomatic enteric (e.g., Ulster 2C, Queensland V4), lentogenic (e.g., Hitchner Bl, F (e.g., Asplin), La Sota), mesogenic (e.g., strain H, Mukteswar, Roakin, Beaudette C) or velogenic (e.g., Texas GB, NY parrot 70181, Italien, Milano, Herts 33/56). Advantages of herpesvirus vaccines based on the NDV vector include, but are not limited to, (1) induce a broad immunity, including humoral, cellular and mucosal responses (2) do not express NP and M proteins and therefore is compatible with the DIVA (differentiate infected from vaccinated animals) strategy, (3) induce rapid onset of immunity, (4) bivalent, and (5) production poses less risk for the environment than inactivated vaccines in case of accidental release.

Certain characteristics of NDV suggest that recombinant NDV (rNDV) or engineered NDV expressing a foreign protein would be very good vaccine candidates. NDV grows to very high titers in many cell lines and eggs, and it elicits strong humoral and cellular immune responses in vivo. NDV naturally infects via respiratory and alimentary tract mucosal surfaces, so it is especially useful to deliver protective antigens of respiratory disease pathogens such as FHV. In addition, commercially available live NDV vaccines are widely used in the United States and most other countries. Vaccines based on live NDV recombinants may also have advantages over other live recombinant vaccine vectors. First, the foreign protein is expressed with only a few NDV proteins. In contrast, pox and herpes virus vectors express a large number of additional proteins from their large-size genomes. For the generation of specific immune responses in vaccine applications, it may be advantageous to have only a limited number of proteins expressed. Second, NDV replicates in the cytoplasm of the infected cells without a DNA phase, which eliminates the problem of integration of viral genome into the host cell DNA. The virus does not undergo detectable genetic recombination.

In one embodiment, the NDV vector is NDV AVINEW® as described in US 2010/0255029. The NDV vector may also be the vector of U.S. Patent No. 5,118,502, in particular the strain deposited as ATCC No. VR 2239.

In one aspect, the present invention relates to a pharmaceutical composition or vaccine for inducing an immunological response in a host animal inoculated with the vaccine
or composition, the vaccine or composition including one or more modified AVINEW recombinant viral vectors. In yet another aspect of the invention, the engineered or recombinant AVINEW viral vector includes, within a non-essential region of the virus genome, a herpesvirus DNA sequence which encodes a herpesvirus antigenic protein derived from a pathogen wherein the composition or vaccine when administered to a host, is capable of inducing an immunological response specific to the protein encoded by the pathogen. The composition optionally comprises a pharmaceutically or veterinarily acceptable carrier or vehicle or adjuvant or excipient.

[0095] The term "nonessential region" refers to a region of a virus genome which is not essential for replication and propagation of the virus in tissue culture and whose deletion or inactivation may reduce virulence in a variety of animal systems. Any nonessential region or portion thereof can be deleted from the AVINEW genome or a foreign sequence can be inserted in it, and the viability and stability of the engineered AVINEW resulting from the deletion or insertion can be used to ascertain whether a deleted region or portion thereof is indeed nonessential. In another embodiment, the nonessential region of the AVINEW genome is the region between P gene and M gene, or the region between M gene and F gene of AVINEW genome. In one embodiment, the nonessential region is located upstream of the NP gene on the AVINEW genome. In another embodiment, the nonessential region is located downstream of the L gene on the AVINEW genome. In yet another embodiment, the nonessential region is a non-coding or intergenic region. In this aspect, the non-coding or intergenic region may be a region between NP and P genes, between P and M genes, between M and F genes, or between F and FIN genes on the AVFNEW genome. In another embodiment, the nonessential region may be in the region of lnt-121nt, 1591nt-1886nt, 3074nt-3289nt, 4384nt-4543nt, 6205nt-641lnt, 8262nt-8380nt, or 14995nt-15186nt of

SEQ ID NO:27.

[0096] One aspect of the invention relates to engineered or recombinant NDV vectors expressing herpesvirus antigens. The antigen may be herpesvirus glycoprotein, such as gB or gD protein aforementioned. The engineered NDV vector may comprise one or more polynucleotides encoding one or more herpesvirus antigens. In another aspect, the engineered NDV-Herpesvirus vector comprises one or more polynucleotides encoding a Herpesvirus gB antigen or variant thereof, a Herpesvirus gD antigen or variant thereof, or a combination thereof.
In one embodiment, the invention provides for the administration of a therapeutically effective amount of a formulation for the delivery and expression of a protein, antigen, epitope or immunogen in a target cell. Determination of the prophylactically or therapeutically effective amount is routine experimentation for one of ordinary skill in the art.

In another embodiment, the formulation comprises an expression vector comprising a polynucleotide that expresses a herpesvirus antigen, epitope or immunogen and a pharmaceutically or veterinarily acceptable carrier, vehicle, adjuvant or excipient. In another embodiment, the pharmaceutically or veterinarily acceptable carrier, vehicle, adjuvant or excipient facilitates transfection and/or improves preservation of the vector or protein.

The pharmaceutically or veterinarily acceptable carriers or vehicles or adjuvant or excipients are well known to the one skilled in the art. For example, a pharmaceutically or veterinarily acceptable carrier or vehicle or adjuvant or excipient can be sterile water, a 0.9% NaCl (e.g., saline) solution or a phosphate buffer. Other pharmaceutically or veterinarily acceptable carrier or vehicle or adjuvant or excipients that can be used for methods of this invention include, but are not limited to, poly-(L-glutamate) or polyvinylpyrrolidone. The pharmaceutically or veterinarily acceptable carrier or vehicle or adjuvant or excipients may be any compound or combination of compounds facilitating the administration of the vector (or protein expressed from an inventive vector in vitro); advantageously, the carrier, vehicle or adjuvant or excipient may facilitate transfection and/or improve preservation of the vector (or protein). Doses and dose volumes are herein discussed in the general description and can also be determined by the skilled artisan from this disclosure read in conjunction with the knowledge in the art, without any undue experimentation.

The cationic lipids containing a quaternary ammonium salt which are but not exclusively suitable for plasmids, are those having the following formula:

\[
\begin{align*}
R_1 \rightarrow O \rightarrow CH_2 \rightarrow CH_2 \rightarrow N \rightarrow R_2 \rightarrow X \\
| \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \ quad
Among these cationic lipids, preference is given to DMRIE (N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propane ammonium; WO96/34109), advantageously associated with a neutral lipid, advantageously DOPE (dioleoyl-phosphatidyl-ethanol amine; Behr, 1994), to form DMRIE-DOPE.

The plasmid mixture with the adjuvant is formed extemporaneously and/or contemporaneously with administration of the preparation or shortly before administration of the preparation; for instance, shortly before or prior to administration, the plasmid-adjuvant mixture is formed, advantageously so as to give enough time prior to administration for the mixture to form a complex, e.g. between about 10 and about 60 minutes prior to administration.

When DOPE is present, the DMRIE:DOPE molar ratio may be about 95:about 5 to about 5:about 95, or about 1:about 1, e.g., 1:1.

The DMRIE or DMRIE-DOPE adjuvant: plasmid weight ratio can be between about 50:about 1 and about 1:about 10, such as about 10:about 1 and about 1:about 5, and advantageously about 1:about 1 and about 1:about 2, e.g., 1:1 and 1:2.

In another embodiment, pharmaceutically or veterinarily acceptable carrier, adjuvant, excipient, or vehicle may be a water-in-oil emulsion. Examples of suitable water-in-oil emulsions include oil-based water-in-oil vaccinal emulsions which are stable and fluid at 4°C containing: from 6 to 50 v/v % of an antigen-containing aqueous phase, preferably from 12 to 25 v/v %, from 50 to 94 v/v % of an oil phase containing in total or in part a non-metabolizable oil (e.g., mineral oil such as paraffin oil) and/or metabolizable oil (e.g., vegetable oil, or fatty acid, polyl or alcohol esters), from 0.2 to 20 p/v % of surfactants, preferably from 3 to 8 p/v %, the latter being in total or in part, or in a mixture either polyglycerol esters, said polyglycerol esters being preferably polyglycerol (poly)ricinoleates, or polyoxyethylene ricin oils or else hydrogenated polyoxyethylene ricin oils. Examples of surfactants that may be used in a water-in-oil emulsion include ethoxylated sorbitan esters (e.g., polyoxyethylene (20) sorbitan monooleate (TWEEN 80®), available from AppliChem, Inc., Cheshire, CT) and sorbitan esters (e.g., sorbitan monooleate (SPAN 80®), available from Sigma Aldrich, St. Louis, MO). In addition, with respect to a water-in-oil emulsion, see also US Patent No. 6,919,084. In some embodiments, the antigen-containing aqueous phase comprises a saline solution comprising one or more buffering agents. An example of a suitable buffering solution is phosphate buffered saline. In one embodiment, the water-in-oil emulsion may be a water/oil/water (W/O/W) triple emulsion (see, e.g., U.S. Patent No.
Examples of other suitable emulsions are described in U.S. Patent No. 7,371,395.

[0105] The immunological compositions and vaccines according to the invention may comprise or consist essentially of one or more adjuvants. Suitable adjuvants for use in the practice of the present invention are (1) polymers of acrylic or methacrylic acid, maleic anhydride and alkenyl derivative polymers, (2) immunostimulating sequences (ISS), such as oligodeoxyribonucleotide sequences having one or more non-methylated CpG units (Klinman et al., 1996; W098/16247), (3) an oil in water emulsion, such as the SPT emulsion described on p 147 of "Vaccine Design, The Subunit and Adjuvant Approach" published by M. Powell, M. Newman, Plenum Press 1995, and the emulsion MF59 described on p183 of the same work, (4) cation lipids containing a quaternary ammonium salt, e.g., DDA (5) cytokines, (6) aluminum hydroxide or aluminum phosphate, (7) saponin or (8) other adjuvants discussed in any document cited and incorporated by reference into the instant application, or (9) any combinations or mixtures thereof.

[0106] The oil in water emulsion (3), which is especially appropriate for viral vectors, can be based on: light liquid paraffin oil (European pharmacopoeia type), isoprenoid oil such as squalane, squalene, oil resulting from the oligomerization of alkenes, e.g. isobutene or decene, esters of acids or alcohols having a straight-chain alkyl group, such as vegetable oils, ethyl oleate, propylene glycol, di(caprylate/caprate), glycerol tri(caprylate/caprate) and propylene glycol dioleate, or esters of branched, fatty alcohols or acids, especially isostearic acid esters.

[0107] The oil is used in combination with emulsifiers to form an emulsion. The emulsifiers may be nonionic surfactants, such as: esters of on the one hand sorbitan, mannide (e.g. anhydromannitol oleate), glycerol, polyglycerol or propylene glycol and on the other hand oleic, isostearic, ricinoleic or hydroxystearic acids, said esters being optionally ethoxylated, or polyoxypropylene-polyoxyethylene copolymer blocks, such as Pluronic, e.g., L121.

[0108] Among the type (1) adjuvant polymers, preference is given to polymers of cross linked acrylic or methacrylic acid, especially cross linked by polyalkenyl ethers of sugars or polyalcohols. These compounds are known under the name carbomer (Pharmeuropa, vol. 8, no. 2, June 1996). One skilled in the art can also refer to U.S. Patent No. 2,909,462, which provides such acrylic polymers cross linked by a polyhydroxyl compound having at least three hydroxyl groups, preferably no more than eight such groups, the hydrogen atoms of at least three hydroxyl groups being replaced by unsaturated, aliphatic radicals having at least
two carbon atoms. The preferred radicals are those containing 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals can also contain other substituents, such as methyl. Products sold under the name Carbopol (BF Goodrich, Ohio, USA) are especially suitable. They are cross linked by allyl saccharose or by allyl pentaerythritol. Among them, reference is made to Carbopol 974P, 934P and 971P.

As to the maleic anhydride-alkenyl derivative copolymers, preference is given to EMA (Monsanto), which are straight-chain or cross linked ethylene-maleic anhydride copolymers and they are, for example, cross linked by divinyl ether. Reference is also made to J. Fields et al., 1960.

With regard to structure, the acrylic or methacrylic acid polymers and EMA are preferably formed by basic units having the following formula:

\[
\begin{align*}
\text{R}_1 & \quad \text{CH}_2 \quad \text{R}_2 \\
\text{COOH} & \quad \text{COOH}
\end{align*}
\]

in which:
- \( \text{R}_1 \) and \( \text{R}_2 \), which can be the same or different, represent H or CH3
- \( x = 0 \) or 1, preferably \( x = 1 \)
- \( y = 1 \) or 2, with \( x + y = 2 \).

For EMA, \( x = 0 \) and \( y = 2 \) and for carbomers \( x = y = 1 \).

These polymers are soluble in water or physiological salt solution (20 g/l NaCl) and the pH can be adjusted to 7.3 to 7.4, e.g., by soda (NaOH), to provide the adjuvant solution in which the expression vector(s) can be incorporated. The polymer concentration in the final immunological or vaccine composition can range between 0.01 and 1.5% w/v, 0.05 to 1% w/v or 0.1 to 0.4% w/v.

The cytokine or cytokines (5) can be in protein form in the immunological or vaccine composition, or can be co-expressed in the host with the immunogen or immunogens or epitope(s) thereof. Preference is given to the co-expression of the cytokine or cytokines, either by the same vector as that expressing the immunogen or immunogens or epitope(s) thereof, or by a separate vector thereof.
The invention comprehends preparing such combination compositions; for instance by admixing the active components, advantageously together and with an adjuvant, carrier, cytokine, and/or diluent.

Cytokines that may be used in the present invention include, but are not limited to, granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage colony stimulating factor (GM-CSF), interferon α (IFNa), interferon β (IFNP), interferon γ (IFNy), interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11), interleukin-12 (IL-12), tumor necrosis factor α (TNFa), tumor necrosis factor β (TNFP), and transforming growth factor β (TGFP). It is understood that cytokines can be co-administered and/or sequentially administered with the immunological or vaccine composition of the present invention. Thus, for instance, the vaccine of the instant invention can also contain an exogenous nucleic acid molecule that expresses in vivo a suitable cytokine, e.g., a cytokine matched to this host to be vaccinated or in which an immunological response is to be elicited (for instance, a feline cytokine for preparations to be administered to a feline).

In another embodiment, the composition of the present invention may be prepared using the chemical or physical procedure as described by Stauffer et al. (Recent Patents on Anti-Infective Drug Discovery, 1, 291-296, 2006). Some of the inactivation techniques are summarized in the table below.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Physical</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic Acid</td>
<td></td>
<td>Ascorbic Acid + UV</td>
</tr>
<tr>
<td>b-Propiolactone</td>
<td>Heat</td>
<td>Beta Propiolactone + UV</td>
</tr>
<tr>
<td>b-aminophenylketone</td>
<td>Pressure</td>
<td>Formalin + Heat</td>
</tr>
<tr>
<td>diethylpyrocarbonate</td>
<td>UV</td>
<td>Formalin + UV</td>
</tr>
<tr>
<td>Ethyleneimine</td>
<td>Non Ionic Detergents</td>
<td>Heat + Low Pressure</td>
</tr>
<tr>
<td>Formalin/Formaldehyde</td>
<td></td>
<td>Pressure + Heat or Cold</td>
</tr>
<tr>
<td>Phenol</td>
<td></td>
<td>Psoralen + UV</td>
</tr>
</tbody>
</table>

The immunological composition and/or vaccine according to the invention comprise or consist essentially of or consist of an effective quantity to elicit a protective or therapeutic response of one or more expression vectors and/or polypeptides as discussed herein; and, an
effective quantity can be determined from this disclosure, including the documents
incorporated herein, and the knowledge in the art, without undue experimentation.

[0117] The compositions or vaccines of the present invention may be administered to an
animal \textit{in ovo}, via drinking water, oro-nasal, sprays, aerosols, intranasal instillation, eye drop,
beak-dipping, by wing-web stabbing, transdermal, subcutaneous or intramuscular injection.
Advantageously, the vaccines are administered by oro-nasal, subcutaneous, eye drop, spray
or drinking water.

[0118] The present invention contemplates at least one administration to an animal of an
efficient amount of the therapeutic composition made according to the invention. The
therapeutic composition according to the invention can be administered by a needleless
apparatus (as, for example with a Pigjet, Dermojet, Biojector, Vetjet or Vitajet apparatus
(Bioject, Oregon, USA)).

[0119] In one embodiment of the invention, a prime-boost regimen can be employed, which
is comprised of at least one primary administration and at least one booster administration
using at least one common protein, polypeptide, antigen, epitope or immunogen. The
immunological composition or vaccine used in primary administration is different in nature
from those used as a booster. However, it is noted that the same composition can be used as
the primary administration and the boost administration. This administration protocol is
called "prime-boost".

[0120] In another aspect of the prime-boost protocol of the invention, a composition
comprising the engineered Avinew NDV Herpesvirus vaccine or composition is administered
followed by the administration of vaccine or composition comprising a recombinant viral
vector that contains and expresses a herpesvirus antigen \textit{in vivo}, or an inactivated viral
vaccine or composition comprising the herpesvirus antigen, or a vaccine or composition
comprising a herpesvirus subunit (protein), or a DNA plasmid vaccine or composition that
contains or expresses a herpesvirus antigen. Likewise, a prime-boost protocol may comprise
the administration of vaccine or composition comprising a recombinant viral vector that
contains and expresses a herpesvirus antigen \textit{in vivo}, or an inactivated viral vaccine or
composition comprising the herpesvirus antigen, or a vaccine or composition comprising a
herpesvirus subunit (protein), or a DNA plasmid vaccine or composition that contains or
expresses a herpesvirus antigen, followed by the administration of a composition comprising
the engineered Avinew NDV Herpesvirus vaccine or composition. It is noted that both the
primary and the secondary administrations may comprise the composition comprising the
engineered Avinew NDV Herpesvirus vaccine or composition. It is further noted that both the primary and the secondary administrations may comprise one or more compositions comprising the engineered NDV-HV vectors of the present invention.

A prime-boost protocol comprises at least one prime-administration and at least one boost administration using at least one common antigen. The vaccine or composition used in prime-administration may be different in nature from those used as a later booster vaccine or composition. The prime-administration may comprise one or more administrations. Similarly, the boost administration may comprise one or more administrations.

The various administrations are preferably carried out about 1 to about 6 weeks apart, or about 2 to about 4 weeks apart. Repeated booster every 2 to 6 weeks or an annual booster is also contemplated. The animals are preferably at least one day old at the time of the first administration.

The immunological composition and/or vaccine contains per dose from about 10^4 to about 10^{11}, advantageously from about 10^5 to about 10^{10} and more advantageously from about 10^6 to about 10^9 viral particles of recombinant adenovirus expressing a herpesvirus antigen, epitope or immunogen. In the case of immunological composition and/or vaccine based on a poxvirus, a dose can be between about 10^2 pfu and about 10^9 pfu. The immunological composition and/or vaccine contains per dose from about 10^2 to about 10^7, advantageously from about 10^3 to about 10^5 pfu of poxvirus or herpesvirus recombinant expressing the herpesvirus antigen, epitope or immunogen.

The viral vector may be an attenuated avipox expression vector. In one embodiment, the avipox expression vector may be a fowlpox vector, for example, TROVAC®. In another embodiment, the avipox expression vector may be a canarypox vector, for example, ALVAC®. The herpesvirus antigen, epitope or immunogen may be a herpesvirus glycoprotein, such as gB or gD. Other viruses that may be used in methods of the invention include, but are not limited to, vaccinia viruses, such as an attenuated vaccinia virus, for instance NYVAC, adenoviruses and herpesviruses.

The efficacy of the vaccines may be tested about 2 to 4 weeks after the last immunization by challenging animals with a virulent strain of herpesvirus. Both homologous and heterologous strains may be used for challenge to test the efficacy of the vaccine. The animal may be challenged by spray, intra-nasal, eye drop, oculo-nasal, IM, intra-tracheal, and/or oral. The challenge viral may be about 10^3 to about 10^8 in a volume depending upon the route of administration. For example, if the administration is by spray, a virus suspension
is aerosolized to generate about 1 to 100 µl droplets, if the administration is intra-nasal, intra-tracheal or oral, the volume of the challenge virus is about 0.05 to about 5 ml. The dose volume of compositions for target species, e.g., the dose volume of feline compositions, may be about 50 µl for in ovo, about 20 to about 50 µl for eye drop, about 0.25ml to about 1 ml for spray. Animals may be observed daily for 14 days following challenge for clinical signs and mortality. In addition, the groups of animals may be euthanized and evaluated for pathological findings. Oropharyngeal, tracheal or cloacal swabs may be collected from all animals post challenge for virus detection. The presence or absence of viral antigens in tissues may be evaluated by immunohistochemistry, viral isolation or titration, or nucleic acid detection such as reverse-transcriptase polymerase chain reaction (RT-PCR). Blood samples may be collected post-challenge and may be analyzed for the presence of anti-herpesvirus gB or gD virus-specific antibody.

[0126] It should be understood by one of skill in the art that the disclosure herein is provided by way of example and the present invention is not limited thereto. From the disclosure herein and the knowledge in the art, the skilled artisan can determine the number of administrations, the administration route, and the doses to be used for each immunization protocol, without any undue experimentation.

[0127] Another embodiment of the invention is a kit for performing a method of inducing an immunological or protective response against herpesvirus in an animal comprising a recombinant NDV immunological composition or vaccine or an inactivated herpesvirus immunological composition or vaccine and instructions for performing the method of delivery in an effective amount for eliciting an immune response in the animal.

[0128] The invention is further illustrated by the following non-limiting examples.

**EXAMPLES**

[0129] Construction of DNA inserts, plasmids and recombinant viral vectors was carried out using the standard molecular biology techniques known in the art, for example, described by J. Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989).

Example 1 Construction of the NDV transcription plasmids containing feline herpesvirus (FHV) gB gene (pFR14 plasmid) and gD gene (pFR16 plasmid)
The FHV gB gene inserted in the NDV genome was codon-optimized for expression in mammals. The synthetic FHV gB gene (SEQ ID NO:2) was cloned into a pBR322-based vector resulting in plasmid pFR13 which contains an insertion cassette as shown in Figure 3. Plasmid pFR13 was digested with Pad and Fsel generating a Pacl-Fsel fragment of 3105bp in size. Plasmid plIV029 (US2010/0255029) was digested with Pad and Fsel generating a Fsel-Pacl fragment of 19140bp in size. The two fragments were ligated to generate plasmid pFR14 (Figure 4).

The FHV gD gene inserted in the NDV genome was codon-optimized for expression in mammals. The synthetic FHV gD gene (SEQ ID NO:5) was cloned into a pBR322-based vector resulting in plasmid pFR15 which contains an insertion cassette as shown in Figure 3. Plasmid pFR15 was digested with Pad and Fsel generating a Pacl-Fsel fragment of 1373bp in size. Plasmid plIV029 was digested with Pad and Fsel generating a Fsel-Pacl fragment of 19140bp in size. The two fragments were ligated to generate plasmid pFR16 (Figure 4).

Example 2 Generation and characterization of NDV vector expressing FHV gB gene (vAVW07)

The NDV is a negative RNA virus and the generation of genetically modified NDV virus needs a reverse genetics system. The transcription of a full length genomic viral RNA and the simultaneous expression of NP, P and L proteins permit the assembly of RNP and the transcription of positive RNA into negative RNA genome. This initiates the normal replication cycle of NDV virus and permit the generation of infectious particles (see Figure 2) 

To generate engineered NDV vector expressing FHV gB gene, the following reagents and conditions were used. Plasmid pFR14 (see Example 1) was used as the transcription plasmid. Plasmids pIV32, pIV33 and pIV34 (US2010/0255029) were used as the expression plasmids for NP, P and L proteins, respectively. Plasmid pNS151 (US2010/0255029) was used as the T7 RNA polymerase plasmid. These five plasmids were co-transfected together into Chinese hamster ovary (CHO) cells, as shown schematically in Figure 2C. After 72 hours, the CHO supernatants were inoculated in 10-day-old embryonated eggs to amplify the virus. After 3 days, the allantoic fluid was harvested and checked for hemagglutination activity (HA) using chicken red blood cells. The infectious particles of NDV-FHV gB were successfully obtained. RNA was extracted using QuiaAMP viral RNA extraction kit (Qiagen). RT-PCR was performed using One-Step RT-PCR kit (Qiagen). The sequencing
result showed that the gB gene is 100% identical to the original sequence of the gB gene cloned in the transcription plasmid. The recombinant NDV-FHV gB viral vector is designated vAVW07.

Example 3  Generation and characterization of NDV vector expressing FHV gD gene (vAVW08)

[0134] To generate engineered NDV vector expressing FHV gD gene, the following reagents and conditions were used. Plasmid pFR16 (see Example 1) was used as the transcription plasmid. Plasmids pIV32, pIV33 and pIV34 (US2010/0255029) were used as the expression plasmids for NP, P and L proteins, respectively. Plasmid pNS151 (US2010/0255029) was used as the T7 RNA polymerase plasmid. These five plasmids were co-transfected together into Chinese hamster ovary (CHO) cells, as shown schematically in Figure 2C. After 72 hours of transfection of CHO cells, the CHO supernatants were inoculated in 10-day-old embryonated eggs to amplify the virus. After 3 days, the allantoic fluid was harvested and checked for hemagglutination activity (HA) using chicken red blood cells. The infectious particles of NDV-FHV gD were successfully obtained.

[0135] RNA was extracted using QuiaAMP viral RNA extraction kit (Qiagen). RT-PCR was performed using One-Step RT-PCR kit (Qiagen). Two primers were used in the RT-PCR reaction:

FR09: CGCAGCTGCAATCAATTACG (SEQ ID NO:25)
FR10: TGGGTGGACAGGGATCTGCT (SEQ ID NO:26)

[0136] The sequencing result showed that the gD gene is 100% identical to the original sequence of the gD gene cloned in the transcription plasmid. The recombinant NDV-HV gD viral vector is designated vAVW08.

Example 4  Clinical evaluation of NDV-HV vaccine in cats

[0137] Thirty-two SPF (specific pathogen free) cats of 9-11 weeks were included in the study. Cats were randomly assigned to 4 groups of 8 cats (groups A to D) according to litter, sex and age by using a randomization table with 4 elements. Cats were cared and housed according to local husbandry and animal welfare procedures.

[0138] The experimental design is shown in Table 1.
Table 1  experimental design for vaccination in cats

<table>
<thead>
<tr>
<th>group</th>
<th>SPF cat 9-11 w</th>
<th>Treatment on D0 (V1) and D28 (V2) 1 mL</th>
<th>Challenge</th>
<th>Clinical follow-up</th>
<th>Viral shedding</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (NDV-HV by ON)</td>
<td>8</td>
<td>NDV-HV* 1mL by ON**</td>
<td>FHV 1 mL by oculo-nasal route on D45 (~2w post-V2)</td>
<td>typical clinical signs: daily from D45 to D59</td>
<td>nasal swabs: D45, D47, D49, D51, D53, D55, D57, D59</td>
<td>ELISA gB: D0, D28, D45, D59</td>
</tr>
<tr>
<td>B (NDV-HV by SC)</td>
<td>8</td>
<td>NDV-HV* 1mL by SC**</td>
<td></td>
<td>bodyweight: D45, D49, D51, D53, D55, D57 and D59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (positive control)**</td>
<td>8</td>
<td>positive control 1 dose by SC**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D (control)</td>
<td>8</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* NDV-HV=NDV-HV gB and NDV-HV gD, both at \(10^{7.8}\) EID\(_{50}\)/mL

**ON=oro-nasal  SC=subcutaneous

***positive control = vaccine containing attenuated feline Herpesvirus F2 strain, Merial Limited.

[0139] On D0 and D28, NDV-HV gB and NDV-HV gD vaccines were diluted 1/25 and 1/35, respectively, in order to reach a titer of \(10^{7.8}\) EID\(_{50}\)/mL for both vaccines. Then, each cat from group A received under general anesthesia 1mL of the NDV-HV vaccine (NDV-HV gB and NDV-HV gD) by oro-nasal route (0.25mL per nostril and 0.5mL in the mouth). Cats from group B received 1mL of the NDV-HV vaccine by subcutaneous route between the shoulders. Cats from group C received one dose of the control vaccine by subcutaneous route between the shoulders. Cats from group D were not vaccinated.

[0140] On D45, each cat was administered under general anesthesia 1mL of diluted 1/50 challenge strain \(10^{5.5}\)CCID50/mL (0.25mL per nostril and 0.25mL per eye).

[0141] The rectal temperature test is shown in Figure 5. Group A is NDV-HV by ON, group B is NDV-HV by SC, group C is positive control (vaccine containing attenuated feline Herpesvirus F2 strain, Merial Limited), group D is control (no vaccination). The result showed that in the control group, 7/8 cats had hyperthermia. In the vaccination groups, there was no hyperthermia with positive control and NDV-HV by ON, there was hyperthermia in 4/8 cats vaccinated with NDV-HV by SC.

[0142] The bodyweight result is shown in Figure 6 and Table 2. All cats gained weight during immunization phase and growth was similar between groups.
Post challenge (pc), in group D, all cats lost weight from day 4 pc to day 8 pc. Some cats (3 out of 8) lost weight until day 10 pc. Then all cats gained weight. During the post challenge monitoring period, a weight loss >5% was recorded in 6 out of 8 cats on one or two occasions. In group C, a weight loss was observed in 6 out of 8 cats between day 4 pc and day 6 or day 8 pc. This weight loss was > 5% in 4 cats. In group B, all cats lost weight between day 4 and day 6 or day 8 pc. A weight loss >5% was observed once in only 2 cats.

Table 2 weight loss observed during the post challenge monitoring period

<table>
<thead>
<tr>
<th>group</th>
<th># cats with weight loss (# cats with weight loss&gt;5%)</th>
<th>Average weight loss observed Between D49 and D51</th>
<th>Between D51 and D53</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (NDV-HV by ON)</td>
<td>5/8 (1/8)</td>
<td>+1%</td>
<td>+0%</td>
</tr>
<tr>
<td>B (NDV-HV by SC)</td>
<td>6/8 (2/8)</td>
<td>-1%</td>
<td>-3%</td>
</tr>
<tr>
<td>C (positive control)</td>
<td>6/8 (4/8)</td>
<td>-3%</td>
<td>-2%</td>
</tr>
<tr>
<td>D (controls)</td>
<td>8/8 (6/8)</td>
<td>-2.5%</td>
<td>-7%</td>
</tr>
</tbody>
</table>

Figure 7 shows the mean clinical scores per group following challenge and table 3 summarizes the clinical symptoms observed. Group A is NDV-HV by ON, group B is NDV-HV by SC, group C is positive control (vaccine containing attenuated feline Herpesvirus F2 strain, Merial Limited), group D is control (no vaccination). In group D, all cats developed clinical signs post challenge. In group A, one cat did not show any clinical sign post challenge and 3 cats presented only slight nasal discharge for one day or slight ocular discharge for 2 days. The other cats from group A, cats from group B and cats from group C presented less severe and more transient clinical signs than cats on group D.

Table 3 summary of the clinical signs observed per group post challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Nasal discharge (copious)</th>
<th>Ocular discharge (copious)</th>
<th>sneezing</th>
<th>cough</th>
<th>apathy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># cat occurrence</td>
<td># cat occurrence</td>
<td># cat occurrence</td>
<td># cat occurrence</td>
<td># cat occurrence</td>
</tr>
</tbody>
</table>
**Table 4** mean Area Under Curve (AUC) per group

<table>
<thead>
<tr>
<th>Group</th>
<th>Average AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>47.2</td>
</tr>
<tr>
<td>B</td>
<td>48.3</td>
</tr>
<tr>
<td>C</td>
<td>49.9</td>
</tr>
<tr>
<td>D</td>
<td>59.6</td>
</tr>
</tbody>
</table>

[0145] Figure 8 shows the distribution of global clinical score per group. The mean global clinical score was: 7.5 in group A, 18.6 in group B, 17.4 in group C, and 33.8 in group D. There was a significant difference between group D and the three vaccinated groups. There was a significant difference on the clinical global score between the three vaccinated groups (ANOVA, p=0.018). Cats from group A showed a significantly reduced clinical global score than cats from groups B and C. There was no significant difference for the global clinical score between groups B and C.

[0146] Figure 9 shows the mean viral shedding per group post challenge and table 4 summarizes the mean AUC per group. Group A is NDV-HV by ON, group B is NDV-HV by SC, group C is positive control (vaccine containing attenuated feline Herpesvirus F2 strain, Merial Limited), group D is control (no vaccination).

[0147] No cats shed feline Herpesvirus before challenge. Post challenge, FHV was isolated in all cats. In group D, excretion increased rapidly and peaked at day 4 pc, then regularly decreased until day 14 pc. On day 14 pc, 5 out of 8 cats still shed low quantity of virus. In the vaccinated groups, viral excretion peaked at day 4 pc in groups B and C or at day 6 pc in group A, then decreased more rapidly than in group D. On day 14 pc, no cat shed virus.
Figure 10 shows the distribution of global viral shedding score per group. Viral shedding was significantly reduced in vaccinated groups compared to group D (no vaccination). Although cats from group A shed virus later than the other vaccinated groups, there was no statistically significant difference on the viral excretion between the three vaccinated groups (ANOVA, p=0.464).

The serology (anti-gB FHV Ab) data is shown in Figure 11. Group A is NDV-HV by ON, group B is NDV-HV by SC, group C is positive control (vaccine containing attenuated feline Herpesvirus F2 strain, Merial Limited), group D is control (no vaccination). All cats were seronegative for gB-FHV on DO. All cats in group D remained seronegative until challenge day. All cats in group D were positive for gB FHV Ab after D28. One injection of NDV-HV by SC or ON was sufficient to induce a seroconversion in all cats. Challenge induced a booster effect in all vaccines and the production of FHV Ab in all control cats. The serology data correlate well with the clinical results.

* * *

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

All documents cited or referenced in the application cited documents, and all documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention.
CLAIMS

WHAT I CLAIMED IS:

1. A composition or vaccine comprising (i) one or more recombinant Newcastle Disease Virus (NDV)-Herpesvirus vectors and (ii) a pharmaceutically or veterinarily acceptable carrier.

2. The composition or vaccine of claim 1, wherein the recombinant NDV-Herpesvirus vector comprises one or more heterologous polynucleotides encoding one or more Herpesvirus antigen, polypeptide, or variant thereof.

3. The composition or vaccine of claim 2, wherein the Herpesvirus antigen or polypeptide is a Herpesvirus glycoprotein B (gB) or glycoprotein D (gD) or variant thereof.

4. The composition or vaccine of claim 2 or 3, wherein the recombinant NDV-Herpesvirus vector comprises a polynucleotide encoding a polypeptide having at least 90% sequence identity to SEQ ID NO:1 or SEQ ID NO:4.

5. The composition or vaccine of claim 2 or 3, wherein the recombinant NDV-Herpesvirus vector comprises a polynucleotide having at least 90%> sequence identity to SEQ ID NO:2, 3, 5, or 6.

6. The composition or vaccine of any one of claims 1-5, wherein the composition or vaccine comprises one or two NDV-Herpesvirus vectors.

7. The composition or vaccine of claim 6, wherein the composition or vaccine comprises a first NDV-Herpesvirus recombinant vector comprising a Herpesvirus gB antigen or variant thereof and a second NDV-Herpesvirus recombinant vector comprising a Herpesvirus gD antigen or variant thereof.

8. The composition or vaccine of claim 6, wherein the composition or vaccine comprises a NDV-Herpesvirus recombinant vector comprising a Herpesvirus gB antigen or variant thereof, a Herpesvirus gD antigen or variant thereof, or a combination thereof.

9. The composition or vaccine of any one of claims 6-8, wherein the NDV-Herpesvirus recombinant vector comprises a polynucleotide encoding a polypeptide having at least 90% sequence identity to SEQ ID NO:1 or SEQ ID NO:4.

10. The composition or vaccine of any one of claims 6-8, wherein the recombinant NDV-Herpesvirus vector comprises a polynucleotide having at least 90%> sequence identity to SEQ ID NO:2, 3, 5, or 6.
11. A recombinant NDV-Herpesvirus vector comprising one or more polynucleotides encoding a Herpesvirus gB antigen or variant thereof, a Herpesvirus gD antigen or variant thereof, or a combination thereof.

12. The recombinant NDV-Herpesvirus vector of claim 11, wherein the one or more polynucleotides encode one or more polypeptides having at least 90% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 4.

13. The recombinant NDV-Herpesvirus vector of claim 11, wherein the one or more polynucleotides have at least 90% sequence identity to SEQ ID NO: 2, 3, 5, or 6.

14. The recombinant NDV-Herpesvirus vector of any one of claims 11-13, wherein the NDV-Herpesvirus vector comprises a polynucleotide encoding a polypeptide having at least 90% sequence identity to SEQ ID NO: 1.

15. The recombinant NDV-Herpesvirus vector of any one of claims 11-13, wherein the NDV-Herpesvirus vector comprises a polynucleotide encoding a polypeptide having at least 90% sequence identity to SEQ ID NO: 4.

16. The recombinant NDV-Herpesvirus vector of any one of claims 11-13, wherein the NDV-Herpesvirus vector comprises two polynucleotides encoding polypeptides having at least 90% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 4.

17. The recombinant NDV-Herpesvirus vector of any one of claims 11-16, wherein the one or more polynucleotides are inserted in the non-essential regions of the NDV Avinew genome.

18. A method of eliciting a protective response in an animal against Herpesvirus comprising administering to the animal a recombinant NDV-Herpesvirus vector expressing at least one Herpesvirus antigen and a pharmaceutically or veterinarily acceptable carrier, adjuvant, excipient or vehicle.

19. The method of claim 18, wherein the NDV-Herpesvirus vector comprises one or more polynucleotides encoding one or more polypeptides having at least 90% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 4.

20. The method of any one of claim 18 or 19, wherein the administering is by oro-nasal, eye drop, spray, drinking water, in ovo, intramuscular, or subcutaneous administration, intradermal, transdermal.

21. The method of any one of claims 18-20, wherein the administering is prime-boost.

22. The method of any one of claims 18-21, wherein the animal is a feline or canine.
<table>
<thead>
<tr>
<th>SEQ ID NO.</th>
<th>Type</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>protein</td>
<td>FHV gB protein</td>
</tr>
<tr>
<td>2</td>
<td>DNA</td>
<td>Codon-optimized FHV gB DNA</td>
</tr>
<tr>
<td>3</td>
<td>DNA</td>
<td>Wild-type FHV gB DNA (GenBank No. FJ478159 encoding AAB28559.3)</td>
</tr>
<tr>
<td>4</td>
<td>Protein</td>
<td>FHV gD protein (GenBank No. AAB30980.1)</td>
</tr>
<tr>
<td>5</td>
<td>DNA</td>
<td>Codon-optimized FHV gD DNA</td>
</tr>
<tr>
<td>6</td>
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Figure 2 (1/2)

Fig 2A

NP  P  M  F  HN  L

Fig 2B

1. NP  P  gB or M  F  HN  L
   gD
   or

2. NP  P  M  gB or F  HN  L
   gD
Fig 2C

NDV Reverse Genetics system

3. T7 RNA polymerase

2. expression plasmids
   pr T7
   NP
   L
   RNP proteins

1. transcription plasmid
   NDV genome as cDNA
   pr T7
   52

T7 RNA pol
NP, P and L proteins
TRANSCRIPTION IN RNA (+)
Formation of RNP + TRANSCRIPTION IN RNA (-)
Complex RNP (-)

Cell cytoplasm
Viral replication
Infectious virus

"Reverse genetic for RNA (-) viruses as Paramyxoviruses"
Figure 5

Ch
Figure 6

![Graph showing average bodyweight (in g) over days. The graph includes four different lines labeled A, B, C, and D. Key markers are labeled V1, V2, and Ch.]
Figure 8

![Box plot diagram showing four groups: Group A (NDV-FHV ON), Group B (NDV-FHV SC), Group C (Positive control), and Group D (Controls). The box plot compares the CGS values across these groups.]
Figure 9

[Graph showing the PHV excretion (in log10 CCID50/mL) over days 45 to 59 for different groups labeled A, B, C, and D.]
Figure 10

1 – group A; 2 – group B; 3 – group C; 4 – group D
Figure 11

Sero logic: Anti-FHV-1 gB Ab titers determined by ELISA.
Figure 12 (1/3)

gB protein alignment

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1911192A (gB) (1)
AAB24381 (gB) (1)
AAK51052 (gB) (1)
AAK93732 (gB) (1)
CAA92272 (gB) (1)
AAB28559 (gB) (1)
SEQ 1 (gB protein) (1)

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AAB24381 (gB) (51)
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AAK93732 (gB) (3)
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SEQ 1 (gB protein) (51)

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AAB28559 (gB) (101)
SEQ 1 (gB protein) (101)

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AAK93732 (gB) (88)
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AAB28559 (gB) (151)
SEQ 1 (gB protein) (151)

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AAK93732 (gB) (138)
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AAB28559 (gB) (201)
SEQ 1 (gB protein) (201)

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AAB28559 (gB) (251)
SEQ 1 (gB protein) (251)

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AAB28559 (gB) (301)
SEQ 1 (gB protein) (301)

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AAK93732 (gB) (350)
CAA92272 (gB) (350)
AAB28559 (gB) (350)
SEQ 1 (gB protein) (350)
Figure 12 (3/3)

SEQ 1 (gB protein) (sequence identity percentage)

1911192A(gB) SEQ ID NO:7 94.2%
AAB24381(gB) SEQ ID NO:9 99.6%
AAX51052(gB) SEQ ID NO:11 71.7%
AAT93732(gB) SEQ ID NO:13 71.6%
CAA92272(gB) SEQ ID NO:15 70.2%
AAB28559(gB) SEQ ID NO:18 100%
Figure 13 (1/2)

gD protein alignment

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Figure 13 (2/2)

SEQ ID NO: 4 (gD protein) (sequence identity percentage)

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/145 C07K14/035

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No.


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Further documents are listed in the continuation of Box C.

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"Z" document member of the same patent family

Date of the actual completion of the international search
11 January 2012

Date of mailing of the international search report
30/01/2012

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

Authorized officer
Heder, Andreas

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### INTERNATIONAL SEARCH REPORT

**C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

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