Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2321903 C 2011/05/31

(11)(21) 2 321 903

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 1999/03/01
(87) Date publication PCT/PCT Publication Date: 1999/09/10
(45) Date de délivrance/Issue Date: 2011/05/31
(85) Entrée phase nationale/National Entry: 2000/08/28
(86) N° demande PCT/PCT Application No.: FR 1999/000453
(87) N° publication PCT/PCT Publication No.: 1999/044633
(30) Priorité/Priority: 1998/03/03 (FR98/02800)

(51) Cl.Int./Int.Cl. *A61K 39/39* (2006.01),
A61K 48/00 (2006.01)

(72) Inventeurs/Inventors:
AUDONNET, JEAN-CHRISTOPHE FRANCIS, FR;
MINKE, JULES MAARTEN, FR

(73) Propriétaire/Owner:
MERIAL, FR

(74) Agent: SMART & BIGGAR

(54) Titre : VACCINS VIVANTS RECOMBINANTS COMPOSES D'UN ADJUVANT ET D'UN VECTEUR VIRAL CONTENANT UN GENE DE VIRUS HERPES ANIMAL OU UN GENE DE VIRUS INFLUENZA

(54) Title: LIVE RECOMBINANT VACCINES COMPRISING AN ADJUVANT COMPOUND AND A VIRAL VECTOR COMPRISING A GENE OF AN ANIMAL HERPES OR INFLUENZA VIRUS

(57) Abrégé/Abstract:
There is provided a live recombinant vaccine comprising (i) a viral vector incorporating and expressing in vivo a heterologous nucleotide sequence selected from a gene of an animal herpes virus and a gene of an influenza virus, and (ii) at least one adjuvant compound chosen from the acrylic or methacrylic acid polymers and the maleic anhydride and alkenyl derivative copolymers.

30754-34

ABSTRACT

There is provided a live recombinant vaccine comprising (i) a viral vector incorporating and expressing in vivo a heterologous nucleotide sequence selected from a gene of an animal herpes virus and a gene of an influenza virus, and (ii) at least one adjuvant compound chosen from the acrylic or methacrylic acid polymers and the maleic anhydride and alkenyl derivative copolymers.

30754-34

LIVE RECOMBINANT VACCINES COMPRISING AN ADJUVANT COMPOUND AND A VIRAL VECTOR COMPRISING A GENE OF AN ANIMAL HERPES OR INFLUENZA VIRUS

The present invention relates to an improvement to recombinant live vaccines integrating and expressing in vivo one or more heterologous genes. It relates in particular to such adjuvant-containing vaccines, to the use of particular adjuvant compounds for using such vaccines as well as to vaccination methods relating thereto. Its subject is also a method of preparing these vaccines.

It is conventional to incorporate into inactivated or subunit vaccines adjuvants intended to increase the immune response towards the antigens which these vaccines contain.

It has also been found to incorporate adjuvants into attenuated live vaccines when the attenuation of microorganisms leads to a reduction in the immune response.

Recently, combined vaccinations against several pathogens using an inactivated vaccine for one valency and an attenuated live vaccine for the other valency have also been proposed. It has thus been proposed to reconstitute the attenuated live vaccine, preserved in freeze-dried form, in the composition of an adjuvant-containing inactivated vaccine. The said composition acts as reconstitution vehicle for the live vaccine, without any adjuvant effect being sought for it.

EP-A-532 833 proposes a vaccine against horse rhinopneumonia, a pathology caused by the equine herpesvirus (EHV). The vaccine is an inactivated and adjuvant-containing vaccine, grouping together the inactivated EHV-4 virus and EHV-1 virus, containing the adjuvant Havlogen®, based on a polyacrylic polymer.

As for most herpesviruses, there is currently no effective vaccine allowing rapid elimination of the virus after infection. The known vaccines attempt to protect against the appearance of clinical signs. In general, the effect on viral excretion remains limited.

According to EP-A-532 833, the vaccine developed is thought to lead to a drop in viral excretion ranging from 79 to 93% (see results section). Eight control animals out of nine excreted virus after a challenge over an average of 1.4 days whereas the normal duration of excretion after challenge is usually greater than or equal to 5 days. This represents a challenge of low intensity which artificially increases the protection of vaccinated horses compared with the controls. The reduction in viral excretion is not therefore significant in this experiment.

Adjuvants of the carbomer type have also been used in inactivated virus-containing equine influenza vaccines (IEV).

30754-34

Mumford et al (Epidemiol. Infect. (1994), 112, 421-437) recall that two equine IEV vaccine doses are required to induce a transient humoral response and a weak protection against the virus in horses. The authors compare the adjuvant effects of carbomer and of aluminum phosphate on inactivated vaccines in the presence or otherwise of tetanus toxoid. In all cases, a low antibody titre measured by the SRH (single radial haemolysis) technique with respect to the strains H7N7 and H3N8 is obtained after a first vaccination and a second and then third vaccination are necessary to see the appearance of stronger transient responses.

US-A-4 500 513 also presents vaccination trials against the equine influenza virus with an inactivated vaccine in the presence of a carbomer. The origin of the animals and their medical status is not indicated precisely and it appears that they are ground animals (column 11, 2nd paragraph). The high antibody titres, measured by a haemagglutination inhibition technique, indicate that the animals had probably already been infected with influenza and that the response induced after vaccination was of the booster type, and not of the primary vaccination type.

Finally, Fort Dodge Solvay markets inactivated equine influenza vaccines (Duvaxyn® IE and IE-T plus) and an inactivated equine rhinopneumonia vaccine (Duvaxyn® $EHV_{1,4}$), in a carbomer adjuvant.

Commercial inactivated vaccines against equine influenza, containing the adjuvant aluminium hydroxide (for example Tetagripiffa®, Merial, Lyons, France) are also known.

A large number of other adjuvants are used in the context of conventional inactivated or subunit vaccines. There may be mentioned, for example, aluminum hydroxide, aluminum phosphate, Avridine®., DDA, monophosphoryl lipid A, Pluronic L121 and other block polymers, muramyl peptides, saponins, trehalose dimycolate, copolymers of maleic anhydride and ethylene, copolymers of styrene and acrylic or methacrylic acid, polyphosphazene, oily emulsions and the like.

WO-A-94 16681 suggests supplementing a recombinant live vaccine expressing a heterologous gene of an enveloped virus with an adjuvant vaccine composition in the form of a water-in-oil, oil-in-water or water-in-oil-in-water emulsion.

Such a solution may however have a number of disadvantages.

30754-34

In practice, the final user should have available, on the one hand, a freeze-dried active ingredient and, on the other hand, an already constituted emulsion which should make it possible to reconstitute the freeze-dried active ingredient.

30754-34

Lack of stability of the emulsion during storage could be detrimental to the efficacy and safety of the reconstituted vaccine.

The activity of attenuated live microorganisms could be called into question following their instability in the oily phase. This may in particular be the case for viruses which may thereby lose their viability.

Vaccines in emulsion can also pose problems of safety at the site of injection.

The present invention is therefore given with the objective of providing new vaccine compositions based on recombinant live vaccine expressing at least one heterologous nucleotide sequence, especially a heterologous gene, containing an adjuvant which is capable of remarkably increasing the immunity conferred relative to the same vaccine with no adjuvant and which is perfectly suitable for this type of vaccine.

The Applicant has found the carbomer class of compounds were capable of acting as adjuvant under the required conditions for this type of vaccine and this in unexpected proportions. Trials carried out on animal herpesviruses (EHV-1, Equine Herpesvirus) have shown that the supply of carbomer could reduce viral excretion during an experimental infection, in unexpected proportions. Other trials carried out on the equine influenza A virus have made it possible to obtain, surprisingly, in horses, early and very high serological titres, better than those obtained with the best commercial vaccines.

The subject of the present invention is therefore a recombinant live vaccine comprising a viral vector incorporating and expressing in vivo a heterologous nucleotide sequence, preferably a gene for a pathogenic agent, and at least one adjuvant compound chosen from the polymers of acrylic or methacrylic acid and the copolymers of maleic anhydride and alkenyl derivative.

The invention will now be described in greater detail with the aid of the embodiments taken by way of non-limiting examples and referring to the drawings.

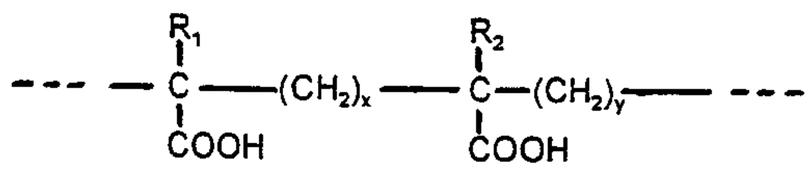
The preferred compounds are the polymers of acrylic or methacrylic acid which are cross-linked, especially with polyalkenyl ethers of sugars or polyalcohols. These compounds are known by the term carbomer (Pharmeuropa Vol. 8, No. 2, June 1996). Persons skilled in the art can also refer to US-A-2 909 462 which describes such acrylic

polymers cross-linked with a polyhydroxylated compound having at least 3 hydroxyl groups, preferably not more than 8, the hydrogen atoms of at least three hydroxyls being replaced by unsaturated aliphatic radicals having at least 2 carbon atoms. The preferred radicals are those

containing from 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals may themselves contain other substituents, such as methyl. The products sold under the name Carbopol® (BF Goodrich, Ohio, USA) are particularly appropriate. They are cross-linked with an allyl sucrose or with allyl pentaerythritol. Among them, there may be mentioned Carbopol® 974P, 934P and 971P.

Among the copolymers of maleic anhydride and alkenyl derivative, the copolymers EMA® (Monsanto) which are copolymers of maleic anhydride and ethylene, linear or cross-linked, for example cross-linked with divinyl ether, are preferred. Reference may be made to J. Fields et al., Nature, 186: 778-780, Jun. 4, 1960.

From the point of view of their structure, the polymers of acrylic or methacrylic acid and the copolymers EMA® are preferably formed of basic units of the following formula:



in which:

- R_1 and R_2, which are identical or different, represent H or CH_3
- x = 0 or 1, preferably x = 1
- y = 1 or 2, with x + y = 2

For the copolymers EMA®, x = 0 and y = 2. For the carbomers, x = y = 1.

The dissolution of these polymers in water leads to an acid solution which will be neutralized, preferably to physiological pH, in order to give the adjuvant solution into which the vaccine itself will be incorporated. The carboxyl groups of the polymer are then partly in COO^- form.

Preferably, a solution of adjuvant according to the invention, especially of carbomer, is prepared in distilled water, preferably in the presence of sodium chloride, the solution obtained being at acidic pH. This stock solution is diluted by adding it to the required quantity (for obtaining the desired final concentration), or a substantial part thereof, of water charged with NaCl, preferably physiological saline (NaCl 9 g/l), all at once or in several portions with concomitant or subsequent neutralization (pH 7.3 to 7.4), preferably with NaOH. This solution at physiological pH will be used as is to reconstitute the vaccine, especially stored in freeze-dried form.

The polymer concentration in the final vaccine composition will be 0.01% to 2% w/v, more particularly 0.06 to 1% w/v, preferably 0.1 to 0.6% w/v.

The invention proves particularly useful for vaccination against animal herpesviruses. The invention relates most particularly to the equine herpesvirus (EHV-1 and EHV-4 in particular), feline herpesvirus (FHV), canine herpesvirus (CHV), avian herpesvirus (Marek and ILTV), bovine herpesvirus (BHV) and porcine herpesvirus (PRV = Aujeszky's disease virus or pseudorabies virus).

The subject of the invention is therefore recombinant live vaccines comprising at least one viral vector incorporating and expressing at least one gene of such a herpesvirus and at least one adjuvant in accordance with the invention.

By way of example, persons skilled in the art may refer to WO-A-92 15672, which describes the production of expression vectors based on poxviruses capable of expressing such genes. There will be found for example a canarypox expressing the gB, gC and gD genes of EHV-1 (vCP132), which is also applicable to EHV-4, a vaccinia virus expressing these same genes (vP 1043), a vaccinia virus expressing the gI(gB), gIII(gC) and gIV(gD) genes of BHV-1, a canarypox expressing gD of FHV-1, or alternatively recombinants expressing the gII(gB), gIII(gC) and gp50(gD) of PRV. They can also refer to WO-A-95/26 751 (incorporated by reference), which describes recombinant viruses vCP320, vCP322 and vCP294 expressing the gB, gC and gD genes, respectively, of CHV. They can also refer to the recombinants expressing FHV, PRV and BHV genes in FR-A-2 647 808, or WO-A-9012882.

The invention also proves particularly advantageous for vaccination against influenza viruses, as demonstrated here for EIV (equine influenza virus). There may also be mentioned avian influenza (AIV) and, porcine influenza (swine influenza virus).

By way of example, persons skilled in the art can refer to the recombinant canarypox expressing the HA gene of EIV in WO-A-92 15 672.

The subject of the invention is therefore recombinant live vaccines comprising at least one viral vector incorporating and expressing at least one gene of such an influenza virus, and at least one adjuvant in accordance with the invention. In particular, the vaccine comprises a mixture of two or three vectors each incorporating and expressing an HA gene, the genes being obtained from different strains, for example equine influenza strains Prague, Kentucky and

Newmarket. Similarly, a single vector may be used to incorporate and express the HAs of 2 or of the 3 strains.

The invention also applies to other animal pathogens, such as in particular FeLV (see also canarypox recombinants in WO-A-92 15672 by way of example, expressing env, gag = vCP93 and vCP97), tetanus (see also WO-A-92 15672 and the recombinants vCP161 and vP1075, canarypox and vaccinia, expressing the tetanus toxin), Carre's disease virus (canine distemper virus or CDV) (see recombinant vCP 258 in WO-A-95 27780.)

The subject of the invention is therefore recombinant live vaccines comprising at least one viral vector incorporating and expressing at least one gene of such a virus.

The subject of the invention is also multivalent recombinant vaccines, that is to say containing two or more recombinant vectors expressing antigens of two or more diseases, in the form of a mixture in an adjuvant solution in accordance with the invention.

Moreover, the invention applies to the use of any type of viral expression vector, such as poxvirus (vaccinia virus, including NYVAC according to WO-A-92/15672, fowlpox, canarypox, pigeonpox, swinepox and the like), adenovirus, herpesvirus. Canarypox, e.g. ALVAC (WO-A-95/27780 and WO-A-92/15672) is found to be particularly appropriate in the context of the present invention.

In a ready-for-use, especially reconstituted, vaccine, the viral vector is present in the quantities normally used and described in the literature.

The recombinant live vaccines generally exist in a freeze-dried form allowing their storage and are reconstituted immediately before use in a solvent or excipient, which will be here the solution of adjuvant in accordance with the invention.

The subject of the invention is therefore also a vaccination set comprising, packaged separately, freeze-dried vaccine and a solution of the adjuvant compound according to the invention for the reconstitution of the freeze-dried vaccine.

The subject of the invention is also a method of vaccination consisting in administering, by the parenteral, preferably subcutaneous, intramuscular or intradermal, route or by the mucosal route a vaccine in accordance with the invention at the rate of one or more administrations, optionally with a preliminary step of reconstituting the freeze-dried vaccine (the recombinant vector) in a solution of adjuvant compound.

One objective of such a method may be to protect animals from the clinical point of view and to reduce viral excretion, which corresponds in particular to the case of herpesviruses.

Another objective may be to increase the immune response and to make it occur earlier, especially by inducing antibodies starting from the first administration.

The subject of the invention is also the use of the adjuvant compounds in accordance with the invention for the production of recombinant live vaccines, especially conferring an improved and earlier immune response and/or an increased reduction in viral excretion. Reference may be made to what was said above.

The invention will now be described in greater detail with the aid of the embodiments taken by way of non-limiting examples and referring to the drawings:

- Figure 1 depicts the nucleotide sequence of the EIV HA gene of the EIV Newmarket 2/93 strain ;
- Figure 2 depicts the nucleotide sequence of the feline herpesvirus-1 (FHV-1) gc gene ;
- Figure 3 depicts a graph showing the variation of viral excretion after experimental injection in horses vaccinated with the aid of different vaccines against EHV.

EXAMPLES

Example 1 : Generation of the Donor Plasmids for the Sites of Insertion C3, C5 and C6 Into the Canarypox Virus "ALVAC"

The donor plasmids for the different sites of insertion into the canarypox virus "ALVAC" (Tartaglia et al. Virology, 1992. 188. 217-232) are described in Application WO-A-95/27780, Example 20.

These plasmids were designated in this application in the following manner:

"plasmid VQH6CP3LSA.2" for the "C3" site

"plasmid HC5LSP28" for the "C5" site

"plasmid pC6L" for the "C6" site.

Example 2 : Generation of the Recombinant Virus vCP258 (ALVAC/CDV HA+F)

The Onderstepoort strain of the CDV virus was used to isolate the HA and F genes (sequence of the HA gene described by Curran et al. Virology. 1991. 72. 443-447, and sequence

of the F gene described by Barrett et al. Virus Research. 1987. 8. 373-386).

The construction of the donor plasmid pMM103 for the insertion of the expression cassettes H6 vaccinia promoter-CDV F gene and H6 vaccinia promoter-CDV HA gene into the C6 locus of the ALVAC virus is described in Example 19 of Application WO-A-95/27780.

This plasmid was used as donor plasmid for in vitro recombination (Piccini et al. Methods in Enzymology. 1987. 153. 545-563), with the ALVAC virus to generate the recombinant virus designated vCP258 as in Example 19 of the abovementioned application.

Example 3 : Generation of the Recombinant Virus vCP1502 (ALVAC/EIV HA Prague)

The sequence of the HA gene (EIV Prague strain) is presented in Figure No. 23 of Application WO-A-92/15672. The viral RNA of the genome of the equine influenza virus strain Prague 56 was extracted from 100 µl of a viral suspension of this virus with the "Total RNA Separator kit" extraction kit from CLONTECH (Palo Alto, Calif.) (Cat #K1042-1). The RNA pellet was taken up in 10 µl of ultrapure water and a complementary DNA synthesis reaction, followed by a PCR reaction (= "RT-PCR" reaction) was carried out taking as template 2 µl of purified viral RNA and the following oligonucleotides:

TAY51A (SEQ ID NO:1) (70 mer)

5' CGCGGCCATCGCGATATCCGTTAAGTTTGTATCGTAATGAACACTCAAATTCTAAT ATTAGCCACTTCGG 3'

and TAY53A (SEQ ID NO:2) (36 mer) 5'

CGCGCGGCGGTACCTTATATACAAATAGTGCACCGC 3'

in order to amplify the Prague EIV HA gene. The PCR fragment thus obtained was ligated into the vector pCRII (Invitrogen, San Diego, Calif.) to give the plasmid pJT007.

The plasmid pJT007 was digested with NruI and Asp718 in order to isolate an NruI-718 fragment of about 1800 bp containing the end of the H6 promoter and the Prague 56 HA gene in its entirety. This fragment was ligated with the donor plasmid C5 HC5LSP28, previously digested with NruI and Asp718, to finally give the plasmid pJT008. This plasmid contains the

expression cassette H6-Prague 56 HA gene in the C5 locus of the ALVAC virus. The structure of this plasmid was verified by sequencing and complete restriction map.

This plasmid is the donor plasmid for the insertion of the expression cassette H6-Prague 56 HA gene into the 5 locus.

After linearizing with NotI, the plasmid pJT008 was used as donor plasmid for in vitro recombination (Piccini et al. Methods in Enzymology. 1987. 153. 545-563) with the ALVAC virus in order to generate the recombinant virus designated vCP1502.

Example 4 : Generation of the Recombinant Virus vCP1529 (ALVAC/EIV HA Kentucky 1/94)

The viral RNA of the genome of the equine influenza virus strain Kentucky 1/94 (Daly et al. J. Gen. Virol. 1996. 77. 661-671) was extracted with 100 µl of a viral suspension of this virus with the "Total RNA Separator kit" extraction kit from CLONTECH (Palo Alto, Calif.) (Cat #K1042-1). The RNA pellet was taken up in 10 µl of ultrapure water and RT-PCR reaction was carried out taking as template 2 µl of purified viral RNA and the following oligonucleotides:

TAY55A (SEQ ID NO:3) (70 mer)

5' CGCGGCCATCGCGATATCCGTTAAGTTTGTATCGTAATGAAGACAACCATTATTTT GATACTACTGACCC 3'

and TAY57A (SEQ ID NO:4) (42 mer) 5'

CGCGCGGCGGTACCTCAAATGCAAATGTTGCATCTGATGTTG 3'

in order to amplify the HA gene. The PCR fragment thus obtained was ligated into the vector pCRII (Invitrogen, San Diego, Calif.) to give the plasmid pJT001. The sequence of the Kentucky 1/94 strain EIV HA gene cloned into the plasmid pJT001 is not different from the sequence of the Kentucky 1/94 strain EIV HA gene available in the GenBank databank (accession number L39914).

The plasmid pJT001 containing the HA gene (Kentucky 1/94) was digested with NruI and Asp718 in order to isolate an NruI-Asp718 fragment of 1800 bp (containing the end of the H6 promoter and the Kentucky 1/94 HA gene in its entirety). This fragment was ligated with the donor plasmid C5 HC5LSP28, previously digested with NruI and Asp718, to finally give the plasmid pJT005. This plasmid contains the expression cassette H6-Kentucky 1/94 HA gene in

the C5 locus of the ALVAC virus. The structure of this plasmid was verified by sequencing and complete restriction mapping.

This plasmid is a donor plasmid for the insertion of the expression cassette H6-Kentucky 1/94 HA gene into the C5 locus.

After linearizing with NotI, the plasmid pJT005 was used as donor plasmid for in vitro recombination (Piccini et al. Methods in Enzymology. 1987. 153. 545-563) with the ALVAC virus in order to generate the recombinant virus designated vCP1529.

Example 5: Generation of the Recombinant Virus vCP1533 (ALVAC/EIV HA Newmarket 2/93)

The viral RNA of the genome of the equine influenza virus strain Newmarket 2/93 (Daly et al. J. Gen. Virol. 1996. 77. 661-671) was extracted from 100 µl of a viral suspension of this virus with the "Total RNA Separator kit" extraction kit from CLONTECH (Cat #K1042-1). The RNA pellet was taken up in 10 µl of ultrapure water and an RT-PCR reaction was carried out taking as template 2 µl of purified viral RNA and the following oligonucleotides:

CCL007 (SEQ ID NO:5) (40 mer)

5' TTGTCGACTCAATCATGAAGACAACCATTATTTTGATACT 3'

and CCL0018 (SEQ ID NO:6) (34 mer)

5' TTGGATCCTTACTCAAATGCAAATGTTGCAYCTG 3'

in order to amplify the HA gene. The PCR fragment thus obtained was ligated to the vector pCRII (InVitrogen, San Diego, Calif.) to give the plasmid pCCL026.

The sequence of the HA gene (EIV Newmarket 2/93 strain) is presented in Figure No. 1 (SEQ ID NO:7).

The plasmid pCCL026 containing the HA gene (Newmarket 2/93 strain) was digested with SpeI and AccI. The following oligonucleotides:

30754-34

TAY99N (SEQ ID NO:8) (74 mer)

5'
CTAGTTCGCGATATCCGTTAAGTTTGTATCGTAATGAAGACAACCATTATTTTGAT
ACTACTGACCCATTGGG T 3'

and TAY100N (SEQ ID NO:9) (72 mer)

5'
AGACCCAATGGGTCAGTAGTATCAAAATAATGGTTGTCTTCATTACGATACAAAC
TTAACGGATATCGCGAA 3'

were annealed and ligated with the plasmid pCCL026 digested with SpeI+AccI to give the plasmid pJT003. The double-stranded oligonucleotide TAY99N/TAY100N contains the 3' region of the H6 promoter up to the NruI site and the first 40 coding bases of the HA gene.

The plasmid JT003 was digested with NruI and XhoI in order to isolate an NruI-XhoI fragment of about 1800 bp containing the end of the H6 promoter and the HA gene in its entirety. This fragment was ligated with the donor plasmid C5 HC5LSP28, previously digested with NruI and XhoI, to finally give the plasmid pJT004. This plasmid contains the expression cassette H6-Newmarket 2/93 HA gene in the C5 locus of the ALVAC virus. The structure of this plasmid was verified by sequencing and complete restriction mapping.

This plasmid is the donor plasmid for the insertion of the expression cassette H6-Newmarket 2/93 HA gene into the C5 locus.

After linearizing with PvuI, the plasmid pJT004 was used as donor plasmid for the in vitro recombination (Puccini et al. Methods in Enzymology. 1987. 153. 545-563) with the ALVAC virus in order to generate the recombinant virus designated vCP1533.

Example 6 : Generation of the Recombinant Virus VCP132 (ALVAC/EHV-1 gB+gC+gD)

The construction of the recombinant virus is described in Examples 25 and 26 of Application WO-A-92/15672. This virus was generated by in vitro recombination between the ALVAC virus and the donor plasmid pJCA049. This plasmid contains the following 3 expression cassettes cloned into the site of insertion C3:

I3L vaccinia promoter-EHV-1 gB gene

H6 vaccinia promoter-EHV-1 gC gene

42K entomopox promoter-EHV-1 gD gene

The sequences of the EHV-1 gB, gC and gD genes are described in Application WO-A-92/15672 in Figures No. 2 (sequence of the EHV-1 gene gp13=gC), No. 6 (sequence of the EHV-1 gene gp14=gB) and No. 12 (sequence of the EHV-1 genes gD, gp63 and gE).

Example 7 : Generation of the Recombinant Virus vCP243 (ALVAC/FHV-1 gB+gC+gD)

30754-34

The sequence of the FHV-1 gB gene (CO strain) is presented in Figure No. 34 of Application WO-A-90/12882.

The FHV-1 gC gene (CO strain) (sequence presented in Figure No. 2) (SEQ ID NO: 10) was cloned from the EcoRI F fragment (7.6 kbp). It has a size of 1599 bp and encodes a protein of 533 amino acids.

The FHV-1 gD gene (CO strain) (sequence presented in Figure No. 28 of Application WO-A-92/15672) was cloned from the EcoRI M fragment (4.4 kbp) (plasmid pFHVEcoRIM).

Construction of the Expression Cassette I3L-FHV-1 gB Gene Mutated at the Level of the Signals for Early Termination of Transcription (TTTTTNT)

The following oligonucleotides:

MP287 (SEQ ID NO: 11) (20 mer)

5' GATTAAACCTAAATAATTGT 3'

and JCA158 (SEQ ID NO:12) (21 mer)

5' TTTTTCTAGACTGCAGCCCGGGACATCATGCAGTGGTTAAAC 3'

were used for a PCR amplification with the template of a plasmid containing the I3L vaccinia promoter (Riviere et al. J. Virol. 1992. 66. 3424-3434) in order to generate a blunt-ended XbaI fragment of 120 bp (containing the I3L vaccinia promoter) = fragment A. The following oligonucleotides:

JCA213 (SEQ ID NO: 13) (18 mer)

5' GGGTTTCAGAGGCAGTTC 3'

and JCA238 (SEQ ID NO: 14) (21 mer)

5' ATGTCCACTCGTGGCGATCTT 3'

were used to generate, by PCR from the template of the plasmid pJCA001, a blunt-ended BamHI fragment of 720 bp (containing the 5' part of the FHV-1 gB gene) = fragment B.

Fragment A was digested with XbaI, and then phosphorylated. Fragment B was digested with BamHI, and then phosphorylated. Fragments A and B were then ligated together with the vector pBluescript SK+, previously digested with XbaI and BamHI, to give the plasmid pJCA075.

The following oligonucleotides:

JCA158 (SEQ ID NO: 15) and JCA211 (SEQ ID NO: 16) (21 mer):

5' GTGGACACATATAGAAAGTCG 3'

30754-34

were used to generate, by PCR from the template of the plasmid pJCA075, a blunt-ended XbaI fragment of 510 bp (containing the I3L promoter fused to the 5' part of the FHV-1 gB gene mutated at the level of the signal TTTTTNT) = fragment C.

The following oligonucleotides:

JCA212 (SEQ ID NO: 17) (21 mer)

5' CACCTTCAGGATCTACTGTCG 3'

and JCA213 (SEQ ID NO: 13) (18 mer)

were used to generate, by PCR from the template of the plasmid pJCA001, a blunt-ended BamHI fragment of 330 bp (containing the central part of the FHV-1 gB gene) = fragment D.

Fragment C was digested with XbaI, and then phosphorylated. Fragment D was digested with BamHI, and then phosphorylated. Fragments C and D were then ligated together with the vector pBluescript SK+, previously digested with XbaI and BamHI to give the plasmid pJCA076.

The following oligonucleotides:

JCA239 (SEQ ID NO: 18) (24 mer)

5' ACGCATGATGACAAGATTATTATC 3'

and JCA249 (SEQ ID NO: 19) (18 mer)

5' CTGTGGAATTCGCAATGC 3'

were used to generate, by PCR from the template of the plasmid pJCA001, a blunt-ended EcoRI fragment of 695 bp (containing the first 3' part of the FHV-1 gB gene) = fragment E. The following oligonucleotides:

JCA221 (SEQ ID NO: 20) (48 mer)

5' AAAACTGCAGCCCGGGAAGCTTACAAAAATTAGATTTGTTTCAGTATC 3'

and JCA247 (SEQ ID NO: 21) (36 mer)

30754-34

5' GGTATGGCAAATTTCTTTCAGGGACTCGGGGATGTG 3'

were used to generate, by PCR from the template of the plasmid pJCA001, a blunt-ended PstI fragment of 560 bp (containing the second 31 part of the FHV-1 gB gene mutated at the level of the signal TTTTTNT) = fragment F.

Fragment E was digested with EcoRI, and then phosphorylated. Fragment F was digested with PstI, and then phosphorylated. Fragments E and F were then ligated together with the vector pIBI24 (International Biotechnologies Inc., New Haven, Conn.), previously digested with EcoRI and PstI, to give the plasmid pJCA077 (containing the cassette I3L vaccinia promoter FHV-1 B gene).

Construction of the Expression Cassette 42K-FHV-1 gD

The following oligonucleotides:

RG286 (SEQ ID NO: 22) (17 mer)

5' TTTATATTGTAATTATA 3'

and M13F (SEQ ID NO: 23) (17 mer)

5' GTAAAACGACGGCCAGT 3'

were used to generate, by PCR from the template of the plasmid containing the 42K Entomopoxvirus AmEPV promoter (described in Example 21 of Patent US-A-5,505,941), a blunt-ended EcoRI fragment of 130 bp (containing the 42K entomopox promoter) = fragment A. The following oligonucleotides:

JCA234 (SEQ ID NO: 24) (21 mer)

5' ATGATGACACGTCTACATTTT 3'

and JCA235 (SEQ ID NO: 25) (21 mer)

5' TGTTACATAACGTACTTCAGC 3'

30754-34

were used to generate by PCR from the template of the plasmid pFHVEcoRIM, a blunt-ended BamHI fragment of 185 bp (containing the 5' part of the FHV-1 gD gene) = fragment B. Fragment A was digested with EcoRI, and then phosphorylated. Fragment B was digested with BamHI, and then phosphorylated. Fragments A and B were then ligated together with the vector pBluescript SK+, previously digested with EcoRI and BamHI, to give the plasmid pJCA078.

The plasmid pFHVEcoRIM (see above) was digested with BamHI and XhoI in order to isolate the BamHI-XhoI fragment of 1270 bp (containing the 3' part of the FHV-1 gD gene). This fragment was then ligated with the vector pIBI24, previously digested with BamHI and XhoI, in order to give the plasmid pJCA072. The following oligonucleotides:

JCA242 (SEQ ID NO:26) (18 mer)

5' GAGGATTCGAAACGGTCC 3'

and JCA237 (SEQ ID NO:27) (53 mer)

5' AATTTTCTCGAGAAGCTTGTTAACAAAAATCATTAAGGATGGTAGATTGCATG 3'

were used to generate, by PCR from the pFHVEcoRIM template, an XbaI-XhoI fragment of 290 bp. This fragment was digested with XbaI and XhoI=fragment C (containing the end of the FHV-1 gD gene).

The plasmid pJCA072 was digested with XbaI and XhoI in order to isolate the XbaI-XhoI fragment of 3575 bp (vector pIBI24+start of the 3' part of the FHV-1 gD gene) = fragment D. Fragments C and D were then ligated together in order to give the plasmid pJCA073.

The plasmid pJCA073 was digested with BamHI and XhoI in order to isolate the BamHI-XhoI fragment of 960 bp (containing the 3' part of the FHV-1 gD gene) = fragment A. The plasmid pJCA078 was digested with HpaI and BamHI in order to isolate the HpaI-BamHI fragment of 310 bp (containing the 42K promoter fused to the 5' part of the FHV-1 gD gene) = fragment B. Fragments A 5 and B were ligated together with the vector pBluescript SK+, previously digested with EcoRV and XhoI, in order to give the plasmid pJCA080 (containing the cassette 42K promoter-FHV-1 gD gene).

30754-34

Construction of the Cassette H6-FHV-1 gC

The genomic DNA of the FHV-1 virus (CO strain) was digested with EcoRI and the EcoRI F fragment of about 7500 bp was cloned into pBluescript SK+ to give the plasmid pFHVEcoRIF. The following oligonucleotides:

JCA274 (SEQ ID NO: 28) (55 mer)

5' CATTATCGCGATATCCGTTAAGTTTGTATCGTAATGAGACGATATAGGATGGGAC 3'

and JCA275 (SEQ ID NO: 29) (18 mer)

5' ACTATTTTCAATACTGAC 3'

were used to generate, by PCR from the pFHVEcoRIF template, a fragment which was digested with NruI and SalI in order to give an NruI-SalI fragment of 107 bp (containing the 3' part of the H6 vaccinia promoter fused to the 5' part of the FHV-1 gC gene) = fragment A.

The following oligonucleotides:

JCA276 (SEQ ID NO: 30) (18 mer)

5' AAATGTGTACCACGGGAC 3'

and JCA277 (SEQ ID NO: 31) (54 mer)

5' AAGAAGCTTCTGCAGAATTCGTTAACAAAAATCATTATAATCGCCGGGGATGAG 3'

were used to generate, by PCR from the pFHVEcoRIF template, a fragment which was digested with EcoRV and HindIII in order to give an EcoRV-HindIII fragment of 370 bp (containing the 3' part of the FHV-1 gC gene and the HpaI-EcoRI-PstI-HindIII sites) = fragment B.

The plasmid pJCA020 (see above) was digested with NruI and HindIII in order to isolate the HindIII-NruI fragment (containing the 5' part of the H6 vaccinia promoter) = fragment C. The plasmid pFHVEcoRIF was digested with BamHI and EcoRV in order to isolate the BamHI-EcoRV fragment of 580 bp (containing the central part of the FHV-1 gC gene) = fragment D. Fragments A and C were ligated together with the vector pBluescript SK+, previously digested with HindIII and SalI in order to give the plasmid pJCA097. Fragments B and D were ligated

together with the vector pBluescript SK+, previously digested with BamHI and HindIII, to give the plasmid pJCA099.

The plasmid pJC097 was digested with PstI and SalI in order to isolate the PstI-SalI fragment of 200 bp (containing the cassette H6-5' part of gC) = fragment E. The plasmid pFHV1EcoRIF was digested with BamHI and SalI in order to isolate the SalI-BamHI fragment of 600 bp (2nd central part of FHV-1 gC) = fragment F. Fragments E and F were then ligated together with the vector pBluescript SK+, previously digested with BamHI and PstI, in order to give the plasmid pJCA098. The plasmid pJCA098 was then digested with EcoRI and BamHI in order to isolate the EcoRI-BamHI fragment of 820 bp (containing the cassette H6-5' part of FHV-1gC) = fragment G. The plasmid pJCA099 (see above) was digested with BamHI and HindIII in order to isolate the BamHI-HindIII fragment of 960 bp (containing the 3' part of the FHV-1 gC gene) = fragment H. Fragments G and H were then ligated together with the vector pBluescript SK+, previously digested with EcoRV and HindIII, in order to give the plasmid pJCA100 (containing the expression cassette H6 vaccinia promoter-FHV-1 gC gene).

The plasmid pJCA100 was digested with NruI and EcoRI in order to isolate the NruI-EcoRI fragment of 1650 bp containing the 3' part of the H6 promoter fused with the FHV-1 gC gene. This fragment, was ligated with the plasmid pJCA053 (cassette VQH6-IBV M in the vector pBluescript SK+), previously digested with NruI and EcoRI, in order to give the plasmid pJCA108 (containing the cassette VQH6-gC in pBluescript SK+). The plasmid pJCA079 (see above) was digested with SmaI and BamHI in order to isolate the BamHI-SmaI fragment of 840 bp (containing the cassette I3L-5' part of the FHV-1 gB gene) = fragment A. The plasmid pJCA079 was also digested with BamHI and HindIII in order to isolate the BamHI-HindIII fragment of 2155 bp (containing the 3' part of the FHV-1 gB gene) = fragment B. The plasmid pJCA108 (see above) was digested with HindIII and EcoRI in order to isolate the HindIII-EcoRI fragment of 1830 bp (containing the cassette VQH6-FHV-1 gC) = fragment C. The plasmid pJCA080 (see above) was digested with EcoRI and XhoI in order to isolate the EcoRI-XhoI fragment of 1275 bp (containing the cassette 42K-FHV-1 gD gene) = fragment D. Fragments A, B, C and D were then ligated together with the donor plasmid pC6L in order to give the plasmid pJCA109.

30754-34

This plasmid contains the expression cassettes H6-FHV-1 gene gC, I3L-FHV-1 gB gene and 42K-FHV-1 gD gene in the C6 locus of the ALVAC virus. The structure of this plasmid was verified by sequencing and complete restriction map.

This plasmid is the donor plasmid for the insertion of the expression cassettes H6-FHV-1 gC gene, I3L-FHV-1 gB gene and 42K-FHV-1 gD gene in the C6 locus of the ALVAC virus.

After linearizing with NotI, the plasmid pJCA109 was used as donor plasmid for in vitro recombination (Piccini et al. Methods in Enzymology. 1987. 153. 545-563) with the ALVAC virus in order to generate the recombinant virus designated vCP243.

Example 8 : Adjuvant

The carbomer used in the vaccines in accordance with the present invention is Carbopol® 974P manufactured by the company BF Goodrich (MW about 3 million).

A stock solution containing 1.5% w/v of Carbopol® 974P was first prepared in distilled water containing sodium chloride at 1 g/l.

This stock solution is then used for the manufacture of a solution of Carbopol® in physiological saline at 4 mg/ml. The stock solution is poured into the entire physiological saline (or optionally into most of it) all at once or optionally in several portions with, each time, adjustment of the pH with the aid of NaOH (for example 1 N or more concentrated) to a value of about 7.3 to 7.4.

A ready-for-use solution of Carbopol® is thereby obtained which can be used by the final user to reconstitute a freeze-dried recombinant vaccine.

Example 9 : Vaccination of Horses With the Aid of the Recombinant Canarypox Vector vCP132 (See Example 6) Expressing the Glycoproteins gB, gC and gD of the Type I Equine Herpesvirus (EHV-1)

1. Protocol for Immunization and Challenge:

30754-34

20 ponies (Welsh mountain ponies) exhibiting no serological signs indicating a recent exposure to EHV-1 and EHV-4 were randomly distributed into 4 groups (A to D) of 5 ponies.

Groups A and B were vaccinated with the recombinant canarypox vCP132 expressing the glycoproteins gB, gC and gD of the Kentucky D strain of EHV-1. The vaccine was reconstituted in sterile water (group A) or in a solution of carbomer 4 mg/ml (group B) according to Example 8.

Group C was vaccinated with a commercial inactivated whole EHV vaccine containing, in a dose volume of 1.5 ml, inactivated EHV-1 and EHV-4 valencies and 6 mg of carbomer.

Group D is the control group in which the animals were vaccinated with a recombinant canarypox virus vCP1502 expressing the HA glycoprotein of the Influenza A/equi-1/Prague56 virus (see Example 3) reconstituted in carbomer under the same conditions as for group B.

The vaccines are described in detail in Table 1:

Groups	Vaccines	Antigens	Diluent/Adjuvant	Dose (1ml)
A	vCP132	EHV-1		$10^{8.0}$ $TCID_{50}$
B	vCP132	EHV-1	Carbopol® 974P	$10^{8.0}$ $TCID_{50}$
C	Commercial vaccine	EHV-1 EHV-4	Carbopol®	$10^{7.3}$ TD_{50} before activation EHV-1 $10^{7.3}$ $TCID_{50}$ before activation EHV-4
D	vCP1502	HA Prague 56	Carbopol® 974P	$10^{8.0}$ $TCID_{50}$

30754-34

Each animal received 1 dose of vaccine corresponding to D0 and D35 by deep intramuscular injection into the neck.

On D56, the ponies were challenged by intranasal instillation of 10^5 $TCID_{50}$ of the Ab4/8 strain of EHV-1.

2. Serological Tests

Neutralization tests SN were carried out according to the technique described in Thompson et al., Equine Vet. J., 8, 58-65, 1976. The EHV-1 virus (RACH) was used as antigen.

The SN titres are expressed as the reciprocal of the serum dilution giving 50% neutralization (log_{10}).

3. Virological Monitoring:

The expression of the virus was monitored daily over 10 days using nasopharyngeal swabs which were collected in virus transporting medium. The swab extracts were titrated on rabbit kidney cells RK13 in microtitre plates. The titres were calculated using the Karber formula expressed in log_{10} $TCID_{50}$ per 1 ml.

4. Results:

No significant local or systemic reaction was noted following these vaccinations.

The seroneutralization SN antibody mean responses (log 10 of the dilution causing 50% neutralization) are:

Group	Titre on D.0	Titre on D.56 (before challenge)
A	1.69 ± 0.49	1.93 ± 0.15
B	1.69 ± 0.47	2.61 ± 0.42
C	1.19 ± 0.30	2.47 ± 0.32
D	1.57 ± 0.45	1.55 ± 0.37

A significant increase in the antibody titre is observed with the vaccine vCP132 in carbomer.

30754-34

All the 5 control ponies excrete the virus through the nasopharynx. The viral excretion in these nonvaccinated ponies continued for an average of 5 days, with a maximum viral excretion at 4 days post-infection.

All the ponies in groups A, C and D excrete a virus after challenge. By contrast only two ponies out of the 5 ponies in group B vaccinated according to the invention excrete the virus. In addition, the quantity of virus excreted in group B is significantly less than the quantity excreted by the other groups including group C. Likewise, the duration of excretion in the animals in group B is much shorter than in the other groups.

Reference may be made to Figure 1 and to the area under the curve values given below, which show very clearly the virtual absence of viral excretion in the ponies vaccinated with vCP132 in the presence of carbomer. The result is very significant if it is compared in particular with the commercial vaccine. A significant reduction in viral excretion is observed in the animals in group B compared with the controls, whereas no significant difference is observed between the animals in group C and the controls. This reduction by a remarkable and unexpected level in the excretion of virus is particularly advantageous because of its very favourable indications on the limitation of the transmission of the virus from horse to horse.

Total virus per pony (area under the curve):

A: 17.1

B: 3.0

C: 9.7

D: 16.3

Example 10 : Vaccination of Horses With the Aid of the Canarypox Vector vCP1533 (See Example 5) Recombinant Expressing the HA Glycoprotein of the Influenza A/equi-2/Newmarket/2/93 Virus in the Presence of Carbomer

1. Protocol for Immunization and Challenge:

30754-34

20 ponies (Welsh mountain ponies), 7 to 8 months old having no detectable antibodies against the H3N8 and H7N7 viruses, measured by the SRH (for single radial haemolysis) test were used in this study. The negative status of the animals makes it possible to study, under the best conditions, the efficacy of the various vaccines in terms of humoral response. The ponies were randomly distributed into 4 groups (A to D) of 5 to 6 ponies.

The ponies in group A were vaccinated with the aid of a recombinant canarypox (vCP1533) expressing the HA glycoprotein of the influenza A/equi-2/Newmarket/2/93 virus. This vaccine was reconstituted in a solution containing 4 mg/ml of carbomer, Carbopol® 974P.

Group B was vaccinated with a commercial vaccine containing, in a dose volume of 1.5 ml, a mixture of 3 inactivated strains of influenza, namely Prague/56, Suffolk/89 and Miami/63, tetanus toxoid, as well as carbomer (4 mg) and aluminium hydroxide (2.2 mg) as adjuvants.

Group C was vaccinated with the aid of a vaccine C comprising 2 influenza inactivated valencies, namely Prague/56, Newmarket/2/93 as well as tetanus toxoid in aluminium hydroxide.

Group D was vaccinated with the aid of a recombinant canarypox vector vCP132 seen above and reconstituted with a solution containing 4 mg/ml of carbomer 974P. The latter group served as control for the challenge.

The vaccines are described in detail in Table II:

Groups	Vaccines	Antigens	Diluent/Adjuvant	Dose (1ml)
A	vCP1533	HA-Newmarket/2/93	Carbopol® 974P	$10^{7.7}$ $TCID_{50}$
B	Commercial vaccine	Prague/56; Suffolk/89 Miami/63; tetanus toxoid	Carbopol® $Al(OH)_3$	15 µg HA of each strain
C	Vaccine C	Prague/56; Newmarket/2/93	$Al(OH)_3$	15 µg HA of

30754-34

		tetanus toxoid		each strain
D	vCP132	gB, gC, gD – EHV-1	Carbopol® 974P	$10^{8.0}$ $TCID_{50}$

2 doses of 1 ml of each vaccine were administered to each animal at an interval of 5 weeks by deep intramuscular injection into the neck.

2 weeks after the second vaccination, each pony was infected by exposure to an aerosol obtained from about 20 ml of allantoic fluid for a total of $10^{7.3}$ EID_{50} of influenza A-equi-2/Sussex/89 virus, using an ULTRA 2000 model spraying device (De Villbiss, Somerset Pa.) as described by Mumford et al, Equine Vet, J., 22: 93-98, 1990.

2. Serological Test:

Samples of whole blood were collected on the following days: 0 (the same day as and before the first vaccination), 7, 14, 35 (the same day as and before the second vaccination), 49 (the same day as and before the challenge), 56 and 63.

The serum was prepared and stored and preserved by freezing at -20° C. until it is used. All the sera were tested for the presence of SRH antibody against Influenza A/equi-1/Prague/56 and Influenza A/equi-2/Newmarket/2/93 as described by Wood et al. (J. Hyg., 90: 371-384, 1983).

The diameters of the haemolysis zones were measured in two directions at right angles using an automated reader. The surface area of the zones was calculated and an increase of 50% was considered as being significant. The titres were expressed in mm^2 of haemolysis.

3. Virological Monitoring.

Viral excretion was monitored daily over 10 days by collecting naso pharyngeal swabs in a virus transporting medium. The exudate from each swab was diluted by 10-fold serial dilutions in PBS at pH 7.2 and 0.1 ml of each dilution was inoculated into the allantoic space of 10 day-old embryonated eggs. The viral titre (EID_{50} /ml) in the swab extracts was calculated from the

30754-34

haemagglutinating activity in the allantoic fluids collected after incubating the eggs at 34° C. for 72 hours.

4. Results.

No significant local or systemic reaction was observed following the first vaccination with the exception of one horse in group B.

It should be noted that the strains Suffolk and Newmarket are similar (Daly et al., J. Gen. Virol. 1996, 661-671) which makes comparison with the commercial vaccine perfectly valid under the trial conditions.

None of the ponies had a detectable SRH antibody against Influenza A/equi-2/Newmarket/2/93 or Influenza A/equi-1/Prague/56 at the beginning of the study. The serological results 1 week after the first vaccination showed that none of the ponies was previously infected with Influenza (no observable booster effect).

2 weeks after the first vaccination, none of the ponies developed a detectable antibody response against Influenza A/equi-1/Prague/56. In addition, there was no detectable SRH antibody against Influenza A/equi-2/Newmarket/93 in 6 animals out of 6 vaccinated with vaccine C, in 4 animals out of 5 vaccinated with the commercial vaccine B and in the control group D. By contrast, a very high SRH antibody titre was observed in all the 5 ponies vaccinated with canarypox in the presence of the carbomer adjuvant: mean 155.4.+-.32.9. Table III below presents the results obtained animal per animal as regards the SRH antibody titres.

TABLE III SRH results (mm^2)

Ponies	Group	PRAGUE	NEWMARKET		
		(D0, D7, D14)	D0	D7	D14
M26	A	0	0	0	158.0
M27	A	0	0	0	104.2
M28	A	0	0	0	160.3

30754-34

M29	A	0	0	0	196.4
M30	A	0	0	0	158.0
M31	B	0	0	0	0
M32	B	0	0	0	0
M33	B	0	0	0	0
M34	B	0	0	0	0
M35	B	0	0	0	81.2
M36	C	0	0	0	0
M37	C	0	0	0	trace
M38	C	0	0	0	0
M39	C	0	0	0	0
M40	C	0	0	0	trace
M41	C	0	0	0	0
M42	D	0	0	0	0
M43	D	0	0	0	0
M44	D	0	0	0	0
M45	D	0	0	0	0
M46	D	0	0	0	0

The vaccine according to the invention leads to the appearance of a high antibody titre from 14 days after the first vaccination whereas, overall, for vaccines B and C, the first

30754-34

vaccination does not cause on this date the appearance of antibodies at detectable levels. Such an early production of such a titre is a remarkable and unexpected result which has never been observed before.

Example 11 : Vaccination of Horses With the Aid of the Canarypox Vector vCP1502 (See Example 3) Recombinant Expressing the HA Glycoprotein of the Influenza A/equi-1/Prague 56 Virus in the Presence of Carbomer

The controls of Example 1, vaccinated with vCP1502, were also monitored from the serological point of view.

Table IV below shows the IHA (inhibition of haemagglutination) titres obtained in the animals immunized with 10^8 pfu of vCP1502 with Carbopol® 974P at 0 (1st injection V1) and 35 (2nd injection V2) days.

Table IV

	titres anti-H7N7 IHA				
Ponies	Day 0 (V1)	Day 7	Day 14	Day 35 (V2)	Day 56
RM16	0	0	128	64	**128**
RM17	0	0	32	128	**256**
RM18	0	0	16	64	**512**
RM19	0	0	32	32	**256**
RM20	0	0	128	64	**128**

As in the preceding example, it is observed that the injection of a canarypox-EIV (expressing the HA gene of the A equi-1/Prague virus) mixed with carbomer allows high specific IHA titres to be obtained from D14 after a vaccination. Remarkably, these high titres are further significantly increased after a booster, reaching a very high mean titre, of a level which has never

30754-34

been observed before for the HA antigen of EIV H7N7 virus on horses which have not undergone promostimulation.

Example 12 : Application in Cats

The recombinant virus tested is a recombinant canarypox virus expressing the gB, gC and gD genes of the feline herpesvirus (Feline Herpesvirus = FHV). This recombinant virus is identified vCP243 (see Example 7).

The protocol for vaccination/challenge in the FHV model is the following.

Groups	Number of cats	Vaccine	Diluent/Adjuvant	Dose
A	6	vCP243	water	$10^{7.5}$ pfu
B	6	vCP243	Carbopol® 974	$10^{7.5}$ pfu
C	6	CORIFELIN®	---	1 commercial dose
D (controls)	6	---	---	---

The cats are vaccinated on D0 and D28 by the subcutaneous route.

The vaccine CORIFELIN® is a subunit FHV vaccine marketed by Merial, Lyon, France, comprising at least 200 IDR units of FHV viral fractions, 25 µg of purified feline calicivirus antigen, 0.1 mg of thiomersal and the oily excipient QS 1 ml.

The challenge is carried out on D49, by the oronasal route for an FHV challenge strain.

The clinical monitoring is carried out for 14 days after challenge, noting the clinical signs (noting of the clinical signs according to the rules of the European Pharmacopoeia).

Protection is assessed after challenge on the following criteria:

30754-34

- mean clinical scores for each group, compared with each other and with the mean clinical score for the control group

- level of FHV viral excretion after challenge (measurement of the viral load in pharyngeal swabs prepared daily from D0 to D10 after challenge)

- FHV virus neutralizing antibody titres on blood samples collected on D0, D28, D49, D63.

For all these criteria, the mean levels for each group are also compared with each other and with the mean level for the control group.

Example 13 : Application in Dogs

The recombinant virus tested is a recombinant canarypox virus expressing the HA and F genes of the Carre's disease virus (Canine Distemper Virus, CDV). This recombinant virus is identified vCP258 (see Example 2).

The protocol for vaccination/challenge in the CDV model is the following.

Groups	Number of dogs	Vaccine	Diluent/Adjuvant	Dose
A	6	vCP258	water	$10^{7.0}$ pfu
B	6	vCP258	Carbopol® 974	$10^{7.0}$ pfu
C	6	EURICAN®	---	1 commercial dose
D (controls)	6	---	---	---

The dogs are vaccinated on D0 and D28 by the subcutaneous route.

The vaccine EURICAN® (CHPPI2) is a live vaccine marketed by Merial, Lyon, France. One commercial dose contains a minimum of 10^4 pfu of the CDV Onderstepoort vaccinal strain.

30754-34

The challenge is made on D56 by intracranial administration of a 1/10 dilution of the CDV "Snyder-Hill" challenge strain (batch prepared and provided by USDA). Clinical monitoring is performed for 21 days after challenge, noting the clinical signs (noting of the clinical signs according to the rules of the European Pharmacopoeia).

Protection is assessed after challenge on the following criteria:

- mean clinical scores for each group, compared with each other and with the mean clinical score of the control group

- CDV viraemia level after challenge (measurement of the viral load in the lymphocytes on D56, D61, D63, D66, D70, D77)

- CDV virus neutralizing antibody titres on D0, D14, D28, D42, D56, D63, D77.

For all these criteria, the mean levels for each group are also compared with each other and with the mean level for the control group.

30754-34

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 30754-34 Seq 27-07-09 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

<110> JEAN CHRISTOPHE AUDONNET ET AL.

<120> Live Recombinant Vaccines Comprising an Adjuvant Compound and a Viral Vector comprising a Gene of an Animal Herpes or Influenza Virus

<130> 454313-3159
<140> 09/622,951
<141> 2000-08-24

<150> PCT/FR99/00453
<151> 1999-03-01

<160> 31

<170> PatentIn Ver. 2.1

<210> 1
<211> 70
<212> DNA
<213> Equine influenza virus

<400> 1
cgcggccatc gcgatatccg ttaagtttgt atcgtaatga acactcaaat tctaatatta 60
gccacttcgg 70

<210> 2
<211> 36
<212> DNA
<213> Equine influenza virus

<400> 2
cgcgcggcgg taccttatat acaaatagtg caccgc 36

<210> 3
<211> 70

30754-34

<212> DNA
<213> Equine influenza virus

<400> 3
cgcggccatc gcgatatccg ttaagtttgt atcgtaatga agacaaccat tattttgata 60
ctactgaccc 70

<210> 4
<211> 42
<212> DNA
<213> Equine influenza virus

<400> 4
cgcgcggcgg tacctcaaat gcaaatgttg catctgatgt tg 42

<210> 5
<211> 40
<212> DNA
<213> Equine influenza virus

<400> 5
ttgtcgactc aatcatgaag acaaccatta ttttgatact 40

<210> 6
<211> 34
<212> DNA
<213> Equine influenza virus

<400> 6
ttggatcctt actcaaatgc aaatgttgca yctg 34

<210> 7
<211> 1698
<212> DNA
<213> Equine Influenza Virus, Newmarket 2/93 Strain

<400> 7
atgaagacaa ccattatttt gatactactg acccattggg tctacagtca aaacccaacc 60
agtggcaaca acacagccac attatgtctg ggacaccatg cagtagcaaa tggaacattg 120
gtaaaaacaa taactgatga ccaaattgag gtgacaaatg ctactgaatt agttcagagc 180
atttcaatag ggaaaatatg caacaactca tatagagttc tagatggaag aaattgcaca 240
ttaatagatg caatgctagg agacccccac tgtgatgtct ttcagtatga gaattgggac 300
ctcttcatag aaagaagcag cgctttcagc aattgctacc catatgacat ccctgactat 360
gcatcgctcc ggtccattgt agcatcctca ggaacattgg aattcacagc agagggattc 420
acatggacag gtgtcactca aaacggaaga agtggagcct gcaaaagggg atcagccgat 480
agtttcttta gccgactgaa ttggctaaca aaatctggaa actcttaccc cacattgaat 540
gtgacaatgc ctaacaataa aaatttcgac aaactataca tctgggggat tcatcacccg 600
agctcaaacc aacagcagac agaattgtac atccaagaat caggacgagt aacagtctca 660
acaaaaagaa gtcaacaaac gataatccct aatatcggat ctagaccatg ggtcaggggt 720
caatcaggca ggataagcat atactggacc attgtaaaac ctggagatat cctaatgata 780
aacagtaatg gcaacttagt tgcaccgcgg ggatatttta aattgaaaac agggaaaagc 840
tctgtaatga gatcagatgc acccatagac atttgtgtgt ctgaatgtat tacaccaaat 900
ggaagcatcc ccaacgacaa accatttcaa aatgtgaaca aagttacata tggaaaatgc 960
cccaagtata tcaggcaaaa cactttaaag ctggccactg ggatgaggaa tgtaccagaa 1020
aagcaaatca gaggaatctt tggagcaata gcgggattca tagaaaacgg ctgggaagga 1080
atggttgatg ggtggtatgg attccgatat caaaactcgg aaggaacagg acaagctgca 1140

30754-34

gatctaaaga gcactcaagc agccatcgac cagattaatg gaaaattaaa cagagtgatt 1200
gaaaggacca atgagaaatt ccatcaaata gagaaggaat tctcagaagt agaagggaga 1260
atccaggact tggagaagta tgtagaagac accaaaatag acctatggtc ctacaatgca 1320
gaattgctgg tggctctaga aaatcaacat acaattgact taacagatgc agaaatgaat 1380
aaattattcg agaagactag acgccagtta agagaaaacg cggaagacat gggaggtgga 1440
tgtttcaaga tttaccacaa atgtgataat gcatgcattg gatcaataag aaatgggaca 1500
tatgaccatt acatatacag agatgaagca ttaaacaacc gatttcaaat caaaggtgtt 1560
gagttgaaat caggctacaa agattggata ctgtggattt cattcgccat atcatgcttc 1620
ttaatttgcg ttgttctatt gggtctcatt atgtgggctt gccaaaaagg caacatcaga 1680
tgcaacattt gcatttga 1698

<210> 8
<211> 74
<212> DNA
<213> Equine influenza virus

<400> 8
ctagttcgcg atatccgtta agtttgtatc gtaatgaaga caaccattat tttgatacta 60
ctgacccatt gggt 74

<210> 9
<211> 72
<212> DNA
<213> Equine influenza virus

<400> 9
agacccaatg ggtcagtagt atcaaaataa tggttgtctt cattacgata caaacttaac 60
ggatatcgcg aa 72

<210> 10
<211> 2956
<212> DNA
<213> Feline Herpesvirus

<400> 10
atgtatggtt actggctgga atgtgttatt tgtacagtca tatgtaatgt accactcaac 60
acgatatatt tatatcgcgg ttgtgtctaa taactgtttt taaataaaga gataagtcga 120
aatcacaggc agtgaaatgc cttaaaaatg ggtctcctgt ctatgttagg aatctcttat 180
tttaagtagt cccgcgagac gatttacatc ccgggatcac caacaatctg cgatgagacg 240
atataggatg ggacgcggaa tctaccttct ctatatctgt ctgttatata catatctcca 300
gtttggtact tcgtcgacaa ccgcggtcag tattgaaaat agtgataata gtactgcgga 360
gatgttatca tctaccagca tgtccgctac caccccgata tcccagccaa catctccatt 420
cactactcca actagaagat ctacaaatat agctacaagt tcgagtacca cccaggcatc 480
ccagccaaca tctacattaa ctactctaac tagaagctcg acaactatag ctacaagtcc 540
gagtaccacc caggcagcca cattcatagg atcatctacc gattccaata ccactttact 600
caaaacaaca aaaaaaccaa agcgtaaaaa gaataagaat aacggggcca gatttaaatt 660
agattgtgga tataaggggg ttatctacag accgtatttt agccctcttc agctaaactg 720
tactctaccc acagaacctc atattaccaa ccctattgac ttcgagatct ggtttaaacc 780
acgcaccaga tttggggatt ttcttgggga taaagaagac ttcgtaggga atcatacccg 840
caccagcata ttactattta gcagccgtaa tgggagtgtt aattccatgg atcttgggga 900
cgcgacactc gggatcctac aatctaggat accagattac acattatata atattcccat 960
acaacatacc gaagcgatgt cattgggaat caaatctgtg gaatctgcca cgtccggtgt 1020
ttatacatgg cgggtctatg gtggagatgg actaaataaa acagtgctag gacaggtaaa 1080
tgtatctgta gtggcatatc acccccgag cgtaaatctt acaccacgcg ccagtctatt 1140
taataagacc tttgaggcgg tatgtgcagt ggcgaattac ttcccgcgat ccacgaaact 1200
aacatggtat cttgacggga agccaataga aaggcaatac atttcagata cggcaagtgt 1260
atggatagat ggactcatca ccagaagttc tgtgttggct attccgacaa ctgaaacaga 1320

30754-34

ttccgagaaa ccagatatac gatgtgattt ggaatggcat gaaagtcctg tgtcctataa 1380
gagattcacg aaaagtgtag ccccggacgt ctattaccca cctactgtgt ctgttacctt 1440
cgctgataca cgggctatat gtgatgttaa atgtgtacca cgggacggga tatccttgat 1500
gtggaaaatt ggtaactacc atctaccaaa agcaatgagt gctgatatac tgatcacagg 1560
tccgtgtata gaacgtccag gtttggtcaa cattcagagt atgtgtgata tatcagaaac 1620
ggatggaccc gtgagttata cctgtcagac catcggatac ccaccaattc taccgggatt 1680
ttacgacaca caagtctacg acgcgtcccc tgaaatcgtc agtgaatcaa tgttggttag 1740
tgtcgttgct gtaatactag gagctgttct catcacagtc tttatcttta ttacggcatt 1800
atgtttatat tattctcatc cccggcgatt ataactctta tagttcgtat aaattactta 1860
tcataaccgt gtttcagcgg ttatattttt ataacagtta attgtttact aatagtttac 1920
aaagtccatc gtttataaaa aacaagccca gtggtattat aatcattcgt atggatataa 1980
accgactcca atccgtgatc tttggtaacc cgcgacgtaa ttactctcac acattttaac 2040
tagtctacga tcacccagat ataataaaaa gattcgcgtg gacatgcaag gtatgaggtc 2100
tacgtcacag ccgttggtcg agataccact ggtagatatg gaaccacagc catctataca 2160
ctccaacgag cctaacccac cgaataaaat gttgacgaca gctatttcat cgcgtaggag 2220
tggaattttt ttatttctc tgggtatgtt ttttttcgga gttatcctaa cagctactat 2280
tatagtatgt acattcatat ttacaatacc agtggatatg ctccagatgc cacgctgccc 2340
tgaggaaacg gtgggtatca aaaactgttg tatccgaccg attagacgcc atgttaaatc 2400
acaccaagat ctagttgcca catgtgccga atacatggaa caacccgccg gccgcatctg 2460
ctgttggagc gcttatacca ttattggaca tcttcaatgg agatgggata tctacaaacg 2520
actctcttta cgattgtatt ctctctgatg aaaaaaaatc gtgtaataca tcaatggccg 2580
tatgtcaatc aacatatctt ccaaatcccc taagtgactt tattatgcgc gttaggcaga 2640
tattttctgg aatcctaaat cattaatcca tttactaaat aaataaacaa taccgtttag 2700
gtaattaaac atgattctag tgtttattgt cgtatgtacg ggcgatgggt ggataacaac 2760
tcgacaatga tcaattatat tgattaacct tgtaataaat tcgtcggatt attggatata 2820
tcgagatgat atcacattat tttctaatag cgtgtgtttg aaagtccacc ctactagtgc 2880
catgtgcgcg tttgatcgaa gaggcattta atgttgccag agtttcaatt ccgtatgtat 2940
cgtcgagtaa tctaga 2956

<210> 11
<211> 20
<212> DNA
<213> Feline Herpesvirus

<400> 11
gattaaacct aaataattgt 20

<210> 12
<211> 42
<212> DNA
<213> Feline Herpesvirus

<400> 12
tttttctaga ctgcagcccg ggacatcatg cagtggttaa ac 42

<210> 13
<211> 18
<212> DNA
<213> Feline Herpesvirus

<400> 13
gggtttcaga ggcagttc 18

<210> 14
<211> 21

30754-34

<212> DNA
<213> Feline Herpesvirus

<400> 14
atgtccactc gtggcgatct t 21

<210> 15
<211> 42
<212> DNA
<213> Feline Herpesvirus

<400> 15
tttttctaga ctgcagcccg ggacatcatg cagtggttaa ac 42

<210> 16
<211> 21
<212> DNA
<213> Feline Herpesvirus

<400> 16
gtggacacat atagaaagtc g 21

<210> 17
<211> 21
<212> DNA
<213> Feline Herpesvirus

<400> 17
caccttcagg atctactgtc g 21

<210> 18
<211> 24
<212> DNA
<213> Feline Herpesvirus

<400> 18
acgcatgatg acaagattat tatc 24

<210> 19
<211> 18
<212> DNA
<213> Feline Herpesvirus

<400> 19
ctgtggaatt cgcaatgc 18

<210> 20
<211> 48
<212> DNA
<213> Feline Herpesvirus

<400> 20
aaaactgcag cccgggaagc ttacaaaaat tagatttgtt tcagtatc 48

30754-34

<210> 21
<211> 36
<212> DNA
<213> Feline Herpesvirus

<400> 21
ggtatggcaa atttctttca gggactcggg gatgtg 36

<210> 22
<211> 17
<212> DNA
<213> Feline Herpesvirus

<400> 22
tttatattgt aattata 17

<210> 23
<211> 17
<212> DNA
<213> Feline Herpesvirus

<400> 23
gtaaaacgac ggccagt 17

<210> 24
<211> 21
<212> DNA
<213> Feline Herpesvirus

<400> 24
atgatgacac gtctacattt t 21

<210> 25
<211> 21
<212> DNA
<213> Feline Herpesvirus

<400> 25
tgttacataa cgtacttcag c 21

<210> 26
<211> 18
<212> DNA
<213> Feline Herpesvirus

<400> 26
gaggattcga aacggtcc 18

<210> 27
<211> 53
<212> DNA
<213> Feline Herpesvirus

30754-34

<400> 27
aattttctcg agaagcttgt taacaaaaat cattaaggat ggtagattgc atg 53

<210> 28
<211> 55
<212> DNA
<213> Feline Herpesvirus

<400> 28
cattatcgcg atatccgtta agtttgtatc gtaatgagac gatataggat gggac 55

<210> 29
<211> 18
<212> DNA
<213> Feline Herpesvirus

<400> 29
actattttca atactgac 18

<210> 30
<211> 18
<212> DNA
<213> Feline Herpesvirus

<400> 30
aaatgtgtac cacgggac 18

<210> 31
<211> 54
<212> DNA
<213> Feline Herpesvirus

<400> 31
aagaagcttc tgcagaattc gttaacaaaa atcattataa tcgccgggga tgag 54

30754-34

CLAIMS:

1. Live recombinant vaccine comprising (i) a viral vector incorporating and expressing *in vivo* a heterologous nucleotide sequence selected from a gene of an animal herpes virus and a gene of an influenza virus, and (ii) at least one adjuvant compound chosen from the acrylic or methacrylic acid polymers and the maleic anhydride and alkenyl derivative copolymers.

2. Vaccine according to claim 1, wherein the adjuvant compound comprises an acrylic or methacrylic acid polymer cross-linked by a sugar or polyalcohol polyalkenyl ether.

3. Vaccine according to claim 2, wherein the polymer is cross-linked by a saccharose allyl or by allylpentaerythritol.

4. Vaccine according to claim 1, wherein the adjuvant compound comprises a linear or cross-linked ethylene-maleic anhydride copolymer.

5. The vaccine according to claim 4, wherein the copolymer is cross-linked by divinyl ether.

6. Vaccine according to claim 1, wherein the adjuvant compound comprises a carbomer.

7. Vaccine according to any one of claims 1 to 6, wherein the adjuvant compound is present in the vaccine at a concentration of 0.01% to 2% w/v.

8. Vaccine according to claim 7, wherein the concentration is 0.06% to 1% w/v.

9. Vaccine according to claim 8, wherein the concentration is 0.1% to 0.6% w/v.

10. Vaccine according to any one of claims 1 to 9, comprising a vector incorporating and expressing at least one gene of an animal herpes virus.

11. Vaccine according to claim 10, wherein the gene originates from a herpes virus selected from the group consisting of equine herpes virus EHV-1,

EHV-4, or both EHV-1 and EHV-4, feline herpes virus FHV, canine herpes virus CHV, avian herpes virus ILTV or Marek's disease virus, bovine herpes virus BHV, and porcine herpes virus PHV.

12. Vaccine according to any one of claims 1 to 9, comprising at least one vector incorporating and expressing at least one gene of an influenza virus.

13. Vaccine according to claim 12, wherein the vector expresses a gene of an influenza virus selected from the group consisting of equine influenza virus, avian influenza virus, and porcine influenza virus.

14. Vaccine according to any one of claims 1 to 9, intended for the vaccination of an animal of the equine species and comprising a viral vector incorporating a gene of the equine herpes virus or a gene of an equine influenza virus.

15. Vaccine according to claim 14, comprising a gene of the equine herpes virus EHV-1, EHV-4, or both EHV-1 and EHV-4.

16. Vaccine according to claim 14, comprising a gene of an equine influenza virus.

17. Vaccine according to any one of claims 1 to 16, wherein the viral vector is selected from the group consisting of a pox virus, adenovirus, and herpes virus.

18. Vaccine according to claim 17, wherein the pox virus is selected from the group consisting of vaccinia virus, fowl pox, canary pox, pigeon pox, and swine pox.

19. Vaccine according to claim 18, wherein the vaccinia virus is NYVAC.

20. Vaccine according to claim 15, comprising a canary pox vector incorporating and expressing the genes gB, gC and gD of the equine herpes virus EHV-1, EHV-4, or both EHV-1 and EHV-4.

21. Vaccine according to claim 16, comprising two or three canary pox vectors each comprising an HA gene of the equine influenza virus, each HA gene originating from a different strain.

22. Vaccine according to claim 16, comprising a canary pox vector comprising two or three HA genes of different strains of equine influenza virus.

23. Vaccine according to claim 21 or 22, comprising two or three HA genes originating from the Prague, Kentucky, Newmarket strains, or any combinations thereof.

24. Vaccine according to any one of claims 18 and 20 to 23, wherein the canary pox vector is an ALVAC vector.

25. Vaccine according to any one of claims 1 to 9, for the vaccination of an animal of an avian species against the influenza virus and comprising a viral vector incorporating a gene of an influenza virus.

26. Vaccination pack comprising, packaged separately, (i) in lyophilized form a live recombinant vaccine comprising a viral vector incorporating and expressing *in vivo* a heterologous nucleotide sequence selected from a gene of an animal herpes virus and a gene of an influenza virus, and (ii) a solution of at least one adjuvant compound chosen from the acrylic or methacrylic acid polymers and the maleic anhydride and alkenyl derivative copolymers, as defined in any one of claims 1 to 9, the adjuvant solution being intended for taking up the lyophilized vaccine.

27. Vaccination pack according to claim 26, wherein the gene of an animal herpes virus and the gene of an influenza virus are as defined in any one of claims 10 to 25.

28. Use of the compound chosen from the acrylic or methacrylic acid polymers and the maleic anhydride and alkenyl derivative copolymers, as defined in any one of claims 1 to 9 and a viral vector comprising and expressing *in vivo* the nucleotide sequence of a gene of an animal herpes virus, for the production of an

adsorbed live recombinant vaccine intended to protect an animal from the disease caused by the herpes virus.

29. Use according to claim 28, wherein the gene is a gene of an equine herpes virus EHV-1, EHV-4, or both EHV-1 and EHV-4, for the production of an adsorbed live recombinant vaccine intended to protect an animal of the equine species from the disease caused by the herpes virus.

30. Use of the compound chosen from the acrylic or methacrylic acid polymers and the maleic anhydride and alkenyl derivative copolymers, as defined in any one of claims 1 to 9 and a viral vector incorporating and expressing *in vivo* the nucleotide sequence of a gene of an influenza virus, for the production of a live recombinant vaccine intended to protect an animal from influenza.

31. Use according to claim 30, wherein the gene is a gene of an equine influenza virus, for the production of an adsorbed live recombinant vaccine intended to protect an animal of the equine species from equine influenza.

32. Use of the compound chosen from the acrylic or methacrylic acid polymers and the maleic anhydride and alkenyl derivative copolymers, as defined in any one of claims 1 to 9 and a viral vector incorporating and expressing *in vivo* the nucleotide sequence of a gene of an equine herpes virus, for the production of an adsorbed live recombinant vaccine for inducing in an animal of the equine species a significant reduction in viral excretion relative to the equine herpes virus.

33. Use of the compound chosen from the acrylic or methacrylic acid polymers and the maleic anhydride and alkenyl derivative copolymers, as defined in any one of claims 1 to 9 and a viral vector incorporating and expressing *in vivo* the nucleotide sequence of a gene of an influenza virus, for the production of an adsorbed live recombinant vaccine for inducing in an animal of the equine species a significant reduction in the viral excretion relative to the influenza virus.

34. Use of the compound chosen from the acrylic or methacrylic acid polymers and the maleic anhydride and alkenyl derivative copolymers, as defined in any one of claims 1 to 9, and a viral vector incorporating and expressing *in vivo* the nucleotide sequence of a gene of an influenza virus, for the production of an

adsorbed live recombinant vaccine for inducing in an animal of the equine species the early production of antibodies against the influenza virus.

35. Use according to claim 34, wherein the production of antibodies is observed after a single administration.

36. Use of the compound chosen from the acrylic or methacrylic acid polymers and the maleic anhydride and alkenyl derivative copolymers, as defined in any one of claims 1 to 9 and a viral vector incorporating and expressing *in vivo* the nucleotide sequence of a gene of an influenza virus, for the production of an adsorbed live recombinant vaccine intended to protect an animal of an avian species against avian influenza.

37. Use according to any one of claims 28 to 36, wherein the adjuvant comprises a carbomer.

38. Use according to any one of claims 28 to 37, wherein the viral vector is a pox virus.

39. Use according to claim 38, wherein the viral vector is selected from the group consisting of vaccinia virus, fowl pox, canary pox, pigeon pox, and swine pox.

40. Use according to claim 31, 33, 34, or 35, wherein the viral vector is a canary pox comprising an HA gene of the influenza virus.

41. Use according to claim 40, wherein two or three canary pox vectors comprising an HA gene of the equine influenza virus are used, each HA gene originating from a different strain.

42. Use according to claim 41, wherein the strains are Prague, Kentucky or Newmarket.

43. Use according to claim 29 or 32, wherein the viral vector is a canary pox comprising the genes gB, gC and gD of EHV-1, EHV-4, or both EHV-1 and EHV-4.

FIGURE 1

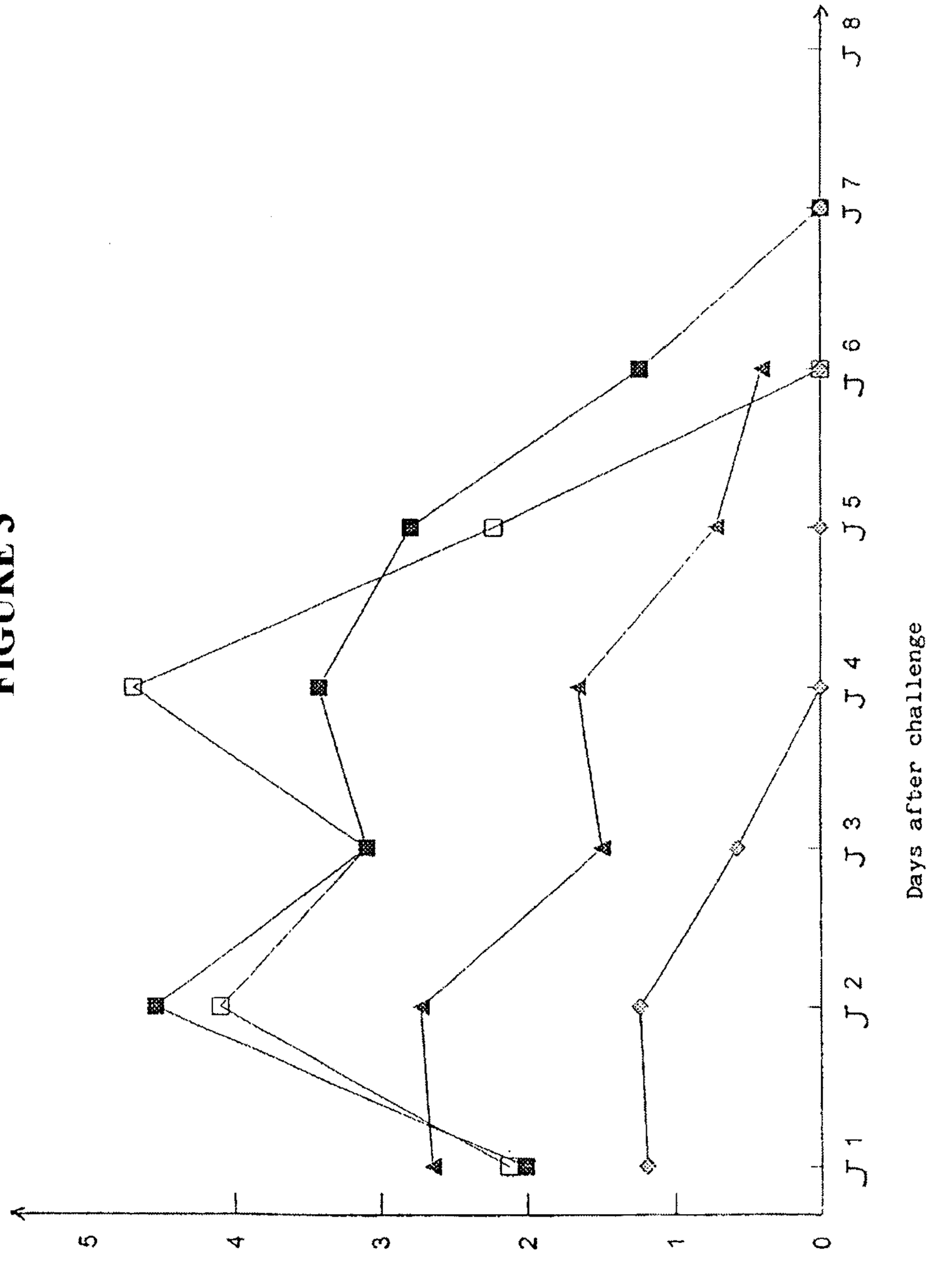
ATGAAGACAACCATTATTTTGATACTACTGACCCATTGGGTCTACAGTCAAAACCCA
ACCAGTGGCAACAACACAGCCACATTATGTCTGGGACACCATGCAGTAGCAAATGG
AACATTGGTAAAAACAATAACTGATGACCAAATTGAGGTGACAAATGCTACTGAAT
TAGTTCAGAGCATTTCAATAGGGAAAATATGCAACAACTCATATAGAGTTCTAGATG
GAAGAAATTGCACATTAATAGATGCAATGCTAGGAGACCCCCACTGTGATGTCTTTC
AGTATGAGAATTGGGACCTCTTCATAGAAAGAAGCAGCGCTTTCAGCAATTGCTACC
CATATGACATCCCTGACTATGCATCGCTCCGGTCCATTGTAGCATCCTCAGGAACAT
TGGAATTCACAGCAGAGGGATTCACATGGACAGGTGTCACTCAAAACGGAAGAAGT
GGAGCCTGCAAAAGGGGATCAGCCGATAGTTTCTTTAGCCGACTGAATTGGCTAAC
AAAATCTGGAAACTCTTACCCCACATTGAATGTGACAATGCCTAACAATAAAAATTT
CGACAAACTATACATCTGGGGGATTCATCACCCGAGCTCAAACCAACAGCAGACAG
AATTGTACATCCAAGAATCAGGACGAGTAACAGTCTCAACAAAAAGAAGTCAACAA
ACGATAATCCCTAATATCGGATCTAGACCATGGGTCAGGGGTCAATCAGGCAGGAT
AAGCATATACTGGACCATTGTAAAACCTGGAGATATCCTAATGATAAACAGTAATG
GCAACTTAGTTGCACCGCGGGGATATTTTAAATTGAAAACAGGGAAAAGCTCTGTA
ATGAGATCAGATGCACCCATAGACATTTGTGTGTCTGAATGTATTACACCAAATGGA
AGCATCCCCAACGACAAACCATTTCAAAATGTGAACAAAGTTACATATGGAAAATG
CCCCAAGTATATCAGGCAAAACACTTTAAAGCTGGCCACTGGGATGAGGAATGTAC
CAGAAAAGCAAATCAGAGGAATCTTTGGAGCAATAGCGGGATTCATAGAAAACGGC
TGGGAAGGAATGGTTGATGGGTGGTATGGATTCCGATATCAAAACTCGGAAGGAAC
AGGACAAGCTGCAGATCTAAAGAGCACTCAAGCAGCCATCGACCAGATTAATGGAA
AATTAAACAGAGTGATTGAAAGGACCAATGAGAAATTCCATCAAATAGAGAAGGAA
TTCTCAGAAGTAGAAGGGAGAATCCAGGACTTGGAGAAGTATGTAGAAGACACCAA
AATAGACCTATGGTCCTACAATGCAGAATTGCTGGTGGCTCTAGAAAATCAACATAC
AATTGACTTAACAGATGCAGAAATGAATAAATTATTCGAGAAGACTAGACGCCAGT
TAAGAGAAAACGCGGAAGACATGGGAGGTGGATGTTTCAAGATTTACCACAAATGT
GATAATGCATGCATTGGATCAATAAGAAATGGGACATATGACCATTACATATACAG
AGATGAAGCATTAAACAACCGATTTCAAATCAAAGGTGTTGAGTTGAAATCAGGCT
ACAAAGATTGGATACTGTGGATTTCATTCGCCATATCATGCTTCTTAATTTGCGTTGT
TCTATTGGGTCTCATTATGTGGGCTTGCCAAAAAGGCAACATCAGATGCAACATTTG
CATTTGA (SEQ ID NO: 1)

FIGURE 2

ATGTATGGTTACTGGCTGGAATGTGTTATTTGTACAGTCATATGTAATGTACCACTCA
ACACGATATATTTATATCGCGGTTGTGTCTAATAACTGTTTTTAAATAAAGAGATAA
GTCGAAATCACAGGCAGTGAAATGCCTTAAAAATGGGTCTCCTGTCTATGTTAGGAA
TCTCTTATTTTAAGTAGTCCCGCGAGACGATTTACATCCCGGGATCACCAACAATCT
GCGATGAGACGATATAGGATGGGACGCGGAATCTACCTTCTCTATATCTGTCTGTTA
TATACATATCTCCAGTTTGGTACTTCGTCGACAACCGCGGTCAGTATTGAAAATAGT
GATAATAGTACTGCGGAGATGTTATCATCTACCAGCATGTCCGCTACCACCCCGATA
TCCCAGCCAACATCTCCATTCACTACTCCAACTAGAAGATCTACAAATATAGCTACA
AGTTCGAGTACCACCCAGGCATCCCAGCCAACATCTACATTAACTACTCTAACTAGA
AGCTCGACAACTATAGCTACAAGTCCGAGTACCACCCAGGCAGCCACATTCATAGG
ATCATCTACCGATTCCAATACCACTTTACTCAAAACAACAAAAAAACCAAAGCGTA
AAAAGAATAAGAATAACGGGGCCAGATTTAAATTAGATTGTGGATATAAGGGGGTT
ATCTACAGACCGTATTTTAGCCCTCTTCAGCTAAACTGTACTCTACCCACAGAACCT
CATATTACCAACCCTATTGACTTCGAGATCTGGTTTAAACCACGCACCAGATTTGGG
GATTTTCTTGGGGATAAAGAAGACTTCGTAGGGAATCATACCCGCACCAGCATATTA
CTATTTAGCAGCCGTAATGGGAGTGTTAATTCCATGGATCTTGGGGACGCGACACTC
GGGATCCTACAATCTAGGATACCAGATTACACATTATATAATATTCCCATACAACAT
ACCGAAGCGATGTCATTGGGAATCAAATCTGTGGAATCTGCCACGTCCGGTGTTTAT
ACATGGCGGGTCTATGGTGGAGATGGACTAAATAAAACAGTGCTAGGACAGGTAAA
TGTATCTGTAGTGGCATATCACCCCCCGAGCGTAAATCTTACACCACGCGCCAGTCT
ATTTAATAAGACCTTTGAGGCGGTATGTGCAGTGGCGAATTACTTCCCGCGATCCAC
GAAACTAACATGGTATCTTGACGGGAAGCCAATAGAAAGGCAATACATTTCAGATA
CGGCAAGTGTATGGATAGATGGACTCATCACCAGAAGTTCTGTGTTGGCTATTCCGA
CAACTGAAACAGATTCCGAGAAACCAGATATACGATGTGATTTGGAATGGCATGAA
AGTCCTGTGTCCTATAAGAGATTCACGAAAAGTGTAGCCCCGGACGTCTATTACCCA
CCTACTGTGTCTGTTACCTTCGCTGATACACGGGCTATATGTGATGTTAAATGTGTAC
CACGGGACGGGATATCCTTGATGTGGAAAATTGGTAACTACCATCTACCAAAAGCA
ATGAGTGCTGATATACTGATCACAGGTCCGTGTATAGAACGTCCAGGTTTGGTCAAC
ATTCAGAGTATGTGTGATATATCAGAAACGGATGGACCCGTGAGTTATACCTGTCAG
ACCATCGGATACCCACCAATTCTACCGGGATTTTACGACACACAAGTCTACGACGCG
TCCCCTGAAATCGTCAGTGAATCAATGTTGGTTAGTGTCGTTGCTGTAATACTAGGA
GCTGTTCTCATCACAGTCTTTATCTTTATTACGGCATTATGTTTATATTATTCTCATCC
CCGGCGATTATAACTCTTATAGTTCGTATAAATTACTTATCATAACCGTGTTTCAGCG
GTTATATTTTTATAACAGTTAATTGTTTACTAATAGTTTACAAAGTCCATCGTTTATA
AAAAACAAGCCCAGTGGTATTATAATCATTCGTATGGATATAAACCGACTCCAATCC
GTGATCTTTGGTAACCCGCGACGTAATTACTCTCACACATTTTAACTAGTCTACGATC
ACCCAGATATAATAAAAAGATTCGCGTGGACATGCAAGGTATGAGGTCTACGTCAC
AGCCGTTGGTCGAGATACCACTGGTAGATATGGAACCACAGCCATCTATACACTCCA
ACGAGCCTAACCCACCGAATAAAATGTTGACGACAGCTATTTCATCGCGTAGGAGT
GGAATTTTTTTATTTTCTCTGGGTATGTTTTTTTTCGGAGTTATCCTAACAGCTACTAT
TATAGTATGTACATTCATATTTACAATACCAGTGGATATGCTCCAGATGCCACGCTG
CCCTGAGGAAACGGTGGGTATCAAAAACTGTTGTATCCGACCGATTAGACGCCATGT
TAAATCACACCAAGATCTAGTTGCCACATGTGCCGAATACATGGAACAACCCGCCG
GCCGCATCTGCTGTTGGAGCGCTTATACCATTATTGGACATCTTCAATGGAGATGGG

ATATCTACAAACGACTCTCTTTACGATTGTATTCTCTCTGATGAAAAAAAATCGTGT
AATACATCAATGGCCGTATGTCAATCAACATATCTTCCAAATCCCCTAAGTGACTTT
ATTATGCGCGTTAGGCAGATATTTTCTGGAATCCTAAATCATTAATCCATTTACTAAA
TAAATAAACAATACCGTTTAGGTAATTAAACATGATTCTAGTGTTTATTGTCGTATGT
ACGGGCGATGGGTGGATAACAACTCGACAATGATCAATTATATTGATTAACCTTGTA
ATAAATTCGTCGGATTATTGGATATATCGAGATGATATCACATTATTTTCTAATAGC
GTGTGTTTGAAAGTCCACCCTACTAGTGCCATGTGCGCGTTTGATCGAAGAGGCATT
TAATGTTGCCAGAGTTTCAATTCCGTATGTATCGTCGAGTAATCTAGA (SEQ ID NO: 2)

FIGURE 3


Mean Viral titre (log 10)
0
1
2
3
4
5
J 1
J 2
J 3
J 4
J 5
J 6
J 7
J 8
Days after challenge