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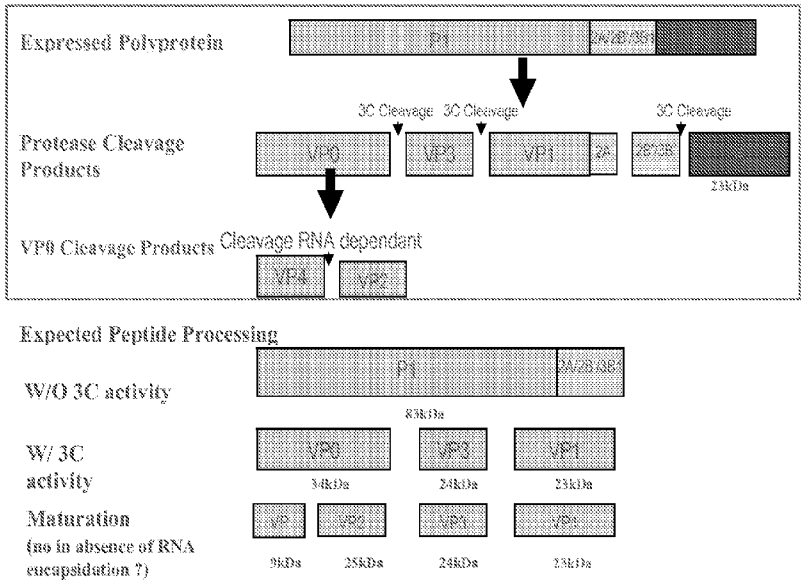
(54) **Title**: FMDV RECOMBINANT VACCINES AND USES THEREOF

Figure 2



(57) **Abstract**: The present invention encompasses FMDV vaccines or compositions. The vaccine or composition may be a vaccine or composition containing FMDV antigens. The invention also encompasses recombinant vectors encoding and expressing FMDV antigens, epitopes or immunogens which can be used to protect animals, in particular ovines, bovines, caprines, or swines, against FMDV.

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FMDV RECOMBINANT VACCINES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional application 62/054,073 filed on September 23, 2014.

FIELD OF THE INVENTION

The present invention relates to compositions for combating Foot and Mouth Disease Virus (FMDV) infection in animals. The present invention provides pharmaceutical compositions comprising an FMDV antigen, methods of vaccination against FMDV, and kits for use with such methods and compositions.

BACKGROUND OF THE INVENTION

Foot-and-mouth disease (FMD) is one of the most virulent and contagious diseases affecting farm animals. This disease is endemic in numerous countries in the world, especially in Africa, Asia and South America. In addition, epidemic outbreaks can occur periodically. The presence of this disease in a country may have very severe economic consequences resulting from loss of productivity, loss of weight and milk production in infected herds, and from trade embargoes imposed on these countries. The measures taken against this disease consist of strict application of import restrictions, hygiene controls and quarantine, slaughtering sick animals and vaccination programs using inactivated vaccines, either as a preventive measure at the national or regional level, or periodically when an epidemic outbreak occurs.

FMD is characterized by its short incubation period, its highly contagious nature, the formation of ulcers in the mouth and on the feet and sometimes, the death of young animals. FMD affects a number of animal species, in particular cattle, pigs, sheep and goats. The agent responsible for this disease is a ribonucleic acid (RNA) virus belonging to the Aphthovirus genus of the *Picornaviridae* family (Cooper *et al.*, Intervirology, 1978, 10, 165-180). At present, at least seven types of foot-and-mouth disease virus (FMDV) are known: the European types (A, O and C), the African types (SAT1, SAT2 and SAT3) and an Asiatic type (Asia 1). Numerous sub-types have also been distinguished (Kleid *et al.* Science (1981), 214, 1125-1129).

FMDV is a naked icosahedral virus of about 25 nm in diameter, containing a single-stranded RNA molecule consisting of about 8500 nucleotides, with a positive polarity. This

RNA molecule comprises a single open reading frame (ORF), encoding a single polyprotein containing, *inter alia*, the capsid precursor also known as protein P1 or P88. Protein P1 is myristylated at its amino-terminal end. During the maturation process, protein P1 is cleaved by protease 3C into three proteins known as VP0, VP1 and VP3 (or 1AB, 1D and 1C respectively; Belsham G. J., Progress in Biophysics and Molecular Biology, 1993, 60, 241-261). In the virion, protein VP0 is then cleaved into two proteins, VP4 and VP2 (or 1A and 1B respectively). The mechanism for the conversion of proteins VP0 into VP4 and VP2, and for the formation of mature virions is not known. Proteins VP1, VP2 and VP3 have a molecular weight of about 26,000 Da, while protein VP4 is smaller at about 8,000 Da.

The simple combination of the capsid proteins forms the protomer or 5S molecule, which is the elementary constituent of the FMDV capsid. This protomer is then complexed into a pentamer to form the 12S molecule. The virion results from the encapsidation of a genomic RNA molecule by assembly of twelve 12S pentamers, thus constituting the 146S particles. The viral capsid may also be formed without the presence of an RNA molecule inside it (hereinafter "empty capsid"). The empty capsid is also designated as particle 70S. The formation of empty capsids may occur naturally during viral replication or may be produced artificially by chemical treatment.

Some studies have been done on natural empty capsids. In particular, Rowlands et al. (Rowlands et al., J. Gen. Virol., 1975, 26, 227-238) have shown that the virions of A10 foot-and-mouth disease comprise mainly the four proteins VP1, VP2, VP3 and VP4. By comparison, the natural empty capsids (not obtained by recombination but purified from cultures of A10 foot-and-mouth virus) essentially contain the uncleaved protein VP0; identical results with the A-Pando foot-and-mouth virus are described by Rweyemamu (Rweyemamu et al., Archives of Virology, 1979, 59, 69-79). The artificial empty capsids, obtained after dialysis in the presence of Tris-EDTA and after centrifuging, contain no protein VP4. These artificial capsids are slightly immunogenic according to Rowlands et al., and the natural empty capsids are only immunogenic after treatment with formaldehyde to stabilize them, while the antibody response induced by the natural empty capsids in the guinea-pig is nevertheless inconstant, as noted by the author. Moreover, Rowlands *et al.* and Rweyemamu *et al.* do not agree on the need to stabilize the natural empty capsids. For Rweyemamu *et al.*, the absence of treatment with formaldehyde is not prejudicial to the level of antigenicity of the natural empty capsids. The immunogenicity is only tested by the induction of neutralizing antibodies in the guinea-pig.

The expression of the gene coding for the precursor P1 of the capsid proteins by means of a recombinant baculovirus in insect cells is compared with the expression of the gene coding for P1 associated with the protease 3C in E. coli (Grubman et al., Vaccine, 1993, 11, 825-829; Lewis et al., J. Virol., 1991, 65, 6572-6580). The co-expression of P1 and 3C in E. coli results in the assembling of empty capsids 70S. The expression product of these two constructions produces neutralizing antibodies in guinea-pigs and pigs. The titers obtained with the P1/baculovirus construction are low. These same expression products induce partial protection in pigs. However, some pigs protected against the disease are not protected against the replication of the challenge virus. However, the E. coli expression system does not myristylate the proteins and the protease 3C is toxic to this cell. Lewis *et al.* conclude that fundamental questions relating to the make-up of the virus and the structure of the capsid needed to obtain maximum protection in the animal have not been answered. Furthermore, Grubman *et al.* state that it would be necessary to stabilize the empty capsids before formulating the vaccine; on this point they agree about the problems encountered with the empty capsids obtained by extraction from viral cultures (see above).

Fusion proteins containing some or all of protein P1 have also been obtained by the use of viral vectors, namely a herpes virus or vaccinia virus. CA-A-2,047,585 in particular describes a bovine herpes virus used to produce fusion proteins containing a peptide sequence of the foot-and-mouth virus (amino acids 141 to 158 of P1 bound to amino acids 200 to 213 of P1) fused with the glycoprotein gpIII of this bovine herpes virus. Viral vectors have also been used to express stabilized FMDV empty capsid (US 7,531,182). Recently, plants have been investigated as a source for the production of FMDV antigens (US 2011/0236416).

Many hypotheses, research routes, and proposals have been developed in an attempt to design effective vaccines against FMD. Currently, the only vaccines on the market contain inactivated virus. Concerns about safety of the FMDV vaccine exist, as outbreaks of FMD in Europe have been associated with shortcomings in vaccine manufacture (King, A.M.Q. *et al.*, 1981, Nature 293: 479-480). The inactivated vaccines do not confer long-term immunity, thus requiring booster injections given every year, or more often in the event of epidemic outbreaks. In addition, there are risks linked to incomplete inactivation and/or to the escape of virus during the production of inactivated vaccines (King, A.M.Q., *ibid*). A goal in the art has been to construct conformationally correct immunogens lacking the infective FMDV genome to make effective and safe vaccines.

It has been reported that maternally derived antibodies (MDA) are able to inhibit calves' (under 2 years of age cattle) response to vaccination against FMD (Graves, 1963,

Journal of Immunology 91:251-256; Brun et al., 1977, Developments in Biological Standardisation, 25:117-122).

Considering the susceptibility of animals (including humans, albeit rarely), to FMDV, a method of preventing FMDV infection and protecting animals is essential. Accordingly, there is a need for more effective and stable vaccines against FMDV.

SUMMARY OF THE INVENTION

Compositions or vaccines comprising an antigenic FMDV polypeptide and fragments and variants thereof and compositions or vaccines comprising recombinant viral vectors expressing FMDV polypeptide and fragments and variants thereof are provided. The FMDV antigens and fragments and variants thereof possess immunogenic and protective properties. The FMDV antigens may be produced by a baculovirus expression vector in insect cells. The FMDV antigens may be modified to enhance the stability of FMDV empty capsids or FMDV VLPs (virus-like particles). The recombinant viral vectors may be adenovirus vectors expressing FMDV antigens.

The antigenic polypeptides and fragments and variants thereof or recombinant viral vectors can be formulated into vaccines and/or pharmaceutical compositions. Such vaccines or compositions can be used to vaccinate an animal and provide protection against homologous and heterologous FMDV strains.

Methods for enhanced protection in conventional animals and maternally derived antibody-positive (MDA-positive) animals against FMDV infections are provided. Kits comprising at least one antigenic polypeptide or fragment or variant thereof and instructions for use are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

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

FIG. 1 depicts a table summarizing the DNA and Protein sequences.

FIG. 2 represents the expressed FMDV polyprotein and the process by 3C.

FIG.3 depicts the plasmid map of pMEB097.

FIG.4 depicts the result of electronic microscopy of MacMEB097.

FIG.5 depicts the western blot results of FMDV capsid protein of A24 strain.

FIGs. 6A and 6B depict the electronic microscopy and specific ELISA of BacMEB099.

FIGs. 7A-7D depict the sequence alignments of the protein sequences.

FIG. 8 depicts the FMDV VLPs.

FIG. 9 depicts the evolution of mean FMDV A24 Cruzeiro neutralizing antibody titers.

FIG. 10 depicts the FMDV A24 Cruzeiro neutralizing antibody titers.

FIGs. 11A and 11B depict the evolution of mean FMDV A24 Cruzeiro neutralizing antibody titers.

FIGs.12A -12C depict the evolution of mean rectal temperature after challenge.

FIG. 13 depicts the EM analysis of A24 Cruzeiro VLPs with or without covalent cage mutation in the presence or absence of heat or acid.

FIG. 14 depicts the ELISA analysis of VLPs with or without the covalent cage mutation for the A24 Cruzeiro serotype after heating.

FIG. 15 depicts the ELISA analysis of A24 Cruzeiro VLPs with or without the covalent cage mutation stored at 5 °C over time.

FIG. 16 depicts the ELISA results and EM pictures showing O1 Manisa covalent cage VLPs are resistant to heat.

FIG. 17 depicts the ELISA results showing O1 Manisa covalent cage VLP stability in acid (above) and heat (below).

FIG. 18A depicts the vaccination and analysis scheme. FIG. 18B depicts the evolution of neutralizing antibody titers against FMD Asia1 Shamir and FMD A22 Iraq.

FIG. 19A depicts the humoral response (memory B cells detection) scheme. FIG. 19B depicts the Asia Shamir covalent cage & Iraq A22 covalent cage VLP serology data.

FIG. 20 depicts the B cell ELISPOT assay in the FMDV VLP vaccinated animals on day 27.

FIG. 21 depicts the B cell ELISPOT assay in the FMDV VLP vaccinated animals on day 43 (measuring B Memory cells).

FIG. 22 depicts the specific γ Interferon (IFNγ) secreting cell assay in the FMDV VLP vaccinated animals on day 27.

FIG. 23 depicts FMDV SVN log10 titer by Groups for Day 42.

FIG.24 depicts mean FMDV VN log10 titer over the course of study (Day 0 – day 42).

FIG. 25 depicts plasmid pAD3027 map.

FIG. 26 depicts Western Blot of vAD3027.

DETAILED DESCRIPTION

Compositions comprising an FMDV polypeptide, antigen and fragments and variants thereof and compositions comprising recombinant viral vectors expressing FMDV antigens that elicit an immunogenic response in an animal are provided. The antigenic polypeptides or fragments or variants thereof are produced by a baculovirus expression vector in insect cells. The recombinant viral vectors may be adenovirus vectors expressing FMDV antigens. The antigenic polypeptides or fragments or variants or recombinant viral vectors expressing the antigens may be formulated into vaccines or pharmaceutical compositions and used to elicit or stimulate a protective response in an animal. In one embodiment the polypeptide antigen is an FMDV P1, VP2 or 3C polypeptide or active fragment or variant thereof. The FMDV antigens may be modified to enhance the stability of FMDV empty capsids or FMDV VLPs (virus-like particles).

It is recognized that the antigenic polypeptides of the invention may be full length polypeptides or active fragments or variants thereof. By "active fragments" or "active variants" is intended that the fragments or variants retain the antigenic nature of the polypeptide. Thus, the present invention encompasses any FMDV polypeptide, antigen, epitope or immunogen that elicits an immunogenic response in an animal. The FMDV polypeptide, antigen, epitope or immunogen may be any FMDV polypeptide, antigen, epitope or immunogen, such as, but not limited to, a protein, peptide or fragment or variant thereof, that elicits, induces or stimulates a response in an animal, such as an ovine, bovine, caprine or porcine.

Particular FMDV antigenic polypeptides include P1, VP2 and 3C. FMDV is a non-enveloped icosahedral virus of about 25 nm in diameter, containing a single-stranded RNA molecule consisting of about 8500 nucleotides, with a positive polarity. This RNA molecule comprises a single open reading frame (ORF), encoding a single polyprotein containing, *inter alia*, the capsid precursor also known as protein P1 or P88. Protein P1 is myristylated at its amino-terminal end. During the maturation process, protein P1 is cleaved by the protease 3C into three proteins known as VP0, VP1 and VP3 (or 1AB, 1D and 1C respectively; Belsham G. J., Progress in Biophysics and Molecular Biology, 1993, 60, 241-261). In the virion,

protein VP0 is then cleaved into two proteins, VP4 and VP2 (or 1A and 1B respectively). Proteins VP1, VP2 and VP3 have a molecular weight of about 26,000 Da, while protein VP4 is smaller at about 8,000 Da. FMDV sequences are also described in US 7,527,960 and US 7,531,182, which documents are herein incorporated in their entirety.

The simple combination of the capsid proteins forms the protomer or 5S molecule, which is the elementary constituent of the FMDV capsid. This protomer is then complexed into a pentamer to form the 12S molecule. The virion results from the encapsidation of a genomic RNA molecule by assembly of twelve 12S pentamers, thus constituting the 146S particles. The viral capsid may also be formed without the presence of an RNA molecule inside it (hereinafter "empty capsid"). The empty capsid is also designated as particle 70S. The formation of empty capsids may occur naturally during viral replication or may be produced artificially by chemical treatment.

The present invention relates to bovine, ovine, caprine, or swine vaccines or compositions which may comprise an effective amount of a recombinant FMDV antigen or a recombinant viral vector expressing FMDV antigen, and a pharmaceutically or veterinarily acceptable carrier, excipient, adjuvant, or vehicle.

In some embodiments, the vaccines further comprise adjuvants, such as the oil-in-water (O/W) emulsions described in US Patent 7371395.

In still other embodiments, the adjuvants include EMULSIGEN, Aluminum Hydroxide, Saponin, and CpG, or combinations thereof.

In some embodiments, the response in the animal is a protective immune response.

By "animal" it is intended mammals, birds, and the like. Animal or host includes mammals and human. 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 felines including cheetahs and lynx), ovine (e.g., sheep), bovine (e.g., cattle, cow), swine (e.g., pig), caprine (e.g., goat), 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.

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 indicate otherwise.

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.

The antigenic polypeptides of the invention are capable of protecting against FMDV. That is, they are capable of stimulating an immune response in an animal. By "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 with immunogenic properties; a piece or fragment of DNA capable of inducing an immune response upon presentation to a host animal; a polypeptide, an epitope, a hapten, or any combination thereof. Alternately, the immunogen or antigen may comprise a toxin or antitoxin.

The term "immunogenic protein, polypeptide, or peptide" as used herein includes 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. 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, PNAS USA, 81(13): 3998-400; Geysen *et al.,* 1985,

PNAS USA, 82(1): 178-82. 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. Methods especially applicable to the proteins of *T. parva* are fully described in PCT/US2004/022605 incorporated herein by reference in its entirety.

As discussed the invention encompasses active fragments and variants of the antigenic polypeptide. Thus, the term "immunogenic protein, polypeptide, 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. The term "conservative variation" denotes the replacement of an amino acid residue by another biologically similar residue, or the replacement of a nucleotide in a nucleic acid sequence such that the encoded amino acid residue does not change or is another biologically similar residue. 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. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another hydrophobic residue, or the substitution of one polar residue for another polar residue, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like; or a similar conservative replacement of an amino acid with a structurally related amino acid that 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. All of the polypeptides produced by these modifications are included herein. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

The term "epitope" refers to the site on an antigen or hapten to which specific B cells and/or T cells respond. 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.

Synthetic antigens are also included within the definition, for example, polyepitopes, flanking epitopes, and other recombinant or synthetically derived antigens. See, e.g., Bergmann et al., 1993; Bergmann et al., 1996; Suhrbier, 1997; Gardner et al., 1998. Immunogenic fragments, for purposes of the present invention, will usually include at least about 3 amino acids, at least about 5 amino acids, at least about 10-15 amino acids, or 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 encoding an epitope or antigenic determinant of an FMDV polypeptide. A polynucleotide encoding a fragment of an FMDV polypeptide may comprise or consist essentially of or consist of a minimum of 15 nucleotides, about 30-45 nucleotides, about 45-75, or at least 57, 87 or 150 consecutive or contiguous nucleotides of the sequence encoding the polypeptide.

The term “nucleic acid” and “polynucleotide” refers to RNA or DNA that is linear or branched, single or double stranded, or a hybrid thereof. The term also encompasses RNA/DNA hybrids. The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, 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, uracyl, 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.

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.

The invention further comprises a complementary strand to a polynucleotide encoding an FMDV antigen, epitope or immunogen. The complementary strand can be polymeric and of any length, and can contain deoxyribonucleotides, ribonucleotides, and analogs in any combination.

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 analogs, 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.

An "isolated" biological component (such as a nucleic acid or protein or organelle) refers to a component that has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, for instance, other chromosomal and extra-chromosomal DNA and RNA, proteins, and organelles. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant technology as well as chemical synthesis.

The term "purified" as used herein does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified polypeptide preparation is one in which the polypeptide is more enriched than the polypeptide is in its natural environment. That is the polypeptide is separated from cellular components. By "substantially purified" it

is intended that such that the polypeptide represents several embodiments at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%, or more of the cellular components or materials have been removed. Likewise, the polypeptide may be partially purified. By "partially purified" is intended that less than 60% of the cellular components or material is removed. The same applies to polynucleotides. The polypeptides disclosed herein can be purified by any of the means known in the art.

As noted above, the antigenic polypeptides or fragments or variants thereof are FMDV antigenic polypeptides that are produced by a baculovirus expression vector in insect cells *in vitro* or by a viral vector *in vivo*. Fragments and variants of the disclosed polynucleotides and polypeptides encoded thereby are also encompassed by the present invention. By "fragment" is intended a portion of the polynucleotide or a portion of the antigenic amino acid sequence encoded thereby. Fragments of a polynucleotide may encode protein fragments that retain the biological activity of the native protein and hence have immunogenic activity as noted elsewhere herein. Fragments of the polypeptide sequence retain the ability to induce a protective immune response in an animal.

"Variants" is intended to mean substantially similar sequences. For polynucleotides, a variant comprises a deletion and/or addition of one or more nucleotides at one or more sites within the native polynucleotide and/or a substitution of one or more nucleotides at one or more sites in the native polynucleotide. As used herein, a "native" polynucleotide or polypeptide comprises a naturally occurring nucleotide sequence or amino acid sequence, respectively. Variants of a particular polynucleotide of the invention (i.e., the reference polynucleotide) can also be evaluated by comparison of the percent sequence identity between the polypeptide encoded by a variant polynucleotide and the polypeptide encoded by the reference polynucleotide. "Variant" protein is intended to mean a protein derived from the native protein by deletion or addition of one or more amino acids at one or more sites in the native protein and/or substitution of one or more amino acids at one or more sites in the native protein. Variant proteins encompassed by the present invention are biologically active, that is they the ability to elicit an immune response.

In one aspect, the present invention provides FMDV polypeptides from ovine, bovine, caprine, or swine FMDV isolates. In another aspect, the present invention provides a polypeptide having a sequence as set forth in SEQ ID NOs:1, 2, 4, 5, 6, 8, 10, 12, 13 or 16 and variant or fragment thereof.

In another aspect, the invention relates to improving the temperature and/or acid stability of FMDV empty capsids or FMDV VLPs (virus-like particles). The temperature

and/or acid stability of the empty capsids is advantageously ensured by the formation of disulfide bridges.

In particular, this improvement is obtained by replacing an amino acid of the original sequence with cysteine in the polypeptide sequence of a structural protein of the capsid, protein VP2 (derived from P1), for example, at position 179 of the amino acid sequence SEQ ID NO:2, 4, 6, 8, 10, or 16 (P1 of FMDV A24 strain, FMDV O1 manisa strain, FMDV Iraq strain, or FMDV Asia strain). As a general rule, the position of this amino acid is identical in VP2 protein derived from other foot-and-mouth viruses (as is the case particularly with the strains described in the examples). The region containing this amino acid corresponds to an alpha helix. To identify or confirm the amino acid which is to be mutated, the amino acid sequences of this region are aligned with the corresponding region (for example of the order of about ten or slightly more--e.g. 10 to 20--amino acids) on the sequence SEQ ID NO:2, 4, 6, 8, 10, or 16 taking into account of the fact that the sequences are well conserved in structure among the different FMDV. The amino acid to mutate is located at position 179 of the FMDV P1 (SEQ ID NO:2, 4, 6, 8, 10, or 16). By convention, the methionine corresponding to the initiation codon (which is not present in the natural sequence and is therefore added) is numbered 1.

Moreover, homologs of FMDV polypeptides from ovine, bovine, caprine, or swine are intended to be within the scope of the present invention. As used herein, the term "homologs" includes orthologs, analogs and paralogs. The term "analogs" refers to two polynucleotides or polypeptides that have the same or similar function, but that have evolved separately in unrelated organisms. The term "orthologs" refers to two polynucleotides or polypeptides from different species, but that have evolved from a common ancestral gene by speciation. Normally, orthologs encode polypeptides having the same or similar functions. The term "paralogs" refers to two polynucleotides or polypeptides that are related by duplication within a genome. Paralogs usually have different functions, but these functions may be related. Analogs, orthologs, and paralogs of a wild-type FMDV polypeptide can differ from the wild-type FMDV polypeptide by post-translational modifications, by amino acid sequence differences, or by both. In particular, homologs of the invention will generally exhibit at least 80-85%, 85-90%, 90-95%, or 95%, 96%, 97%, 98% , 99% sequence identity, with all or part of the wild-type FMDV polypepetide or polynucleotide sequences, and will exhibit a similar function. Variants include allelic variants. The term "allelic variant" refers to a polynucleotide or a polypeptide containing polymorphisms that lead to changes in the amino acid sequences of a protein and that exist within a natural population (e.g., a virus

species or variety). Such natural allelic variations can typically result in 1- 5% variance in a polynucleotide or a polypeptide. Allelic variants can be identified by sequencing the nucleic acid sequence of interest in a number of different species, which can be readily carried out by using hybridization probes to identify the same gene genetic locus in those species. Any and all such nucleic acid variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation and that do not alter the functional activity of gene of interest, are intended to be within the scope of the invention.

As used herein, the term "derivative" or “variant” refers to a polypeptide, or a nucleic acid encoding a polypeptide, that has one or more conservative amino acid variations or other minor modifications such that (1) the corresponding polypeptide has substantially equivalent function when compared to the wild type polypeptide or (2) an antibody raised against the polypeptide is immunoreactive with the wild-type polypeptide. These variants or derivatives include polypeptides having minor modifications of the FMDV polypeptide primary amino acid sequences that may result in peptides which have substantially equivalent activity as compared to the unmodified counterpart polypeptide. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. The term “variant” further contemplates deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein.

The term "conservative variation" denotes the replacement of an amino acid residue by another biologically similar residue, or the replacement of a nucleotide in a nucleic acid sequence such that the encoded amino acid residue does not change or is another biologically similar residue. In this regard, particularly preferred substitutions will generally be conservative in nature, as described above.

The polynucleotides of the disclosure include sequences that are degenerate as a result of the genetic code, e.g., optimized codon usage for a specific host. As used herein, “optimized” refers to a polynucleotide that is genetically engineered to increase its expression in a given species. To provide optimized polynucleotides coding for FMDV polypeptides, the DNA sequence of the FMDV protein gene can be modified to 1) comprise codons preferred by highly expressed genes in a particular species; 2) comprise an A+T or G+C content in nucleotide base composition to that substantially found in said species; 3) form an initiation sequence of said species; or 4) eliminate sequences that cause destabilization, inappropriate polyadenylation, degradation and termination of RNA, or that form secondary structure hairpins or RNA splice sites. Increased expression of FMDV protein in said species can be achieved by utilizing the distribution frequency of codon usage in eukaryotes and

prokaryotes, or in a particular species. The term "frequency of preferred codon usage" refers to the preference exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the disclosure as long as the amino acid sequence of the FMDV polypeptide encoded by the nucleotide sequence is functionally unchanged.

The sequence identity between two amino acid sequences may be established by the NCBI (National Center for Biotechnology Information) pairwise blast and the blosum62 matrix, using the standard parameters (see, e.g., the BLAST or BLASTX algorithm available on the "National Center for Biotechnology Information" (NCBI, Bethesda, Md., USA) server, as well as in Altschul *et al.*; and thus, this document speaks of using the algorithm or the BLAST or BLASTX and BLOSUM62 matrix by the term "blasts").

The "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. The sequence identity or sequence similarity of two amino acid sequences, or the sequence identity between two nucleotide sequences can be determined using Vector NTI software package (Invitrogen, 1600 Faraday Ave., Carlsbad, CA). 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.

Hybridization reactions can be performed under conditions of different "stringency." Conditions that increase stringency of a hybridization reaction are well known. See for example, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook et al., 1989).

The invention further encompasses the FMDV 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 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 includes cloning vectors and viral vectors.

The term "recombinant" means a polynucleotide semisynthetic, or synthetic origin which either does not occur in nature or is linked to another polynucleotide in an arrangement not found in nature.

"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 placed by genetic engineering techniques into a plasmid or vector derived from a different source, and is 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.

The present invention relates to ovine, bovine, caprine and swine vaccines or pharmaceutical or immunological compositions which may comprise an effective amount of a recombinant FMDV antigens and a pharmaceutically or veterinarily acceptable carrier, adjuvant, excipient, or vehicle.

The subject matter described herein is directed in part, to compositions and methods related to the FMDV antigen prepared in a baculovirus/insect cell expression system that is highly immunogenic and protects animals against challenge from homologous and heterologous FMDV strains.

Compositions

The present invention relates to an FMDV vaccine or composition which may comprise an effective amount of a recombinant FMDV antigen and a pharmaceutically or veterinarily acceptable carrier, excipient, adjuvant, or vehicle. In one embodiment, the recombinant FMDV antigen is expressed by a baculovirus expression vector in insect cells. In another embodiment, the FMDV vaccine or composition comprises a recombinant viral vector expressing FMDV antigens.

One embodiment of the invention relates to a vaccine or composition comprising FMDV empty capsids. In another embodiment, the invention relates to a vaccine or composition comprising a viral vector expressing FMDV empty capsids. The FMDV empty capsids are obtained by expression of the cDNA of regions P1, 2A/2B'/3B' and 3C. The FMDV empty capsids or FMDV VLPs (virus-like particles) may be modified with enhanced heat and/or acid (low PH) stability.

The present invention relates to vaccines against foot-and-mouth disease and in particular to improving their heat and/or acid (low PH) stability. It also relates to processes for preparing these vaccines, the use of antigens for producing these vaccines and vaccination methods using them.

The present invention also relates to nucleotide sequences, in particular cDNA, and to amino acid sequences, modified compared with natural sequences of the virus. The invention also relates to the expression products of the modified nucleotide sequences and to the FMDV antigens and virus incorporating these modifications.

The present invention encompasses any FMDV polypeptide, antigen, epitope or immunogen that elicits an immunogenic response in an animal, such as an ovine, bovine, caprine or swine. The FMDV polypeptide, antigen, epitope or immunogen may be any FMDV polypeptide, antigen, epitope or immunogen, such as, but not limited to, a protein, peptide or fragment thereof, that elicits, induces or stimulates a response in an animal, such as an ovine, bovine, caprine or swine.

In an embodiment wherein the FMDV immunological composition or vaccine is a recombinant immunological composition or vaccine, the composition or vaccine comprises a recombinant vector and a pharmaceutical or veterinary acceptable excipient, carrier, adjuvant or vehicle; the recombinant vector is a baculovirus expression vector which may comprise a polynucleotide encoding an FMDV polypeptide, antigen, epitope or immunogen. The FMDV polypeptide, antigen, epitope or immunogen, may be VP1, VP2, VP3, VP4, VP5, NS1, VP7, NS2, VP6, NS3, NS3a, P1, VP0, 3C, or any fragment thereof.

In another embodiment, the FMDV antigen is P1, VP0, VP3, VP1, VP2, VP4, 2A, 2B, or 3C.

In one embodiment, the nucleic acid molecule encoding one or more FMDV antigen(s) is a cDNA encoding FMDV P1 region and a cDNA encoding FMDV 3C protease of FMDV.

In one embodiment, the FMDV antigen may be a P1-3C polypeptide. In another embodiment, the FMDV antigen may be P1 alone, or P1-2A/2B1. In yet another embodiment, the FMDV antigen may be VP0-VP3. In another embodiment, the FMDV antigen may be VP4-VP2. In still another embodiment, the FMDV antigen may be 3C, or may be 3C with a 5'UTR optimized for expression in insect cells. In one embodiment, both P1-2A/2B1 and 3C polypeptides may be expressed in insect cells using a single construct and the expression may be regulated by one or more promoter sequences. In another embodiment, the FMDV antigen is a modified P1 or VP2.

In another embodiment, the FMDV antigen may be derived from *FMDV O1 Manisa, O1 BFS or Campos, A24 Cruzeiro, Asia 1 Shamir, A Iran'96, A22 Iraq, SAT2 Saudi Arabia.*

The present invention relates to an FMDV vaccine which may comprise an effective amount of a recombinant FMDV antigen or a recombinant viral vector expressing an FMDV antigen, and a pharmaceutically or veterinarily acceptable carrier, excipient, adjuvant, or vehicle.

In another embodiment, pharmaceutically or veterinarily acceptable carrier, excipient, adjuvant, or vehicle may be a water-in-oil emulsion. In yet another embodiment, the water-in-oil emulsion may be an oil-in-water emulsion.

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

In one aspect, the present invention provides FMDV polypeptides, particularly ovine, bovine, caprine or swine polypeptides having a sequence as set forth in SEQ ID NO:1, 2, 4, 5, 6, 8, 10, 12, 13, or 16 and variants or fragments 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 an antigenic polypeptide of the invention, particularly to the polypeptides having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 6, 8, 10, 12, 13, or 16.

In yet another aspect, the present invention provides fragments and variants of the FMDV polypeptides identified above (SEQ ID NO: 1, 2, 4, 5, 6, 8, 10, 12, 13, or 16) 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 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 6, 8, 10, 12, 13, or 16.

An immunogenic fragment of an FMDV 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 an FMDV polypeptide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 6, 8, 10, 12, 13, or 16, or variants thereof. In another embodiment, a fragment of an FMDV polypeptide includes a specific antigenic epitope found on a full-length FMDV polypeptide.

In another aspect, the present invention provides a polynucleotide encoding an FMDV polypeptide, such as a polynucleotide encoding a polypeptide having a sequence as set forth

in SEQ ID NO: 1, 2, 4, 5, 6, 8, 10, 12, 13, or 16. 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, 2, 4, 5, 6, 8, 10, 12, 13, or 16, 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.

In another aspect, the present invention provides a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:3, 7, 9, 11, 14, 15, 17, 18, 19, or 20, 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%, 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: 3, 7, 9, 11, 14, 15, 17, 18, 19, or 20, or a variant thereof.

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, enhancer, 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.

Elements for the expression of an FMDV 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, e.g., viral vectors other than poxviruses. When the polynucleotide encodes a polyprotein fragment, e.g. an FMDV 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 signal sequences permitting the secretion of the protein.

The present invention also relates to preparations comprising vectors, such as expression vectors, e.g., therapeutic compositions. The preparations can comprise one or more vectors, e.g., expression vectors, such as *in vivo* expression vectors, comprising and expressing one or more FMDV polypeptides, antigens, epitopes or immunogens. In one embodiment, the vector contains and expresses a polynucleotide that comprises, consists

essentially of, or consists of a polynucleotide coding for (and advantageously expressing) an FMDV antigen, epitope or immunogen, in a pharmaceutically or veterinarily acceptable carrier, excipient or vehicle. Thus, according to an embodiment of the invention, the other vector or vectors in the preparation comprises, consists essentially of or consists of a polynucleotide that encodes, and under appropriate circumstances the vector expresses one or more other proteins of an FMDV polypeptide, antigen, epitope or immunogen, or a fragment thereof.

According to another embodiment, the vector or vectors in the preparation comprise, or consist essentially of, or consist of polynucleotide(s) encoding one or more proteins or fragment(s) thereof of an FMDV polypeptide, antigen, epitope or immunogen, the vector or vectors expressing the polynucleotide(s). In another embodiment, the preparation comprises one, two, or more vectors comprising polynucleotides encoding and expressing, advantageously *in vivo*, an FMDV polypeptide, antigen, fusion protein or an epitope thereof. The invention is also directed at mixtures of vectors that comprise polynucleotides encoding and expressing different FMDV polypeptides, antigens, epitopes or immunogens, e.g., an FMDV polypeptide, antigen, epitope or immunogen from different animal species such as, but not limited to, ovine, bovine, caprine or swine.

According to a yet further embodiment of the invention, the expression vector is a plasmid vector or a DNA plasmid vector, in particular an *in vivo* expression vector. In a specific, non-limiting example, the pVR1020 or 1012 plasmid (VICAL Inc.; Luke et al., 1997; Hartikka et al., 1996, Hum Gene Ther, **7**(10): 1205-17; see, e.g., U.S. Patent Nos. 5,846,946 and 6,451,769) can be utilized as a vector for the insertion of a polynucleotide sequence. The pVR1020 plasmid is derived from pVR1012 and contains the human tPA signal sequence. In one embodiment the human tPA signal comprises from amino acid M(1) to amino acid S(23) in Genbank under the accession number HUMTPA14. In another specific, non-limiting example, the plasmid utilized as a vector for the insertion of a polynucleotide sequence can contain the signal peptide sequence of equine IGF1 from amino acid M(24) to amino acid A(48) in Genbank under the accession number U28070. Additional information on DNA plasmids which may be consulted or employed in the practice are found, for example, in U.S. Patent Nos. 6,852,705; 6,818,628; 6,586,412; 6,576,243; 6,558,674; 6,464,984; 6,451,770; 6,376,473 and 6,221,362.

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 or non-

supercoiled, circular plasmid, as well as a linear form, are intended to be within the scope of the invention.

Each plasmid comprises or contains or consists essentially of, in addition to the polynucleotide encoding an FMDV antigen, epitope or immunogen, optionally fused with 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 functional in eukaryotic cells. The strong promoter may be, but not limited to, 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 Super promoter (Ni, M. et al., Plant J. 7, 661-676, 1995.). The CMV-IE promoter can comprise the actual promoter part, which may or may not be associated with the enhancer part. Reference can be made to EP-A-260 148, EP-A-323 597, U.S. Patents Nos. 5,168,062, 5,385,839, and 4,968,615, as well as to PCT Application No WO87/03905. The CMV-IE promoter is advantageously a human CMV-IE (Boshart et al., 1985, Cell, 41(2): 521-30) or murine CMV-IE.

In more general terms, the promoter has either a viral, a plant, 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, Vaccine, 18(22): 2337-44), or the actin promoter (Miyazaki *et al.,* 1989, Gene, 79(2): 269-77).

The plasmids may comprise other expression control elements. It is particularly advantageous to incorporate stabilizing sequence(s), e.g., intron sequence(s), for example, maize alcohol dehydrogenase intron (Callis *et al.* Genes & Dev.1(10):1183-1200, Dec. 1987), the first intron of the hCMV-IE (PCT Application No. WO1989/01036), the intron II of the rabbit β-globin gene (van Ooyen et al., 1979, Science, 206(4416): 337-44). In another embodiment, the plasmids may comprise 3' UTR. The 3' UTR may be, but not limited to, *agrobacterium* nopaline synthase (Nos) 3' UTR (Nopaline synthase: transcript mapping and DNA sequence. Depicker, A. et al. J. Mol. Appl. Genet., 1982; Bevan, NAR, 1984, 12(22): 8711-8721).

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. 5,122,458), or the poly(A) signal of the rabbit β-globin gene or the poly(A) signal of the SV40 virus.

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.

In one embodiment, the recombinant FMDV antigen is expressed in insect cells.

Methods of Use

In an embodiment, the subject matter disclosed herein is directed to a method of vaccinating an ovine, bovine, caprine, or swine comprising administering to the ovine, bovine, caprine, or swine an effective amount of a vaccine which may comprise an effective amount of a recombinant FMDV antigen or a recombinant viral vector expressing an FMDV antigen, and a pharmaceutically or veterinarily acceptable carrier, excipient, adjuvant, or vehicle.

In one embodiment of the present invention, the method comprises a single administration of a vaccine composition formulated with an emulsion according to the invention. For example, in one embodiment, the immunological or vaccine composition comprises baculovirus expressed FMDV antigens, including polypeptides and VLPs (virus-like particles) or empty capsids, or a recombinant viral vector expressing an FMDV antigen. Electron microscopy indicates the insect cells transformed with baculovirus expression vectors produce FMDV VLPs or FMDV empty capsids, and so immunological or vaccine compositions according to the instant invention encompass those comprising FMDV VLPs or FMDV empty capsids.

In another embodiment of the present invention, the method comprises a single administration of two heterologous vaccine compositions. The heterologous vaccines or compositions may be different types of vaccines, such as FMDV VLPs vaccine or FMDV viral vector vaccines. The heterologous vaccines may also be the same type of vaccines expressing the capsids of different FMDV serotypes, such as A24, O1 Manisa, Asia or Iraq strains.

In an embodiment, the subject matter disclosed herein is directed to a method of vaccinating an ovine, bovine, caprine, or swine comprising administering to the ovine,

bovine, caprine, or swine the FMDV antigen produced by a baculovirus vector in insect cells or a recombinant viral vector expressing an FMDV antigen

In an embodiment, the subject matter disclosed herein is directed to a method of eliciting an immune response comprising administering to the ovine, bovine, caprine, or swine a vaccine comprising the FMDV antigen produced by a baculovirus vector in insect cells or a recombinant viral vector expressing an FMDV antigen.

In an embodiment, the subject matter disclosed herein is directed to a method of preparing a vaccine or composition comprising isolating an FMDV antigen produced by a baculovirus vector in insect cells or a recombinant viral vector expressing an FMDV antigen and optionally combining with a pharmaceutically or veterinarily acceptable carrier, excipient, adjuvant, or vehicle.

Both homologous and heterologous FMDV strains are used for challenge to test the efficacy of the vaccine. The administering may be subcutaneously or intramuscularly. The administering may be needle free (for example Pigjet or Bioject).

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 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. This administration protocol is called “prime-boost”.

A prime-boost according to the present invention can include a recombinant viral vector that is used to express an FMDV coding sequence or fragments thereof encoding an antigenic polypeptide or fragment or variant thereof. Specifically, the viral vector can express an FMDV gene or fragment thereof that encodes an antigenic polypeptide. Viral vector contemplated herein includes, but not limited to, poxvirus [e.g., vaccinia virus or attenuated vaccinia virus, avipox virus or attenuated avipox virus (e.g., canarypox, fowlpox, dovepox, pigeonpox, quailpox, ALVAC, TROVAC; see e.g., US 5,505,941, US 5,494,8070), raccoonpox virus, swinepox virus, etc.], adenovirus (e.g., human adenovirus, canine adenovirus), herpesvirus (e.g. canine herpesvirus, herpesvirus of turkey, Marek’s disease virus, infectious laryngotracheitis virus, feline herpesvirus, laryngotracheitis virus (ILTV), bovine herpesvirus, swine herpesvirus), baculovirus, retrovirus, etc. In another embodiment, the avipox expression vector may be a canarypox vector, such as, ALVAC. In yet another embodiment, the avipox expression vector may be a fowlpox vector, such as, TROVAC. The FMDV antigen of the invention to be expressed is inserted under the control of a specific

poxvirus promoter, e.g., the entomopoxvirus *Amsacta moorei* 42K promoter (Barcena, Lorenzo et al., 2000, J Gen Virol., 81(4): 1073-85), the vaccinia promoter 7.5 kDa (Cochran *et al.*, 1985, J Virol, 54(1): 30-7), the vaccinia promoter I3L (Riviere et al., 1992, J Virol, 66(6): 3424-34), the vaccinia promoter HA (Shida, 1986, Virology, 150(2): 451-62), the cowpox promoter ATI (Funahashi et al., 1988, J Gen Virol, 69 (1): 35-47), the vaccinia promoter H6 (Taylor et al., 1988, Vaccine, 6(6): 504-8; Guo *et al.,* 1989, J Virol, 63(10): 4189-98; Perkus et al., 1989, J Virol, 63(9): 3829-36.), *inter alia*.

In another embodiment, the avipox expression vector may be a canarypox vector, such as, ALVAC. The FMDV antigen, epitope or immunogen may be FMDV P1-3C. The FMDV viral vector may be a canarypox virus such as vCP2186, vCP2181, or vCP2176, or a fowlpox virus such as vFP2215 (see US 7,527,960). In yet another embodiment, the FMDV antigen, epitope or immunogen may be produced in duckweed (US 2011/0236416).

In another aspect of the prime-boost protocol of the invention, a composition comprising the FMDV antigen of the invention is administered followed by the administration of vaccine or composition comprising a recombinant viral vector that contains and expresses the FMDV antigen *in vivo*, or an inactivated viral vaccine or composition comprising the FMDV antigen, or a DNA plasmid vaccine or composition that contains or expresses the FMDV antigen. Likewise, a prime-boost protocol may comprise the administration of vaccine or composition comprising a recombinant viral vector that contains and expresses an FMDV antigen *in vivo*, or an inactivated viral vaccine or composition comprising an FMDV antigen, or a DNA plasmid vaccine or composition that contains or expresses an FMDV antigen, followed by the administration of a composition comprising the FMDV antigen of the invention. It is further noted that both the primary and the secondary administrations may comprise the composition comprising the FMDV antigen of the invention.

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

The dose volume of compositions for target species that are mammals, e.g., the dose volume of ovine, bovine, caprine or swine compositions, based on viral vectors, e.g., non-

poxvirus-viral-vector-based compositions, is generally between about 0.1 to about 5.0 ml, between about 0.1 to about 3.0 ml, and between about 0.5 ml to about 2.5 ml.

The efficacy of the vaccines may be tested about 2 to 4 weeks after the last immunization by challenging animals, such as ovine, bovine, caprine or swine, with a virulent strain of FMDV, advantageously the FMDV *O1 Manisa, O1 BFS or Campos, A24 Cruzeiro, Asia 1 Shamir, A Iran'96, A22 Iraq, SAT2 Saudi Arabia* strains.

Still other strains may include FMDV strains A10-61, A5, A12, A24/Cruzeiro, C3/Indaial, O1, C1-Santa Pau, C1-C5, A22/550/Azerbaijan/65, SAT1-SAT3, A, A/TNC/71/94, A/IND/2/68, A/IND/3/77, A/IND/5/68, A/IND/7/82, A/IND/16/82, A/IND/17/77, A/IND/17/82, A/IND/19/76, A/IND/20/82, A/IND/22/82, A/IND/25/81, A/IND/26/82, A/IND/54/79, A/IND/57/79, A/IND/73/79, A/IND/85/79, A/IND/86/79, A/APA/25/84, A/APN/41/84, A/APS/44/05, A/APS/50/05, A/APS/55/05, A/APS/66/05, A/APS/68/05, A/BIM/46/95, A/GUM/33/84, A/ORS/66/84, A/ORS/75/88, A/TNAn/60/947/Asia/1, A/IRN/05, Asia/IRN/05, O/HK/2001, O/UKG/3952/2001, O/UKG/4141/2001, Asia 1/HNK/CHA/05 (GenBank accession number EF149010, herein incorporated by reference), Asia I/XJ (Li, ZhiYong *et al.* Chin Sci Bull, 2007), HK/70 (Chin Sci Bull, 2006, 51(17): 2072—2078), O/UKG/7039/2001, O/UKG/9161/2001, O/UKG/7299/2001, O/UKG/4014/2001, O/UKG/4998/2001, O/UKG/9443/2001, O/UKG/5470/2001, O/UKG/5681/2001, O/ES/2001, HKN/2002, O5India, O/BKF/2/92, K/37/84/A, KEN/1/76/A, GAM/51/98/A, A10/Holland, O/KEN/1/91, O/IND49/97, O/IND65/98, O/IND64/98, O/IND48/98, O/IND47/98, O/IND82/97, O/IND81/99, O/IND81/98, O/IND79/97, O/IND78/97, O/IND75/97, O/IND74/97, O/IND70/97, O/IND66/98, O/IND63/97, O/IND61/97, O/IND57/98, O/IND56/98, O/IND55/98, O/IND54/98, O/IND469/98, O/IND465/97, O/IND464/97, O/IND424/97, O/IND423/97, O/IND420/97, O/IND414/97, O/IND411/97, O/IND410/97, O/IND409/97, O/IND407/97, O/IND399/97, O/IND39/97, O/IND391/97, O/IND38/97, O/IND384/97, O/IND380/97, O/IND37/97, O/IND352/97, O/IND33/97, O/IND31/97, O/IND296/97, O/IND23/99, O/IND463/97, O/IND461/97, O/IND427/98, O/IND28/97, O/IND287/99, O/IND285/99, O/IND282/99, O/IND281/97, O/IND27/97, O/IND278/97, O/IND256/99, O/IND249/99, O/IND210/99, O/IND208/99, O/IND207/99, O/IND205/99, O/IND185/99, O/IND175/99, O/IND170/97, O/IND164/99, O/IND160/99, O/IND153/99, O/IND148/99, O/IND146/99, O/SKR/2000, A22/India/17/77.

Further details of these FMDV strains may be found on the European Bioinformatics Information (EMBL-EBI) web pages, and all of the associated nucleotide sequences are

herein incorporated by reference. The inventors contemplate that all FMDV strains, both herein listed, and those yet to be identified, could be expressed according to the teachings of the present disclosure to produce, for example, effective vaccine compositions. Both homologous and heterologous strains are used for challenge to test the efficacy of the vaccines. The animal may be challenged intradermally, subcutaneously, spray, intra-nasally, intra-ocularly, intra-tracheally, and/or orally.

The prime-boost administrations may be advantageously carried out 1 to 6 weeks apart, for example, about 3 weeks apart. According to one embodiment, a semi-annual booster or an annual booster, advantageously using the viral vector-based vaccine, is also envisaged. The animals are advantageously at least 6 to 8 weeks old at the time of the first administration.

The compositions comprising the recombinant antigenic polypeptides of the invention used in the prime-boost protocols are contained in a pharmaceutically or veterinary acceptable vehicle, diluent, adjuvant, or excipient. The protocols of the invention protect the animal from ovine, bovine, caprine or porcine FMDV and/or prevent disease progression in an infected animal.

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 injection protocol, without any undue experimentation.

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 animal may be male, female, pregnant female and newborn. This administration may be via various routes including, but not limited to, intramuscular (IM), intradermal (ID) or subcutaneous (SC) injection or via intranasal or oral administration. The therapeutic composition according to the invention can also be administered by a needleless apparatus (as, for example with a Pigjet, Dermojet, Biojector, Avijet (Merial, GA, USA), Vetjet or Vitajet apparatus (Bioject, Oregon, USA)). Another approach to administering plasmid compositions is to use electroporation (see, e.g. Tollefsen et al., 2002; Tollefsen et al., 2003; Babiuk et al., 2002; PCT Application No. WO99/01158). In another embodiment, the therapeutic composition is delivered to the animal by gene gun or gold particle bombardment.

In one embodiment, the invention provides for the administration of a therapeutically effective amount of a formulation for the delivery and expression of an FMDV antigen or

epitope in a target cell. Determination of the therapeutically effective amount is routine experimentation for one of ordinary skill in the art. In one embodiment, the formulation comprises an expression vector comprising a polynucleotide that expresses an FMDV antigen or epitope and a pharmaceutically or veterinarily acceptable carrier, vehicle or excipient. In another embodiment, the pharmaceutically or veterinarily acceptable carrier, vehicle or excipient facilitates transfection or other means of transfer of polynucleotides to a host animal and/or improves preservation of the vector or protein in a host.

In one embodiment, the subject matter disclosed herein provides a detection method for differentiation between infected and vaccinated animals (DIVA).

It is disclosed herein that the use of the vaccine or composition of the present invention allows the detection of FMDV infection in an animal. It is disclosed herein that the use of the vaccine or composition of the present invention allows the detection of the infection in animals by differentiating between infected and vaccinated animals (DIVA). A method is disclosed herein for diagnosing the infection of FMDV in an animal using an FMDV non-structural protein (e.g. a FMDV 3ABC or 3D-specific ELISA).

Article of Manufacture

In an embodiment, the subject matter disclosed herein is directed to a kit for performing a method of eliciting or inducing an immune response which may comprise any one of the recombinant FMDV immunological compositions or vaccines, or inactivated FMDV immunological compositions or vaccines, recombinant FMDV viral compositions or vaccines, and instructions for performing the method.

Another embodiment of the invention is a kit for performing a method of inducing an immunological or protective response against FMDV in an animal comprising a composition or vaccine comprising an FMDV antigen of the invention and a recombinant FMDV viral immunological composition or vaccine, and instructions for performing the method of delivery in an effective amount for eliciting an immune response in the animal.

Another embodiment of the invention is a kit for performing a method of inducing an immunological or protective response against FMDV in an animal comprising a composition or vaccine comprising an FMDV antigen of the invention and an inactivated FMDV immunological composition or vaccine, and instructions for performing the method of delivery in an effective amount for eliciting an immune response in the animal.

Yet another aspect of the present invention relates to a kit for prime-boost vaccination according to the present invention as described above. The kit may comprise at least two

vials: a first vial containing a vaccine or composition for the prime-vaccination according to the present invention, and a second vial containing a vaccine or composition for the boost-vaccination according to the present invention. The kit may advantageously contain additional first or second vials for additional prime-vaccinations or additional boost-vaccinations.

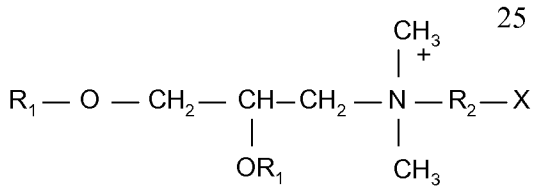
The following embodiments are encompassed by the invention. In an embodiment, a composition comprising an FMDV antigen or fragment or variant thereof and a pharmaceutically or veterinarily acceptable carrier, excipient, adjuvant, or vehicle is disclosed. In another embodiment, a composition comprising a recombinant viral vector expressing FMDV antigens and a pharmaceutically or veterinarily acceptable carrier, excipient, adjuvant, or vehicle is disclosed. In another embodiment, the composition described above wherein the FMDV antigen or fragment or variant thereof comprises an immunogenic fragment comprising at least 15 amino acids of an ovine, bovine, caprine, or swine FMDV antigen is disclosed. In an embodiment, the above compositions wherein the FMDV antigen or fragment or variant thereof is partially purified are disclosed. In an embodiment, the above compositions wherein the FMDV antigen or fragment or variant thereof is substantially purified are disclosed.

In an embodiment, the above compositions wherein the FMDV antigen or fragment or variant thereof is an ovine, bovine, caprine, or swine FMDV polypeptide are disclosed. In an embodiment, the above compositions wherein the FMDV polypeptide is a P1-3C polypeptide, P1 polypeptide, VP0 polypeptide, VP1 polypeptide, VP3 polypeptide, VP2 polypeptide, VP4 polypeptide, 2A polypeptide, 2B1 polypeptide, or 3C polypeptide are disclosed. In an embodiment, the above compositions wherein the FMDV antigen or fragment or variant thereof has at least 80% sequence identity to the sequence as set forth in SEQ ID NO:1, 2, 4, 5, 6, 8 ,10, 12, 13 or 16 are disclosed. In one embodiment, the above compositions wherein the FMDV antigen is encoded by a polynucleotide having at least 70% sequence identity to the sequence as set forth in SEQ ID NO:3, 7, 9, 11, 14, 15, 17, 18, 19 or 20 are disclosed. In an embodiment, the above compositions wherein the pharmaceutically or veterinarily acceptable carrier, excipient, adjuvant, or vehicle is a water-in-oil emulsion or an oil-in-water emulsion are disclosed. In another embodiment, a method of vaccinating an animal susceptible to ovine, bovine, caprine, or swine FMDV comprising administering the compositions above to the animal is disclosed. In an embodiment, a method of vaccinating an animal susceptible to ovine, bovine, caprine, or swine FMDV comprising a prime-boost regimen is disclosed. In an embodiment, a substantially purified antigenic polypeptide

expressed in insect cells, wherein the polypeptide comprises: an amino acid sequence having at least 80% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 6, 8 10, 12, 13, or 16 is disclosed. In any embodiment the animal is preferably an ovine, a bovine, a swine, or a caprine. In one embodiment, a method of diagnosing FMDV infection in an animal is disclosed. In yet another embodiment, a kit for prime-boost vaccination comprising at least two vials, wherein a first vial containing the composition comprising an FMDV antigen or fragment or variant thereof, and a second vial containing a recombinant viral vector that contains or expresses the FMDV antigen is disclosed.

The pharmaceutically or veterinarily acceptable carriers or vehicles or adjuvants or excipients are well known to the one skilled in the art. For example, a pharmaceutically or veterinarily acceptable carrier or vehicle or excipient can be a 0.9% NaCl (e.g., saline) solution or a phosphate buffer. Other pharmaceutically or veterinarily acceptable carrier or vehicle 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 adjuvants 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 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 advantageously but not exclusively suitable for plasmids, are advantageously those having the following formula:



in which R1 is a saturated or unsaturated straight-chain aliphatic radical having 12 to 18 carbon atoms, R2 is another aliphatic radical containing 2 or 3 carbon atoms and X is an amine or hydroxyl group, e.g. the DMRIE. In another embodiment the cationic lipid can be associated with a neutral lipid, e.g. the DOPE.

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.

Advantageously, the plasmid mixture with the adjuvant is formed extemporaneously and advantageously 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, such as approximately 30 minutes prior to administration.

When DOPE is present, the DMRIE:DOPE molar ratio is advantageously about 95: about 5 to about 5: about 95, more advantageously 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 about 1: about 1 and about 1: about 2, e.g., 1:1 and 1:2.

In another embodiment, pharmaceutically or veterinarily acceptable carrier, excipient, adjuvant, 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, polyol 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, e.g., Example 8 thereof, incorporated herein by reference. 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 an advantageous embodiment, the water-in-oil emulsion may be a water/oil/water (W/O/W) triple emulsion (U.S. Patent No. 6,358,500). Examples of other suitable emulsions are described in U.S. Patent No. 7,371,395.

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, PNAS USA, 93(7): 2879-83; WO98/16247), (3) an oil in water emulsion, such as the SPT emulsion described on page 147 of "Vaccine Design, The Subunit and Adjuvant Approach" published by M. Powell, M. Newman, Plenum Press 1995, 6: 147, 183, and the emulsion MF59 described on page 183 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.

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.

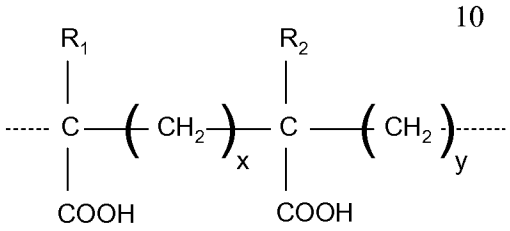
The oil is used in combination with emulsifiers to form an emulsion. The emulsifiers may be nonionic surfactants, such as: esters of on the one hand sorbitan, mannide (e.g. anhydromannitol oleate), glycerol, polyglycerol or propylene glycol and on the other hand oleic, isostearic, ricinoleic or hydroxystearic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

Among the type (1) adjuvant polymers, preference is given to polymers of crosslinked acrylic or methacrylic acid, especially crosslinked 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 crosslinked 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 crosslinked 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 crosslinked ethylene-maleic anhydride copolymers and they are, for example, crosslinked 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:



in which:

- R1 and R2, 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 about 0.01 to about 1.5% w/v, about 0.05 to about 1% w/v, and about 0.1 to about 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 (IFNα), interferon β (IFNβ), interferon γ, (IFNγ), 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 (TNFα), tumor necrosis factor β (TNFβ), polyinosinic and polycytidylic acid, cytidine–phosphate–guanosine oligodeoxynucleotides (CpG ODN), and transforming growth factor β (TGFβ). 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 bovine cytokine for preparations to be administered to bovines).

In a particular embodiment, the adjuvant may include TS6, TS7, TS8 and TS9 emulsions (US 7,371,395); LR3 and LR4 (US7,691,368); TSAP (US20110129494); TRIGEN™ (Newport Labs); synthetic dsRNAs (e.g. poly-IC, poly-ICLC [HILTONOL®]); and MONTANIDE™ adjuvants (W/O, W/O/W, O/W, IMS and Gel; all produced by SEPPIC).

In the case of immunological composition and/or vaccine based on a baculovirus/insect cell-expressed polypeptides, a dose may include, about 1 μg to about 2000 μg, about 50 μg to about 1000 μg, and from about 100 μg to about 500 μg of FMDV antigen, epitope or immunogen. The dose may include about 10^2 to about 10^{20}, about 10^3 to about 10^{18}, about 10^4 to about 10^{16}, about 10^5 to about 10^{12} VLPs. In the case of immunological composition and/or vaccine based on a viral vector expressing FMDV antigens, a dose may include, about 10^3 $TCID_{50}$ to about 10^{15} $TCID_{50}$ (50% Tissue Culture Infective Dose), about 10^3 $TCID_{50}$ to about 10^{14} $TCID_{50}$, about 10^3 $TCID_{50}$ to about 10^{13} $TCID_{50}$, about 10^3 $TCID_{50}$ to about 10^{12} $TCID_{50}$. The dose volumes can be between about 0.1 and about 10 ml, advantageously between about 0.2 and about 5 ml.

The invention will now be further described by way of the following non-limiting examples.

EXAMPLES

Construction of DNA inserts, plasmids and recombinant viral or baculovirus vectors was carried out using the standard molecular biology techniques 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 and expression of FMDV antigens in baculovirus/insect cells system

The positive strain RNA genome of serotype A FMDV is composed of a single ORF (1662 amino acids) flanked by two non-coding regions. The ORF holds 3 parts. The first part is Polyprotein P1-2A that leads to the expression of 4 capsids components (VP4, VP2, VP3 and VP1) after maturation and cleavage. The second part is P2 containing 2 proteins (2B and 2C) and is involved in RNA synthesis and cell membrane vesicle proliferation. The first part is P3 containing 4 proteins (3A, 3B, 3C and 3D). 3C is the major one involved in the cleavage of the polyprotein. 3D is another protease and 3A/3B are involved in the membrane anchorage, pathogenesis, RNA synthesis and encapsidation. P1-2A and 3C are necessary for expression and cleavage of all the proteins making up the FMDV capsid particles or FMDV VLPs (see FIG. 2). Potential functional domains are shown in table 1 below.

Table 1 Potential functional domains annotated on GenBank Accession No. AAT01711

Putative domains	From to (or position)	Length
Non coding sequence 5′	1-201	201
VP4	202-286	85
VP2	287-504	218
VP3	505-725	221
VP1	726-938	213
2A	939-954	16
2B	955-1108	154
2C3A+beginning2B	1109-1587	479
3B′ (end)	1588-1650	63
3C	1651-1863	213
Signal sequence	no	
N-glycosylation	14,18,24,25,33,42,133,277,625,764	
Disulfide bridge	No	

Example 1.1 Construction of plasmid pMEB097 containing polynucleotide encoding polyprotein of FMDV A24 Cruzeiro Strain with a mutation in VP2 (H93C) for creation of a

disulfide bridge (+optimized translation initiation context) and generation of corresponding recombinant baculovirus BacMEB097

Generation of plasmid pMEB097

The plasmid pMEB096 containing wild-type polynucleotide encoding polyprotein of FMDV A24 Cruzeiro strain was mutated to generate plasmid pMEB097. Plasmid pMEB097 contains the polynucleotide (SEQ ID NO:3) encoding a modified polyprotein (SEQ ID NO:2) containing a mutation in VP2 (H93C) or P1 (H179C). The modified polyprotein (SEQ ID NO:2) contains a substitution of Histidine by Cysteine at position 93 of VP2 or position 179 of P1.

Generation of recombinant baculovirus BacMEB097

Plasmid pMEB097 (see FIG. 3) was used to generate a recombinant baculovirus, encoding FMDV P1/2A/2B'3B'/3C gene (with cysteine at position 93 of VP2) of FMDV A24 Cruzeiro strain under control of polyhedrin promoter, by homologous recombination. *Spodoptera frugiperda* (Sf) 9 insect cells from ATCC were co-transfected with plasmid pMEB097 and Bsu36I triple-cut linearized AcNPV DNA, according to manufacturer's protocol (Baculogold, Pharmingen). Recombinant baculovirus from co-transfection supernatant were plaque purified twice. Five clones were amplified (passage 1) at 28°C at a 25 cm^2 monolayer flask scale. Five clones were amplified in Sf9 insect cells and recombinant clones were analysed by Western blot using monoclonal antibody which is specific to FMDV A24 serotype. The clone **2** showed a good level of expression. This clone was further amplified (passage 2) at 28°C at a 50mL scale in Erlenmeyers (suspension) at 105 rpm. A third passage (passage 3) at a 200mL scale was performed to obtain virus stock used for protein expression. This virus stock was then titrated by plaque assay. The obtention of the virus stock was performed using SF900III media, supplemented with 2% of FCS. After titration recombinant baculovirus stock (Passage 3) was used for protein production in serum free medium.

Expression analysis of baculovirus BacMEB097

Insect cells (Sf9-Invitrogen) were infected by the generated baculovirus BacMEB097 and by BacMEB084 (as reference without mutation) at a Multiplicity Of Infection (MOI) of 1 pfu/ml. Insect cells were grown at 105 rpm in Sf900II medium without FCS during 4 days at 28°C. Supernatants were concentrated around factor 4 and treated as: A = no treatment; B = 1h at 56°C; C = HCl added to reach a pH below 5.

The correct assembly of the capsid protein into VLPs was assessed by Electronic microscopy (see FIG. 4, column "A"). Particles of 25-30 nm showed very uniform round

to eicosahedral morphology, a constant size of 31 nm and was characterised by penetration of stain and hence was interpreted as FMDV-like. The number of particles was estimated at about 10^8 per ml.

Moreover, the stability of VLPs was clearly increased with the formation of the disulfide bridge brought by the mutation in BacMEB097 as seen after treatment 1 hour at 56°C (column "B") and by acidification of the medium (column "C").

The identity of FMDV protein was confirmed by analysis of the supernatant by Western blot using monoclonal antibody which is specific to FMDV A24 serotype (see FIG.5).

In conclusion, baculovirus BacMEB097, generated with transfer plasmid pMEB097, induce FMDV capsid expression and processing in Sf9 insect cells. These FMDV expressed capsids auto-assembled into VLPs with characteristic morphology of FMDV like virions. The mutation involving a formation of a disulfide bridge increased the stability of the VLPs (after heat treatment or acidification) compared to VLPs obtained by BacMEB084 (containing plasmid pMEB096 containing wild-type polynucleotide encoding polyprotien of FMDV A24 Cruzeiro strain).

Example 1.2 Construction of plasmid pMEB099 containing polynucleotide encoding polyprotein of FMDV O1 manisa strain with a mutation in VP2 (S93C) for creation of a disulfide bridge (+optimized translation initiation context) and generation of recombinant baculovirus BacMEB099 expressing FMDV capsid proteins

The plasmid pMEB095 containing wild-type polynucleotide encoding polyprotien of FMDV O1 manisa strain was mutated to generate plasmid pMEB099. Plasmid pMEB099 contains the polynucleotide (SEQ ID NO:7) encoding a modified polyprotien (SEQ ID NO:6) containing a mutation in VP2 (S93C) or P1 (S179C). The modified polyprotein (SEQ ID NO:6) contains a substitution of Serine by Cysteine at position 93 of VP2 or position 179 of P1.

Generation and expression of recombinant baculovirus BacMEB099 were carried out according to the procedures described in example 1.1 for BacMEB097. Insect cells (Sf9-Inv) were infected by the baculovirus BacMEB099 at a Multiplicity Of Infection (MOI) of 0.5 pfu/ml. Insect cells were grown at 105 rpm in Sf900II medium without FCS during 4 days at 28°C. Protein production was done after treatment of the supernatant: concentration and with or without heating 1h at 56°C (to see the effect of the covalent cage mutation). Supernatant treated were analysed directly by electronic microscopy and by specific ELISA (see FIG.6).

The result (FIG. 6A and 6B) shows that a significant titer with the ELISA method was obtained. This ELISA is specific for the VLP, suggesting the presence of VLP after infection by BacMEB099.

The correct assembly of the capsid protein into VLPs was assessed by Electronic microscopy (FIG.6B). Particles of 25-30 nm showed very uniform round to eicosahedral morphology, a constant size of 31 nm and was characterised by penetration of stain and hence was interpreted as FMDV-like. The number of particles was estimated at about 10^8 per ml.

In conclusion, baculovirus BacMEB099, generated with transfer plasmid pMEB099, induced FMDV capsid expression and processing in Sf9 insect cells. These FMDV expressed capsids auto-assembled into VLPs with characteristic morphology of FMDV like virions.

Example 1.3 Construction of plasmid pMEB106 containing polynucleotide encoding polyprotein of FMDV Iraq strain and plasmid pMEB104 containing polynucleotide encoding polyprotein of FMDV Asia strain with a mutation in VP2 for creation of a disulfide bridge (+optimized translation initiation context) and generation of recombinant baculovirus expressing FMDV capsid proteins

The plasmids containing the polynucleotide (SEQ ID NO:9) encoding a modified polyprotien (SEQ ID NO:8) containing a mutation in P1 (C at position 179) of FMDV Iraq strain and the polynucleotide (SEQ ID NO:11) encoding a modified polyprotien (SEQ ID NO:10) containing a mutation in P1 (C at position179) of FMDV Asia strain were constructed according to the procedure outlined in example 1.1.

Generation and expression of recombinant baculovirus expressing modified polyprotein (SEQ ID NO:8) of FMDV Iraq strain and polyprotein (SEQ ID NO:10) of FMDV Asia strain were carried out according to the procedures described in example 1.1 for BacMEB097.

The baculovirus BacMEB106 that was generated with transfer plasmid pMEB106 induced FMDV capsid expression and processing in Sf9 insect cells. The baculovirus BacMEB104, generated with transfer plasmid pMEB104, induced FMDV capsid expression and processing in Sf9 insect cells. These FMDV expressed capsids auto-assembled into VLPs with characteristic morphology of FMDV like virions.

Example 2 Stability of Baculovirus-expressed "caged" FMDV VLP

The ability to produce the FMDV VLP in large scale (150 L) was demonstrated. When grown in 150 L batch, manufacturing scale, satisfactory titers of baculovirus-expressed covalent cage and wildtype A24 FMDV-VLPs were obtained (log10$CCID_{50}$ 7.14 / mL) (CCID: cell culture infective dose). Surprisingly, large quantities of stable FMD VLPs were present, even after heating the samples (2.14 log10 $CCID_{50}$ / ml heated against 2.19 log10 $CCID_{50}$ /ml unheated) (see FIG. 8). Moreover, as indicated in FIG. 8, EM counting revealed excellent numbers of VLPs from both the 4 L and 150 L batches.

Example 3 Vaccination of Cattle with Baculovirus, duckweed and Canarypox expressed FMDV, and subsequent virulent challenge

The purpose of this study was to test, in cattle, the efficacy against an FMD virulent challenge of 3 experimental FMD A24 *Cruzeiro* antigens, formulated in TS6 adjuvant.

Vaccines containing either Lemna (duckweed, see US2011/0236416), Baculovirus or vCP (canarypox virus) expressed antigens were administered on D0 to bovines (Table 2.1). Vaccine protection was assessed according to the relevant European Pharmacopoeia Monograph, through a virulent FMDV A24 Cruzeiro challenge performed on D21 followed by a clinical monitoring of the bovines (Table 2.2). FMD lesions in the controls validated the challenge. Two animals in the Baculovirus vaccinated group were fully protected from lesions indicating full protection, while the other three presented very limited extension of lesions representing partial protection. The animals in G2 and G3 all presented lesions though the intensity of the lesions varied. Neutralizing titer data are shown in FIG. 9 and FIG. 10. Three weeks after vaccination (D21), a clear sero-conversion was observed in all calves in G2. In G1 and G3, little or no sero-conversion was observed. Controls were negative on that date. All calves strongly sero-converted after challenge (D25 or D28). The sero-response appeared more intense on D28 than D25 and in the vaccinated animals in G2 than in the controls. This observation suggests a priming effect of the vaccination in G2. The results indicate that the Baculovirus FMDV A24 (wildtype A24) vaccine group provided highest SN titers when compared to the other two groups and provided the protection against virulent FMDV A24 Cruzeiro challenge.

Table 2.1 Vaccination scheme

Nature of antigen	Volume of antigen blended	Volume of TS6 adjuvant blended	Volume of vaccine injected per bovine
G1: Lemna FMDV 5x Concentrated (see US 2011/0236416)	4 mL	8 mL	2 mL
G2: Baculovirus FMDV (wildy-type) 14.4 x Concentrated	4 mL	8 mL	2 mL
G3: vCP2186 (canarypox-vectored FMDV) (see US 7,527,960)	2.5 mL	8 mL	2 mL

Table 2.2 Challenge study design

Group	# of animals	Vaccine on Do	FMD A24 Cruzeiro challenge on D21
G1	5	Lemna FMDV + TS6	Yes
G2	5	Baculovirus FMDV + TS6	Yes
G3	5	vCP2186 + TS6	Yes
G4	2	-	Yes

Example 4 Assessment of protection in cattle of different experimental FMD A24 Cruzeiro vaccines (Baculovirus expressing wildtype FMDV) in FMDV challenge study

The goal of the study was to test, in cattle, the efficacy against an FMD virulent challenge of 3 experimental FMD A24 Cruzeiro antigens, formulated in TS6/saponin adjuvant. Vaccines containing FMD A24 Cruzeiro antigen expressed in either, Baculovirus (BacMEB084, filtered or not filtered) or Canarypoxvirus were administered on D0 to bovines. Vaccine protection was assessed (according to the relevant European Pharmacopoeia Monograph), against a virulent FMDV A24 Cruzeiro challenge performed on D21.

Table 3.1 Vaccination scheme

Group	No. Cattle (7-9 months of age on D0)	Vaccine administered on D0	Volume injected	FMD A24 Cruzeiro challenge on D21
G1	5	A24 (BAC filtered* + TS6/Saponin)	2 mL	Yes
G2	5	A24 (BAC filtered + TS6/ Saponin)	0.5 mL	Yes
G3	5	A24 (BAC filtered + TS6/Sap)	0.125 mL	Yes

G4	2	- (Controls)	-	Yes
G5	5	A24 (BAC not filtered** + TS6/Sap)	2 mL	Yes
G6	5	A24 (BAC not filtered + TS6/Sap)	0.5 mL	Yes
G7	5	(vCP2166*** + TS6/Sap)	2 mL	Yes

A24 BAC filtered*: baculo infectiour titer 8.71 $log_{10}CCID_{50}$/ml
A24 BAC not filtered**: baculo infectiour titer 5.58 $log_{10}CCID_{50}$/ml, 10^8 VLPs
vCP2166***: baculo infectiour titer 8.8 $log_{10}CCID_{50}$/ml

Table 3.2 Summary of the FMD feet lesions observed at necropsy and percentages of protection

Group	FMD specific lesions Number of animal with feet lesions (number of feet affected per animal)	Percent Protection
G1	0 (0, 0, 0, 0, 0)	100
G2	0 (0, 0, 0, 0, 0)	100
G3	0 (0, 0, 3, 4, 0)	60
G4	2 (4, 4)	0
G5	0 (0, 0, 0, 0, 0)	100
G6	1 (0, 0, 1, 0, 0)	100
G7	1 (0, 0, 1, 0, 0)	80

The two Baculovirus vaccines were demonstrated protective in 100% of the animals at full and quarter vaccine dose. The filtered Baculovirus vaccine was still protective in 60% of the animals at a 1/16 vaccine dose. The Canarypoxvirus vaccine was demonstrated protective in 80% of the animals at full dose. FIGs. 11A, 11B and FIG. 12C present graphs and table depicting the FMDV A24 Cruzeiro antibody titers over time. The results show that three weeks after vaccination (D21), a clear sero-conversion was observed in all vaccinated calves. There was a dose/effect relation among the baculo vaccinated groups (G1-G2-G3 and G5-G6). Controls were negative on both dates. All vaccinated calves strongly sero-convereted after challenge (D25 and D28). The sero-response appeared more intense on D28 than D25. Sero-conversion in the controls was rather weak. FIGs. 12A and 12B present the evolution of temperature over time. The results show that only control presented significant temperature increase. The vaccines didn't increase the rectal temperature.

Using these data, the PD50 was calculated using logistic regression and Spearman-Kärber methods. The logistic regression method estimated the strength of vaccine A > 16 PD_{50}. The Karber method estimated the strength of vaccine A > 26PD_{50}. While there was a difference between the estimates, both methods suggest a highly significant and substantial efficacy of vaccine A. Given the clinical results, similar estimates for vaccine B also suggested a great efficacy of vaccine B.

Example 5 Immunogenicity in piglets of experimental FMDV formulations using Baculovirus

The objective of this study was to assess the immunogenicity of 4 FMDV antigens expressed in Baculovirus and formulated in TS6 or TS6+Saponin. The vaccines were prepared according to the following proportions (Tables 4.1).

Table 4.1 vaccine formulation

BACMEB097	FMD A24 modified (covalent cage) antigen (aqueous solution, 656.5 µL), saponin (10 hemaglutinin units), ester of fatty acids and or polyols (12 µL), triester of fatty acids and of ethoxylated polyols (68 µL), light paraffin oil (586.7 µL), ester of fatty acids and of ethoxylated polyols (20% solution, 75 µL), and phosphate buffer (to a 2 mL dose).
BACMEB095	FMD O1 Manisa wild-type antigen (aqueous solution, 656.5 µL), saponin (10 hemaglutinin units), ester of fatty acids and or polyols (12 µL), triester of fatty acids and of ethoxylated polyols (68 µL), light paraffin oil (586.7 µL), ester of fatty acids and of ethoxylated polyols (20% solution, 75 µL), and phosphate buffer (to a 2 mL dose).
BACMEB084	FMD A24 wild-type antigen (aqueous solution, 656.5 µL), saponin (10 hemaglutinin units), ester of fatty acids and or polyols (12 µL), triester of fatty acids and of ethoxylated polyols (68 µL), light paraffin oil (586.7 µL), ester of fatty acids and of ethoxylated polyols (20% solution, 75 µL), and phosphate buffer (to a 2 mL dose).
BACMEB084	FMD A24 wild-type antigen, aqueous solution, 656.5 µL), ester of fatty acids and or polyols (12 µL), triester of fatty acids and of ethoxylated polyols (68 µL), light paraffin oil (586.7 µL), ester of fatty acids and of

	ethoxylated polyols (20% solution, 75 µL), and phosphate buffer (to a 2 mL dose).

The antigens studied differed by the serotype (A24 Cruzeiro or O1-Manisa), the method of production (insect cells or silkworm) or the insert (standard or covalent cage). The term "covalent cage" refers the establishment of a non-naturally occurring disulfide bond in the FMDV P1 peptide, which is accomplished by substitution of a cysteine residue. The assessment was performed in young piglets, vaccinated intramuscularly twice, at a 21 days interval. The piglets were monitored for general reactions following each vaccination and were monitored for FMDV-A24 or FMDV-O1M neutralizing antibody titers on D1, D20 and D42.

Table 4.2 vaccination groups

Group	**# Pigs**	**Active Ingredient (AI)**	**Insert**	**AI Culture**	**Adjuvant**
G1	7	A24 Cruzeiro	Wildtype	SF9 cells	TS6
G2	7	A24 Cruzeiro	Wildtype	SF9 cells	TS6+Saponin
G3	7	A24 Cruzeiro	Covalent cage	SF9 cells	TS6+Saponin
G4	7	A24 Cruzeiro	Wildtype	Silkworm	TS6+Saponin
G5	7	O1-Manisa	Wildtype	SF9 cells	TS6+Saponin
G6	7	-	-	-	-

The O1-Manisa baculovirus-expressed antigens did not elicit sero-conversion in any of the animals in G5. These data demonstrated, for the first time, that baculovirus-produced FMDV subunits containing the "covalent cage" mutation were immunogenic (see Table 5 below). Moreover, the data here strongly showed that the "caged" FMDV subunits were significantly more productive, effective and stable, when compared to the unmodified wildtype FMDV. Further, the silkworm-based vaccine did not appear to contain significantly more antigen than the other vaccines tested.

Table 5

Group	FMD-A24 Neutralizing titer ($Log10PD_{50}$)	Number exceeding threshold

	D1		D20		D42		(associated with protective immunity)*	
	Mean	sd**	Mean	sd	Mean	sd	D20	D42
G1	0.90	0.12	1.26	0.19	1.56	0.17	1/7	6/7
G2	1.03	0.13	1.29	0.17	1.67	0.20	2/7	7/7
G3	>0.96	0.24	1.46	0.27	2.08	0.29	3/7	6/6
G4	>1.09	0.17	1.48	0.13	1.58	0.23	4/7	4/6
G6 control	0.92	0.18	1.07	0.13	0.94	0.17	0/7	0/7

*: titer $\geq$ 1.35 log10PD_{50}; sd**: standard deviation.

In conclusion, as indicated by the results in Table 5, all SF9 cell-cultured, baculovirus-expressed A24 VLPs (groups G1, G2 and G3) yielded a significant "booster" effect (i.e. second dose significantly increased the number of seroconverting animals.) In contrast, the silkworm cultured A24 VLPs appear to have elicited an initial and persistent serological response, without having elicited an apparent booster effect.

Example 6 Acid and heat stability of the baculovirus-expressed A24 FMDV VLPs

A24 FMDV VLPs were subjected to acid and heat treatments, and their stability was evaluated using EM (FIG. 13) and ELISA analysis (FIG. 14). As indicated in the figures, the covalent cage VLPs, but not the wild type VLPs, were significantly resistant to both low pH and heat. Moreover, as indicated in FIG. 15 and Table 6, the covalent cage VLP were significantly more stable over time when stored at 5°C, relative to their wild type counterparts.

Table 6 Stability of A24 VLPs (covalent cage vs. wild type)

	T0	T1month	T6months	T9months	T12months
VLPs FMDV A24 Covalent cage	10E9	5.10E8	3.10E8	3.10E9	10E8
VLPs FMDV A24 Wild Type	10E9	5.10E8	5.10E7	No VLPs observed	No VLPs observed

In conclusion, the stabilization of VLPs from the A24 Cruzeiro serotype with the "covalent cage mutation" was highly effective regarding resistance to heating or acidification treatments. Covalent cage stabilized VLPs after an 18 month storage. Several FMDV serotypes have now been shown to be effectively stabilized by the introduction of the covalent cage mutation, including: A24 Cruzeiro; O1 Manisa (FIG. 16, EM & FIG. 17, ELISA); Asia 1 Shamir; and A22 Iraq. FIG. 17 shows no impact of heating on stabilized VLPs (covalent cage, BacMEB099) whereas no signal was detected for standard FMDV O1-Manisa active ingredient after heating indicating no assembled VLPs for standard FMDV O1 Manisa after heating.

Example 7 Immunogenicity of vaccines formulated with baculovirus FMD O1 Manisa (modified, covalent cage) in conventional piglets

The objective of the study was to assess the safety and efficacy in conventional pigs, of two Baculovirus FMD O1-Manisa antigens, formulated in TS6 and one Adenoviral-vectored FMDV O1-Manisa. Each formulation was administered in two injections three weeks apart (D0 – D21). Pigs were 11-12 weeks of age on D0 and received 2mL intra-muscular infection of the vaccines. Safety was assessed through monitoring of local and general reactions. Efficacy was assessed through serological (Sero-neutralization titers) monitoring (D1, D20, D43) and cell mediated immunity (CMI) assays (D27, D43).

Table 7.1 Characteristics of the antigens and vaccines

Group	vaccine	type	adjuvant
G1	Bac099 FMDV VLP covalent cage batch 1	O1-Manisa (covalent cage)	TS6
G2	Bac099 FMDV VLP covalent cage batch 2	O1-Manisa (covalent cage)	TS6
G3	AFTOPOR™ Classical Inactivated FMDV vaccine (O1 manisa)	O1-Manisa O Thailand O1 BSF	-
G4	Adenoviral-vectored FMDV	O1-Manisa	-
G5	control	-	-

Blood samples were collected on D27 and D43 for Cell Mediated Immunity (CMI) assays. Samples were assayed after re-stimulation either for γ Interferon (IFNγ) secreting cell quantification, plasma cell quantification or memory B cell quantification (see Table 7.2). All quantifications were performed by ELISpot.

Table 7.2 CMI assays

Quantification assays	Ex vivo re-stimulation with	D27	D43
IFNγ secreting cells	VLP FMDV O1 M + irrelevant control	+	NT
	Peptide pool O1 M + irrelevant control	+	NT
Specific IgG secreting plasma cell	VLP FMDV O1 M + irrelevant control	+	NT
Specific IgG secreting memory B cells	VLP FMDV O1 M + irrelevant control	NT	+

+: tested; NT: not tested

The results showed surprisingly that unlike baculovirus expressed wild-type FMDV O1 M (see example 5), the baculovirus expressed modified (covalent cage) FMDV O1 M elicited secoconversion in piglets. As shown in Table 7.3 below, after first vaccination (D0), seroconversion was observed in the 2 baculovirus groups (G1 and G2) on D20. After second vaccination, booster effect was observed in both groups (G1 and G2).

Table 7.3 Serology study of baculovirus FMD O1 Manisa in piglets

Serology Evaluation	# piglets	SN titer (log 10) D1	SN titer (log 10) D20	SN titer (log 10) D43
G1	7	1.19	1.65	1.91
G2	7	1.48	1.49	1.80
G3	4	1.50	2.22	2.67
G4	5	1.32	1.74	1.77
G5	1	NT	NT	1.27

As shown in Figure 20, specific IgG secreting plasma cells were detected in both baculovirus expressed FMDV-VLP (modified, covalent cage)/TS6 vaccinated groups (G1 and G2). The specific IgG secreting plasma cells were significantly higher in G1 and G2 than in G4. No specific IgG secreting plasma cells were detected in G3.

Figure 21 shows that high numbers of specific IgG secreting memory B cells were detected in all vaccinated pigs in groups G1 and G2, a weak portion of specific IgG secreting

memory B cells were detected in G3, and no specific IgG secreting memory B cells were detected in G4.

Figure 22 shows that specific IFNγ secreting cells were detected in all vaccinated groups in the order of G1 and G2 > G4 > G3. There is no difference between G1 and G2. The specific IFNγ secreting cells were significantly higher in G1 and G2 than in G3.

The high levels of specific IFNγ secreting cells, specific IgG secreting plasma cells and specific IgG secretingmemory B cells in both baculovirus expressed FMDV-VLP (modified, covalent cage)/TS6 vaccinated groups (G1 and G2) indicate good levels of protection against FMDV infections.

Example 8 Asia Shamir BacMEB102 (wild type) & BacMEB104 (covalent cage)

Table 8 Stability of Asia Shamir VLPs (BacMEB102, wild type and BacMEB104, covalent cage) measured by EM

	EM
A Shamir BacMEB102 (WT), P3 D4 pH7, heated	No VLPs
A Shamir BacMEB104 (covalent cage), P3 D4 pH7, heated	5.10E7; 1.0E9 (7 month); 2.10E9 (9 month); 10E8 (15 month)

The results showed that large quantities of stable FMD VLPs for BacMEB104 (covalent cate) were present after heating, and the VLPs were stable up to at least 15 months while the BacMEB102 (WT) didn't form any detectable VLPs after heating.

Example 9 Iraq 22 BacMEB106 (covalent cage) stability

Table 9 Stability of Iraq 22 VLPs (BacMEB105, wild type and BacMEB106, covalent cage) measured by EM after heating

AI	EM	Biacore
A22-Iraq BacMEB106	3 x 10E9; 5 x10E8 (3 months)	+

Compared to covalent cage Iraq22 VLPs, wild type Iraq22 VLPs were not stable after heating at Day 0.

Example 10 Asia Shamir covalent cage & Iraq A22 covalent cage serology study in pigs

The objective of the study was to assess the immunogenicity in conventional pigs, of FMD Asia1 Shamir and FMD A22 Iraq VLP antigens, produced in Baculovirus. Two batches of Asia1 Shamir antigen and 2 batches of A22 Iraq antigens were tested in TS6 formulations. Each formulation was administered in two injections three weeks apart (D0 – D21) to pigs. Pigs were 8-9 weeks of age on D0. Immunogenicity was assessed through serological monitoring (D-1, D20, D42) and cell mediated immunity (CMI) assays (D26, D41).

Table 10 Vaccination scheme of Asia Shamir VLPs (BacMEB104, covalent cage) and A22 IraqVLPs (BacMEB106, covalent cage), two injections at Do and D21

Group	Antigen	Adjuvant
G1 (n=5)	Asia1 Shamir VLP CC	TS6
G2 (n=5)	A22 Iraq VLP CC	TS6
G3 (n=4)	Asia1 Shamir VLP CC	TS6
G4 (n=4)	A22 Iraq VLP CC	TS6
G5 (n=2)	Unvaccinated	-

As indicated in FIG. 18, covalent cage VLPs from both strains elicited strong, serotype-specific, serological responses.

All pigs vaccinated with the VLP Asia1 Shamir (G1 & G3) had clearly sero-responded to first vaccination, with an average titer close to 1.20 Log10 PD50. Further, there was a clear booster effect following second vaccination, with most pigs exceeding a titer of 1.8 Log10 PD50. The controls (G5) and most of the A22 Iraq vaccinated pig (G2 & G4) did not show any sero-response against Asia1 Shamir, even after a booster vaccination.

All pigs vaccinated with the VLP A22 Iraq (G2 & G4) had sero-responded to first vaccination, with an average titer close to 1.15 Log10 PD50. Further, there was a strong booster effect following second vaccination, with most pigs exceeding a titer of 2.0 Log10 PD50. The controls (G5) and most of the Asia1 Shamir vaccinated pig (G1 & G3) did not show any sero-response against A22 Iraq, even after a booster vaccination.

Surprisingly, cross-reactivity was observed, as indicated in FIG. 19. The results have shown that specific IFNγ response (cellular response) was detected in both Asia1 Shamir VLP groups when Asia1 Shamir FMDV peptide pool was used, and specific IFNγ response (cellular response) was detected in both A22 Iraq VLP groups when A22 Iraq FMDV peptide pool was used. The Asia1 Shamir VLP group showed cross-immunogenicity when A22 Iraq FMDV peptide pool was used. Specific plasma cells (humoral response) were detected in both Asia1 Shamiar VLP groups and both A22 Iraq VLP groups with Asia1 Shamir inactivated AI coating and A22 Iraq inactivated AI coating, respectively. Cross-immunogenicity (plasma cells) in Asia1 Shamir VLP groups was observed with A22 Iraq inactivated AI coating. Specific memory B cells (humoral response) were detected in both Asia1 Shamiar VLP groups and both A22 Iraq VLP groups with Asia1 Shamir AI coating and A22 Iraq AI coating, respectively. Good cross-immunogenicity (B cells) in Asia1 Shamir VLP groups was observed with A22 Iraq AI coating. Some cross-immunogenicity (B cells) in A22 Iraq VLP groups was also observed with Asia1 Shamir AI coating. Taken together, the results indicate the VLPs could elicit an immune response sufficient to protect against heterologous challenge.

In conclusion, the 4 FMDV serotypes-VLPs antigens (A24, O1 Manisa, Asia1 Shamir and A22 Iraq) adjuvanted in TS6 induced humoral and cellular responses with strong, serotype-specific, neutralizing antibody responses, consistent proportion of specific IFNγ responses, presence of memory B cells (with cross-reactivity between the 2 serotypes), indicating good levels of protection against homologous and heterologous FMDV infections.

Example 11 Construction of human adenovirus 5 vectored recombinant FMDV

The goal of the study is to generate an adenovirus recombinant expressing the codon optimized FMDV structural proteins and non-optimized 3C protease with a C142T site mutation for serotype A24 Cruzeiro. The FMDV antigen contains FMDV capsid precursor (VP1, VP2 (with H93C site mutation, covalent cage), VP3, VP4, 2A, and full 2B codon optimized) and a non-optimized partial 3B and full length 3C protease with C142T site mutation (SEQ ID NO:16).

HEK 293 cells (ATCC) were maintained in MEM (Gibco #11095) with 10% Fetal Bovine serum (Moregate Batch #81827101) at 37 °C in 5% CO2. These cells were used to rescue the recombinant adenovirus vAD3027 and make virus stocks.

The expression plasmid pAD3027 (see FIG.25) contains codon-optimized polynucleotide (SEQ ID NO:17 encoding FMDV capsid protein SEQ ID NO:16) linked to CMV promoter and SV40 polyA tail. The polynucleotide includes a synthetic intron.

The expression clones were generated by LR recombination of entry vector with destination vector using Gateway technology (Invitrogen). Recombinant adenovirus vAD3027 were generated by transfection of linearized expression clones in HEK 293 cells with transfection reagent. After rescue, each virus was harvested by freeze-thaw cycle and clarification the cell debris by centrifugation. For passage, each virus was inoculated into monolayer of HEK 293 cells and approximately 3-4 days post infection, virus was harvested by freeze-thaw cycle and clarification by centrifugation. Five passages were conducted to make virus stock, which was stored at -80 °C.

Sequece analysis of the recombinant clone has confirmed that sequences from the beginning of the CMV promoter to the end of the SV40 tail were correct (SEQ ID NO:20). The immunofluorescence testing showed that all examined plaques of vAD3027 were found to express FMDV capsid protein. Western Blot using the antibody against VP2 protein detected the liner epitope of VP2 either as VP2 (fully processed ~25 kDA), VP0 (partially processed transgene ~37 kDa) or P1 (unprocessed transgene ~80 kDa) expressed by vAD3027 (see FIG. 26).

The adenovirus recombinants expressing the codon-optimized FMDV modified (covalent cage) capsid protein for O1 Manisa, Asia and Iraq strains were constructed according to the precedures described above.

Example 12 Serology Assessment of Various Foot and Mouth Disease Virus (FMDV) Vaccine Candidates Following Vaccination in Pigs

Example 12.1 Serology Assessment of FMDV A24 vaccines

The goal of the study is to evaluate the immunogenicity of various FMDV A24 vaccine formulations including human adenovirus 5-vectored FMDV vaccine and Baculovirus expressed FMD A24 recombinant VLP (covalent cage) vaccine in conjunction with different adjuvants (or without) following vaccination in pigs.

Sixty conventionally reared piglets (approximately 1 month of age) were each randomized to one of nine treatment groups each containing 4-7 pigs. The resulting group composition is presented in Table 11 below.

Table 11 Vaccination scheme of human adenovirus 5-vectored FMDV vaccine and Baculovirus expressed FMD A24 recombinant VLP vaccine, two injections at D0 and D21

Group	Vaccine**	Adjuvant (s)	No.of Animals
1	BacA24VLP[1] (TV1)	TS6	5
2	BacA24VLP (TV2)	TS6 +Carbopol	6
3	BacA24VLP (TV3)	TS6 +polymer	6
4	BacA24VLP (TV4)	ISA206 + carbopol	6
5	BacA24VLP (TV5)	ISA206 + polymer	3
6	vAD3027[2] (TV6)	Poly IC	7
7	vAD3027 (TV8)	Poly ICLC	7
8*	vAD3027 TV7 (D0); BacA24VLP TV1(D21)	N/A(D0); TS6 (D21)	7
9	control	N/A	4

TV: Test Vaccine.
BacA24VLP[1]: Baculovirus expressed FMD A24 recombinant VLP (covalent cage) vaccine.
vAD3027[2]: human adenovirus 5-vectored FMDV A24 (covalent cage) vaccine.
*Group 8 received the TV7 at D0 and the TV1 at D21.
**The target dose per piglet was: $10^{3.9}$ VLPs in 0.6 mls of active ingredient for those vaccinated with the BacA24VLP and $10^{10.35}$ $TCID_{50}$ for those vaccinated with the vAD3027 construct.

Each piglet was vaccinated, except those from Group 9 (control), twice at a 21- day interval, with 2 ml of the test vaccine. All injections were given via the intramuscular route (IM) in the neck region, caudal to the ear, alternately on the right and left sides.

Blood samples were collected from all piglets on Days 0 (prior to vaccination), 7, 15, 21 (prior to vaccination), 28, 35 and 42. Day 42 serum samples from all piglets were tested for FMDV antibodies by Serum Virus Neutralization (SVN). Samples from those vaccinated with the vAD3027 construct and control (Groups 6-9) were subject to SVN assay on all collection days.

All piglets from Groups 1-9 tested negative for FMDV antibodies prior to the start of the study. All piglets in control were negative for FMDV antibodies throughout the study.

The FMDV SVN results are shown in FIG. 23. The results demonstrated that both FMD A24 recombinant VLP vaccine and human adenovirus 5-vectored FMDV vaccine induced immune response in animals. At a low dose of $10^{3.9}$ VLPs, FMD A24 recombinant VLP vaccine induced good immune response in the animals. Animals vaccinated with the human adenovirus 5-vectored FMDV vaccine (groups 6-7) had a higher antibody response following a two-dose vaccination regimen than those vaccinated with the low dose FMD A24

recombinant VLP vaccine. Surprisingly, heterologous prime-boost regimen with viral vectored FMDV vaccine and BacA24 VLP vaccine demonstrated stronger immune response than prime-boost with the same vaccines.

FIG. 24 shows the FMDV antibody titers in Groups 6-9 over the course of study (day 0 – day 42). By Day 15 (2 weeks following the 1st vaccination), all vAD3027 vaccinates from Group 8 seroconverted to FMDV. Those in Groups 6-7 had between 29-57% of the piglets seroconvert. In addition, the mean antibody titer per group was higher in group 8 (heterologous prime-boost regimen with viral vectored FMDV vaccine and BacA24 VLP vaccine) as compared to those in Groups 6-7.

Antibody responses to Adenovirus (SVN) were determined in all animals from all groups in samples collected on Day 42. The results were reported in Log_{10} and a value ≤ 0.6 Log_{10} was considered negative for serum antibody.

The results showed that all control and piglets vaccinated with the BacA24VLP formulated vaccines were negative to Adenovirus by SN antibody titers on Day 42 (≤ 0.6 Log_{10}). All animals vaccinated with the vAD3027 in groups 6 and 7 seroconverted with titers ranging between 1.8 and 3.0, while 50% of the piglets in group 8 seroconverted. Overall, animals in group 6 had a higher antibody response to adenovirus.

Example 12.2 Serology Assessment of FMDV O1 Manisa, Asia and Iraq vaccines

The goal of the study is to evaluate the immunogenicity of various FMDV O1 Manisa, Asia and Iraq vaccine formulations including human adenovirus 5-vectored FMDV vaccine and Baculovirus expressed FMD O1 Manisa, Asia and Iraq recombinant VLP (covalent cage) vaccine in conjunction with different adjuvants (or without) following vaccination in pigs.

The study design is as described in Example 12.1. The blood samples from the vaccinated animals are taken at various stages as described in Example 12.1 and tested for serology. The results show that the composition or vaccine of the present invention is immunogenic and provides protection in animals.

Example 14 Serology Assessment and efficacy of Various Foot and Mouth Disease Virus (FMDV) Vaccine Candidates Following Vaccination in in conventional Swine and Cattle and MDA-positive Swine and Cattle

The goal of the study is to evaluate the prime-boost administration (two administrations) of two heterologous vaccines or administration at the same time (one administration) of two heterologous vaccines in conventional swine or cattle to increase immune response, and also in MDA-positive pigs and cattle to overcome MDA and increase immune response. The heterologous vaccines may be different types of vaccines, such as FMDV VLPs vaccine or FMDV viral vector vaccine expressing the capsid from the same FMDV serotype. The heterologous vaccines may also be the same type of vaccines expressing the capsid of different FMDV serotypes, such as A24, O1 Manisa, Asia or Iraq strains. The heterologous vaccines may also be different types of vaccines expressing the capsids of different FMDV serotypes, such as A24, O1 Manisa, Asia or Iraq strains. The heterologous vaccines may also be different types of vaccines, i.e. FMDV VLPs vaccine or FMDV viral vector vaccine, expressing the capsids of different FMDV serotypes, such as A24, O1 Manisa, Asia or Iraq strains.

In one group, conventional pigs or cattle are vaccinated twice at an interval of between 3-5 weeks with FMDV VLPs vaccine and followed by recombinant viral FMDV vaccine, or primed with recombinant viral FMDV vaccine and boosted with FMDV VLPs vaccine. In another group, conventional pigs or cattle are vaccinated once with both FMDV VLPs and recombinant viral FMDV vaccines at the same time.

In one group, pigs or cattle which are MDA-positive are vaccinated twice at an interval of between 3-5 weeks with FMDV VLPs vaccine and followed by recombinant viral FMDV vaccine, or primed with recombinant viral FMDV vaccine and boosted with FMDV VLPs vaccine. In another group, pigs or cattle which are MDA-positive are vaccinated once with both FMDV VLPs and recombinant viral FMDV vaccines at the same time.

The animals are challenged with homologous or heterologous FMDV strains after the vaccination.

The protective efficacy induced by the composition or vaccine is evaluated against the FMDV pathogen by vaccination challenge in conventional animals or MDV-positive animals. The protective effect is evaluated by clinical observations and/or viral load of the specific pathogen in tissues and blood. The blood samples from the vaccinated animals are taken at various stages and tested for serology. The results show that the composition or vaccine of the present invention is immunogenic and provides protection in in conventional animals and MDV-positive animals.

***

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above examples 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 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 we claim is:

1. A composition or vaccine comprising a foot and mouth Disease Virus (FMDV) antigen or a recombinant viral vector expressing an FMDV antigen.
2. The composition or vaccine of claim 1, wherein the FMDV antigen forms FMDV VLPs or empty capsids.
3. The composition or vaccine of claim 1 or 2, wherein the FMDV antigen is expressed by a baculovirus vector in insect cells.
4. The composition or vaccine of claim 1, wherein the viral vector is an adenovirus.
5. The composition or vaccine of any one of claims 1-4, wherein the FMDV antigen is a wild-type P1 polypeptide.
6. The composition or vaccine of any one of claims 1-5, wherein the FMDV antigen is a modified P1 polypeptide.
7. The composition or vaccine of any one of claims 1-6, wherein the FMDV antigen comprises a polypeptide having the sequence as set forth in SEQ ID NO:1, 2, 4, 5, 6, 8, 10, 12, 13 or 16.
8. The composition or vaccine of any one of claims 1-7, wherein the FMDV antigen is encoded by a polynucleotide having the sequence as set forth in SEQ ID NO:3, 7, 9, 11, 14, 15, 17 or 20.
9. The composition or vaccine of any one of claims 1-8, wherein the FMDV antigen forms a non-natually occurring disulfide bridge that provides enhaced heat and acid stability of VLPs or empty capsids.
10. The composition or vaccine of claim 1 or 4, wherein the modified P1 polypeptide comprises a cysteine substitution at position corresponding to amino acid 179 of SEQ ID NO:2, 4, 6, 8, 10, or 16.
11. The composition or vaccine of any one of claims 1-10, wherein the composition or vaccine further comprises a pharmaceutically or veterinarily acceptable carrier, excipient, adjuvant, or vehicle.
12. A plasmid comprising a polynucleotide encoding an FMDV antigen having the sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 6, 8, 10, 12, 13, or 16.
13. The plasmid of claim 12, wherein the polynucleotide has the sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 14, 15, 17, or 20.
14. The plasmid of claims 12 or 13, wherein the polynucleotide is operably linked to a promoter.

15. A stably transformed insect cell expressing FMDV empty capsids or FMDV VLPs.
16. A recombinant viral vector comprising one or more heterologous polynucleotides coding for and expressing one or more FMDV antigens.
17. The recombinant viral vector of claim 16, wherein the viral vector is an adenovirus.
18. The recombinant vial vector of claim 16 or 17, wherein the FMDV antigen comprises a polypeptide having the sequence as set forth in SEQ ID NO:1, 2, 4, 5, 6, 8, 10, 12, 13 or 16.
19. The recombinant viral vector of any one of claims 16-18, wherein the FMDV antigen is encoded by a polynucleotide having the sequence as set forth in SEQ ID NO:3, 7, 9, 11, 14, 15, 17 or 20.
20. A substantially purified FMDV empty capsid or FMDV VLP expressed in insect cells, wherein the FMDV empty capsid or VLP comprises a polypeptide having the sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 6, 8, 10, 12, 13, or 16.
21. The FMDV empty capsid or VLP of claim 20, wherein the polypeptide is a modified FMDV P1 comprises a cysteine substitution at position corresponding to amino acid 179 of SEQ ID NO:2, 4, 6, 8, 10 or 16.
22. A method of vaccinating an animal susceptible to FMDV infection or eliciting an immune response in the animal against FMDV comprising at least one administration of the composition of any one of claims 1- 11, or the viral vector of claims 16-19, or the FMDV empty capsids or VLPs of any one of claims 20-21.
23. The method of claim 22, wherein the method comprises a prime-boost administration regimen.
24. The method of claim 23, wherein the prime-boost regimen comprises a prime-administration of a composition according to claim 1, and a boost administration of a composition comprising a recombinant viral vector comprising a polynucleotide for expressing, *in vivo*, an FMDV antigen, to protect the animal from FMDV and/or to prevent disease progression in infected animal.
25. The method of claim 23, wherein the prime-boost regimen comprises a prime-administration of a composition comprising a recombinant viral vector comprising a polynucleotide for expressing, *in vivo*, an FMDV antigen, and a boost administration of a composition according to claim 1 to protect the animal from FMDV and/or to prevent disease progression in infected animal.
26. The method of claim 22, wherein the method comprises one or more administrations of same or different FMDV compositions or vaccines.

27. The methoc of claim 26, wherein the FMDV compositions or vaccines comprise the FMDV VLPs or empty capsids expressed *in vitro* and a viral vector expressing FMDV antigens in vivo.
28. The method of any one of claims 22-27, wherein the method protects Maternally Derived Antibody-positive (MDA-positive) animals against FMDV infection.

Figure 1

SEQ ID NO:	type	Gene Description
1	protein	Wild-type polyprotein of FMDV A24 Cruzeiro Strain (GenBank AAT01711)
2	protein	Modified polyprotein of A24 Cruzeiro Strain in VP2 (H93C) or P1 (H179C) (in pMEB097)
3	DNA	Polynucleotide encoding modified polyprotein of FMDV A24 (in pMEB097)
4	protein	Unmodified Polyprotein of A24 Cruzeiro Strain (in pMEB084)
5	protein	Wild-type polyprotein of FMDV O1 manisa strain (GenBank AAT01766) (in pMEB095)
6	protein	Modified polyprotein of FMDV O1 manisa strain in VP2 (S93C) or P1 (S179C) (in pMEB099)
7	DNA	Polynucleotide encoding modified polyprotein of FMDV O1 manisa strain (in pMEB099)
8	protein	Modified polyprotein of FMDV Iraq strain (in pMEB106)
9	DNA	Polynucleotide encoding modified polyprotein of FMDV Iraq strain (in pMEB106)
10	protein	Modified polyprotein of FMDV Asia strain (in pMEB104)
11	DNA	Polynucleotide encoding modified polyprotein of FMDV Asia strain (in pMEB104)
12	protein	Wild-type polyprotein of FMDV Iraq strain (in pMEB105)
13	protein	Wild-type polyprotein of FMDV Asia strain (in pMEB102)
14	DNA	Polynucleotide encoding wild-type polyprotein of FMDV Iraq strain (in pMEB105)
15	DNA	Polynucleotide encoding wild-type polyprotein of FMDV Asia strain (in pMEB102)
16	protein	FMDV capsid precursor (VP1, VP2 (with H93C site mutation), VP3, VP4, 2A, and Full 2B codon optimized) and a non-optimized partial 3B and full length 3C protease with C142T site mutation (in vAD3027)
17	DNA	Codon-optimized polynucleotide encoding FMDV capsid precursor (in vAD3027)
18	DNA	CMV promoter
19	DNA	Synthetic enhancer
20	DNA	CMV promoter –synthetic enhancer- codon optimized FMDV capsid gene – SV40 PolyA in vAD3027

Figure 2

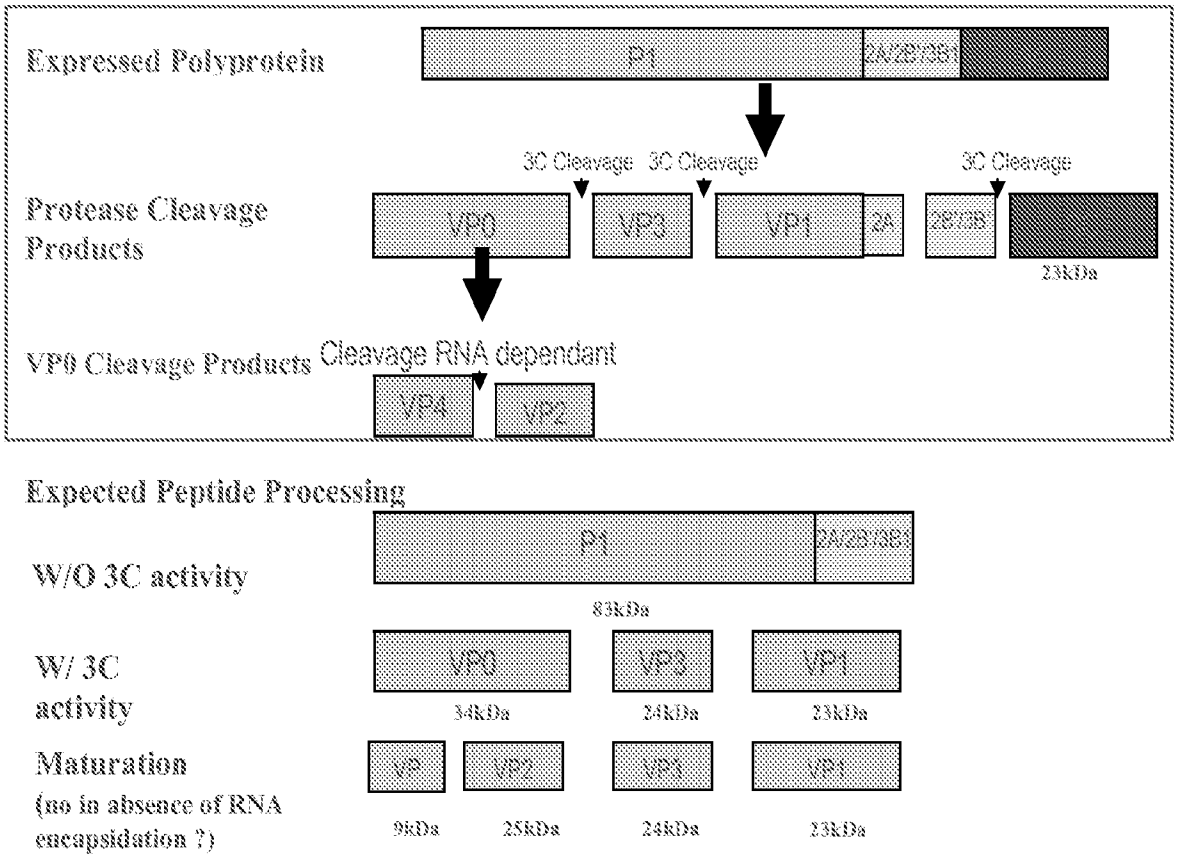

Expressed Polyprotein
P1
2A/2B/3B1
3C Cleavage
3C Cleavage
3C Cleavage
Protease Cleavage Products
VP0
VP3
VP1
2A
2B/3B
23kDa
VP0 Cleavage Products
Cleavage RNA dependant
VP4
VP2
Expected Peptide Processing
W/O 3C activity
P1
2A/2B/3B1
83kDa
W/ 3C activity
VP0
VP3
VP1
34kDa
24kDa
23kDa
Maturation
(no in absence of RNA encapsidation ?)
VP
VP2
VP3
VP1
9kDa
25kDa
24kDa
23kDa

Figure 3

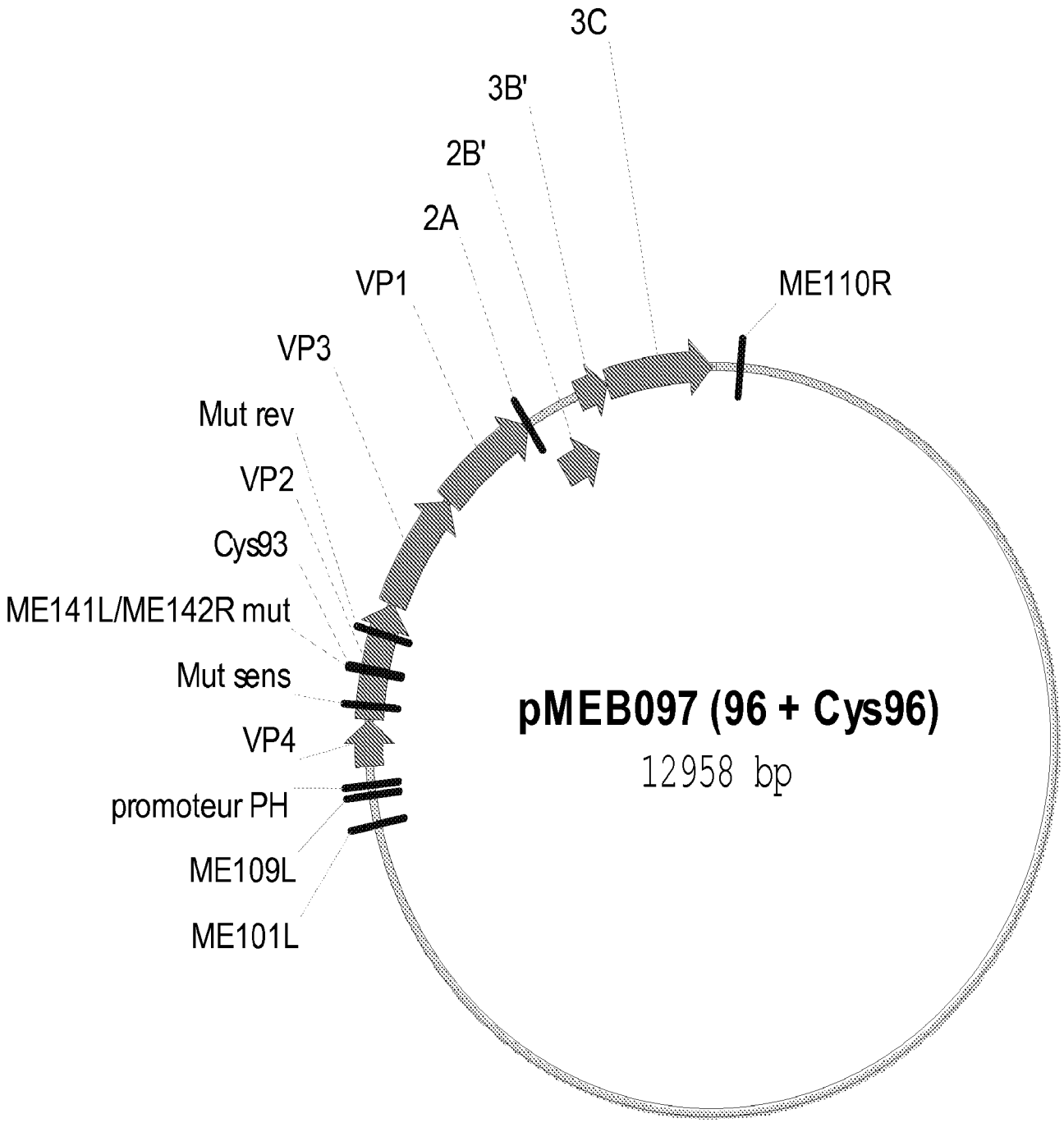

3C
3B'
2B'
2A
VP1
VP3
Mut rev
VP2
Cys93
ME141L/ME142R mut
Mut sens
VP4
promoteur PH
ME109L
ME101L
ME110R
pMEB097 (96 + Cys96)
12958 bp

Figure 4

Result of electronic microscopy of BacMEB097

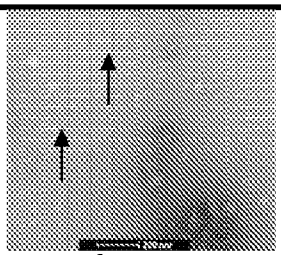
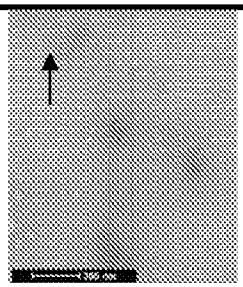
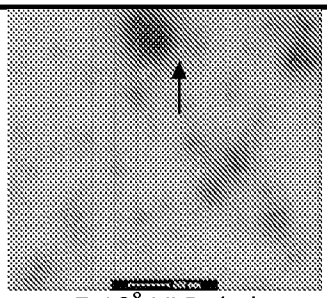
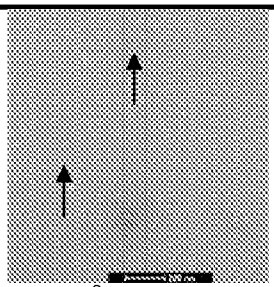
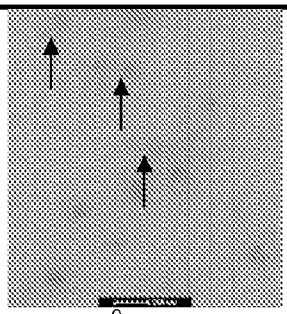
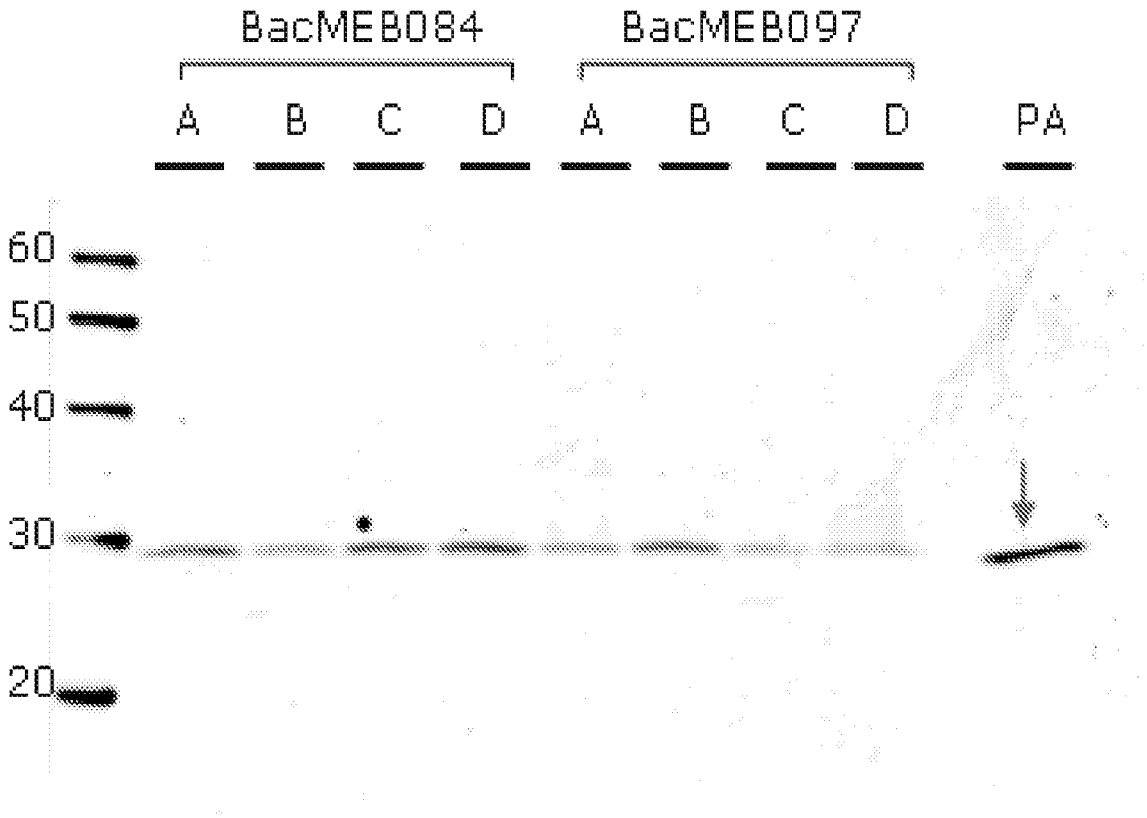
Recombinant Baculovirus Construction	**A** W/O treatment	**B** 1h at 56°C	**C** Acidification: pH=5
"classical A24" =**BacMEB084** Samples [] 4X	 10^8 VLPs/ml	 Only very few particles detected	No VLPs
"stabilized A24" =**BacMEB097** Samples [] 3,3X	 5.10^8 VLPs/ml	 10^9 VLPs/ml	 2.10^9 VLPs/ml

Figure 5

Detection by western blot of capsid protein of FMDV (A24 serotype)



A: no treatment (pH=6.1)
B: 1hr at 56°C
C: 4.7 < pH < 5
D: pH adjusted at 6.5
PA: inactivated FMDV A24

Figure 6

Electronic microscopy and specific ELISA of BacMEB099

Fig. 6A

Construct	Clone	MOI	Harvest at day 4 Num	Viability	[]
BacMEB099	**4**	0,45	2,80E+05	16,9%	12x

Fig. 6B

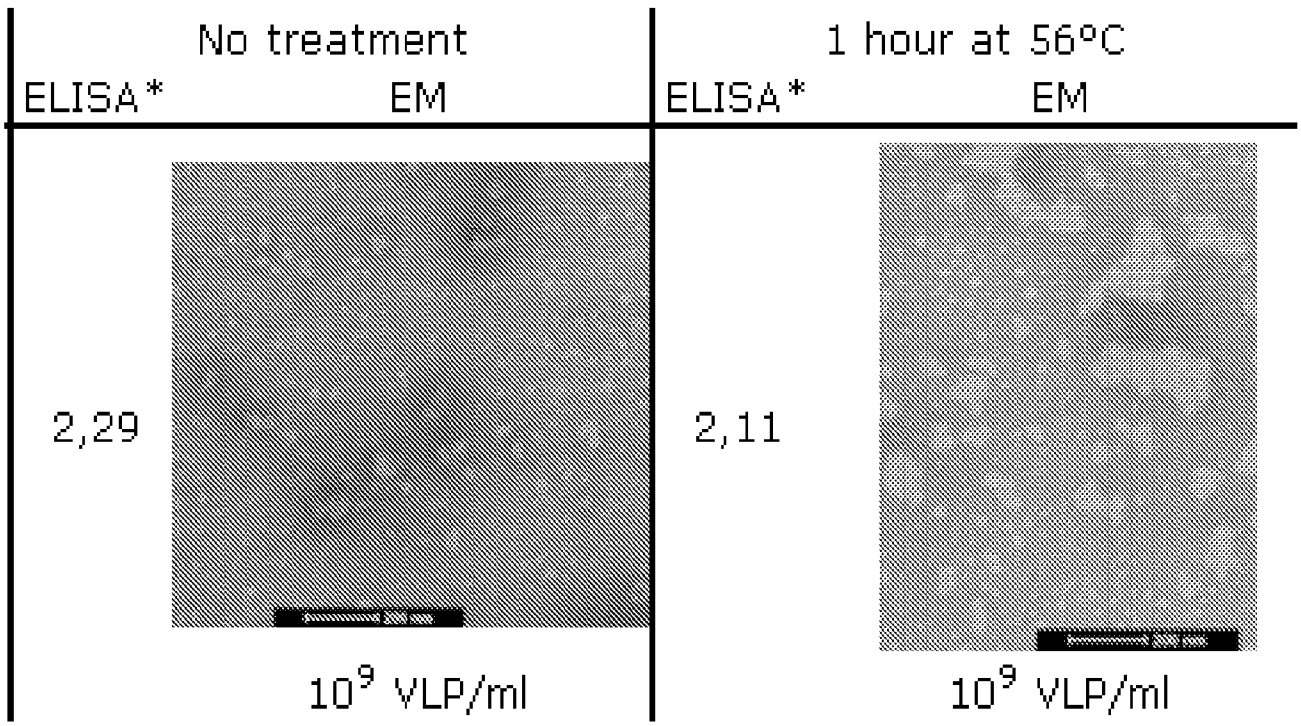


Figure 7A

Alignment of the amino acid sequences of the recombinant polyprotein in pMEB097 and and reference sequence (FMDV serotype A / Genbank (AAT01711) using the vector NTI program

Figure 7A (continued)

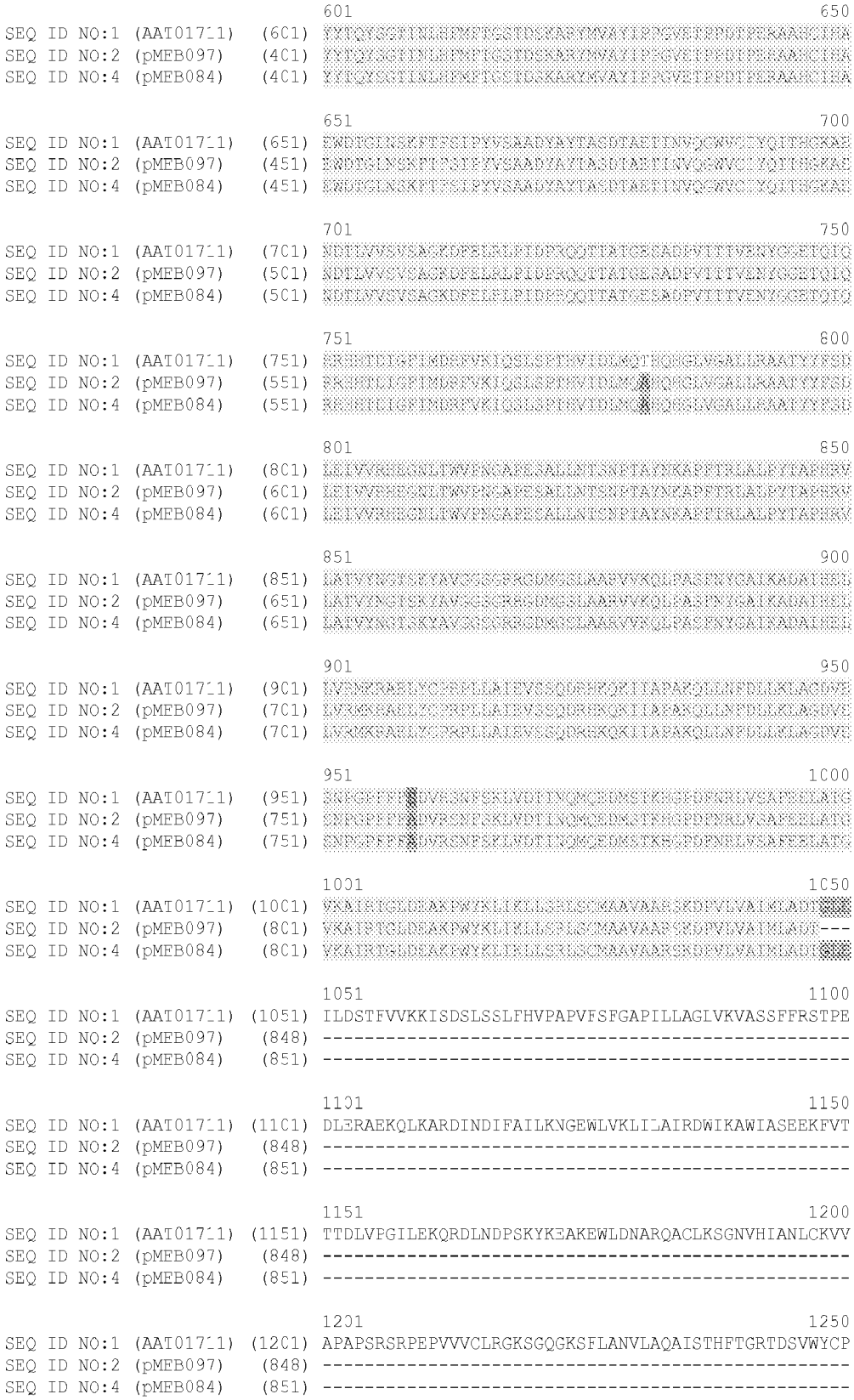


Figure 7A (continued)

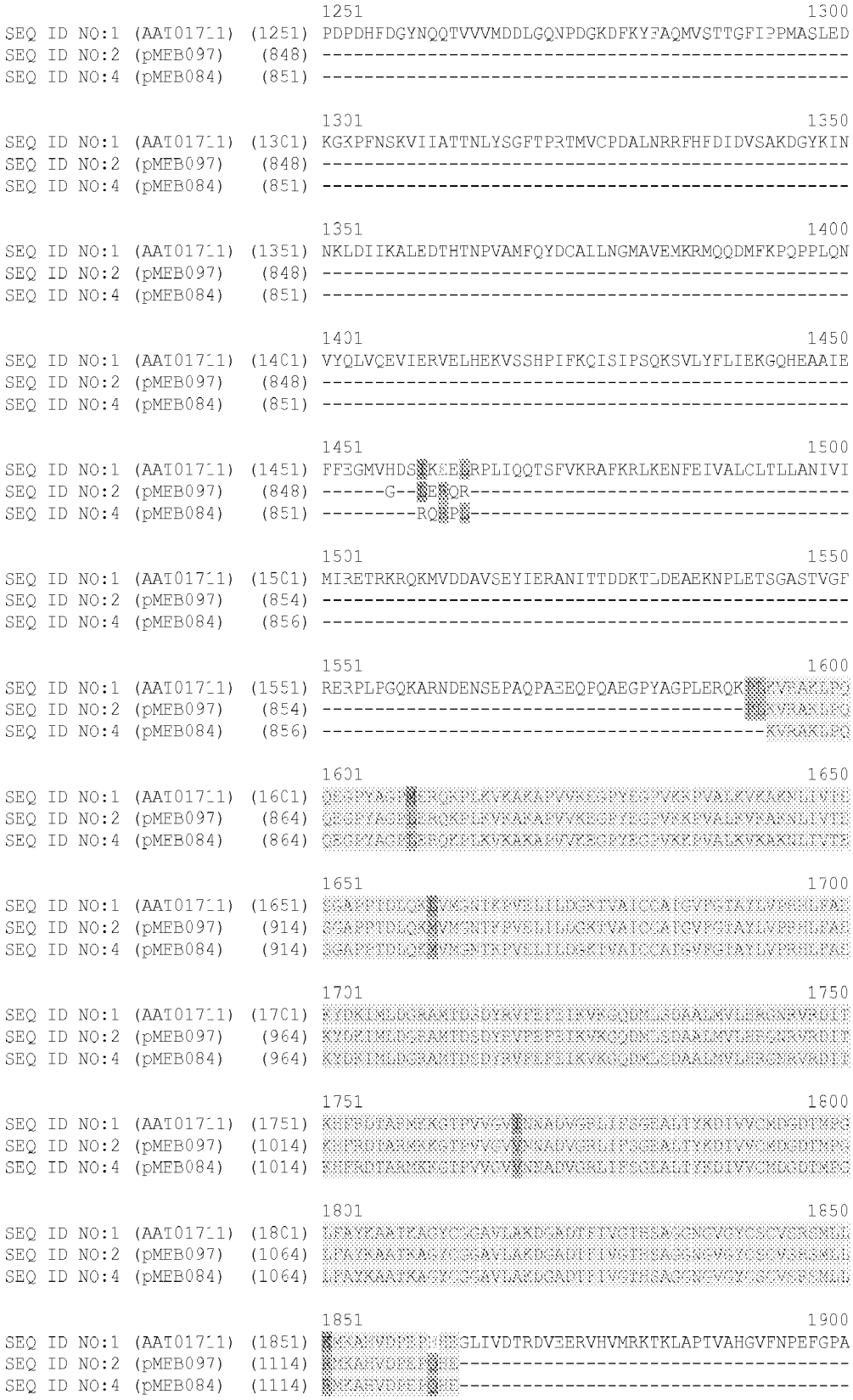


Figure 7A (continued)

```
                                1901                                        1950
SEQ ID NO:1 (AAT01721) (1901) ALSNKDPRLNDGVVLDEVIFSKHKGDTKMSEEDKALFRRCAADYASRLHS
SEQ ID NO:2 (pMEB097)  (1127) --------------------------------------------------
SEQ ID NO:4 (pMEB084)  (1127) --------------------------------------------------

                                1951                                        2000
SEQ ID NO:1 (AAT01721) (1951) VLGTANAPLSIYEAIKGVDGLDAMEPDTAPGLPWALQGKRRGALIDFENG
SEQ ID NO:2 (pMEB097)  (1127) --------------------------------------------------
SEQ ID NO:4 (pMEB084)  (1127) --------------------------------------------------

                                2001                                        2050
SEQ ID NO:1 (AAT01721) (2001) TVGPEVEAALKLMEKREYKFACQTFLKDEIRPMEKVRAGKTRIVDVLPVE
SEQ ID NO:2 (pMEB097)  (1127) --------------------------------------------------
SEQ ID NO:4 (pMEB084)  (1127) --------------------------------------------------

                               2051                                         2100
SEQ ID NO:1 (AAT01721) (2051) HILYTRMMIGRFCAQMFSNNGPQIGSAVGCNPDVDWQRFGTHFAQYRNVW
SEQ ID NO:2 (pMEB097)  (1127) --------------------------------------------------
SEQ ID NO:4 (pMEB084)  (1127) --------------------------------------------------

                                2101                                        2150
SEQ ID NO:1 (AAT01721) (2101) DVDYSAFDANHCSDAMNIMFEEVFRTEFGFHPNAEWILKTLVNTEHAYEN
SEQ ID NO:2 (pMEB097)  (1127) --------------------------------------------------
SEQ ID NO:4 (pMEB084)  (1127) --------------------------------------------------

                                2151                                        2200
SEQ ID NO:1 (AAT01721) (2151) KRITVEGGMPSGCSATSIINTILNNIYVLYALRRHYEGVELDTYTMISYG
SEQ ID NO:2 (pMEB097)  (1127) --------------------------------------------------
SEQ ID NO:4 (pMEB084)  (1127) --------------------------------------------------

                                2201                                        2250
SEQ ID NO:1 (AAT01721) (2201) DDIVVASDYDLDFEALKPHFKSLGQTITPADKSDKGFVLGHSITDVTFLK
SEQ ID NO:2 (pMEB097)  (1127) --------------------------------------------------
SEQ ID NO:4 (pMEB084)  (1127) --------------------------------------------------

                                2251                                        2300
SEQ ID NO:1 (AAT01721) (2251) RHFHMDYGTGFYKPVMASKTLEAILSFARRGTIQEKLISVAGLAVHSGPD
SEQ ID NO:2 (pMEB097)  (1127) --------------------------------------------------
SEQ ID NO:4 (pMEB084)  (1127) --------------------------------------------------

                                2301                          2333
SEQ ID NO:1 (AAT01721) (2301) EYRRLFEPFQGLFEIPSYRSLYLRWVNAVCGDA
SEQ ID NO:2 (pMEB097)  (1127) --------------------------------
SEQ ID NO:4 (pMEB084)  (1127) --------------------------------
```

Figure 7B

Alignment of the amino acid sequences of P1 region of the recombinant polyprotein in pMEB099 and the reference sequence (Genbank AAT01766) using the vector NTI program

```
                    1                                                  50
P1 AAT01766     (1) -GAGQSSPATGSQNQSGNTGSIINNYYMQQYQNSMDTQLGDNAISGGSNE
 p1 pMEB099     (1) MGAGQSSPATGSQNQSGNTGSIINNYYMQQYQNSMDTQLGDNAISGGSNE

                    51                                                 100
P1 AAT01766    (50) GSTDTTSTHTTNTQNNDWFSKLASSAFSGLFGALLADKKTEETTLLEDRI
 p1 pMEB099    (51) GSTDTTSTHTTNTQNNDWFSKLASSAFSGLFGALLADKKTEETTLLEDRI

                   101                                                 150
P1 AAT01766   (100) LTTPNGETTSTTQSSVGVTYGYATAEDFVSGPNTSGLETRVAQAERFFKT
 p1 pMEB099   (101) LTTPNGETTSTTQSSVGVTYGYATAEDFVSGPNTSGLETRVAQAERFFKT

                   151                                                 200
P1 AAT01766   (150) HLFDWVTSDPFGRCHLLELPTDHKGVYGSLTDSYAYMRNGWDVEVTAVGN
 p1 pMEB099   (151) HLFDWVTSDPFGRCHLLELPTDHKGVYGCLTDSYAYMRNGWDVEVTAVGN

                   201                                                 250
P1 AAT01766   (200) QFNGGCLLVAMVPELCSIQKPELYQLTLFPHQFINPRTNMTAHITVPFVG
 p1 pMEB099   (201) QFNGGCLLVAMVPELCSIQKPELYQLTLFPHQFINPRTNMTAHITVPFVG

                   251                                                 300
P1 AAT01766   (250) VNRYDQYKVHKPWTLVVMVVAPLTVNSEGAPQIKVYANIAPTNVHVAGEF
 p1 pMEB099   (251) VNRYDQYKVHKPWTLVVMVVAPLTVNSEGAPQIKVYANIAPTNVHVAGEF

                   301                                                 350
P1 AAT01766   (300) PSKEGIFPVACSDGYGGLVTTDPKTADPAYGKVFNPPRNMLPGRFTNFLD
 p1 pMEB099   (301) PSKEGIFPVACSDGYGGLVTTDPKTADPAYGKVFNPPRNMLPGRFTNFLD

                   351                                                 400
P1 AAT01766   (350) VAEACPTFLHFEGDVPYVTTKTDSDRVLAQFDLSLAAKEMSNTFLAGLAQ
 p1 pMEB099   (351) VAEACPTFLHFEGDVPYVTTKTDSDRVLAQFDLSLAAKEMSNTFLAGLAQ

                   401                                                 450
P1 AAT01766   (400) YYTQYSGTINLHFMFTGPTDAKARYMIAYAPPGMEPPKTPEAAAHCIHAE
 p1 pMEB099   (401) YYTQYSGTINLHFMFTGPTDAKARYMIAYAPPGMEPPKTPEAAAHCIHAE

                   451                                                 500
P1 AAT01766   (450) WDTGLNSKFTFSIPYLSAADYAYTASDTAETTNVQGWVCLFQITHGKADG
 p1 pMEB099   (451) WDTGLNSKFTFSIPYLSAADYAYTASDTAETTNVQGWVCLFQITHGKADG

                   501                                                 550
P1 AAT01766   (500) DALVVLASAGKDFELRLPVDARTQTTSAGESADPVTATVENYGGETQVQR
 p1 pMEB099   (501) DALVVLASAGKDFELRLPVDARTQTTSAGESADPVTATVENYGGETQVQR

                   551                                                 600
P1 AAT01766   (550) RQHTDVSFILDRFVKVTPKDQINVLDLMQTPAHTLVGALLRTATYYFADL
 p1 pMEB099   (551) RQHTDVSFILDRFVKVTPKDQINVLDLMQTPAHTLVGALLRTATYYFADL
```

Figure 7B (continued)

```
                    601                                                 650
P1 AAT01766   (600) EVAVKHEGNLTWVFNGAPEAALDNTTNPTAYHKAPLTPLALPYTAPHRVL
 p1 pMEB099   (601) EVAVKHEGNLTWVFNGAPEAALDNTTNPTAYHKAPLTPLALPYTAPHRVL

                    651                                                 700
P1 AAT01766   (650) ATVYNGNSKYGLGTVANVPGDLQVLAQKAARALPTSFNYGAIKATRVTEL
 p1 pMEB099   (651) ATVYNGNSKYGLGTVANVPGDLQVLAQKAARALPTSFNYGAIKATRVTEL

                    701                                 737
P1 AAT01766   (700) LYRMKRAETYCPRPLLAIHPDQARHKQKIVAPVKQLL
 p1 pMEB099   (701) LYRMKRAETYCPRPLLAIHPDQARHKQKIVAPVKQLL
```

P1 AAT01766: SEQ ID NO:5; p1 pMEB099: part of SEQ ID NO:6

Figure 7C
Alignment of the amino acid sequences of P1 region of the recombinant polyprotein using the vector NTI program

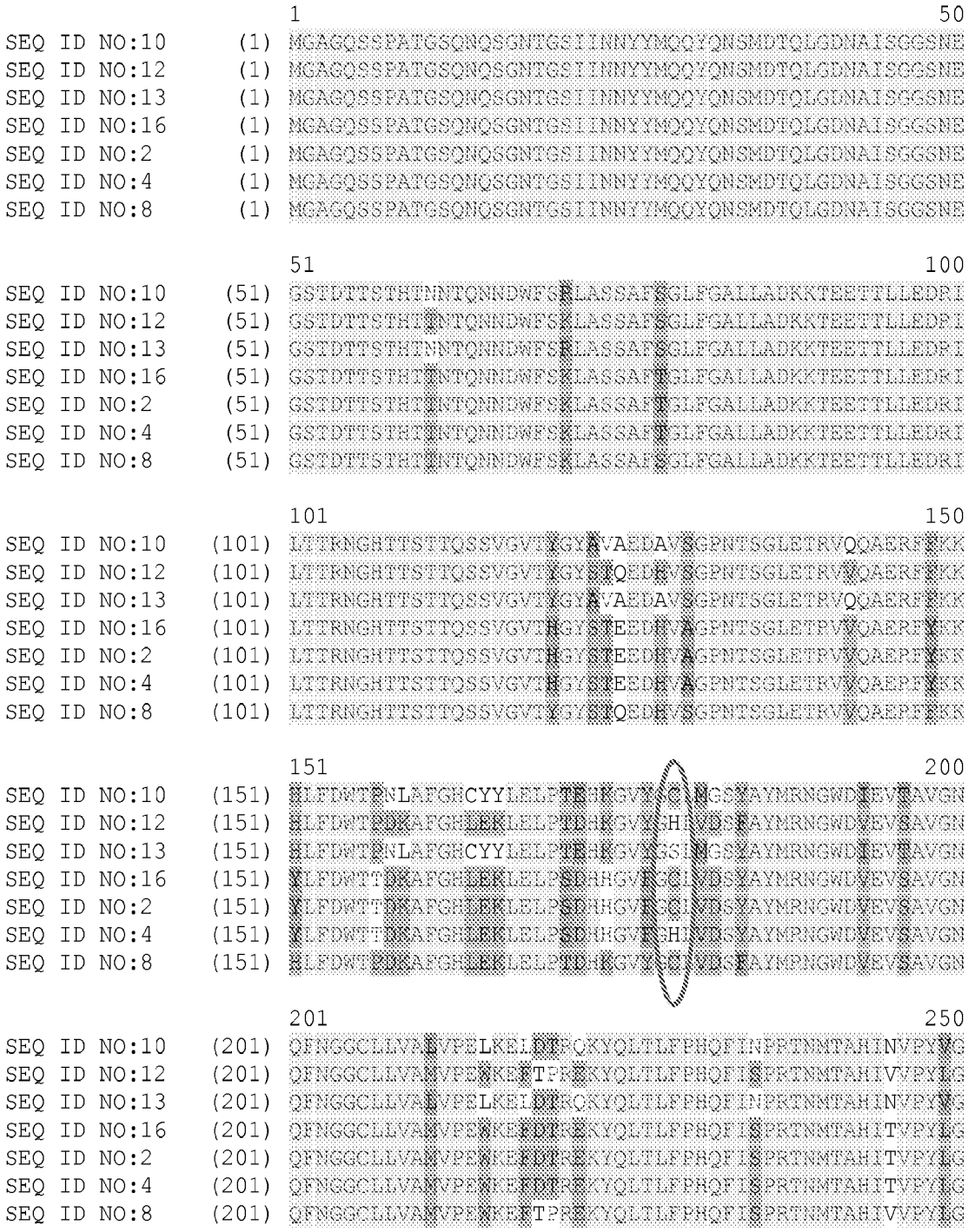


Figure 7C (continued)

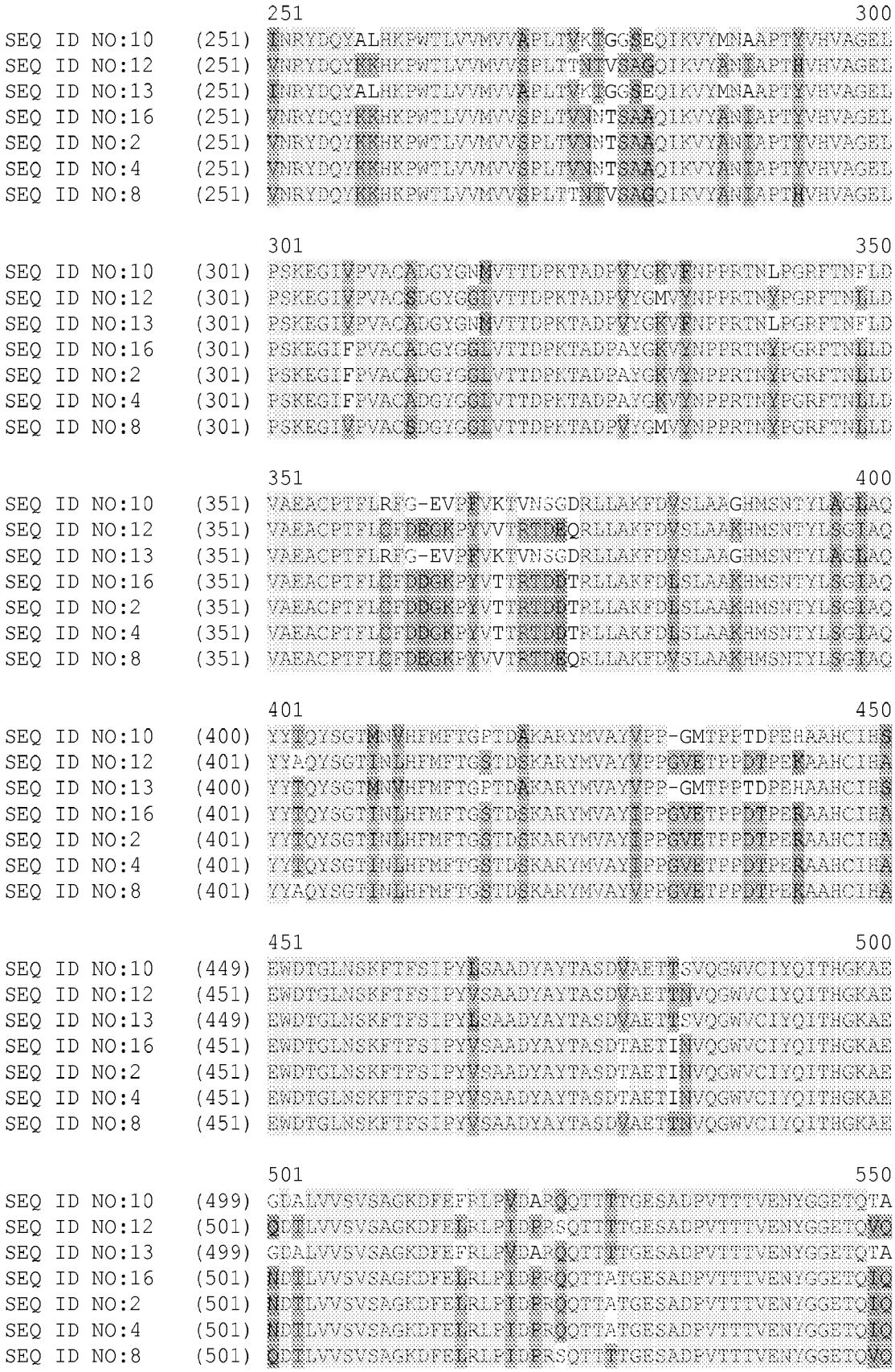


Figure 7C (continued)

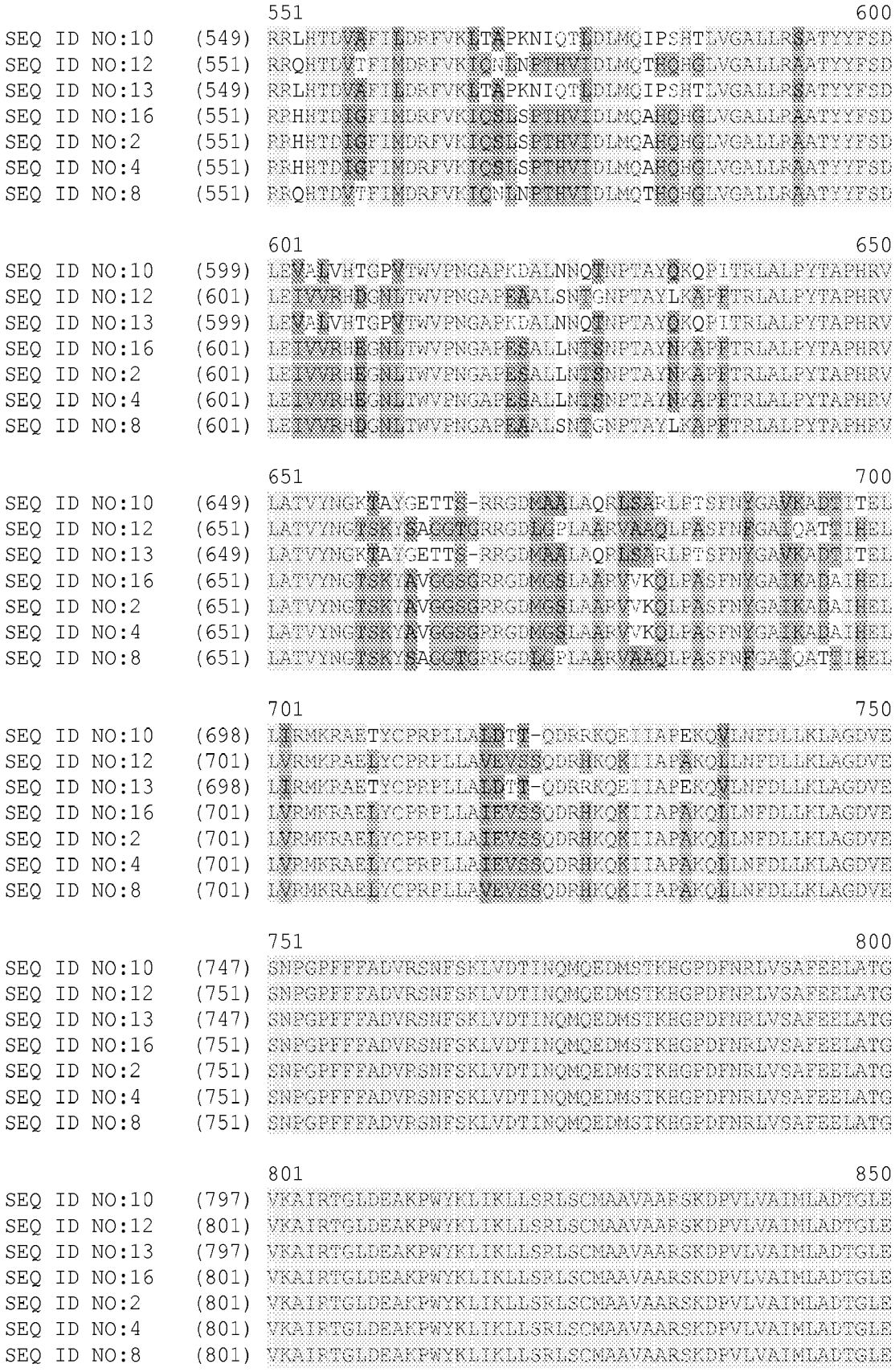


Figure 7C (continued)

```
                          851                                                 900
SEQ ID NO:10    (847) --------------------------------------------------
SEQ ID NO:12    (851) --------------------------------------------------
SEQ ID NO:13    (847) --------------------------------------------------
SEQ ID NO:16    (851) ILDSTFVVKKISDSLSSLFHVPAPVFSFGAPILLAGLVKVASSFFRSTPE
SEQ ID NO:2     (851) --------------------------------------------------
SEQ ID NO:4     (851) --------------------------------------------------
SEQ ID NO:8     (851) --------------------------------------------------

                          901                                                 950
SEQ ID NO:10    (847) --------RQRPLKVRAKLPQQEGPYAGPLERQKPLKVKAKAPVVKEGPY
SEQ ID NO:12    (851) --------RQRPLKVRAKLPQQEGPYAGPLERQKPLKVKAKAPVVKEGPY
SEQ ID NO:13    (847) --------RQRPLKVRAKLPQQEGPYAGPLERQKPLKVKAKAPVVKEGPY
SEQ ID NO:16    (901) DLERAEKQRQRPLKVRAKLPQQEGPYAGPLERQKPLKVKAKAPVVKEGPY
SEQ ID NO:2     (851) --------RQRPLKVRAKLPQQEGPYAGPLERQKPLKVKAKAPVVKEGPY
SEQ ID NO:4     (851) --------RQRPLKVRAKLPQQEGPYAGPLERQKPLKVKAKAPVVKEGPY
SEQ ID NO:8     (851) --------RQRPLKVRAKLPQQEGPYAGPLERQKPLKVKAKAPVVKEGPY

                          951                                                1000
SEQ ID NO:10    (889) EGPVKKPVALKVKAKNLIVTESGAPPTDLQKMVMGNTKPVELILDGKTVA
SEQ ID NO:12    (893) EGPVKKPVALKVKAKNLIVTESGAPPTDLQKMVMGNTKPVELILDGKTVA
SEQ ID NO:13    (889) EGPVKKPVALKVKAKNLIVTESGAPPTDLQKMVMGNTKPVELILDGKTVA
SEQ ID NO:16    (951) EGPVKKPVALKVKAKNLIVTESGAPPTDLQKMVMGNTKPVELILDGKTVA
SEQ ID NO:2     (893) EGPVKKPVALKVKAKNLIVTESGAPPTDLQKMVMGNTKPVELILDGKTVA
SEQ ID NO:4     (893) EGPVKKPVALKVKAKNLIVTESGAPPTDLQKMVMGNTKPVELILDGKTVA
SEQ ID NO:8     (893) EGPVKKPVALKVKAKNLIVTESGAPPTDLQKMVMGNTKPVELILDGKTVA

                         1001                                                1050
SEQ ID NO:10    (939) ICCATGVFGTAYLVPRHLFAEKYDKIMLDGRAMTDSDYPVFEFEIKVKGQ
SEQ ID NO:12    (943) ICCATGVFGTAYLVPRHLFAEKYDKIMLDGRAMTDSDYPVFEFEIKVKGQ
SEQ ID NO:13    (939) ICCATGVFGTAYLVPRHLFAEKYDKIMLDGRAMTDSDYRVFEFEIKVKGQ
SEQ ID NO:16   (1001) ICCATGVFGTAYLVPRHLFAEKYDKIMLDGRAMTDSDYRVFEFEIKVKGQ
SEQ ID NO:2     (943) ICCATGVFGTAYLVPRHLFAEKYDKIMLDGRAMTDSDYRVFEFEIKVKGQ
SEQ ID NO:4     (943) ICCATGVFGTAYLVPRHLFAEKYDKIMLDGRAMTDSDYRVFEFEIKVKGQ
SEQ ID NO:8     (943) ICCATGVFGTAYLVPRHLFAEKYDKIMLDGRAMTDSDYRVFEFEIKVKGQ

                         1051                                                1100
SEQ ID NO:10    (989) DMLSDAALMVLHRGNPVRDITKHFRDTARMKKGTPVVGVVNNADVGRLIF
SEQ ID NO:12    (993) DMLSDAALMVLHRGNPVRDITKHFRDTARMKKGTPVVGVVNNADVGPLIF
SEQ ID NO:13    (989) DMLSDAALMVLHRGNRVRDITKHFRDTARMKKGTPVVGVVNNADVGRLIF
SEQ ID NO:16   (1051) DMLSDAALMVLHPGNRVRDITKHFRDTAPMKKGTPVVGVVNNADVGRLIF
SEQ ID NO:2     (993) DMLSDAALMVLHPGNRVRDITKHFRDTAPMKKGTPVVGVVNNADVGRLIF
SEQ ID NO:4     (993) DMLSDAALMVLHPGNRVRDITKHFRDTAPMKKGTPVVGVVNNADVGRLIF
SEQ ID NO:8     (993) DMLSDAALMVLHPGNRVRDITKHFRDTAPMKKGTPVVGVVNNADVGRLIF

                         1101                                                1150
SEQ ID NO:10   (1039) SGEALTYKDIVVGMDGDTMPGLFAYKAATKAGYCGGAVLAKDGADTFIVG
SEQ ID NO:12   (1043) SGEALTYKDIVVGMDGDTMPGLFAYKAATKAGYCGGAVLAKDGADTFIVG
SEQ ID NO:13   (1039) SGEALTYKDIVVGMDGDTMPGLFAYKAATKAGYCGGAVLAKDGADTFIVG
SEQ ID NO:16   (1101) SGEALTYKDIVVTMDGDTMPGLFAYKAATKAGYCGGAVLAKDGADTFIVG
SEQ ID NO:2    (1043) SGEALTYKDIVVGMDGDTMPGLFAYKAATKAGYCGGAVLAKDGADTFIVG
SEQ ID NO:4    (1043) SGEALTYKDIVVGMDGDTMPGLFAYKAATKAGYCGGAVLAKDGADTFIVG
SEQ ID NO:8    (1043) SGEALTYKDIVVGMDGDTMPGLFAYKAATKAGYCGGAVLAKDGADTFIVG
```

Figure 7C (continued)

```
                        1151                                1184
SEQ ID NO:10   (1089) THSAGGNGVGYCSCVSRSMLLRMKAHVDPEPQHE
SEQ ID NO:12   (1093) THSAGGNGVGYCSCVSRSMLLRMKAHVDPEPQHE
SEQ ID NO:13   (1089) THSAGGNGVGYCSCVSRSMLLRMKAHVDPEPQHE
SEQ ID NO:16   (1151) THSAGGNGVGYCSCVSRSMLLRMKAHVDPEPQHE
SEQ ID NO:2    (1093) THSAGGNGVGYCSCVSPSMLLRMKAHVDPEPQHE
SEQ ID NO:4    (1093) THSAGGNGVGYCSCVSPSMLLRMKAHVDPEPQHE
SEQ ID NO:8    (1093) THSAGGNGVGYCSCVSPSMLLRMKAHVDPEPQHE
```

Figure 7D

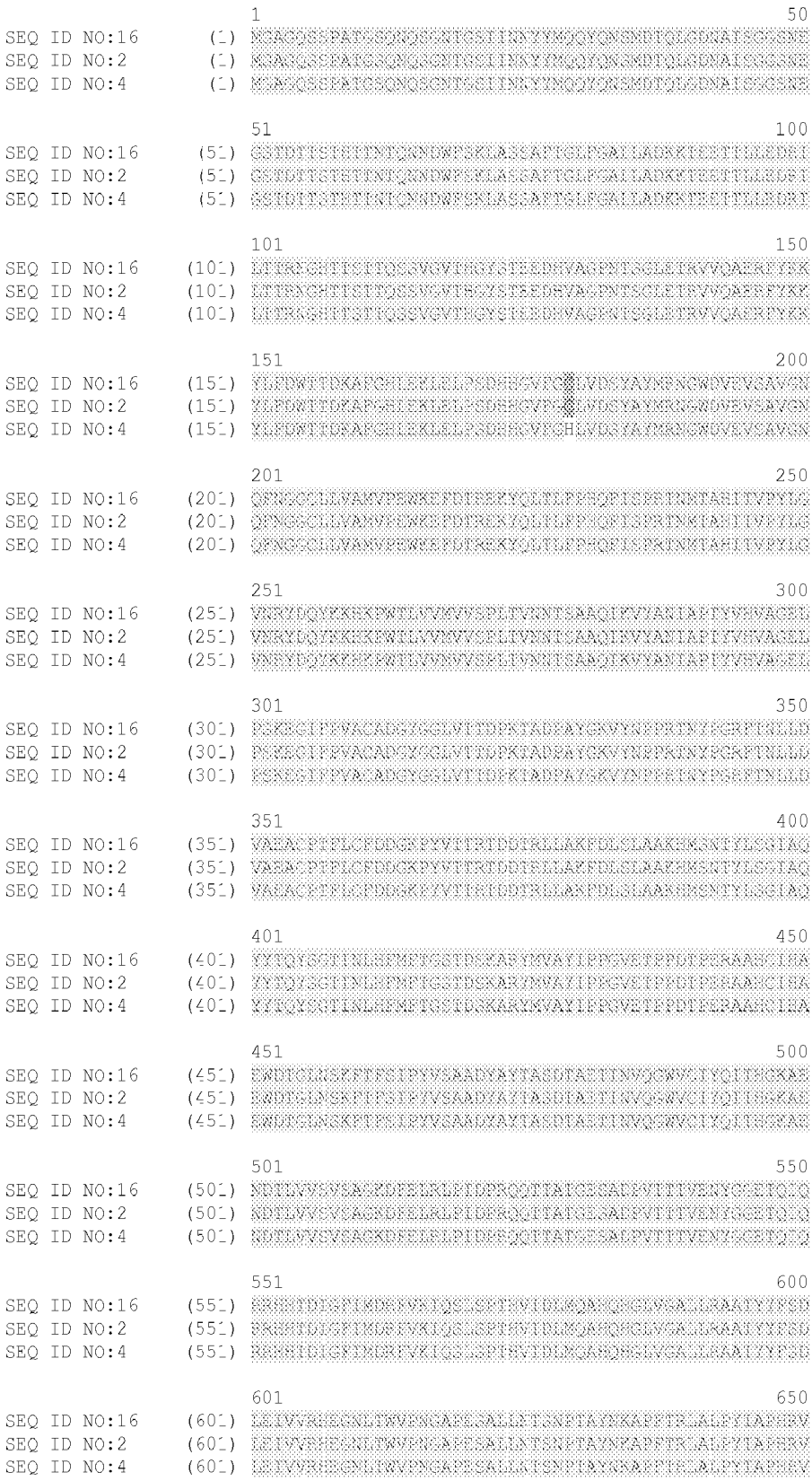


Figure 7D (continued)

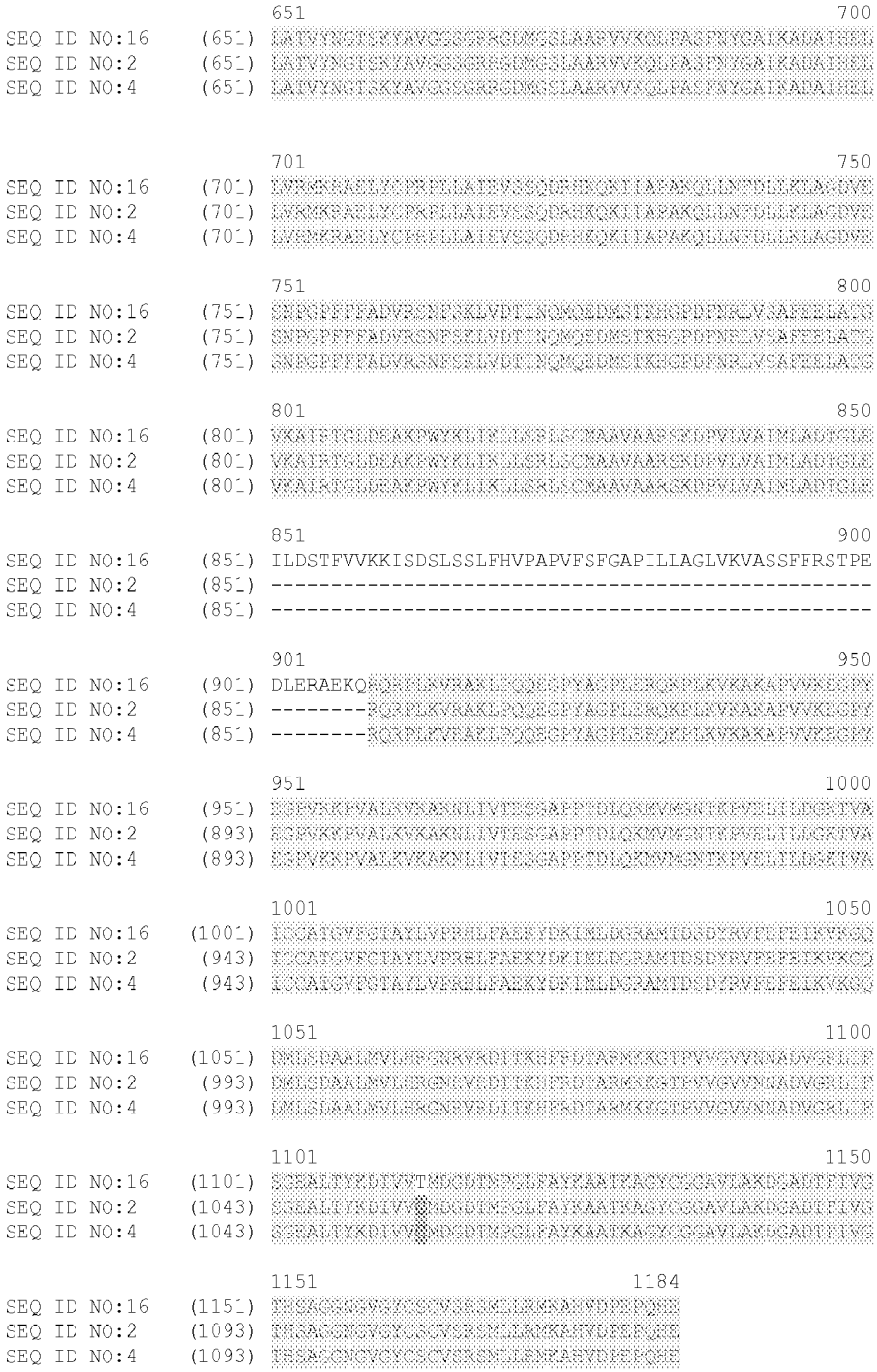
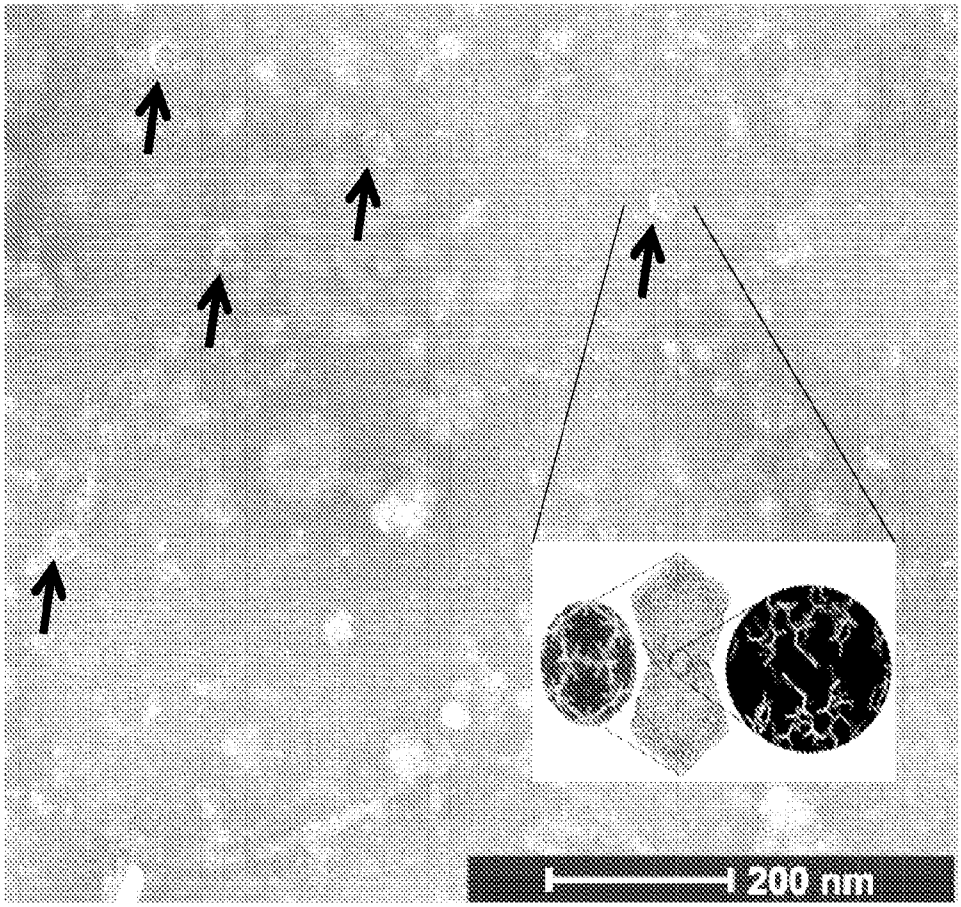


Figure 8

FMDV VLPs

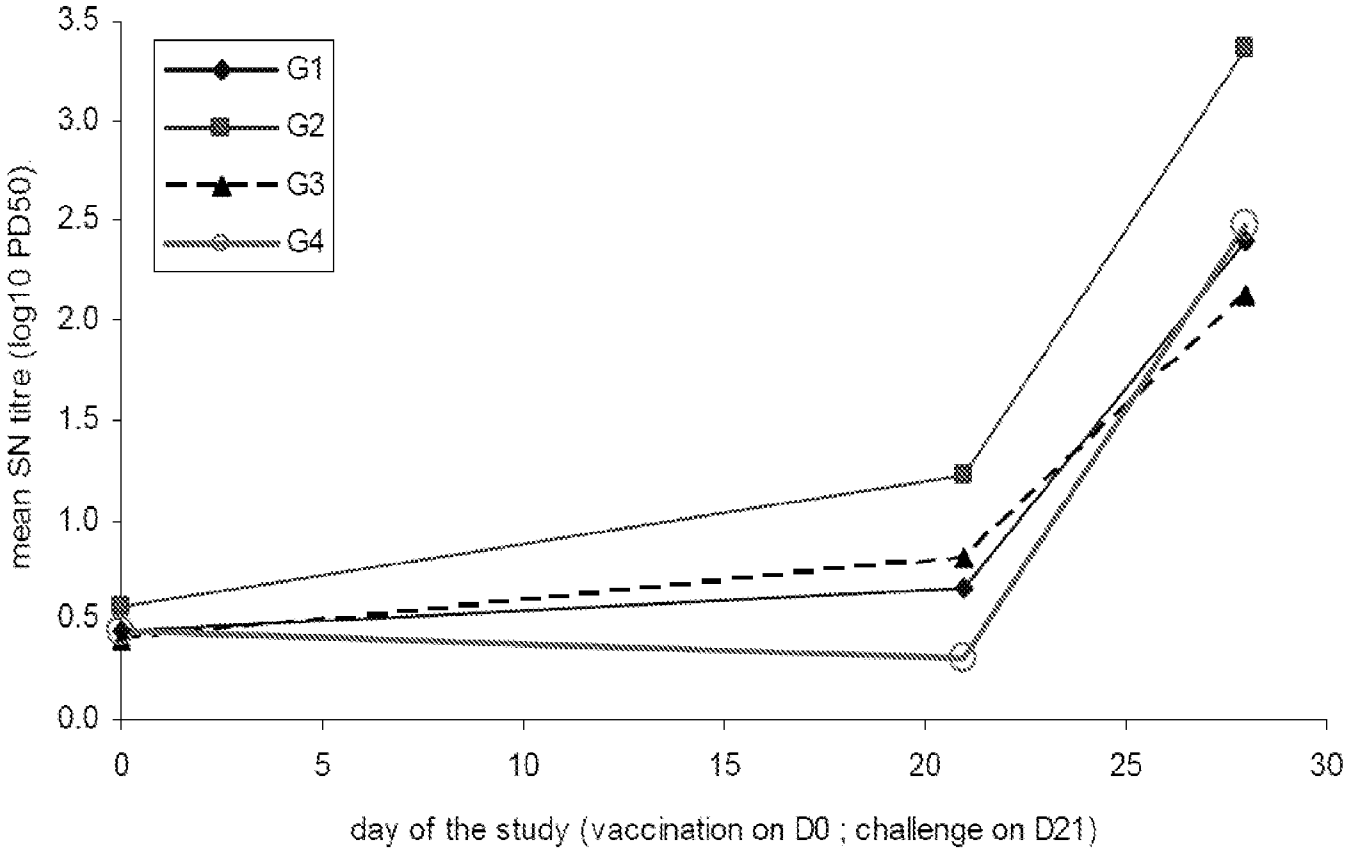


Batch Size	Titer[a] ($Log10CCID_{50}$/mL)	ELISA M326/M326→146S[b] ($Log10CCID_{50}$/mL)	EM (VLPs/mL)
150 L	7.14	2.19; *2.14 (heated)*	5×10^{9} [C]
4 L	7.17	2.09; *1.89 (heated)*	2×10^{9} [d]

a: supernatant D3 1xC
b: supernatant D5 1xC
c: supernatant D5 concentrated 12.8 x g (CENTRICON tube)
d: supernatant D5 concentrated 9.5 x g (VIVAFLOW)

Figure 9

The evolution of mean FMDV A24 Cruzeiro neutralizing antibody titers



G1: Lemna; G2: Baculovirus;
G3: vCP2186; G4 Controls

Figure 10

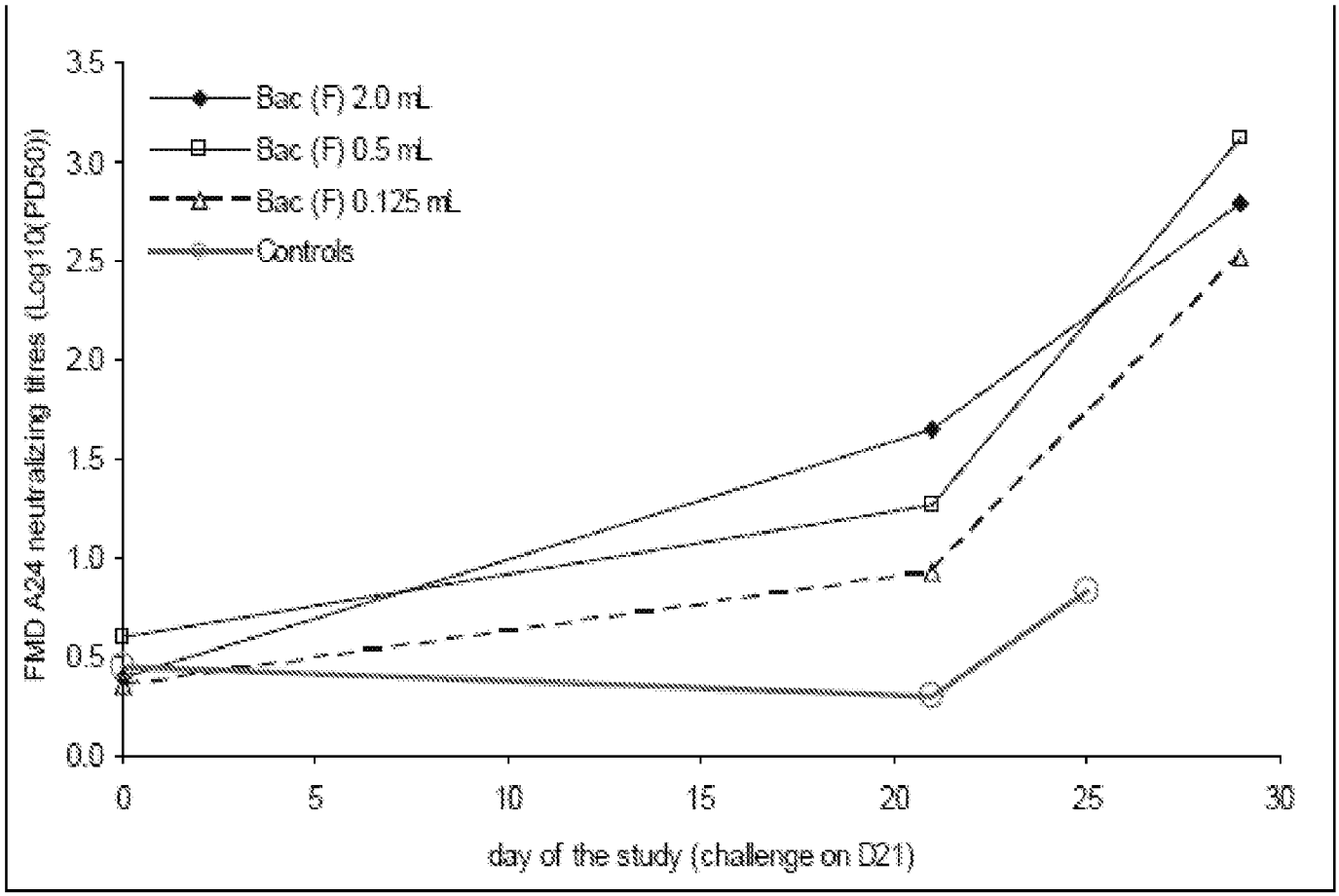
FMDV A24 Cruzeiro neutralizing antibody titers

Group	Cattle	FMD A24 Cruzeiro neutralizing titre (Log10 PD50)			
		D0	D21	D25*	D28*
1 (Lemna-FMDV PE 5X)	3953	0.60	0.60	1.95	-
	3954	0.75	0.75	1.80	-
	3955	<=0.30	0.75	-	2.85
	3956	<=0.30	0.90	-	>=3.45
	3957	0.30	<=0.30	1.95	-
	Mean	**<= 0.45**	**<=0.66**	**1.90**	**>= 3.15**
	S.d.	*0.21*	*0.23*	*0.09*	*0.42*
2 (Batch n°4 Baculo 14.4xC)	3958	1.05	1.35	-	>=3.45
	3959	0.30	1.05	-	3.30
	3960	0.45	1.50	-	3.30
	3961	0.60	1.05	-	3.30
	3962	0.45	1.20	-	>=3.45
	Mean	**0.57**	**1.23**	-	**>=3.36**
	S.d.	*0.29*	*0.20*	-	*0.08*
3 (Batch n°1 vCP 2186-1)	3963	0.30	0.75	2.70	-
	3964	0.30	0.90	2.25	-
	3965	0.60	1.20	2.25	-
	3966	<=0.30	0.90	1.80	-
	3967	0.45	<=0.30	1.65	-
	Mean	**<=0.39**	**<=0.81**	**2.13**	-
	S.d.	*0.13*	*0.33*	*0.42*	-
4 Control	3932	0.45	<=0.30	-	2.10
	3933	0.45	<=0.30	-	2.85
	Mean	**0.45**	**<=0.30**	-	**2.48**
	S.d.	*0.00*	*0.00*	-	*0.53*

G1: Lemna duckweed; G2: Baculo;
G3: vCP2186; G4 Controls)

Figure 11A

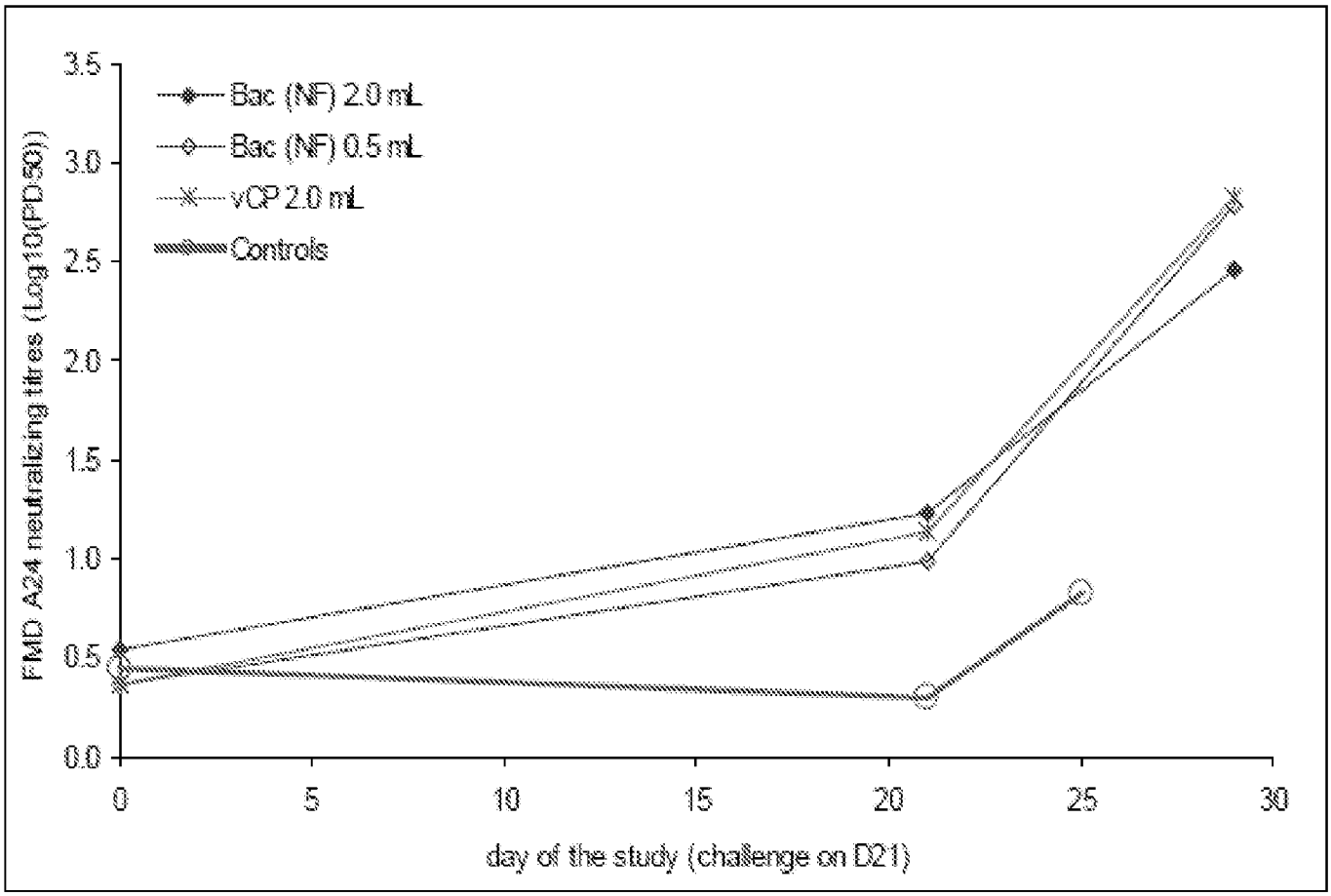
Evolution of mean FMDV A24 Cruzeiro neutralizing antibody titers



G1: baculo filtered 2.0 mL;
G2: baculo filtered 0.5 mL;
G3: baculo filtered 0.125 mL;
G4: controls

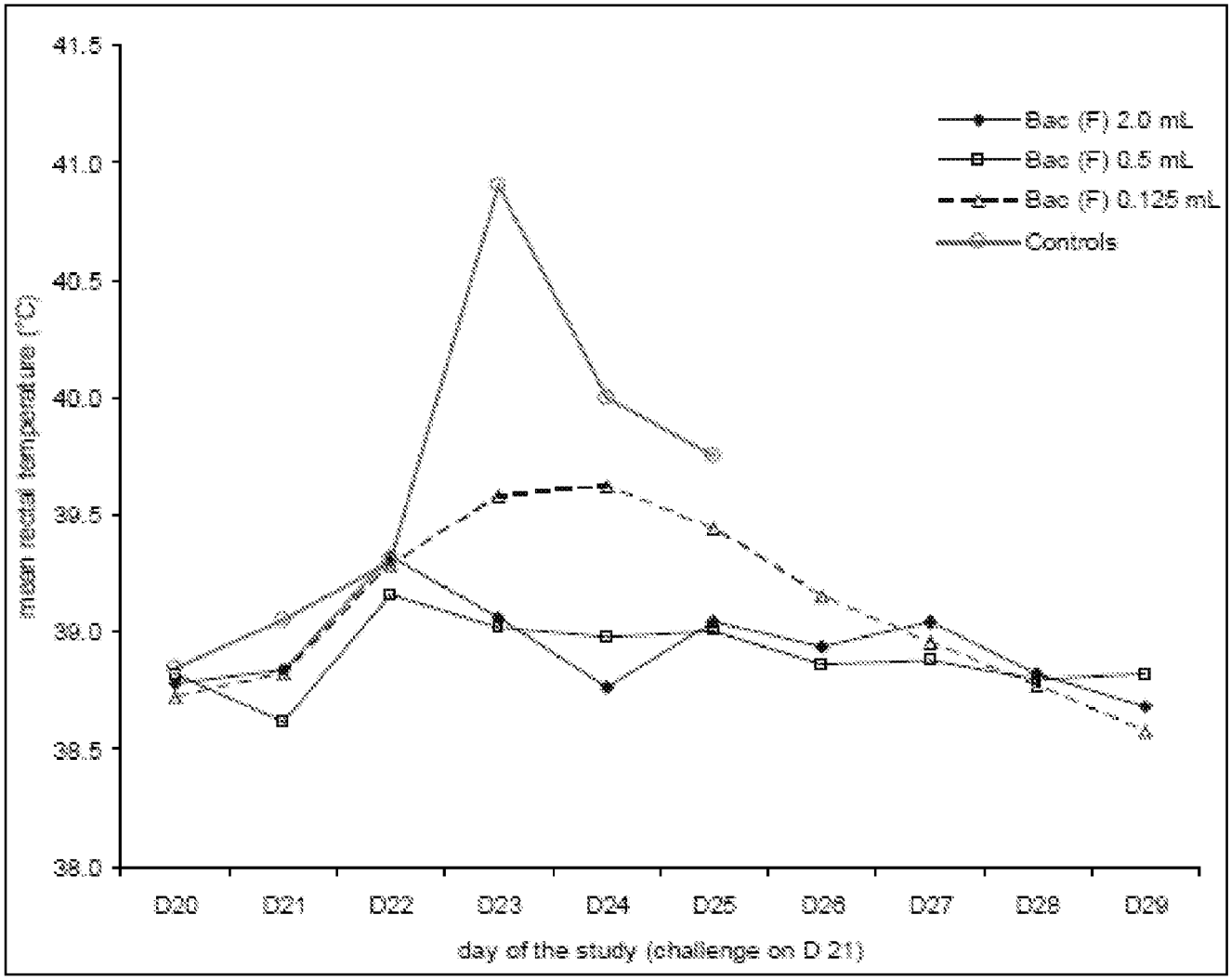
Figure 11B

Evolution of mean FMDV A24 Cruzeiro neutralizing antibody titers



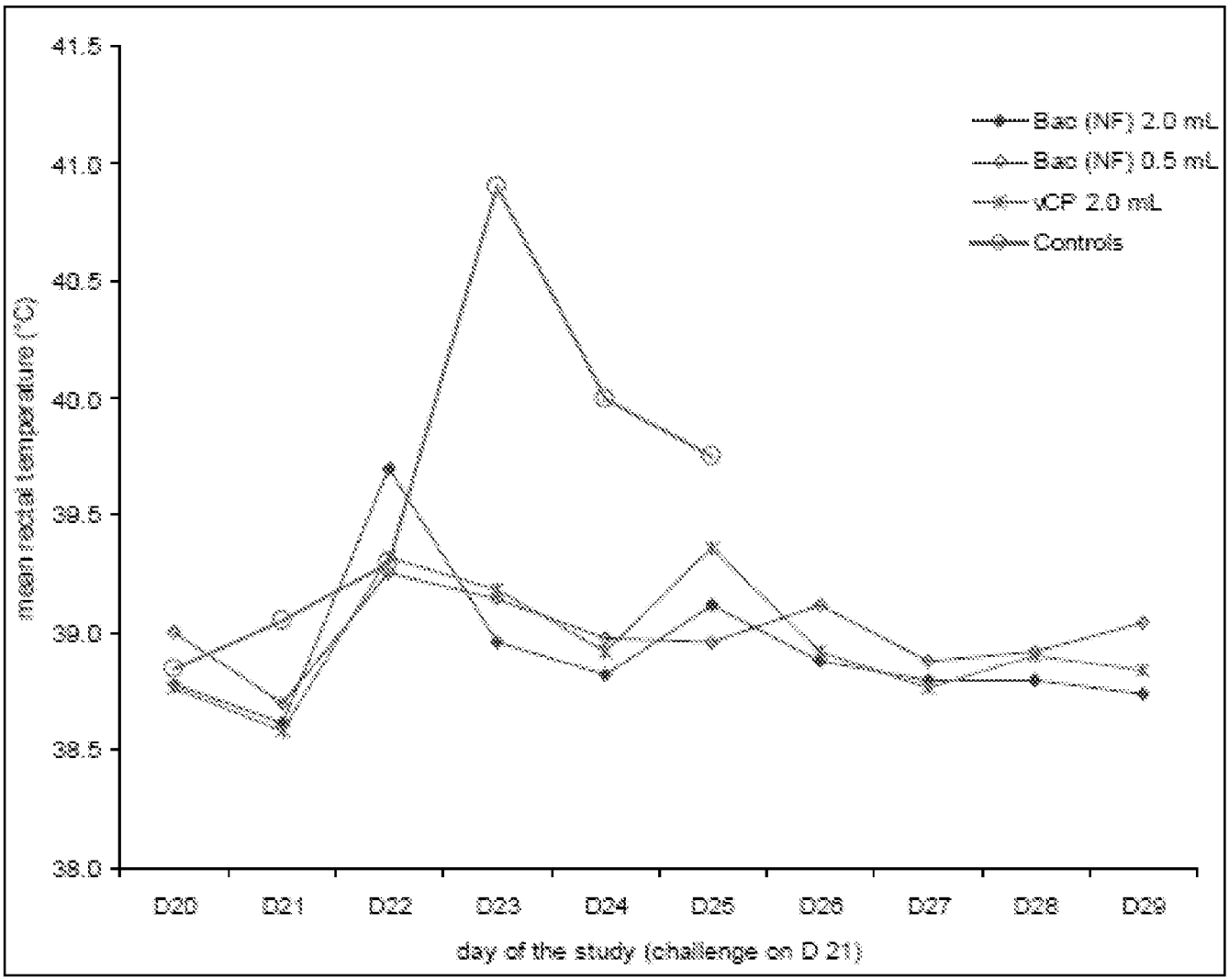
G4: controls;
G5: baculo not filtered 2.0 mL;
G6: baculo not filtered 0.5 mL;
G7: vCP 2.0 mL

Figure 12A
Evolution of mean rectal temperature after challenge



G1: baculo filtered 2.0 mL; G2 baculo filtered 0.5 mL;
G3: baculo filtered 0.125 mL; G4: controls

Figure 12B
Evolution of mean rectal temperature after challenge



G5 baculo not filtered 2.0 mL; G6: baculo not filtered 0.5 mL; G7: vCP 2.0 mL

Figure 12C
Sero-neutralization test results

Group	Cattle	FMD A24 neutralizing titres (Log10(PD50))		
		D0	D21	D28*
1 Filtered BAC 2 mL	4869	0.75	1.65	3.30
	4870	< 0.30	1.35	3.15
	4871	< 0.30	1.95	2.10
	4872	< 0.30	1.35	3.15
	4873	< 0.30	1.95	2.25
	Mean	< 0.39	1.65	2.79
	Sd	0.20	0.30	0.57
2 Filtered BAC 0.5 mL	4874	< 0.30	0.90	3.30
	4875	< 0.30	1.65	3.30
	4876	1.20	1.20	3.30
	4877	0.60	1.35	3.00
	4878	0.60	1.20	2.70
	Mean	< 0.60	1.26	3.12
	Sd	0.37	0.27	0.27
3 Filtered BAC 0.125 mL	4879	< 0.30	0.75	3.00
	4880	< 0.30	1.05	2.70
	4881	0.60	0.75	3.30
	4882	< 0.30	0.75	* 1.80
	4883	< 0.30	1.35	1.80
	Mean	< 0.36	0.93	2.52
	Sd	0.13	0.27	0.69
4 Controls	4884	< 0.30	< 0.30	* 0.90
	4885	0.60	< 0.30	* 0.75
	Mean	< 0.45	< 0.30	0.83
	Sd	0.21	0.00	0.11
5 Not filtered BAC 2 mL	4886	0.75	1.05	3.30
	4887	0.60	1.05	3.30
	4888	< 0.30	1.50	2.10
	4889	0.75	1.05	1.50
	4890	< 0.30	1.50	2.10
	Mean	< 0.54	1.23	2.46
	Sd	0.23	0.25	0.80
6 Not filtered BAC 0.5 mL	4891	< 0.30	1.05	2.70
	4892	0.60	1.05	3.00
	4893	< 0.30	1.05	3.15
	4894	< 0.30	0.75	2.70
	4895	< 0.30	1.05	2.40
	Mean	< 0.36	0.99	2.79
	Sd	0.13	0.13	0.29
7 vCP 2 mL	4896	< 0.30	1.20	2.70
	4897	< 0.30	1.05	2.40
	4898	< 0.30	1.05	3.00
	4899	< 0.30	1.20	3.00
	4900	0.60	1.20	3.00
	Mean	< 0.36	1.14	2.82

Figure 13

EM analysis of A24 Cruzeiro VLPs with (MEB097) or without (MEB084) the covalent cage mutation in the presence or absence of heat or acid

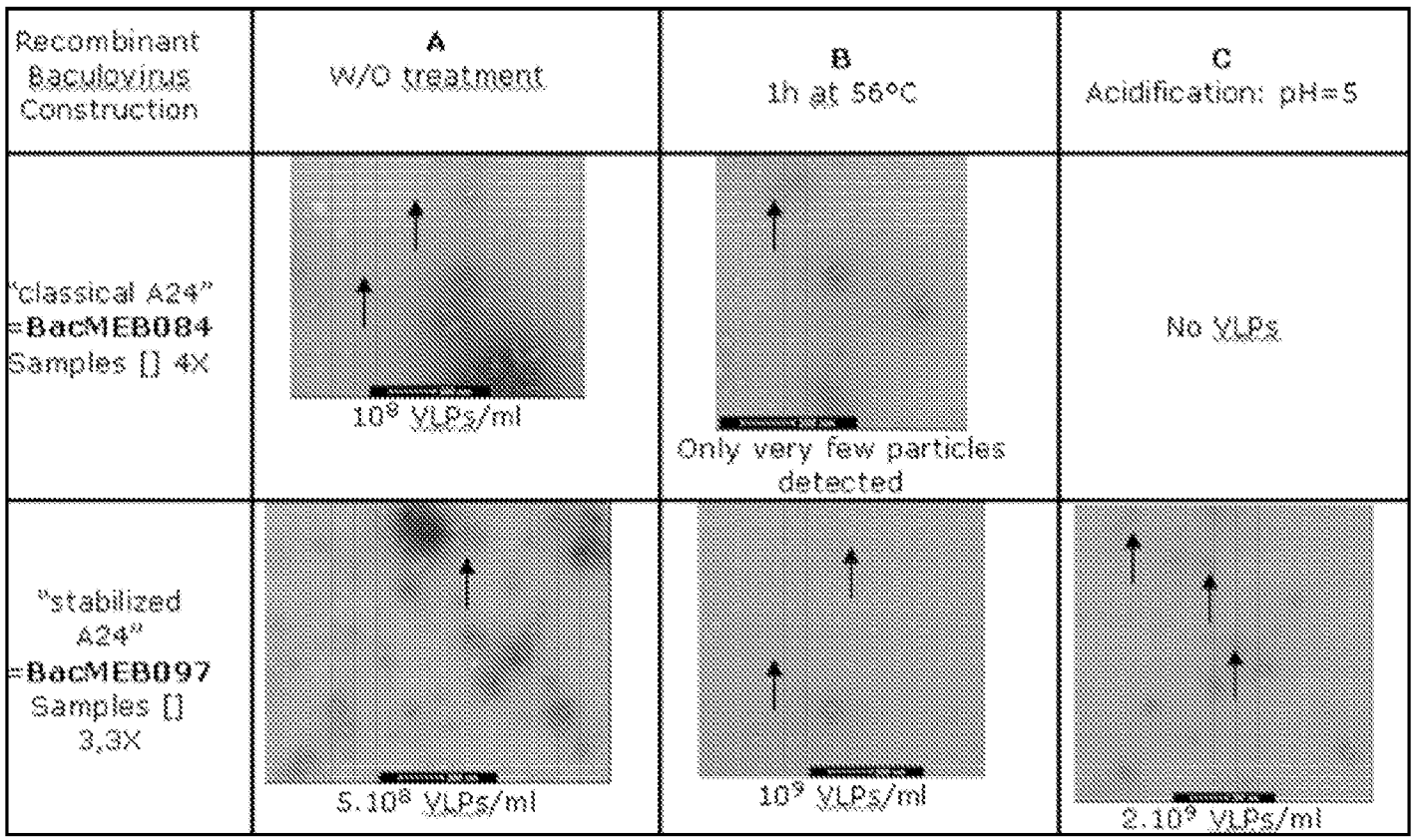
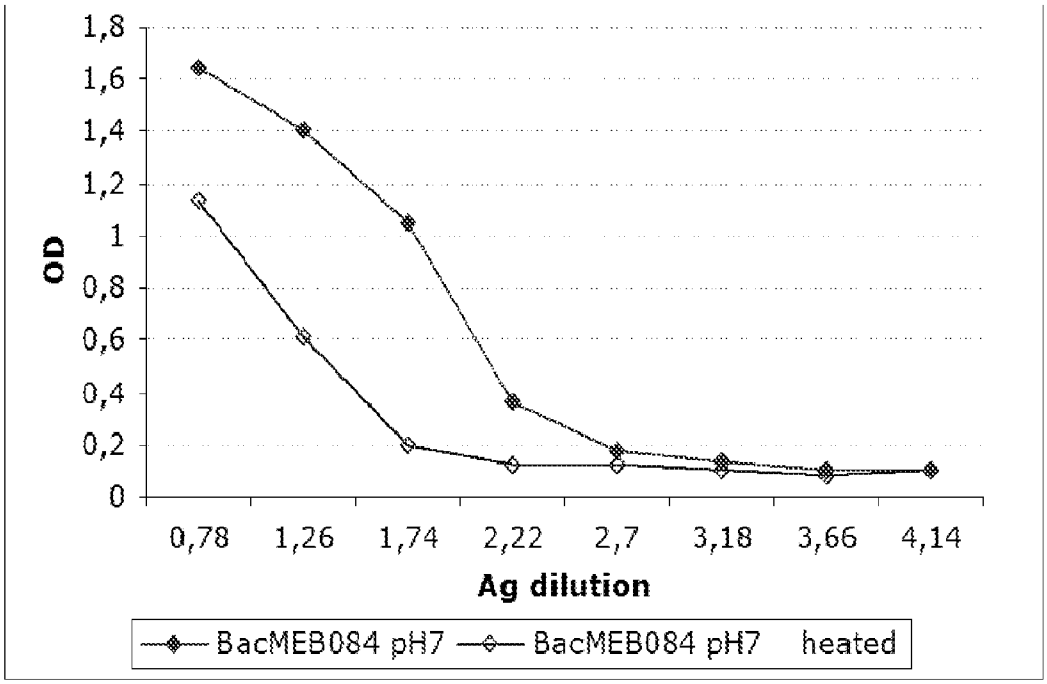


Figure 14
ELISA analysis of VLPs with (MEB097) or without (MEB084) the covalent cage mutation for the A24 Cruzeiro serotype after heating



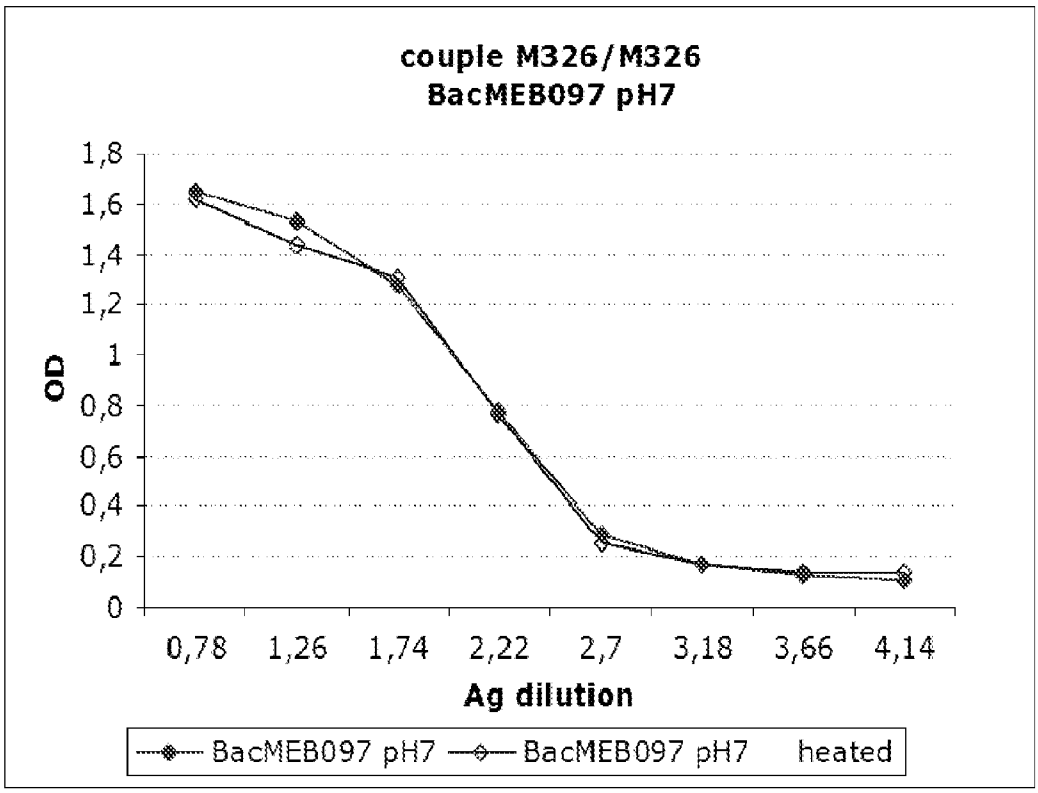


Figure 15
ELISA analysis of A24 Cruzeiro VLPs with (MEB097, covalent cage) or without (MEB084, wild type) the covalent cage mutation stored at 5°C over time

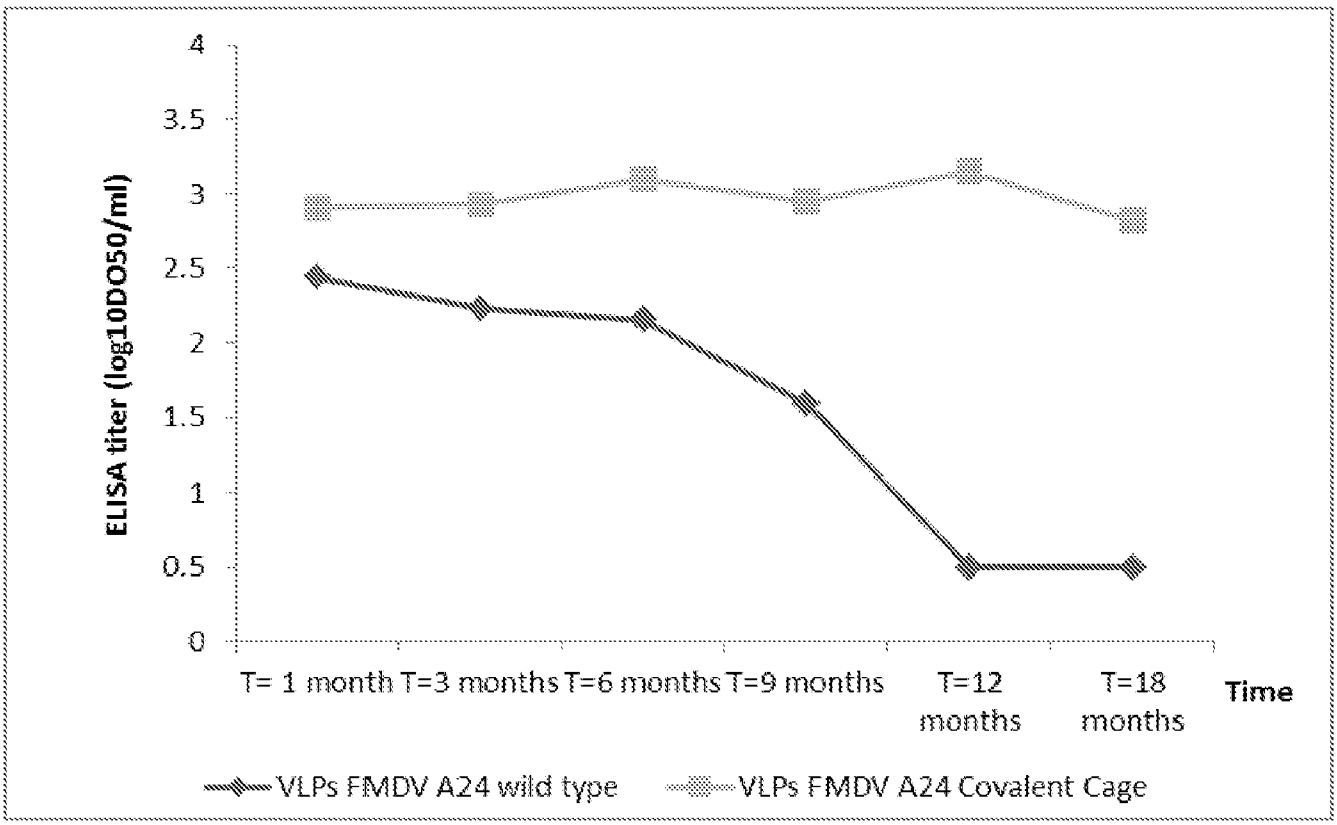


Figure 16

ELISA results and EM pictures showing O1 Manisa covalent cage VLPs are resistant to heat

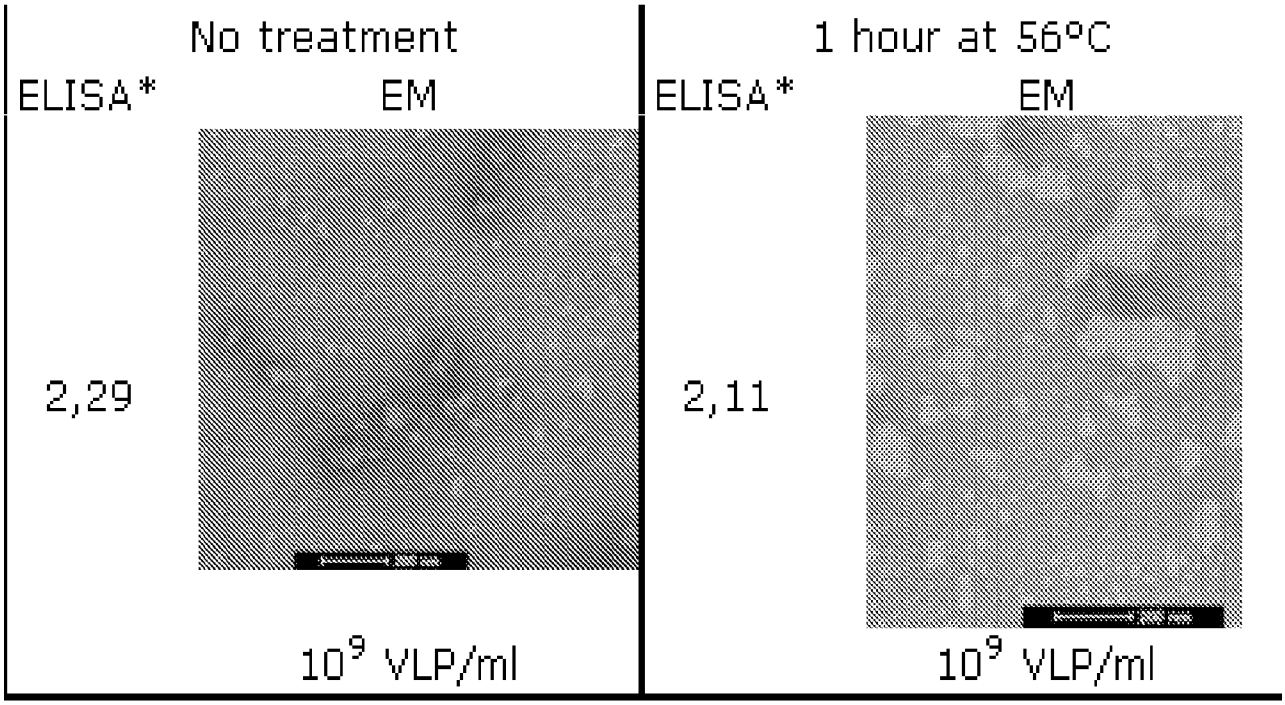
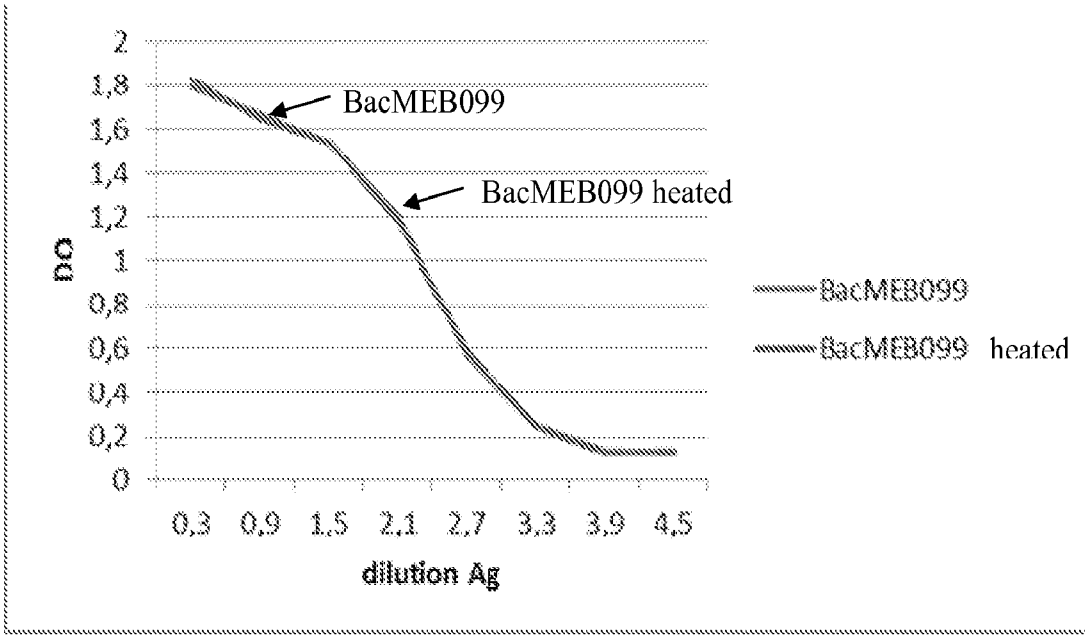


Figure 17

ELISA results showing O1 Manisa covalent cage VLP stability in heat



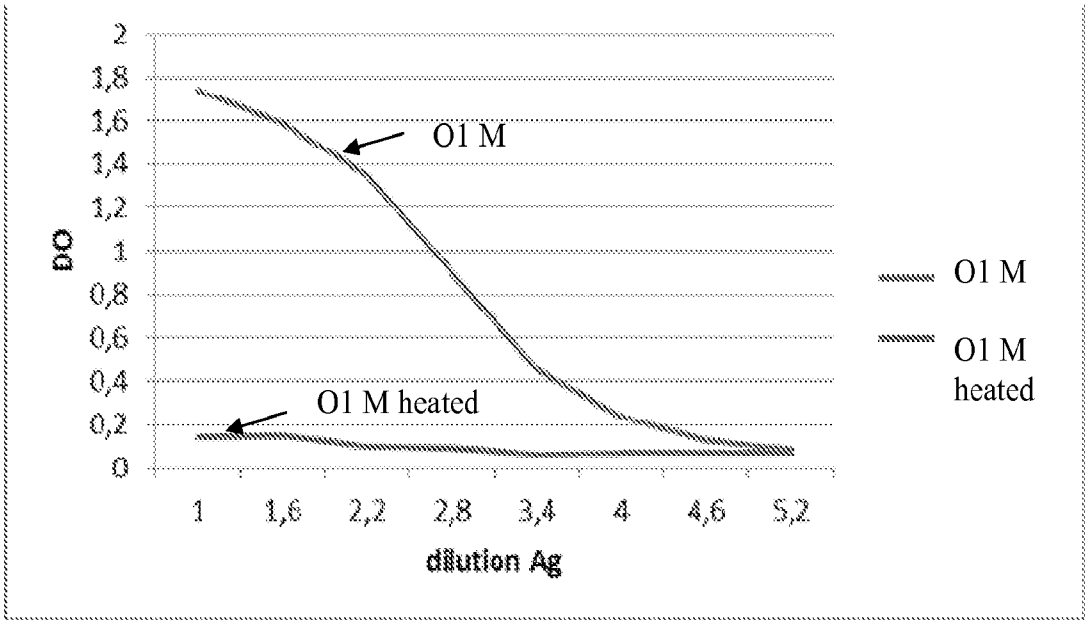


Figure 18A

Vaccination and analysis scheme

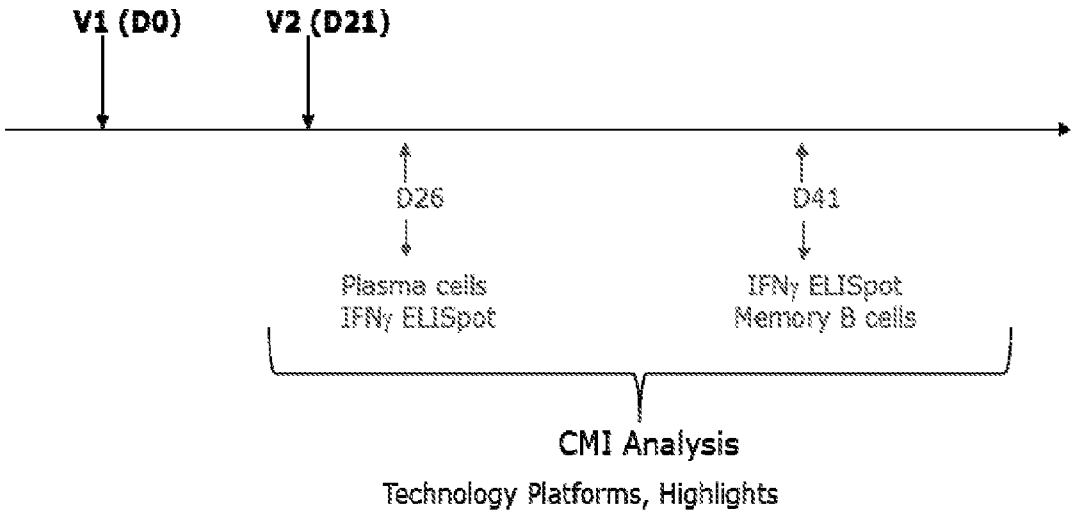


Figure 18B

Evolution of neutralizing antibody titers against FMD Asia1 Shamir and FMD A22 Iraq

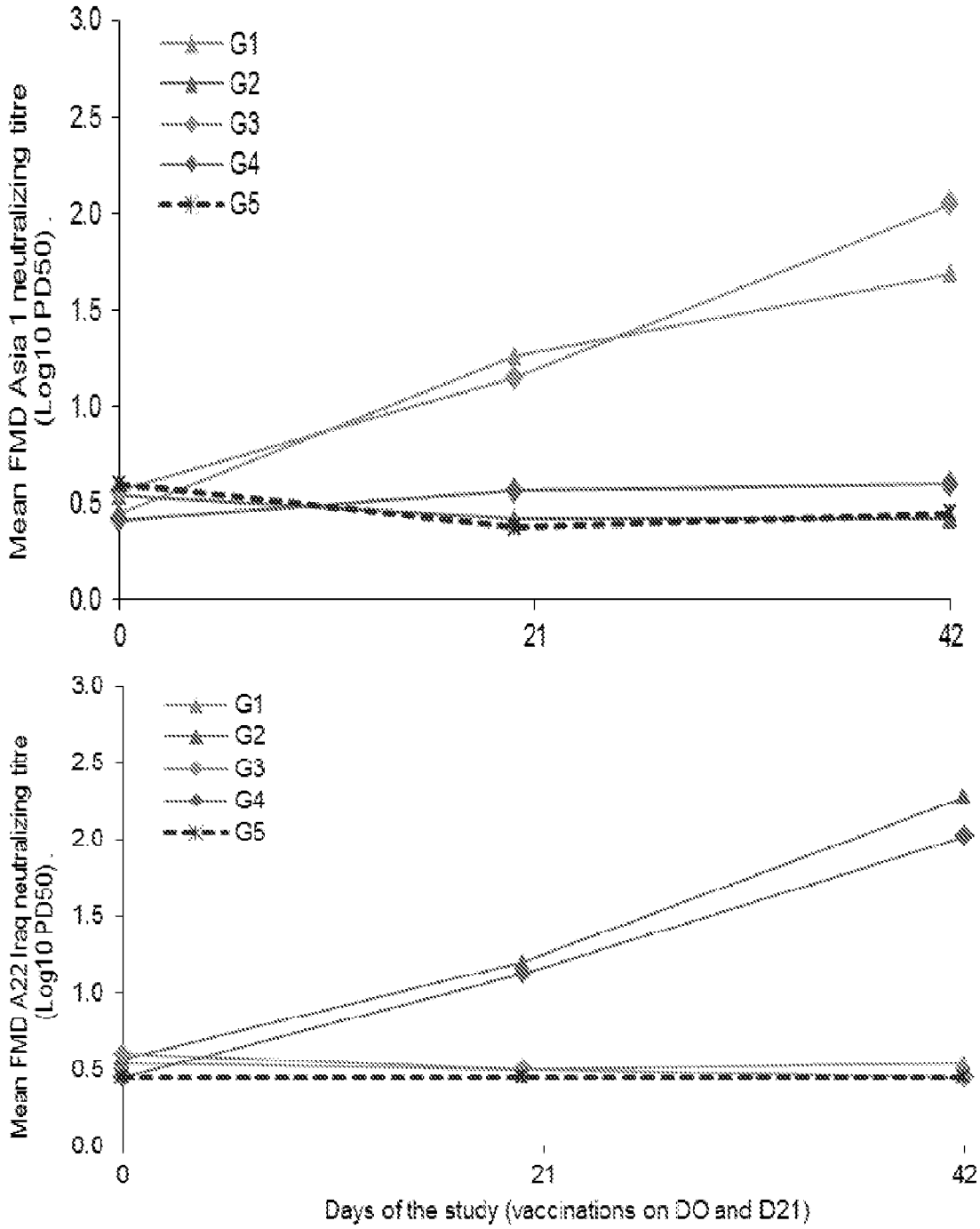


Figure 19A

Humoral response - Memory B cells detection

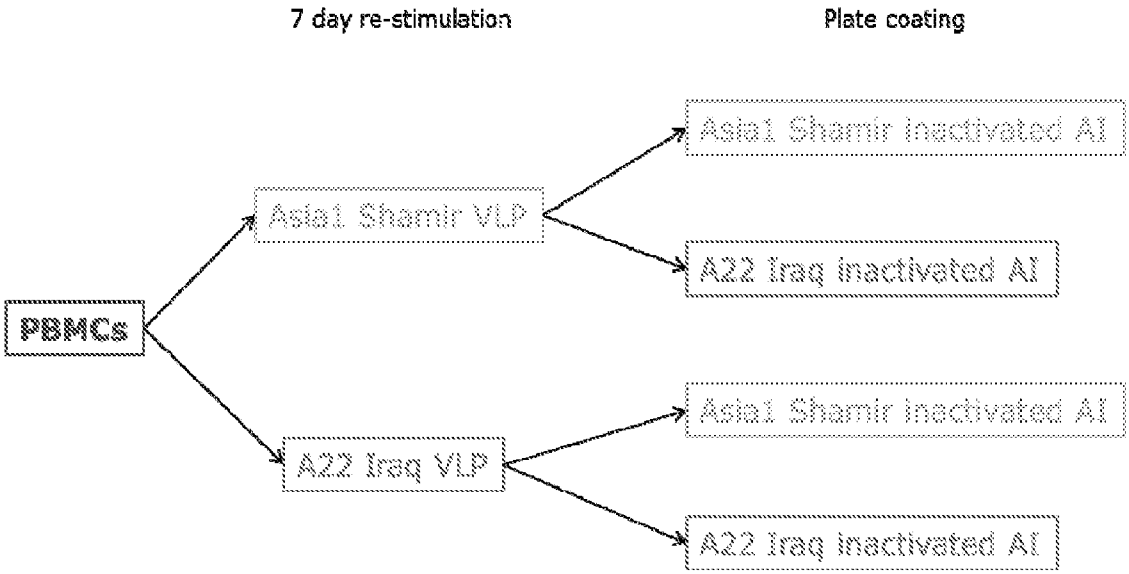


Figure 19B

Asia Shamir covalent cage & Iraq A22 covalent cage VLP serology data

		Antigens specific responses			
		Asia 1 Shamir		A22 Iraq	
Assay	Group ⟶	Asia Shamir VLP +TS6	A22 Iraq VLP +TS6	Asia Shamir VLP +TS6	A22 Iraq VLP +TS6
IFNγ secreting cells	Peptide pool	+	-	+	+
IgG secreting plasma cell	Inactivated AI	++	-	+	+
IgG secreting memory B cells	Coating Asia1 shamir AI	++	+	+	+/-
	Coating A22 Iraq AI	+	+	+	++

Figure 20

Specific IgG secreting plasma cells

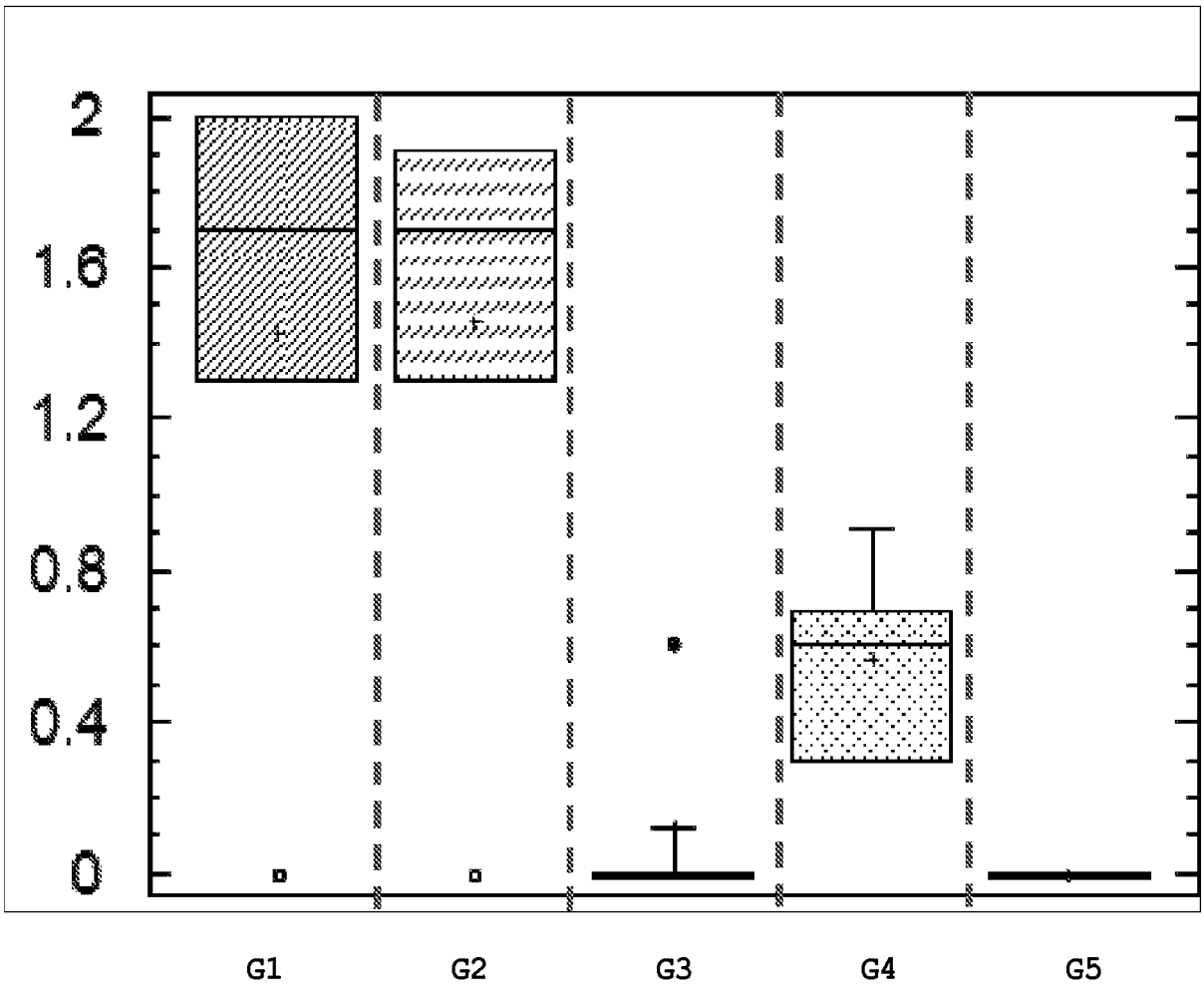


Figure 21

Specific IgG secreting memory B cells

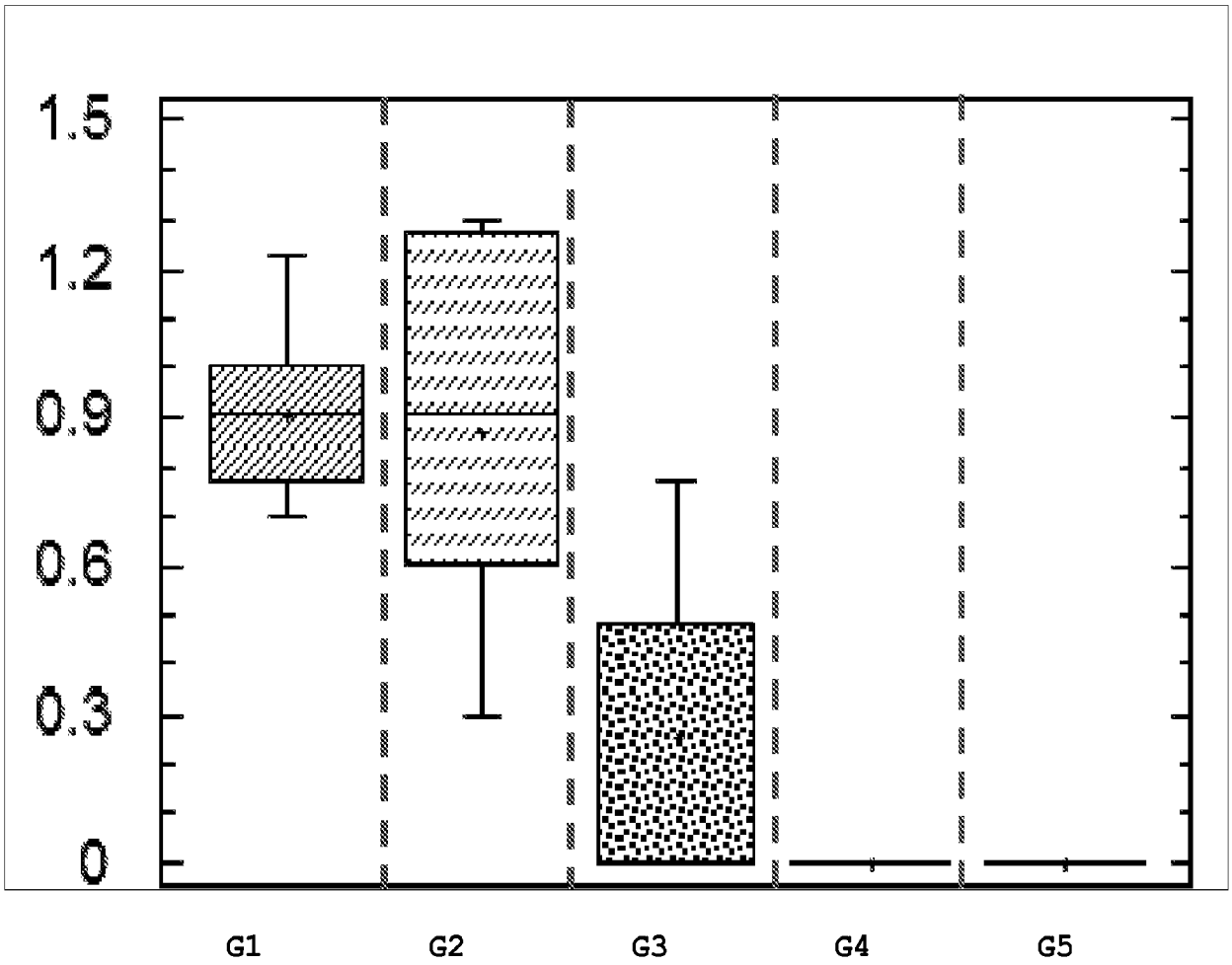


Figure 22

Specific IFNγ secreting cells

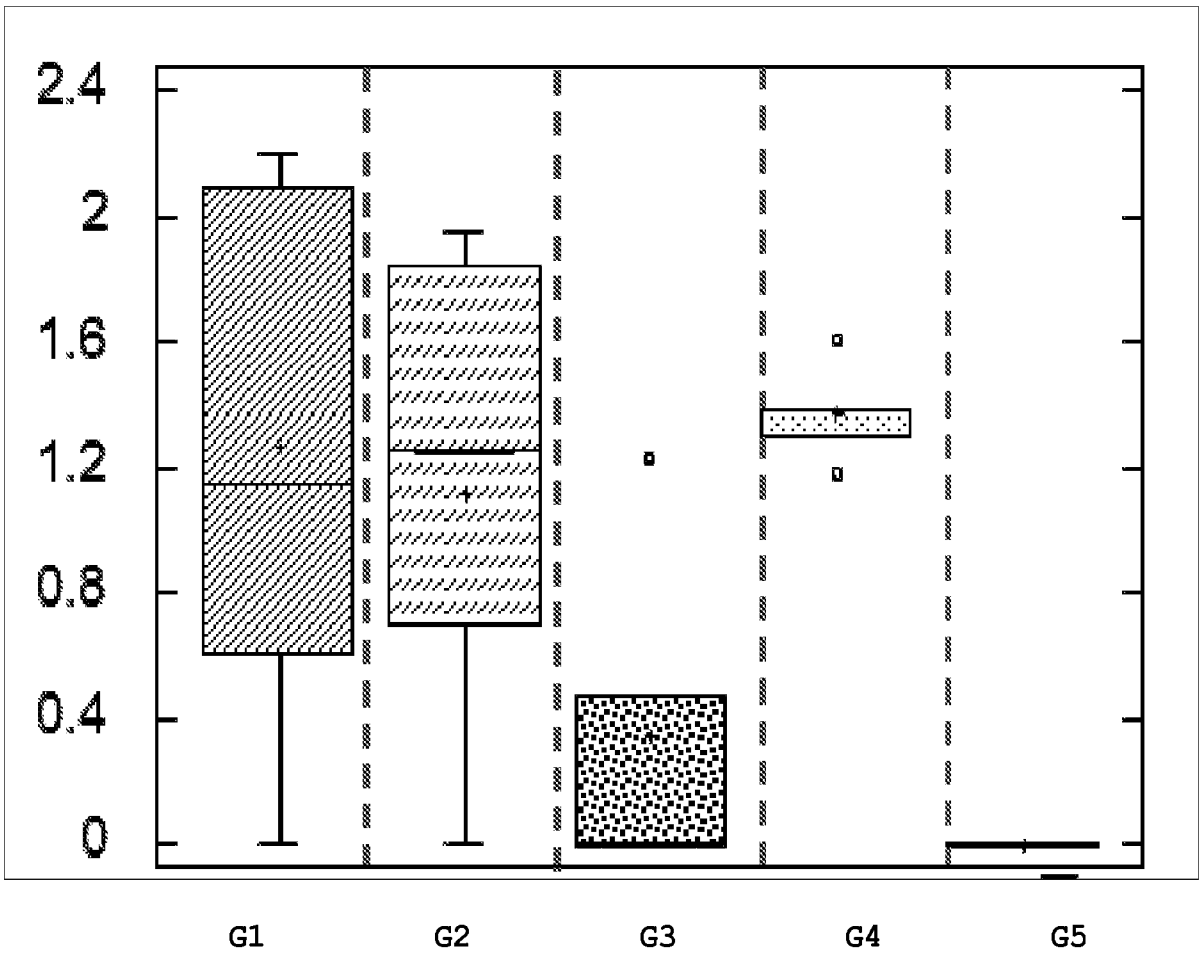


Figure 23

FMDV SVN log10 titer by Groups for Day 42

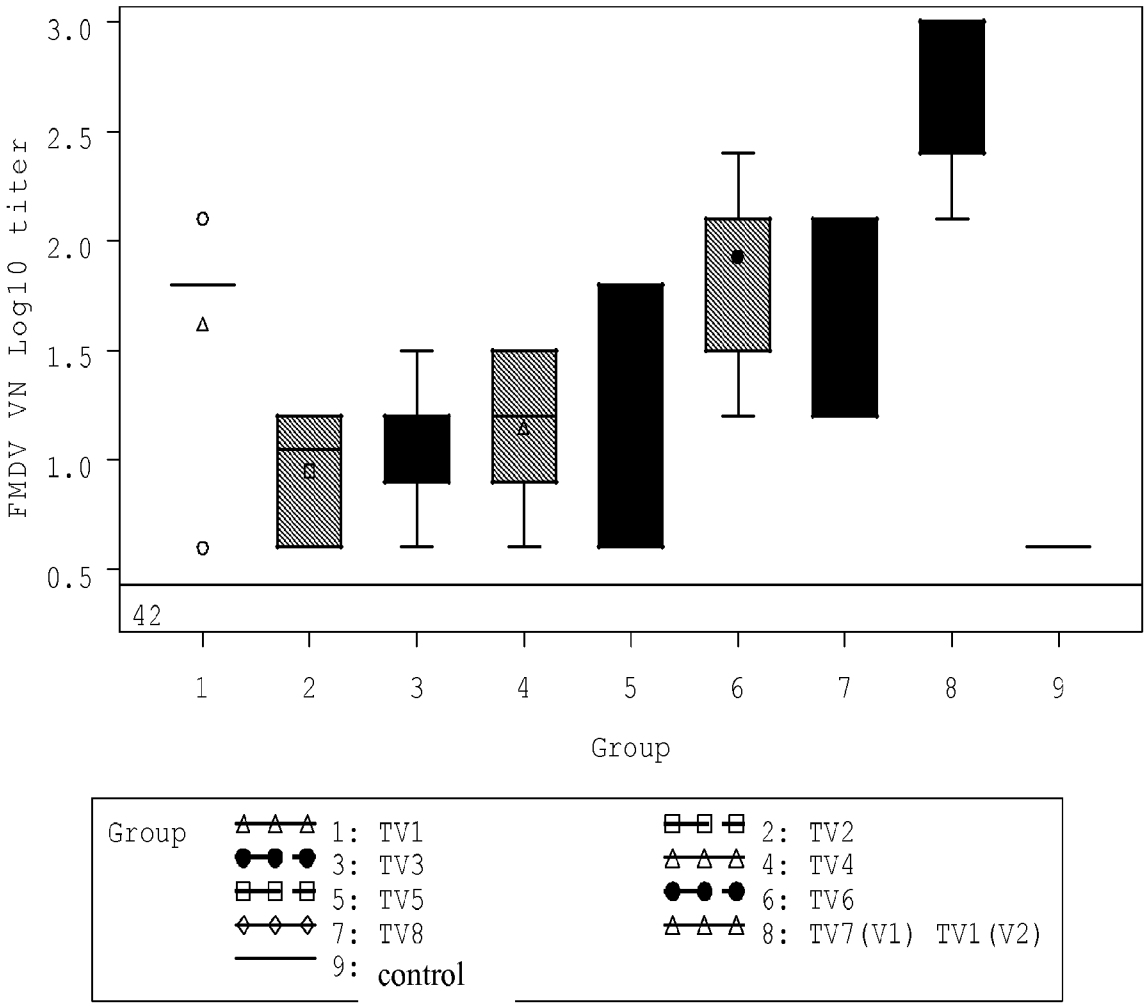


Figure 24

Mean FMDV VN log10 titer

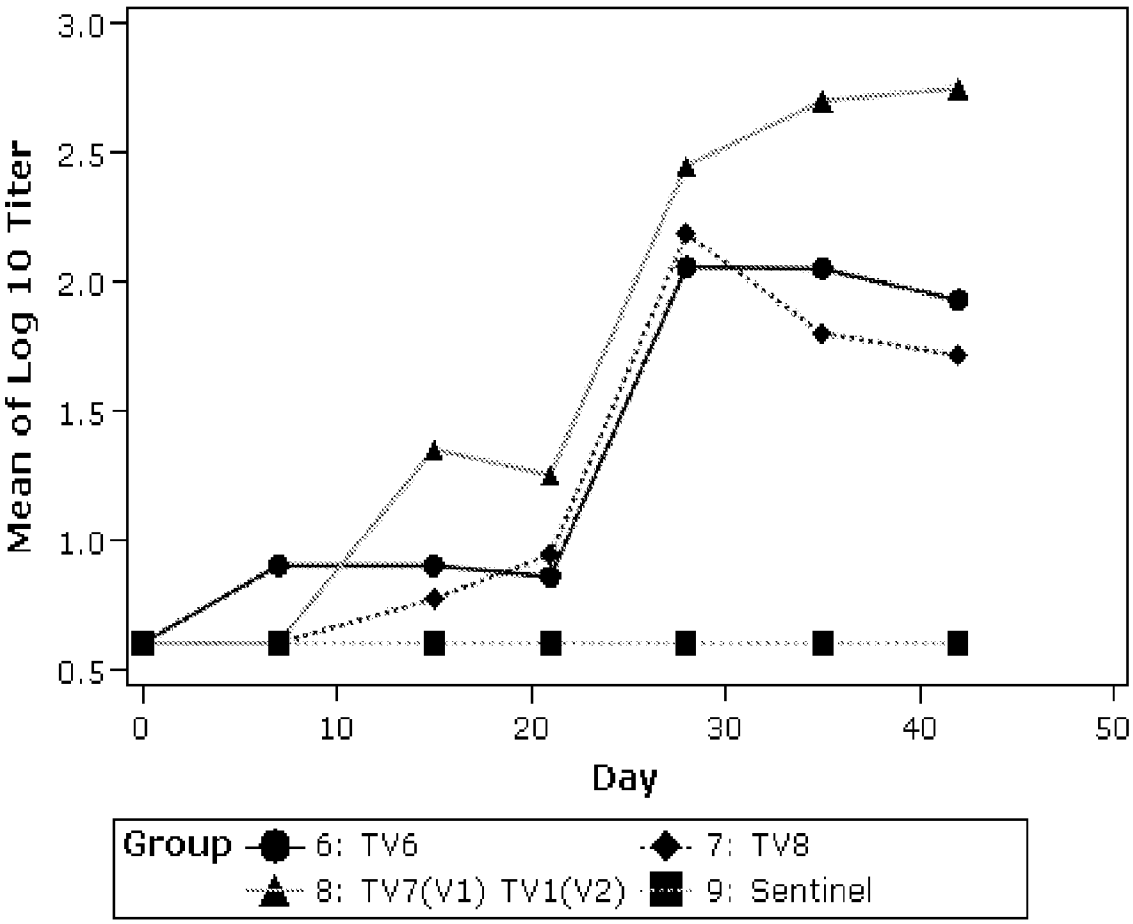


Figure 25

Plasmid pAD3027 map

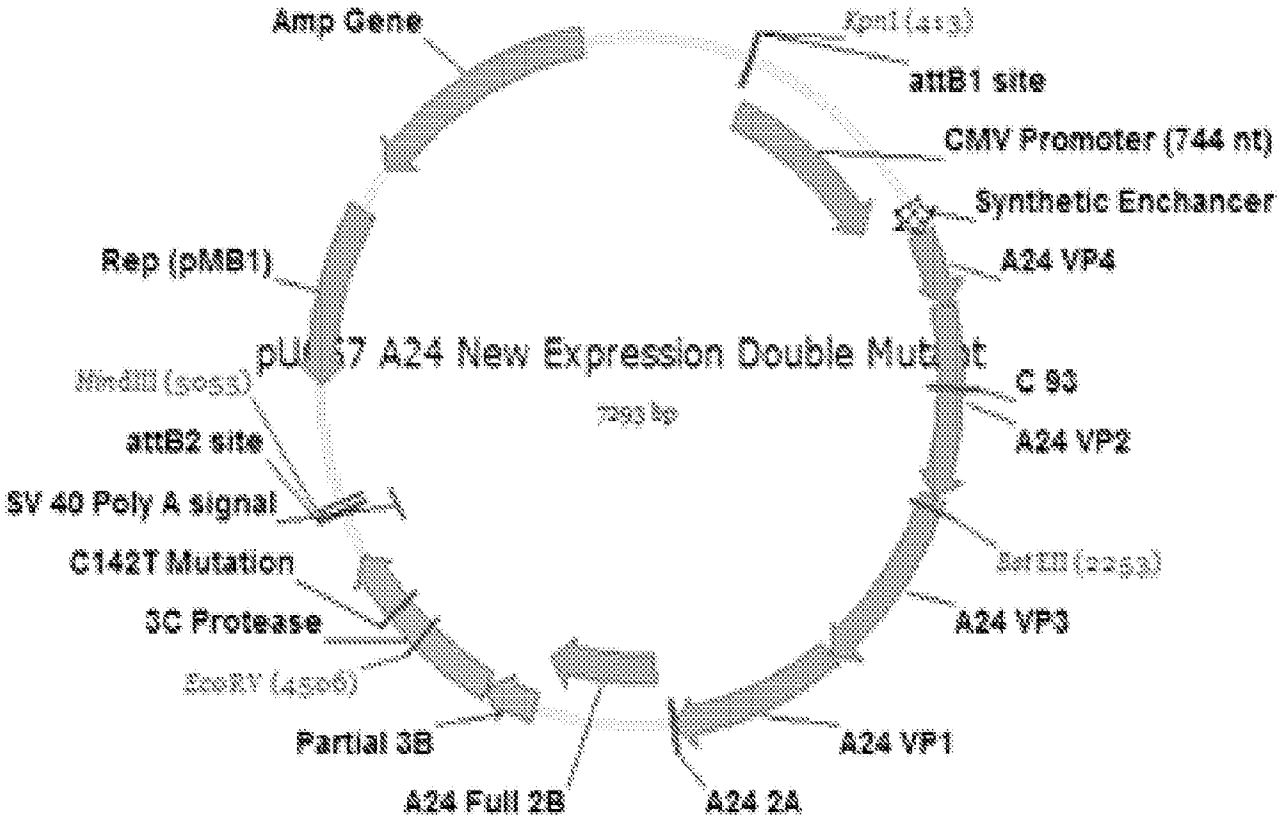
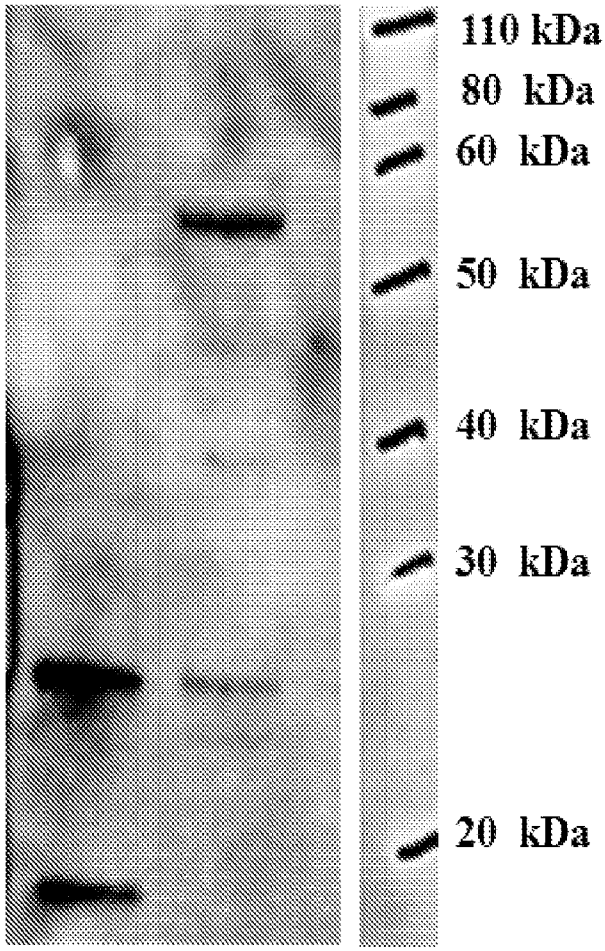


Figure 26

Western Blot of vAD3027



Lane 1: control to show VP2 (fully processed)
Lane 2: vAD3027

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/051755

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/12
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 C12N

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 practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/00251 A1 (MERIAL) 3 January 2002 (2002-01-03)	1,2,5,6, 9,11,16, 20,22-26
Y	page 4, line 23 - line 26 page 5, line 3 - line 7 page 5, line 8 - line 9 page 5, line 18 - line 21 page 5, line 24 - line 29 page 8, line 16 - line 18 page 16, line 14 - line 20 page 17, line 6 - line 13 examples 14-21 ----- -/--	7,8,10, 12-14, 18-21, 27,28

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 December 2015	17/12/2015

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/253185 A1 (NORDGREN ROBERT [US] ET AL) 8 October 2009 (2009-10-08) paragraph [0014] - paragraph [0015] paragraph [0114] paragraph [0120] paragraph [0122] examples 4-10	7,8,10, 12-14, 18-21, 27,28

X	CLAUDINE PORTA ET AL: "Efficient production of foot-and-mouth disease virus empty capsids in insect cells following down regulation of 3C protease activity", JOURNAL OF VIROLOGICAL METHODS, vol. 187, no. 2, 19 November 2012 (2012-11-19), pages 406-412, XP055121131, ISSN: 0166-0934, DOI: 10.1016/j.jviromet.2012.11.011 abstract Material and Methods	3,15

X	B. MOHANA SUBRAMANIAN ET AL: "Development of foot-and-mouth disease virus (FMDV) serotype O virus-like-particles (VLPs) vaccine and evaluation of its potency", ANTIVIRAL RESEARCH, vol. 96, no. 3, 6 October 2012 (2012-10-06), pages 288-295, XP055228469, NL ISSN: 0166-3542, DOI: 10.1016/j.antiviral.2012.09.019 abstract Material and Methods	3,15

X	DIANA M. ALEJO ET AL: "An adenovirus vectored mucosal adjuvant augments protection of mice immunized intranasally with an adenovirus-vectored foot-and-mouth disease virus subunit vaccine", VACCINE, vol. 31, no. 18, 1 April 2013 (2013-04-01) , pages 2302-2309, XP055232707, GB ISSN: 0264-410X, DOI: 10.1016/j.vaccine.2013.02.060 abstract Material and Methods	4,17

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GUOHUI ZHOU ET AL: "Recombinant adenovirus expressing type Asia1 foot-and-mouth disease virus capsid proteins induces protective immunity against homologous virus challenge in mice", RESEARCH IN VETERINARY SCIENCE., vol. 94, no. 3, 1 June 2013 (2013-06-01), pages 796-802, XP055232710, GB ISSN: 0034-5288, DOI: 10.1016/j.rvsc.2012.12.004 abstract Material and Methods -----	4,17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/051755

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

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

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

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

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

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

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-28

   All searched claims

1.1. claims: 1-11

   A composition or vaccine comprising a foot and mouth Disease Virus (FMDV) antigen or a recombinant viral vector expressing an FMDV antigen.

1.2. claims: 12-14

   A plasmid comprising a polynucleotide encoding an FMDV antigen having the sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 6, 8, 10, 12, 13, or 16.

1.3. claim: 15

   A stably transformed insect cell expressing FMDV empty capsids or FMDV VLPs.

1.4. claims: 16-19

   A recombinant viral vector comprising one or more heterologous polynucleotides coding for and expressing one or more FMDV antigens.

1.5. claims: 20, 21

   A substantially purified FMDV empty capsid or FMDV VLP expressed in insect cells, wherein the FMDV empty capsid or VLP comprises a polypeptide having the sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 6, 8, 10, 12, 13, or 16.

1.6. claims: 22-28

   A method of vaccinating an animal susceptible to FMDV infection or eliciting an immune response in the animal against FMDV comprising at least one administration of the composition of any one of claims 1- 11, or the viral vector of claims 16-19, or the FMDV empty capsids or VLPs of any one of claims 20-21.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/051755

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