(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 3 172 319 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
20.11.2019 Bulletin 2019/47

(21) Application number: **15750093.5**

(22) Date of filing: **23.07.2015**

(51) Int Cl.:
C12N 7/04 *(2006.01)* ***C07K 14/165*** *(2006.01)*
A61K 39/00 *(2006.01)* ***A61K 39/215*** *(2006.01)*

(86) International application number:
PCT/GB2015/052124

(87) International publication number:
WO 2016/012793 (28.01.2016 Gazette 2016/04)

(54) **CORONAVIRUS**

CORONAVIRUS

CORONAVIRUS

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **23.07.2014 GB 201413020**

(43) Date of publication of application:
31.05.2017 Bulletin 2017/22

(73) Proprietor: **The Pirbright Institute**
Pirbright
Woking
Surrey GU24 0NF (GB)

(72) Inventors:
- **BICKERTON, Erica**
  Woking
  Surrey GU24 0NF (GB)
- **KEEP, Sarah**
  Woking
  Surrey GU24 0NF (GB)
- **BRITTON, Paul**
  Devon EX16 8NN (GB)

(74) Representative: **D Young & Co LLP**
120 Holborn
London EC1N 2DY (GB)

(56) References cited:
WO-A1-2011/004146 **WO-A2-2004/092360**
WO-A2-2005/049814

- **V. D. MENACHERY ET AL: "Attenuation and Restoration of Severe Acute Respiratory Syndrome Coronavirus Mutant Lacking 2'-O-Methyltransferase Activity", JOURNAL OF VIROLOGY, vol. 88, no. 8, 29 January 2014 (2014-01-29), pages 4251-4264, XP055215583, ISSN: 0022-538X, DOI: 10.1128/JVI.03571-13**
- **Anonymous: "EM_STD:KF377577", , 30 October 2013 (2013-10-30), XP55216202, Retrieved from the Internet: URL:http://ibis/exam/dbfetch.jsp?id=EM_STD :KF377577 [retrieved on 2015-09-25]**
- **PAUL BRITTON ET AL: "Modification of the avian coronavirus infectious bronchitis virus for vaccine development", BIOENGINEERED, vol. 3, no. 2, 1 March 2012 (2012-03-01), pages 114-119, XP055215793, ISSN: 2165-5979, DOI: 10.4161/bbug.18983**
- **MARIA ARMESTO ET AL: "A Recombinant Avian Infectious Bronchitis Virus Expressing a Heterologous Spike Gene Belonging to the 4/91 Serotype", PLOS ONE, vol. 6, no. 8, 30 August 2011 (2011-08-30) , page e24352, XP055215311, DOI: 10.1371/journal.pone.0024352**
- **MARIA ARMESTO ET AL: "The Replicase Gene of Avian Coronavirus Infectious Bronchitis Virus Is a Determinant of Pathogenicity", PLOS ONE, vol. 4, no. 10, 9 October 2009 (2009-10-09), page e7384, XP055215449, DOI: 10.1371/journal.pone.0007384 cited in the application**

Printed by Jouve, 75001 PARIS (FR)

(Cont. next page)

- CAVANAGH ET AL: "Manipulation of the infectious bronchitis coronavirus genome for vaccine development and analysis of the accessory proteins", VACCINE, ELSEVIER LTD, GB, vol. 25, no. 30, 10 July 2007 (2007-07-10) , pages 5558-5562, XP022148593, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2007.02.046
- R. CASAIS ET AL: "Reverse Genetics System for the Avian Coronavirus Infectious Bronchitis Virus", JOURNAL OF VIROLOGY, vol. 75, no. 24, 15 December 2001 (2001-12-15), pages 12359-12369, XP055215746, ISSN: 0022-538X, DOI: 10.1128/JVI.75.24.12359-12369.2001
- YAN-QUAN WEI ET AL: "Development and characterization of a recombinant infectious bronchitis virus expressing the ectodomain region of S1 gene of H120 strain", APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, vol. 98, no. 4, 1 February 2014 (2014-02-01), pages 1727-1735, XP055132063, ISSN: 0175-7598, DOI: 10.1007/s00253-013-5352-5
- WANG ET AL: "Attenuation of porcine reproductive and respiratory syndrome virus strain MN184 using chimeric construction with vaccine sequence", VIROLOGY, ELSEVIER, AMSTERDAM, NL, vol. 371, no. 2, 31 October 2007 (2007-10-31), pages 418-429, XP022439793, ISSN: 0042-6822, DOI: 10.1016/J.VIROL.2007.09.032

Description

FIELD OF THE INVENTION

[0001] The present invention relates to an attenuated coronavirus comprising a variant replicase gene, which causes the virus to have reduced pathogenicity. The present invention also relates to the use of such a coronavirus in a vaccine to prevent and/or treat a disease.

BACKGROUND TO THE INVENTION

[0002] Avian infectious bronchitis virus (IBV), the aetiological agent of infectious bronchitis (IB), is a highly infectious and contagious pathogen of domestic fowl that replicates primarily in the respiratory tract but also in epithelial cells of the gut, kidney and oviduct. IBV is a member of the Order *Nidovirales*, Family *Coronaviridae,* Subfamily *Coronavirinae* and Genus *Gammacoronavirus*; genetically very similar coronaviruses cause disease in turkeys, guinea fowl and pheasants.

[0003] Clinical signs of IB include sneezing, tracheal rales, nasal discharge and wheezing. Meat-type birds have reduced weight gain, whilst egg-laying birds lay fewer eggs and produce poor quality eggs. The respiratory infection predisposes chickens to secondary bacterial infections which can be fatal in chicks. The virus can also cause permanent damage to the oviduct, especially in chicks, leading to reduced egg production and quality; and kidney, sometimes leading to kidney disease which can be fatal.

[0004] IBV has been reported to be responsible for more economic loss to the poultry industry than any other infectious disease. Although live attenuated vaccines and inactivated vaccines are universally used in the control of IBV, the protection gained by use of vaccination can be lost either due to vaccine breakdown or the introduction of a new IBV serotype that is not related to the vaccine used, posing a risk to the poultry industry.

[0005] Further, there is a need in the industry to develop vaccines which are suitable for use *in ovo,* in order to improve the efficiency and cost-effectiveness of vaccination programmes. A major challenge associated with *in ovo* vaccination is that the virus must be capable of replicating in the presence of maternally-derived antibodies against the virus, without being pathogenic to the embryo. Current IBV vaccines are derived following multiple passage in embryonated eggs, this results in viruses with reduced pathogenicity for chickens, so that they can be used as live attenuated vaccines. However such viruses almost always show an *increased* virulence to embryos and therefore cannot be used for *in ovo* vaccination as they cause reduced hatchability. A 70% reduction in hatchability is seen in some cases.

[0006] Attenuation following multiple passage in embryonated eggs also suffers from other disadvantages. It is an empirical method, as attenuation of the viruses is random and will differ every time the virus is passaged, so passage of the same virus through a different series of eggs for attenuation purposes will lead to a different set of mutations leading to attenuation. There are also efficacy problems associated with the process: some mutations will affect the replication of the virus and some of the mutations may make the virus too attenuated. Mutations can also occur in the S gene which may also affect immunogenicity so that the desired immune response is affected and the potential vaccine may not protect against the required serotype. In addition there are problems associated with reversion to virulence and stability of vaccines.

[0007] Menachery, V. D. et al. (2014) J. Virol., vol. 88, no. 8, 4251 - 4264, WO 2005/049814 A2 and WO 2004/092360 already disclosed a coronavirus comprising a mutation in nsp-15 and nsp-16 as well as means and methods of how to arrive at a coronavirus comprising such mutated structural proteins.

[0008] It is important that new and safer vaccines are developed for the control of IBV. Thus there is a need for IBV vaccines which are not associated with these issues, in particular vaccines which may be used for *in ovo* vaccination.

SUMMARY OF ASPECTS OF THE INVENTION

[0009] The present inventors have used a reverse genetics approach in order to rationally attenuate IBV. This approach is much more controllable than random attenuation following multiple passages in embryonated eggs because the position of each mutation is known and its effect on the virus, i.e. the reason for attenuation, can be derived.

[0010] Using their reverse genetics approach, the present inventors have identified various mutations which cause the virus to have reduced levels of pathogenicity. The levels of pathogenicity may be reduced such that when the virus is administered to an embryonated egg, it is capable of replicating without being pathogenic to the embryo. Such viruses may be suitable for *in ovo* vaccination, which is a significant advantage and has improvement over attenuated IBV vaccines produced following multiple passage in embryonated eggs.

[0011] Thus in a first aspect, the present invention provides a live, attenuated coronavirus comprising a variant replicase gene encoding polyproteins comprising a mutation in nsp-14, wherein the variant replicase gene encodes a protein comprising an amino acid mutation of Val to Leu at the position corresponding to position 393 of SEQ ID NO:7.

[0012] The variant replicase gene may further encode a protein comprising one or more amino acid mutations selected from the list of:

Pro to Leu at position 85 of SEQ ID NO: 6,
Leu to lie at position 183 of SEQ ID NO: 8;
Val to lie at position 209 of SEQ ID NO: 9.

[0013] The replicase gene may further encode a protein comprising the amino acid mutation Pro to Leu at position 85 of SEQ ID NO: 6.

[0014] The replicase gene may encode a protein comprising the amino acid mutations Val to Leu at position 393 of SEQ ID NO: 7;Leu to lie at position 183 of SEQ ID NO: 8; and Val to lie at position 209 of SEQ ID NO: 9.

[0015] The replicase gene may encode a protein comprising the amino acid mutations Pro to Leu at position 85 of SEQ ID NO: 6; Val to Leu at position 393 of SEQ ID NO:7; Leu to lie at position 183 of SEQ ID NO:8; and Val to lie at position 209 of SEQ ID NO: 9.

[0016] The replicase gene may comprise one or more nucleotide substitutions selected from the list of:

C to T at nucleotide position 12137;
G to C at nucleotide position 18114;
T to A at nucleotide position 19047; and
G to A at nucleotide position 20139;
compared to the sequence shown as SEQ ID NO: 1.

[0017] The coronavirus may be an infectious bronchitis virus (IBV).

[0018] The coronavirus may be IBV M41.

[0019] The coronavirus may comprise an S protein at least part of which is from an IBV serotype other than M41.

[0020] For example, the S1 subunit or the entire S protein may be from an IBV serotype other than M41.

[0021] The coronavirus according to the first aspect has reduced pathogenicity compared to a coronavirus expressing a corresponding wild-type replicase, such that when the virus is administered to an embryonated egg, it is capable of replicating without being pathogenic to the embryo.

[0022] In a second aspect, a variant replicase gene as defined in the claims is provided.

[0023] In a third aspect, a protein encoded by a variant coronavirus replicase gene as defined in the claims is provided.

[0024] In a fourth aspect, a plasmid comprising a replicase gene as defined in the claims is provided.

[0025] In a fifth aspect, a method for making the coronavirus as defined in the claims is provided which comprises the following steps:

(i) transfecting a plasmid according to the fourth aspect of the invention into a host cell;
(ii) infecting the host cell with a recombining virus comprising the genome of a coronavirus strain with a replicase gene;
(iii) allowing homologous recombination to occur between the replicase gene sequences in the plasmid and the corresponding sequences in the recombining virus genome to produce a modified replicase gene; and
(iv) selecting for recombining virus comprising the modified replicase gene.

[0026] The recombining virus may be a vaccinia virus.

[0027] The method may also include the step:
(v) recovering recombinant coronavirus comprising the modified replicase gene from the DNA from the recombining virus from step (iv).

[0028] A cell capable of producing a coronavirus according to the first aspect is provided.

[0029] In a another aspect, a vaccine comprising a coronavirus as defined in the claims and a pharmaceutically acceptable carrier is provided.

[0030] Also described herein is a method for treating and/or preventing a disease in a subject which comprises the step of administering a vaccine according to the invention to the subject.

[0031] Further aspects of the invention provide:

- the vaccine as defined in the claims for use in preventing a disease in a subject.

[0032] Also described herein is the use of a coronavirus according to the first aspect in the manufacture of a vaccine for treating and/or preventing a disease in a subject.

[0033] The disease may be infectious bronchitis (IB).

[0034] The method of administration of the vaccine may be selected from the group consisting of; eye drop adminis-

tration, intranasal administration, drinking water administration, post-hatch injection and *in ovo* injection.

[0035] Vaccination may be by *in ovo* vaccination.

[0036] The present invention also provides a method for producing a vaccine as defined in the claims which comprises the step of infecting a cell as defined in the claims with a coronavirus as defined in the claims.

DESCRIPTION OF THE FIGURES

[0037]

Figure 1 - Growth kinetics of M41-R-6 and M41-R-12 compared to M41-CK (M41 EP4) on CK cells

Figure 2 - Clinical signs, snicking and wheezing, associated with M41-R-6 and M41-R-12 compared to M41-CK (M41 EP4) and Beau-R (Bars show mock, Beau-R, M41-R 6, M41 - R 12, M41-CK EP4 from left to right of each timepoint).

Figure 3 - Ciliary activity of the viruses in tracheal rings isolated from tracheas taken from infected chicks. 100% ciliary activity indicates no effect by the virus; apathogenic, 0% activity indicates complete loss of ciliary activity, complete ciliostasis, indicating the virus is pathogenic (Bars show mock, Beau-R, M41-R 6, M41-R 12, M41-CK EP4 from left to right of each timepoint).

Figure 4 - Clinical signs, snicking, associated with M41R-nsp10rep and M41R-nsp14,15,16rep compared to M41-R-12 and M41-CK (M41 EP5) (Bars show mock, M41-R12; M41 R-nsp10rep; M41 R-nsp14,15,16rep and M41-CK EP5 from left to right of each timepoint).

Figure 5 - Ciliary activity of M41R-nsp10rep and M41R-nsp14,15,16rep compared to M41-R-12 and M41-CK in tracheal rings isolated from tracheas taken from infected chicks (Bars show mock; M41-R12; M41R-nsp10rep; M41R-nsp14,15,16rep and M41-CK EP5 from left to right of each timepoint).

Figure 6 - Clinical signs, snicking, associated with M41R-nsp10, 15rep, M41R-nsp10, 14, 15rep, M41R-nsp10, 14, 16rep, M41 R-nsp10, 15, 16rep and M41-K compared to M41-CK (Bars show mock, M41R-nsp10,15rep1; M41R-nsp10,14,16rep4; M41R-nsp10,15,16rep8; M41R-nsp10,14,15rep10; M41-K6 and M41-CK EP4 from left to right of each timepoint).

Figure 7 - Clinical signs, wheezing, associated with M41R-nsp10, 15rep, M41R-nsp10, 14, 15rep, M41R-nsp10, 14, 16rep, M41 R-nsp1 0, 15, 16rep and M41-K compared to M41-CK (Bars show mock, M41R-nsp10,15rep1; M41R-nsp10,14,16rep4; M41R-nsp10,15,16rep8; M41R-nsp10,14,15rep10; M41-K6 and M41-CK EP4 from left to right of each timepoint).

Figure 8 - Ciliary activity of M41R-nsp1 0, 15rep, M41 R-nsp1 0, 14, 15rep, M41R-nsp10, 14, 16rep, M41R-nsp10, 15, 16rep and M41-K compared to M41-CK in tracheal rings isolated from tracheas taken from infected chicks (Bars show mock, M41R-nsp10,15rep1; M41R-nsp10,14,16rep4; M41R-nsp10,15,16rep8; M41R-nsp10,14,15rep10; M41-K6 and M41-CK EP4 from left to right of each timepoint).

Figure 9 - Growth kinetics of rIBVs compared to M41-CK on CK cells. Fig 9A shows the results for M41-R and M41-K. Fig 9B shows the results for M41-nsp10 rep; M41 R-nsp14, 15, 16 rep; M41 R-nsp1 0, 15 rep; M41 R-nsp10, 15, 16 rep; M41R-nsp10, 14, 15 rep; and M41R-nsp10, 14, 16.

Figure 10 - Position of amino acid mutations in mutated nsp10, nsp14, nsp15 and nsp16 sequences.

Figure 11 - A) Snicking; B) Respiratory symptoms (wheezing and rales combined) and C) Ciliary activity of rIBV M41R-nsp10,14 rep and rIBV M41R-nsp10,16 rep compared to M41-CK (Bars show mock, M41R-nsp10,14rep; M41R-nsp10,16rep and M41-K from left to right of each timepoint).

DETAILED DESCRIPTION

[0038] The present invention provides a coronavirus comprising a variant replicase gene which, when expressed in the coronavirus, causes the virus to have reduced pathogenicity compared to a corresponding coronavirus which comprises the wild-type replicase gene.

CORONAVIRUS

[0039] *Gammacoronavirus* is a genus of animal virus belonging to the family *Coronaviridae*. Coronaviruses are enveloped viruses with a positive-sense single-stranded RNA genome and a helical symmetry.

[0040] The genomic size of coronaviruses ranges from approximately 27 to 32 kilobases, which is the longest size for any known RNA virus.

[0041] Coronaviruses primarily infect the upper respiratory or gastrointestinal tract of mammals and birds. Five to six different currently known strains of coronaviruses infect humans. The most publicized human coronavirus, SARS-CoV which causes severe acute respiratory syndrome (SARS), has a unique pathogenesis because it causes both upper and lower respiratory tract infections and can also cause gastroenteritis. Middle East respiratory syndrome coronavirus (MERS-CoV) also causes a lower respiratory tract infection in humans. Coronaviruses are believed to cause a significant percentage of all common colds in human adults.

[0042] Coronaviruses also cause a range of diseases in livestock animals and domesticated pets, some of which can be serious and are a threat to the farming industry. Economically significant coronaviruses of livestock animals include infectious bronchitis virus (IBV) which mainly causes respiratory disease in chickens and seriously affects the poultry industry worldwide; porcine coronavirus (transmissible gastroenteritis, TGE) and bovine coronavirus, which both result in diarrhoea in young animals. Feline coronavirus has two forms, feline enteric coronavirus is a pathogen of minor clinical significance, but spontaneous mutation of this virus can result in feline infectious peritonitis (FIP), a disease associated with high mortality.

[0043] There are also two types of canine coronavirus (CCoV), one that causes mild gastrointestinal disease and one that has been found to cause respiratory disease. Mouse hepatitis virus (MHV) is a coronavirus that causes an epidemic murine illness with high mortality, especially among colonies of laboratory mice.

[0044] Coronaviruses are divided into four groups, as shown below:

Alpha

- Canine coronavirus (CCoV)
- Feline coronavirus (FeCoV)
- Human coronavirus 229E (HCoV-229E)
- Porcine epidemic diarrhoea virus (PEDV)
- Transmissible gastroenteritis virus (TGEV)
- Human Coronavirus NL63 (NL or New Haven)

Beta

- Bovine coronavirus (BCoV)
- Canine respiratory coronavirus (CRCoV) - Common in SE Asia and Micronesia
- Human coronavirus OC43 (HCoV-OC43)
- Mouse hepatitis virus (MHV)
- Porcine haemagglutinating encephalomyelitis virus (HEV)
- Rat coronavirus (RCV). Rat Coronavirus is quite prevalent in Eastern Australia where, as of March/April 2008, it has been found among native and feral rodent colonies.
- (No common name as of yet) (HCoV-HKU1)
  Severe acute respiratory syndrome coronavirus (SARS-CoV)
- Middle East respiratory syndrome coronavirus (MERS-CoV)

Gamma

- Infectious bronchitis virus (IBV)
- Turkey coronavirus (Bluecomb disease virus)
- Pheasant coronavirus
- Guinea fowl coronavirus

Delta

- Bulbul coronavirus (BuCoV)
- Thrush coronavirus (ThCoV)
- Munia coronavirus (MuCoV)

- Porcine coronavirus (PorCov) HKU15

[0045] The variant replicase gene of the coronavirus of the present invention may be derived from an alphacoronavirus such as TGEV; a betacoronavirus such as MHV; or a gammacoronavirus such as IBV.

[0046] As used herein the term "derived from" means that the replicase gene comprises substantially the same nucleotide sequence as the wild-type replicase gene of the relevant coronavirus. For example, the variant replicase gene of the present invention may have up to 80%, 85%, 90%, 95%, 98% or 99% identity with the wild type replicase sequence. The variant coronavirus replicase gene encodes a protein comprising a mutation in one or more of non-structural protein (nsp)-10, nsp-14, nsp-15 or nsp-16 when compared to the wild-type sequence of the non-structural protein.

IBV

[0047] Avian infectious bronchitis (IB) is an acute and highly contagious respiratory disease of chickens which causes significant economic losses. The disease is characterized by respiratory signs including gasping, coughing, sneezing, tracheal rales, and nasal discharge. In young chickens, severe respiratory distress may occur. In layers, respiratory distress, nephritis, decrease in egg production, and loss of internal egg quality and egg shell quality are common.

[0048] In broilers, coughing and rattling are common clinical signs, rapidly spreading in all the birds of the premises. Morbidity is 100% in non-vaccinated flocks. Mortality varies depending on age, virus strain, and secondary infections but may be up to 60% in non-vaccinated flocks.

[0049] The first IBV serotype to be identified was Massachusetts, but in the United States several serotypes, including Arkansas and Delaware, are currently circulating, in addition to the originally identified Massachusetts type.

[0050] The IBV strain Beaudette was derived following at least 150 passages in chick embryos. IBV Beaudette is no longer pathogenic for hatched chickens but rapidly kills embryos.

[0051] H120 is a commercial live attenuated IBV Massachusetts serotype vaccine strain, attenuated by approximately 120 passages in embryonated chicken eggs. H52 is another Massachusetts vaccine, and represents an earlier and slightly more pathogenic passage virus (passage 52) during the development of H120. Vaccines based on H120 are commonly used.

[0052] IB QX is a virulent field isolate of IBV. It is sometimes known as "Chinese QX" as it was originally isolated following outbreaks of disease in the Qingdao region in China in the mid 1990s. Since that time the virus has crept towards Europe. From 2004, severe egg production issues have been identified with a very similar virus in parts of Western Europe, predominantly in the Netherlands, but also reported from Germany, France, Belgium, Denmark and in the UK.

[0053] The virus isolated from the Dutch cases was identified by the Dutch Research Institute at Deventer as a new strain that they called D388. The Chinese connection came from further tests which showed that the virus was 99% similar to the Chinese QX viruses. A live attenuated QX-like IBV vaccine strain has now been developed.

[0054] IBV is an enveloped virus that replicates in the cell cytoplasm and contains an non-segmented, single-stranded, positive sense RNA genome. IBV has a 27.6 kb RNA genome and like all coronaviruses contains the four structural proteins; spike glycoprotein (S), small membrane protein (E), integral membrane protein (M) and nucleocapsid protein (N) which interacts with the genomic RNA.

[0055] The genome is organised in the following manner: 5'UTR - polymerase (replicase) gene - structural protein genes (S-E-M-N) - UTR 3'; where the UTR are untranslated regions (each ~ 500 nucleotides in IBV).

[0056] The lipid envelope contains three membrane proteins: S, M and E. The IBV S protein is a type I glycoprotein which oligomerizes in the endoplasmic reticulum and is assembled into homotrimer inserted in the virion membrane via the transmembrane domain and is associated through non-covalent interactions with the M protein. Following incorporation into coronavirus particles, the S protein is responsible for binding to the target cell receptor and fusion of the viral and cellular membranes. The S glycoprotein consists of four domains: a signal sequence that is cleaved during synthesis; the ectodomain, which is present on the outside of the virion particle; the transmembrane region responsible for anchoring the S protein into the lipid bilayer of the virion particle; and the cytoplasmic tail.

[0057] All coronaviruses also encode a set of accessory protein genes of unknown function that are not required for replication *in vitro,* but may play a role in pathogenesis. IBV encodes two accessory genes, genes 3 and 5, which both express two accessory proteins 3a, 3b and 5a, 5b, respectively.

[0058] The variant replicase gene of the coronavirus of the present invention may be derived from an IBV. For example the IBV may be IBV Beaudette, H120, H52, IB QX, D388 or M41.

[0059] The IBV may be IBV M41. M41 is a prototypic Massachusetts serotype that was isolated in the USA in 1941. It is an isolate used in many labs throughout the world as a pathogenic lab stain and can be obtained from ATCC (VR-21™). Attenuated variants are also used by several vaccine producers as IBV vaccines against Massachusetts serotypes causing problems in the field. The present inventors chose to use this strain as they had worked for many years on this virus, and because the sequence of the complete virus genome is available. The M41 isolate, M41-CK, used by the