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(54) **Title:** FELINE CALICIVIRUS VACCINE

(57) **Abstract:** The present invention provides new feline calicivirus vaccines, including multivalent vaccines. The present invention also provides methods of making and using the vaccines.

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FELINE CALICIVIRUS VACCINE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) of provisional application U.S. Serial No. 62/596,508 filed December 8, 2017, U.S. Serial No. 62/582,050 filed November 6, 2017, U.S. Serial No. 62/581,955 filed November 6, 2017, and U.S. Serial No. 62/599,401 filed December 15, 2017, the contents of which are hereby incorporated by reference in their entireties.

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FIELD OF THE INVENTION

The present invention relates to new vaccines for feline calicivirus. Methods of making and using the vaccines alone or in combination with other protective agents are also provided.

BACKGROUND

Feline calicivirus (FCV) is usually associated with upper respiratory disease in cats. FCV together with feline herpesvirus are thought to be responsible for approximately 80% of all feline respiratory disease. The most common characteristic and clinical signs of FCV infection is the development of vesicles (ulcers) on the tongue and oral mucosa. These vesicles begin as small, individual ulcers which may spread and affect a large part of the tongue. The vesicles usually do not interfere with eating and drinking, and normally heal without incident. Fever often also is observed in infected cats.

Certain strains of FCV also cause a disease in cats known as limping syndrome. Limping syndrome is characterized by fever, joint and muscle soreness (limping), and occasional lingual/oral ulceration. In addition, some strains of FCV have been associated with chronic stomatitis in infected cats. Other, less common clinical signs are conjunctivitis, rhinitis, and occasionally pneumonia. Cats infected with FCV may become persistently infected, and may shed infectious virus for long periods of time.

FCV comprises a single-stranded, positive-sense RNA genome consisting of three open reading frames (ORFs). The genome is polyadenylated at the 3' end and bound by a virally-encoded protein at the 5'-end. The first open reading frame encodes a viral protease and an RNA-dependent RNA polymerase, which are expressed on a single polypeptide. This polypeptide then is post-translationally cleaved by the viral protease. The second open reading frame encodes the major capsid protein (*i.e.*, the FCV capsid protein), which has six regions denoted as A-F [Scott *et al.*, 60 *Am. J. Vet. Res.*:652–658 (1999)]. Region A is cleaved to produce the mature capsid protein. Whereas regions B, D, and F of ORF2 are relatively conserved between FCV isolates, regions C and E are variable, with region E of ORF2 containing the major B-cell epitopes [*see*, Radford *et al.*, 38(2) *Vet Res.*:319-335 (2007)]. ORF 3 encodes a minor structural protein [Sosnovtsev and Green, 277 *Virology*:193–203 (2000)].

A number of vector strategies have been employed through the years for vaccines in an effort to protect against certain pathogens. One such vector strategy includes the use of alphavirus-derived replicon RNA particles (RP) [Vander Veen, *et al. Anim Health Res Rev*. 13(1):1-9. (2012) doi: 10.1017/S1466252312000011; Kamrud *et al*., *J Gen Virol.* 91(Pt 7):1723-1727 (2010)] which have been developed from several different alphaviruses, including Venezuelan equine encephalitis virus (VEE) [Pushko *et al., Virology* 239:389-401 (1997)], Sindbis (SIN) [Bredenbeek *et al.*, *Journal of Virology* 67:6439-6446 (1993)], and Semliki Forest virus (SFV) [Liljestrom and Garoff, Biotechnology (NY) 9:1356- 1361 (1991)]. RP vaccines deliver propagation-defective alphavirus RNA replicons into host cells and result in the expression of the desired antigenic transgene(s) *in vivo* [Pushko *et al.*, *Virology* 239(2):389-401 (1997)]. RPs have an attractive safety and efficacy profile when compared to some traditional vaccine formulations [Vander Veen, *et al. Anim Health Res Rev*. 13(1):1-9. (2012)]. The RP platform has been used to encode pathogenic antigens and is the basis for several USDA-licensed vaccines for swine and poultry.

Although, long characterized as belonging to a single serotype, FCV isolates are antigenically highly variable, and antibodies from cats vaccinated with older vaccine strains of FCV, such as FCV F9, do not efficiently neutralize all current field isolates. Moreover, new FCV strains associated with systemic disease and high mortality have been identified [*see e.g.*, U.S. 7,449,323 B2]. These “virulent systemic” (VS-FCV) isolates are responsible for localized outbreaks, and current vaccines also do not appear to protect cats from disease caused by these strains. This has led to concern that cats vaccinated with current vaccine strains are not fully protected from disease caused by such “antigenically heterologous” FCV strains, and that these heterologous strains may be responsible for outbreaks of disease, even in vaccinated cats. It is therefore desirable to develop new vaccines that stimulate more broadly reactive virus-neutralizing (VN) antibodies, and therefore provide better protection against new field isolates.

The citation of any reference herein should not be construed as an admission that such reference is available as "prior art" to the instant application.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides vectors that encode one or more feline calicivirus (FCV) antigens. Such vectors can be used in immunogenic compositions comprising these vectors. The immunogenic compositions of the present invention may be used in vaccines. In one aspect of the present invention, a vaccine protects the vaccinated subject (*e.g.*, mammal) against FCV. In a particular embodiment of this type, the vaccinated subject is a feline. In a more particular embodiment, the vaccinated subject is a domestic cat. The present invention further provides combination vaccines for eliciting protective immunity against FCV and other diseases, *e.g.*, other infectious diseases in cats. Methods of making and using the immunogenic compositions and vaccines of the present invention are also provided.

In specific embodiments, the vector is an alphavirus RNA replicon particle that encodes one or more antigens that originate from a feline pathogen. In particular embodiments, the feline pathogen is a feline calicivirus (FCV). In specific embodiments of this type, the alphavirus RNA replicon particle encodes an FCV capsid protein. In related embodiments, the alphavirus RNA replicon particle encodes an antigenic fragment of an FCV capsid protein. In certain embodiments, the FCV capsid protein is an FCV F9-Like capsid protein. In other embodiments, the alphavirus RNA replicon particle encodes an antigenic fragment of an FCV F9-Like capsid protein. In yet other embodiments, the FCV capsid protein is a virulent systemic FCV (VS-FCV) capsid protein. In still other embodiments, the alphavirus RNA replicon particle encodes an antigenic fragment of a VS-FCV capsid protein. In yet other embodiments, the alphavirus RNA replicon particle encodes both the FCV F9-Like capsid protein or antigenic fragment thereof and the VS-FCV capsid protein or an antigenic fragment thereof.

In still more particular embodiments, the alphavirus RNA replicon particle is a Venezuelan Equine Encephalitis (VEE) alphavirus RNA replicon particle. In yet more specific embodiments the VEE alphavirus RNA replicon particle is a TC-83 VEE alphavirus RNA replicon particle. In other embodiments, the alphavirus RNA replicon particle is a Sindbis (SIN) alphavirus RNA replicon particle. In still other embodiments, the alphavirus RNA replicon particle is a Semliki Forest virus (SFV) alphavirus RNA replicon particle. In an alternative embodiment a naked DNA vector comprises a nucleic acid construct that encodes one or more antigens that originate from a feline pathogen. In particular embodiments of this type, the naked DNA vectors comprise a nucleic acid construct that encodes an FCV capsid protein, or antigenic fragment thereof.

In certain embodiments, an alphavirus RNA replicon particle of the present invention encodes one or more FCV antigens or antigenic fragments thereof. In particular embodiments of this type, the alphavirus RNA replicon particles encode two to four FCV antigens or antigenic fragments thereof. In related embodiments an alphavirus RNA replicon particle of the present invention encodes one or more FCV antigens or antigenic fragments thereof and one or more non-FCV antigens or antigenic fragments thereof. In specific embodiments of this type, the alphavirus RNA replicon particles encode one or more FCV capsid proteins or antigenic fragments thereof and one to three non-FCV antigens or antigenic fragments thereof. In more specific embodiments, the alphavirus RNA replicon particles encode the VS-FCV capsid protein or an antigenic fragment thereof and the

FCV-F9-like capsid protein or an antigenic fragment thereof and one to three non-FCV antigens or antigenic fragments thereof.

In another aspect, the present invention provides immunogenic compositions that comprise alphavirus RNA replicon particles that encode one or more FCV antigens or antigenic fragments thereof. In related embodiments, the immunogenic compositions comprise alphavirus RNA replicon particles that encode two to four FCV antigens or antigenic fragments thereof. In particular embodiments of this type, the alphavirus RNA replicon particle encodes an FCV capsid protein. In other embodiments, the alphavirus RNA replicon particle encodes an antigenic fragment of an FCV capsid protein. In certain embodiments, the immunogenic compositions comprise an alphavirus RNA replicon particle that encodes an FCV F9-Like capsid protein. In other embodiments, the immunogenic compositions comprise an alphavirus RNA replicon particle that encodes an antigenic fragment of an FCV F9-Like capsid protein. In yet other embodiments, the immunogenic compositions comprise an alphavirus RNA replicon particle that encodes a virulent systemic FCV (VS-FCV) capsid protein. In still other embodiments, the immunogenic compositions comprise an alphavirus RNA replicon particle that encodes an antigenic fragment of a VS-FCV capsid protein. In yet other embodiments, the immunogenic compositions comprise an alphavirus RNA replicon particle that encodes both the FCV F9-Like capsid protein or antigenic fragment thereof and the VS-FCV capsid protein or an antigenic fragment thereof. In more particular embodiments, the immunogenic composition comprises alphavirus RNA replicon particles that are Venezuelan Equine Encephalitis (VEE) alphavirus RNA replicon particles. In yet more specific embodiments the immunogenic compositions comprise VEE alphavirus RNA replicon particles that are TC-83 VEE alphavirus RNA replicon particles.

In still other embodiments, the immunogenic composition comprises two or more sets of alphavirus RNA replicon particles. In certain embodiments of this type, one set of alphavirus RNA replicon particles encodes a first antigen, whereas the other set of alphavirus RNA replicon particles encodes a second antigen. In particular embodiments of this type, the first set of alphavirus RNA replicon particles encodes one or more FCV antigens or antigenic fragments thereof, and the second set of alphavirus RNA replicon particles encode one or more FeLV antigens or antigenic fragments thereof. In certain embodiments, the FCV antigen originates from a virulent systemic feline calicivirus (VS-FCV) isolate. In other embodiments the FCV antigen originates from a classical (F9-like) feline calicivirus isolate. In yet other embodiments, the second set of alphavirus RNA replicon particles encode two FCV antigens or antigens thereof, one of which originates from a virulent systemic FCV isolate, whereas the other originates from a F9-like FCV. In still other embodiments, an immunogenic composition comprises a first set of alphavirus RNA replicon particles that encode an FCV F9-Like capsid protein or antigenic fragment thereof and the second set of alphavirus RNA replicon particles encode a VS-FCV capsid protein or an antigenic fragment thereof. In related embodiments, an immunogenic composition comprises a first set of alphavirus RNA replicon particles that encode a VS-FCV capsid protein or antigenic fragment thereof and the second set of alphavirus RNA replicon particles encode a FeLV glycoprotein (*e.g.*, gp85) or an antigenic fragment thereof, (*e.g.*, FeLV glycoprotein gp70 and/or gp45).

In yet other embodiments, the immunogenic composition comprises one set of alphavirus RNA replicon particles that encode a first antigen, another set of alphavirus RNA replicon particles that encode a second antigen, and a third set of alphavirus RNA replicon particles that encode a third antigen. In a particular embodiment of this type, the first set of alphavirus RNA replicon particles encode an FCV antigen (*e.g.*, the capsid protein) which originates from a classical (F9-like) feline calicivirus or an antigenic fragment thereof, the second set of alphavirus RNA replicon particles encode an FCV antigen (*e.g.*, the capsid protein), which originates from a virulent systemic feline calicivirus or an antigenic fragment thereof, and the third set of alphavirus RNA replicon particles encode a FeLV antigen (*e.g.*, the FeLV gp85) or an antigenic fragment thereof.

Accordingly, in particular embodiments in which the immunogenic compositions comprise multiple sets (*e.g.,* 2-10) of alphavirus RNA replicon particles, in which the first set of alphavirus RNA replicon particles encodes an FCV F9-Like capsid protein or antigenic fragment thereof and/or a VS-FCV capsid protein or an antigenic fragment thereof, and the one or more other sets of alphavirus RNA replicon particles encode one or more non-FCV antigens.

In certain embodiments of this type, the non-FCV antigen or antigenic fragment thereof is a protein antigen that originates from feline herpesvirus (FHV). In other embodiments, the non-FCV antigen is a protein antigen that originates from feline leukemia virus (FeLV). In yet other embodiments, the non-FCV antigen is a protein antigen that originates from feline pneumovirus (FPN). In still other embodiments, the non-FCV antigen is a protein antigen that originates from feline parvovirus (FPV). In yet other embodiments, the non-FCV antigen is a protein antigen that originates from rabies virus. In still other embodiments, the non-FCV antigen is a protein antigen that originates from feline infectious peritonitis virus (FIPV). In yet other embodiments, the non-FCV antigen is a protein antigen that originates from feline immunodeficiency virus. In still other embodiments, the non-FCV antigen is a protein antigen that originates from borna disease virus (BDV). In yet other embodiments, the non-FCV antigen is a protein antigen that originates from feline influenza virus. In still other embodiments, the non-FCV antigen is a protein antigen that originates from feline panleukopenia virus (FPLV). In yet other embodiments the non-FCV antigen is a protein antigen that originates from feline coronavirus (FCoV). In still other embodiments the non-FCV antigen is a protein antigen that originates from feline rhinotracheitis virus (FVR). In yet other embodiments the non-FCV antigen is a protein antigen that originates from *Chlamydophila felis*.

The present invention also includes all of the alphavirus RNA replicon particles of the present invention, the naked DNA vectors, the nucleic acid constructs of the present invention including synthetic messenger RNA, and RNA replicons, as well as all of the immunogenic compositions and/or vaccines that comprise the nucleic acid constructs (*e.g.*, synthetic messenger RNA, RNA replicons), the alphavirus RNA replicon particles, and/or the naked DNA vectors of the present invention.

In particular embodiments, a nucleic acid construct of the present invention encodes one or more FCV antigens or antigenic fragments thereof. In related embodiments of this type, the nucleic acid construct encodes two to four FCV antigens or antigenic fragments thereof. In other embodiments, alphavirus RNA replicon particles comprise a nucleic acid construct that encodes one or more FCV antigens or antigenic fragments thereof. In particular embodiments, alphavirus RNA replicon particles comprise a nucleic acid construct that encodes two to four FCV antigens or antigenic fragments thereof.

In still other embodiments, the immunogenic compositions comprise alphavirus RNA replicon particles and/or naked DNA vectors that comprise a nucleic acid construct that encodes two to four FCV antigens or antigenic fragments thereof. In particular embodiments of this type, the alphavirus RNA replicon particles encode an FCV F9-Like capsid protein or antigenic fragment thereof and/or a VS-FCV capsid protein or an antigenic fragment thereof and an FeLV glycoprotein (gp85) or an antigenic fragment thereof. In particular embodiments of this type, the antigenic fragment of gp85 is the FeLV glycoprotein gp70. In other related embodiments, the antigenic fragment of gp85 is the FeLV glycoprotein gp45. In more particular embodiments, the immunogenic composition comprises alphavirus RNA replicon particles that are Venezuelan Equine Encephalitis (VEE) alphavirus RNA replicon particles. In yet more specific embodiments the VEE alphavirus RNA replicon particles are TC-83 VEE alphavirus RNA replicon particles.

In yet other embodiments, the immunogenic composition comprises two or more sets of alphavirus RNA replicon particles and/or naked DNA vectors. In particular embodiments of this type, one set of alphavirus RNA replicon particles and/or naked DNA vectors comprise a first nucleic acid construct, whereas the other set of alphavirus RNA replicon particles and/or naked DNA vectors comprise a second nucleic acid construct. In a specific embodiment of this type the first nucleic acid construct encodes an FCV antigen or an antigenic fragment thereof, and the second nucleic acid construct encodes a feline calicivirus (FCV) antigen or an antigenic fragment thereof. In certain embodiments of this type, the FCV antigen originates from a virulent systemic feline calicivirus (VS-FCV) isolate. In other embodiments the FCV antigen originates from a classical (F9-like) feline calicivirus isolate. In yet other embodiments, the second nucleic acid construct encodes two FCV antigens, one of which originates from a virulent systemic feline calicivirus isolate, whereas the other originates from a classical (F9-like) feline calicivirus isolate.

In still other embodiments, the immunogenic composition comprises one set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a first nucleic acid construct, another set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a second nucleic acid construct, and a third set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a third nucleic acid construct. In a particular embodiment of this type, the first nucleic acid construct encodes a FeLV antigen or an antigenic fragment thereof, the second nucleic acid construct encodes a feline calicivirus (FCV) antigen which originates from a virulent systemic feline calicivirus or an antigenic fragment thereof, and the third nucleic acid construct encodes a feline calicivirus (FCV) antigen which originates from a classical (F9-like) feline calicivirus or an antigenic fragment thereof.

In yet other embodiments, the immunogenic composition comprises one set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a first nucleic acid construct, another set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a second nucleic acid construct, a third set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a third nucleic acid construct, and a fourth set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a fourth nucleic acid construct. In particular embodiments of this type, the first nucleic acid construct encodes both a feline calicivirus (FCV) antigen which originates from a virulent systemic feline calicivirus or an antigenic fragment thereof, and a feline calicivirus (FCV) antigen which originates from a classical (F9-like) feline calicivirus or an antigenic fragment thereof.

In still other embodiments, the immunogenic composition comprises a set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a first nucleic acid construct, another set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a second nucleic acid construct, a third set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a third nucleic acid construct, a fourth set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a fourth nucleic acid construct ,and a fifth set of alphavirus RNA replicon particles and/or naked DNA vectors that comprise a fifth nucleic acid construct. In such embodiments, the nucleotide sequences of the first nucleic acid construct, the second nucleic acid construct, third nucleic acid construct, the fourth nucleic acid construct, and the fifth nucleic acid construct are all different. In particular embodiments of this type, the first nucleic acid construct encodes both a feline calicivirus (FCV) antigen which originates from a virulent systemic feline calicivirus or an antigenic fragment thereof, and a feline calicivirus (FCV) antigen which originates from a classical (F9-like) feline calicivirus or an antigenic fragment thereof.

Accordingly, an immunogenic composition of the present invention can contain alphavirus RNA replicon particles and/or naked DNA vectors that comprise a nucleic acid construct that encodes at least one non-FCV antigen for eliciting protective immunity to a non-FCV pathogen. In particular embodiments of this type, the non-FCV antigen is a protein antigen that originates from feline herpesvirus (FHV). In other embodiments, the non-FCV antigen is a protein antigen that originates from feline leukemia virus (FeLV). In yet other embodiments, the non-FCV antigen is a protein antigen that originates from feline pneumovirus (FPN). In still other embodiments, the non-FCV antigen is a protein antigen that originates from feline parvovirus (FPV). In yet other embodiments, the non-FCV antigen is a protein antigen that originates from feline infectious peritonitis virus (FIPV). In still other embodiments, the non-FCV antigen is a protein antigen that originates from feline immunodeficiency virus. In yet other embodiments, the non-FCV antigen is a protein antigen that originates from rabies virus. In still other embodiments, the non-FCV antigen is a protein antigen that originates from borna disease virus (BDV). In yet other embodiments, the non-FCV antigen is a protein antigen that originates from feline influenza virus. In still other embodiments, the non-FCV antigen is a protein antigen that originates from feline panleukopenia virus (FPLV). In yet other embodiments the non-FCV antigen is a protein antigen that originates from feline coronavirus (FCoV). In still other embodiments the non-FCV antigen is a protein antigen that originates from feline rhinotracheitis virus (FVR). In still other embodiments the non-FCV antigen is a protein antigen that originates from *Chlamydophila felis*.

The present invention further provides combination immunogenic compositions and/or vaccines (multivalent vaccines) that include alphavirus RNA replicon particles that encode one or more antigens or antigenic fragments thereof originating from FCV together (*e.g.*, the FCV capsid protein) and further comprise one or more modified live/attenuated or killed feline pathogens. In particular embodiments, the immunogenic compositions further comprise a modified live or killed *Chlamydophila felis* combined with alphavirus RNA replicon particles that encode an antigen or antigenic fragment thereof originating from FeLV. In other embodiments, the immunogenic compositions further comprise a modified live or killed feline rhinotracheitis Virus (FVR) combined with alphavirus RNA replicon particles that encode an antigen or antigenic fragment thereof originating from FeLV. In yet other embodiments, the immunogenic compositions further comprise a modified live or killed feline panleukopenia virus (FPL) combined with alphavirus RNA replicon particles that encode an antigen or antigenic fragment thereof originating from FeLV. In certain embodiments, a vaccine comprises an immunologically effective amount of one or more of these immunogenic compositions.

In more specific embodiments, the immunogenic compositions comprise alphavirus RNA replicon particles that encode a capsid protein or antigenic fragment thereof originating from VS-FCV and further comprise a modified live or killed F9-like FCV. In still other embodiments, the immunogenic compositions comprise alphavirus RNA replicon particles that encode a capsid protein or antigenic fragment thereof originating from VS-FCV and further comprise a modified live or killed F9-like FCV, a modified live or killed *Chlamydophila felis*, a modified live or killed FVR, and a modified live or killed FPL. In related embodiments, the immunogenic composition also comprises alphavirus RNA replicon particles that encode an antigen or antigenic fragment thereof originating from FeLV. In particular embodiments of this type, the feline antigen of the FeLV is the FeLV viral glycoprotein (gp85). In certain embodiments, the present invention provides vaccines that comprise an immunologically effective amount of one or more of these immunogenic compositions.

In particular embodiments, an alphavirus RNA replicon particle of the present invention encodes a VS-FCV capsid protein or antigenic fragment thereof. In specific embodiments of this type, the VS-FCV capsid protein comprises an amino acid sequence comprising 95% identity or more with the amino acid sequence of SEQ ID NO: 2. In more specific embodiments of this type, the VS-FCV capsid protein comprises an amino acid sequence comprising 98% identity or more with the amino acid sequence of SEQ ID NO: 2. In even more specific embodiments of this type, the VS-FCV capsid protein comprises the amino acid sequence of SEQ ID NO: 2. In specific embodiments of this type the VS-FCV capsid protein is encoded by the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 12.

In related embodiments, an alphavirus RNA replicon particle of the present invention encodes a FCV F9-Like capsid protein or antigenic fragment thereof. In specific embodiments of this type, the FCV F9-Like capsid protein comprises an amino acid sequence comprising 95% identity or more with the amino acid sequence of SEQ ID NO: 4. In more specific embodiments of this type, the FCV F9-Like capsid protein comprises an amino acid sequence comprising 98% identity or more with the amino acid sequence of SEQ ID NO: 4. In even more specific embodiments of this type, the FCV F9-Like capsid protein comprises the amino acid sequence of SEQ ID NO: 4. In specific embodiments of this type the FCV F9-Like capsid protein is encoded by the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 13.

In certain embodiments an alphavirus RNA replicon particle of the present invention encodes a FeLV glycoprotein (gp85). In specific embodiments of this type, the FeLV glycoprotein gp85 comprises an amino acid sequence comprising

95% identity or more with the amino acid sequence of SEQ ID NO: 6. In more specific embodiments of this type, the FeLV glycoprotein (gp85) comprises the amino acid sequence of SEQ ID NO: 6. In even more specific embodiments of this type the FeLV glycoprotein (gp85) is encoded by the nucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 14.

In related embodiments, the FeLV glycoprotein gp70 comprises an amino acid sequence comprising 95% identity or more with the amino acid sequence of SEQ ID NO: 8. In more specific embodiments of this type, the FeLV glycoprotein (gp85) comprises the amino acid sequence of SEQ ID NO: 8. In even more specific embodiments of this type the FeLV glycoprotein (gp70) is encoded by the nucleotide sequence of SEQ ID NO: 7 or SEQ ID NO: 15.

In yet other embodiments an alphavirus RNA replicon particle of the present invention encodes a rabies virus glycoprotein (G). In specific embodiments of this type, the rabies virus glycoprotein comprises an amino acid sequence comprising 95% identity or more with the amino acid sequence of SEQ ID NO: 10. In more specific embodiments of this type, the rabies virus glycoprotein (G) comprises the amino acid sequence of SEQ ID NO: 10. In even more specific embodiments of this type the rabies virus glycoprotein (G) is encoded by the nucleotide sequence of SEQ ID NO: 9 or SEQ ID NO: 16.

The present invention further comprises vaccines, including multivalent vaccines, comprising the immunogenic compositions of the present invention. In particular embodiments, the vaccines are nonadjuvanted vaccine. In certain embodiments, the vaccine aids in the prevention of disease due to FCV. In related embodiments, antibodies are induced in a feline subject when the feline is immunized with the vaccine.

The present invention also provides methods of immunizing a feline against a feline pathogen, *e.g.*, FCV, comprising administering to the feline an immunologically effective amount of a vaccine or multivalent of the present invention. In particular embodiments the vaccine is administered via intramuscular injection. In alternative embodiments the vaccine is administered via subcutaneous injection. In other embodiments the vaccine is administered via intravenous injection. In still other embodiments the vaccine is administered via intradermal injection. In yet other embodiments the vaccine is administered via oral administration. In still other embodiments the vaccine is administered via intranasal administration. In specific embodiments, the feline is a domestic cat.

The vaccines and multivalent vaccines of the present invention can be administered as a primer vaccine and/or as a booster vaccine. In specific embodiments, a vaccine of the present invention is administered as a one shot vaccine (one dose), without requiring subsequent administrations. In certain embodiments, in the case of the administration of both a primer vaccine and a booster vaccine, the primer vaccine and the booster vaccine can be administered by the identical route. In certain embodiments of this type, the primer vaccine and the booster vaccine are both administered by subcutaneous injection. In alternative embodiments, in the case of the administration of both a primer vaccine and a booster vaccine, the administration of the primer vaccine can be performed by one route and the booster vaccine by another route. In certain embodiments of this type, the primer vaccine can be administered by subcutaneous injection and the booster vaccine can be administered orally.

The invention further provides for a method of immunizing a feline against FCV comprising injecting the feline with an immunologically effective amount of the above described vaccines. In particular embodiments the vaccines can include from about 1×10^4 to about 1×10^{10} RPs or higher, for example. In more particular embodiments the vaccines can include from about 1×10^5 to about 1×10^9 RPs. In even more particular embodiments the vaccines can include from about 1×10^6 to about 1×10^8 RPs. In particular embodiments the feline is a domestic cat.

In particular embodiments the vaccines of the present invention are administered in 0.05 mL to 3 mL doses. In more particular embodiments the dose administered is 0.1 mL to 2 mLs. In still more particular embodiments the dose administered is 0.2 mL to 1.5 mLs. In yet other embodiments the dose administered is 0.5 mL to 2.0 mLs. In still other embodiments the dose administered is 0.3 to 1.0

mLs. In yet more particular embodiments the dose administered is 0.4 mL to 0.8 mLs.

These and other aspects of the present invention will be better appreciated by reference to the following Detailed Description.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides efficacious, safe FCV vaccines. In particular embodiments the vaccine is nonadjuvanted. In one aspect, the vaccines of the present invention do not induce feline injection-site sarcomas, yet still aid in the protection of the vaccinates from the upper respiratory disease and/or limping syndrome caused by FCV infection. In a particular embodiment, the FCV capsid protein originates from a virulent systemic FCV (VS-FCV). In related embodiments, the FCV capsid protein originates from an older strain, such as an FCV F9 strain (F9-Like FCV).

Accordingly, the vaccine compositions of the present invention include an immunologically effective amount of a vector encoding an antigen from one or more strains of feline calicivirus that aids in eliciting protective immunity in the recipient vaccinated animal. Furthermore, the present invention provides new immunogenic compositions to improve the reliability of the vaccination to aid in the reduction of upper respiratory disease in cats in a feline infected by FCV and to thereby yield more transient or mild disease and/or lead to the reduction of the infection. In a particular aspect of the present invention, the vaccines comprise an alphavirus RNA replicon particle (RP) encoding an FCV capsid, *e.g.*, originating from a VS-FCV or alternatively, a classical strain, such as an FCV F9-Like strain.

In more specific embodiments, the vaccines comprise alphavirus RNA replicon particles (RPs) that comprise the capsid protein and glycoproteins of Venezuelan Equine Encephalitis Virus (VEE) and encode the FCV capsid protein and/or an antigenic fragment thereof. In even more specific embodiments, the vaccines comprise alphavirus RNA replicon particles (RPs) that comprise the capsid protein and glycoproteins of the avirulent TC-83 strain of VEE and encode one or more FCV capsid proteins and/or one or more antigenic fragments thereof.

In another aspect of the present invention, the vaccines comprise naked DNA vectors that encode one or more FCV capsid proteins and/or one or more antigenic fragments thereof. The vaccines of the present invention can be administered to a feline in the absence of an adjuvant and still effectively aid in the protection of the vaccinated feline against FCV.

In order to more fully appreciate the invention, the following definitions are provided.

The use of singular terms for convenience in description is in no way intended to be so limiting. Thus, for example, reference to a composition comprising "a polypeptide" includes reference to one or more of such polypeptides. In addition, reference to an "alphavirus RNA replicon particle" includes reference to a plurality of such alphavirus RNA replicon particles, unless otherwise indicated.

As used herein the term "approximately" is used interchangeably with the term "about" and signifies that a value is within fifty percent of the indicated value *i.e.*, a composition containing "approximately" 1×10^8 alphavirus RNA replicon particles per milliliter contains from 0.5×10^8 to 1.5×10^8 alphavirus RNA replicon particles per milliliter.

As used herein, the term "feline" refers to any member of the Felidae family. Domestic cats, pure-bred and/or mongrel companion cats, and wild or feral cats are all felines.

As used herein, the term “replicon” refers to a modified RNA viral genome that lacks one or more elements (*e.g.*, coding sequences for structural proteins) that if they were present, would enable the successful propagation of the parental virus in cell cultures or animal hosts. In suitable cellular contexts, the replicon will amplify itself and may produce one or more sub-genomic RNA species.

As used herein, the term “alphavirus RNA replicon particle”, abbreviated “RP”, is an alphavirus-derived RNA replicon packaged in structural proteins, *e.g.,* the capsid and glycoproteins, which also are derived from an alphavirus, *e.g.*, as described by Pushko *et al.*, [*Virology* 239(2):389-401 (1997)]. An RP cannot propagate in cell cultures or animal hosts (without a helper plasmid or analogous component), because the replicon does not encode the alphavirus structural components (*e.g.,* capsid and glycoproteins).

The terms “FCV F9-Like” and “F9-Like FCV” are used interchangeably with each other and with the term “classical FCV” and as used herein is an FCV that can be characterized as an older and formerly, universal vaccine strain of FCV, for which the FCV F9 strain is considered a typical representative. In direct contrast, the FCV termed virulent systemic “VS-FCV” or as used herein interchangeably “(VS) FCV”, is a newer class of FCV, which is unusually virulent, and cannot be neutralized by antibodies raised against the FCV F9-Like strains [*see*, U.S. 7,449,323; Radford *et al.*, 38(2) Vet res. 319-335 (2007)].

The term “non-FCV”, is used to modify terms such as pathogen, and/or antigen (or immunogen) to signify that the respective pathogen, and/or antigen (or immunogen) is neither an FCV pathogen nor an FCV antigen (or immunogen) and that a non-FCV protein antigen (or immunogen) does not originate from an FCV.

The terms “originate from”, “originates from” and “originating from” are used interchangeably with respect to a given protein antigen and the pathogen or strain of that pathogen that naturally encodes it, and as used herein signify that the unmodified and/or truncated amino acid sequence of that given protein antigen is encoded by that pathogen or strain of that pathogen. The coding sequence, within a nucleic acid construct of the present invention for a protein antigen originating from a pathogen may have been genetically manipulated so as to result in a modification and/or truncation of the amino acid sequence of the expressed protein antigen relative to the corresponding sequence of that protein antigen in the pathogen or strain of pathogen (including naturally attenuated strains) it originates from.

As used herein, the terms “protecting”, or “providing protection to”, or “eliciting protective immunity to”, “aids in prevention of disease”, and "aids in the protection" do not require complete protection from any indication of infection. For example, "aids in the protection" can mean that the protection is sufficient such that, after challenge, symptoms of the underlying infection are at least reduced, and/or that one or more of the underlying cellular, physiological, or biochemical causes or mechanisms causing the symptoms are reduced and/or eliminated. It is understood that “reduced,” as used in this context, means relative to the state of the infection, including the molecular state of the infection, not just the physiological state of the infection.

As used herein, a "vaccine" is a composition that is suitable for application to an animal, *e.g.*, feline (including, in certain embodiments, humans, while in other embodiments being specifically not for humans) comprising one or more antigens typically combined with a pharmaceutically acceptable carrier such as a liquid containing water, which upon administration to the animal induces an immune response strong enough to minimally aid in the protection from a disease arising from an infection with a wild-type micro-organism, *i.e.*, strong enough for aiding in the prevention of the disease, and/or preventing, ameliorating or curing the disease.

As used herein, a multivalent vaccine is a vaccine that comprises two or more different antigens. In a particular embodiment of this type, the multivalent vaccine stimulates the immune system of the recipient against two or more different pathogens.

The terms “adjuvant” and "immune stimulant" are used interchangeably herein, and are defined as one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to one or more vaccine antigens/isolates. Accordingly, "adjuvants" are agents that nonspecifically increase an immune response to a particular antigen, thus reducing the quantity of antigen necessary in any given vaccine, and/or the frequency of injection necessary in order to generate an adequate immune response to the antigen of interest. In this context, an adjuvant is used to enhance an immune response to one or more vaccine antigens/isolates. The American Association of Feline Practitioners *Feline Vaccination Guidelines,* for example, suggest the use of nonadjuvanted FeLV vaccines [*AAFP Feline Advisory Panel*, 15: 785-808 (2013)].

As used herein, a “nonadjuvanted vaccine” is a vaccine or a multivalent vaccine that does not contain an adjuvant.

As used herein, the term "pharmaceutically acceptable" is used adjectivally to mean that the modified noun is appropriate for use in a pharmaceutical product. When it is used, for example, to describe an excipient in a pharmaceutical vaccine, it characterizes the excipient as being compatible with the other ingredients of the composition and not disadvantageously deleterious to the intended recipient animal, *e.g.*, feline.

Parenteral administration" includes subcutaneous injections, submucosal injections, intravenous injections, intramuscular injections, intradermal injections, and infusion.

As used herein the term “antigenic fragment” in regard to a particular protein (*e.g.*, a protein antigen) is a fragment of that protein that is antigenic, *i.e.*, capable of specifically interacting with an antigen recognition molecule of the immune system, such as an immunoglobulin (antibody) or T cell antigen receptor. For example, an antigenic fragment of an FCV capsid protein is a fragment of the capsid protein that is antigenic. In specific embodiments, the antigenic fragment of an FCV capsid protein comprises region E of the ORF2, which contains the major B-cell epitopes. Preferably, an antigenic fragment of the present invention is immunodominant for antibody and/or T cell receptor recognition. In particular embodiments, an antigenic fragment with respect to a given protein antigen is a fragment of that protein that retains at least 25% of the antigenicity of the full length protein. In preferred embodiments an antigenic fragment retains at least 50% of the antigenicity of the full length protein. In more preferred embodiments, an antigenic fragment retains at least 75% of the antigenicity of the full length protein. Antigenic fragments can be as small as 20 amino acids or at the other extreme, be large fragments that are missing as little as a single amino acid from the full-length protein. In particular embodiments the antigenic fragment comprises 25 to 150 amino acid residues. In other embodiments, the antigenic fragment comprises 50 to 250 amino acid residues. For FeLV, for example, the FeLV gp45 glycoprotein and the FeLV gp70 glycoprotein are antigenic fragments of the FeLV gp85 glycoprotein.

As used herein one amino acid sequence is 100% "identical" or has 100% “identity” to a second amino acid sequence when the amino acid residues of both sequences are identical. Accordingly, an amino acid sequence is 50% "identical" to a second amino acid sequence when 50% of the amino acid residues of the two amino acid sequences are identical. The sequence comparison is performed over a contiguous block of amino acid residues comprised by a given protein, *e.g.,* a protein, or a portion of the polypeptide being compared. In a particular embodiment, selected deletions or insertions that could otherwise alter the correspondence between the two amino acid sequences are taken into account.

As used herein, nucleotide and amino acid sequence percent identity can be determined using C, MacVector (MacVector, Inc. Cary, NC 27519), Vector NTI (Informax, Inc. MD), Oxford Molecular Group PLC (1996) and the Clustal W algorithm with the alignment default parameters, and default parameters for identity. These commercially available programs can also be used to determine sequence similarity using the same or analogous default parameters. Alternatively, an Advanced Blast search under the default filter conditions can be used, *e.g.,* using the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program using the default parameters.

As used herein, the term "inactivated" microorganism is used interchangeably with the term “killed” microorganism. For the purposes of this invention, an "inactivated" microorganism is an organism which is capable of eliciting an immune response in an animal, but is not capable of infecting the animal. An antigen of the present invention (*e.g.*, an inactivated feline calicivirus) may be inactivated by an agent selected from the group consisting of binary ethyleneimine, formalin, *beta*-propiolactone, thimerosal, or heat. In a particular embodiment, inactivated feline calicivirus isolates combined with an RP of the present invention are inactivated by binary ethyleneimine.

The alphavirus RNA replicon particles of the present invention may be lyophilized and rehydrated with a sterile water diluent. On the other hand, when the alphavirus RNA replicon particles are stored separately, but intended to be mixed with other vaccine components prior to administration, the alphavirus RNA replicon particles can be stored in the stabilizing solution of those components, *e.g.*, a high sucrose solution.

A vaccine of the present invention can be readily administered by any standard route including intravenous, intramuscular, subcutaneous, oral, intranasal, intradermal, and/or intraperitoneal vaccination. The skilled artisan will appreciate that the vaccine composition is preferably formulated appropriately for each type of recipient animal and route of administration.

Thus, the present invention also provides methods of immunizing a feline against FCV and/or other feline pathogens. One such method comprises injecting a feline with an immunologically effective amount of a vaccine of the present invention, so that the feline produces appropriate FCV antibodies.

Multivalent Vaccines:

The present invention also provides multivalent vaccines. For example, the coding sequence of a protein antigen or antigenic fragment thereof, or combination of such coding sequences of protein antigens useful in a feline vaccine can be added to an alphavirus RNA replicon particle (RP) or combined in the same RP as one that encodes a feline antigen of the FCV [*e.g.*, the FCV capsid protein] in the vaccine. In specific embodiments, the alphavirus RNA replicon particle encodes both the FCV F9-Like capsid protein or an antigenic fragment thereof and the

VS-FCV capsid protein or an antigenic fragment thereof and encodes a non-FCV antigen. Accordingly, such multivalent vaccines are included in the present invention.

Examples of pathogens that one or more of such protein antigens can originate from include feline rhinotracheitis Virus (FVR), feline leukemia virus (FeLV), feline panleukopenia Virus (FPL) feline herpesvirus (FHV), other FCV strains, feline parvovirus (FPV), feline infectious peritonitis virus (FIPV), feline immunodeficiency virus, borna disease virus (BDV), rabies virus, feline influenza virus, canine influenza virus, avian influenza, canine pneumovirus, feline pneumovirus, *Chlamydophila felis (FKA Chlamydia psittaci)*, *Bordetella bronchiseptica*, and *Bartonella spp. (e.g., B. henselae).* In particular embodiments, a coding sequence for a capsid protein or analogous protein from one or more of these feline or canine pathogens can be inserted into the same RP as the FCV antigen. Alternatively, or in combination therewith, a coding sequence for a capsid protein or analogous protein from one or more of these feline or canine pathogens can be inserted into one or more other RPs, which can be combined in a vaccine with an RP that encodes the FCV F9-Like capsid protein or an antigenic fragment thereof and/or the VS-FCV capsid protein or an antigenic fragment thereof.

In addition, an alphavirus RNA replicon particle(RP) that encodes one or more antigens of the FCV [*e.g.*, the FCV F9-Like capsid protein or an antigenic fragment thereof and/or the VS-FCV capsid protein or an antigenic fragment thereof] can be added together with one or more other live, attenuated virus isolates, *e.g.*, a live attenuated FCV F9-Like virus (*e.g.*, modified live FCV F9) and/or a live attenuated feline herpesvirus and/or a live attenuated feline parvovirus and/or a live, attenuated feline leukemia virus, and/or a live, attenuated feline infectious peritonitis virus and/or a live, attenuated feline immunodeficiency virus and/or a live, attenuated borna disease virus and/or a live, attenuated rabies virus, and/or a live, attenuated feline influenza virus and/or a live, attenuated canine influenza virus, and/or a live, attenuated avian influenza, and/or a live, attenuated canine pneumovirus, and/or a live, attenuated feline pneumovirus. In addition, a live, attenuated *Chlamydophila felis*, and/or a live, attenuated *Bordetella bronchiseptica* and/or a live, attenuated

Bartonella spp. (e.g., B. henselae) can also be included in such multivalent vaccines.

Furthermore, an alphavirus RNA replicon particle (RP) that encodes one or more antigens of the FCV [*e.g.*, the FCV F9-Like capsid protein or an antigenic fragment thereof and/or the VS-FCV capsid protein or an antigenic fragment thereof] can be added together with one or more other killed virus isolates such as a killed FCV strain, and/or a killed feline herpesvirus and/or a killed feline parvovirus and/or a killed feline leukemia virus, and/or a killed feline infectious peritonitis virus and/or a killed feline immunodeficiency virus and/or a killed borna disease virus and/or a killed rabies virus, and/or a killed feline influenza virus and/or a killed canine influenza virus, and/or a killed avian influenza virus, and/or a killed canine pneumovirus, and/or a killed feline pneumovirus. In addition, bacterins (or subfractions of the bacterins, *e.g.*, the pilus subfraction) of *Chlamydophila felis*, and/or *Bordetella bronchiseptica* and/or *Bartonella spp. (e.g., B. henselae)* can also be included in such multivalent vaccines.

It also is to be understood that this invention is not limited to the particular configurations, process steps, and materials disclosed herein as such configurations, process steps, and materials may vary somewhat. It further is to be understood that the terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

SEQUENCE TABLE

SEQ ID NO:	Description	Type
1	Feline Calicivirus (VS-FCV)	nucleic acid DNA
2	Feline Calicivirus (VS-FCV)	amino acid
3	Feline Calicivirus (F9-like)	nucleic acid DNA
4	Feline Calicivirus (F9-like)	amino acid
5	FeLV viral glycoprotein (gp85)	nucleic acid DNA
6	FeLV viral glycoprotein (gp85)	amino acid
7	FeLV viral glycoprotein (gp70)	nucleic acid DNA
8	FeLV viral glycoprotein (gp70)	amino acid
9	Rabies virus Glycoprotein	nucleic acid DNA
10	Rabies virus Glycoprotein	amino acid
11	GGCGCGCCGCACC	nucleic acid
12	Feline Calicivirus (VS-FCV)	nucleic acid RNA
13	Feline Calicivirus (F9-like)	nucleic acid RNA
14	FeLV viral glycoprotein (gp85)	nucleic acid RNA
15	FeLV viral glycoprotein (gp70)	nucleic acid RNA
16	Rabies virus Glycoprotein	nucleic acid RNA
	TTAATTAA	nucleic acid

SEQUENCES

Feline Calicivirus capsid (VS-FCV) SEQ ID NO: 1

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atggctgacgacggatctgtgaccaccccagaacaaggaacaatggtcggaggagtgatt
gccgaacccagcgctcagatgtcaactgcggcggacatggcctccggaaagtcggtggac
tccgagtgggaagccttcttctcgttccacacgtccgtgaactggagcacctccgaaacc
caaggaaagatcctcttcaagcagtccctgggtcccctgctgaacccgtacctggagcac
atcagcaagctgtacgtcgcttggagcgggtcgatcgaagtgcgattttccatctcggga
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aacagctcgggttgcatcgtgaccgtcgaaacgaagcctggcatcgatttcaagtttcat
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gacagcgatgggttttcattcgtgggcgtgtcgtcatttccaaagctggagtttccgctg
tccgcctcatacatgggcatccagctcgcaaagatccggctggcgtccaacatccggtca
tccatgactaagctgtga

Feline Calicivirus capsid (VS-FCV) SEQ ID NO: 2

MADDGSVTTPEQGTMVGGVIAEPSAQMSTAADMASGKSVDSEWEAFFSFHTSVNWSTSET
QGKILFKQSLGPLLNPYLEHISKLYVAWSGSIEVRFSISGSGVFGGKLAAIVVPPGVDPV
QSTSMLQYPHVLFDARQVEPVIFSIPDLRNSLYHLMSDTDTTSLVIMVYNDLINPYANDS
NSSGCIVTVETKPGIDFKFHLLKPPGSMLTHGSIPSDLIPKSSSLWIGNRHWTDITDFVI
RPFVFQANRHFDFNQETAGWSTPRFRPLAVTVSQSKGAKLGNGIATDYIVPGIPDGWPDT
TIPTKLTPTGDYAITSSDGNDIETKLEYENADVIKNNTNFRSMYICGSLQRAWGDKKISN
TGFITTGVISDNSISPSNTIDQSKIVVYQDNHVNSEVQTSDITLAILGYTGIGEEAIGAN
RDSVVRISVLPETGARGGNHPIFYKNSMKLGYVISSIDVFNSQILHTSRQLSLNNYLLPP
DSFAVYRIIDSNGSWFDIGIDSDGFSFVGVSSFPKLEFPLSASYMGIQLAKIRLASNIRS
SMTKL

Feline Calicivirus (VS-FCV) capsid (SEQ ID NO: 12)

auggcugacgacggaucugugaccaccccagaacaaggaacaauggucggaggagugauu
gccgaacccagcgcucagaugucaacugcggcggacauggccuccggaaagucgguggac
uccgagugggaagccuucuucucguuccacacguccgugaacuggagcaccuccgaaacc
caaggaaagauccucuucaagcagucccugggucccccugcugaacccguaccuggagcac
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uccgccucauacaugggcauccagcucgcaaagauccggcuggcguccaacauccgguca
uccaugacuaagcuguga

Feline Calicivirus (F9-like) capsid (SEQ ID NO: 3)

atgactgccccggaacaaggaacgatggtcggaggagtgattgcagaaccgtcagcacag
atgtccaccgctgccgacatggccactggaaagagcgtggactccgaatgggaagccttc
ttctccttccacacttcggtcaactggtcgactagcgaaacccaggggaagattttgttc
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gcatggtcgggcagcatcgaagtgcgctttagcatttccggctccggagtgttcgggga
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tacatgggtatccagctggccaagatcaggctggcctccaacatccgctcacctatgact
aagctgtga

Feline Calicivirus (F9-like) capsid (SEQ ID NO: 4)

MTAPEQGTMVGGVIAEPSAQMSTAADMATGKSVDSEWEAFFSFHTSVNWSTSETQGKILF
KQSLGPLLNPYLEHLAKLYVAWSGSIEVRFSISGSGVFGGKLAAIVVPPGVDPVQSTSML
QYPHVLFDARQVEPVIFCLPDLRSTLYHLMSDTDTTSLVIMVYNDLINPYANDANSSGCI
VTVETKPGPDFKFHLLKPPGSMLTHGSIPSDLIPKTSSLWIGNRYWSDITDFVIRPFVFQ
ANRHFDFNQETAGWSTPRFRPISVTITEQNGAKLGIGVATDYIVPGIPDGWPDTTIPGEL
IPAGDYAITNGTGNDITTATGYDTADIIKNNTNFRGMYICGSLQRAWGDKKISNTAFITT
ATLDGDNNNKINPCNTIDQSKIVVFQDNHVGKKAQTSDDTLALLGYTGIGEQAIGSDRDR
VVRISTLPETGARGGNHPIFYKNSIKLGYVIRSIDVFNSQILHTSRQLSLNHYLLPPDSF
AVYRIIDSNGSWFDIGIDSDGFSFVGVSGFGKLEFPLSASYMGIQLAKIRLASNIRSPMT
KL

Feline Calicivirus (F9-like) capsid (SEQ ID NO: 13)

augacugccccggaacaaggaacgauggucggaggagugauugcagaaccgucagcacag
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aagcuguga

Feline Leukemia Virus envelope glycoprotein (gp85) SEQ ID NO: 5
atggagtcaccaacacaccctaaaccttctaaagacaaaaccctctcgtggaatctcgccttccttgt
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gcagtacgaccccgatcggccttga

Feline Leukemia Virus envelope glycoprotein (gp85) SEQ ID NO: 6
MESPTHPKPSKDKTLSWNLAFLVGILFTIDIGMANPSPHQIYNVTWVITNVQTNTQANAT
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PFYVCPGHAPSLGPKGTHCGGAQDGFCAAWGCETTGEAWWKPTSSWDYITVKRGSSQDNS
CEGKCNPLVLQFTQKGRQASWDGPKMWGLRLYRTGYDPIALFTVSRQVSTITPPQAMGPN
LVLPDQKPPSRQSQTGSKVATQRPQTNESAPRSVAPTTMGPKRIGTGDRLINLVQGTYLA
LNATDPNKTKDCWLCLVSRPPYYEGIAILGNYSNQTNPPPSCLSTPQHKLTISEVSGQGM
CIGTVPKTHQALCNKTQQGHTGAHYLAAPNGTYWACNTGLTPCISMAVLNWTSDFCVLIE
LWPRVTYHQPEYVYTHFAKAVRFRREPISLTVALMLGGLTVGGIAAGVGTGTKALLETAQ
FRQLQMAMHTDIQALEESISALEKSLTSLSEVVLQNRRGLDILFLQEGGLCAALKEECCF
YADHTGLVRDNMAKLRERLKQRQQLFDSQQGWFEGWFNKSPWFTTLISSIMGPLLILLLI
LLFGPCILNRLVQFVKDRISVVQALILTQQYQQIKQYDPDRP*

Feline Leukemia Virus envelope glycoprotein (gp85) SEQ ID NO: 14

auggagucaccaacacacccuaaaccuucuaaagacaaaacccucucguggaaucucgccuuccuugu
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gggucauuacuaaugugcagacaaacacccaggcaaaugcuacuucuaugcuugguacucugacugau
gcuuauccaacccugcacgucgaccuuugcgaucucgucggugacacaugggagcccaucgugcugaa
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agaaacagcaacaaaccuacccauucuacgugugcccgggucacgcaccgucccugggucc gaaggga
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guggaagccuaccucaucuugggacuacauuacugugaaaagaggcucuagccaggauaacagcugcg
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cucucagcagggaugguucgagggcugguuuaacaagagcccaugguuuaccacucugaucucuucaa
ucaugggu ccacugcucauccugcuucugauucuucucuucggaccguguauucucaacaggcuggug
caguuugucaaggacagaaucucgguggu ccaggcccugauucuuacucagcaguaucagcagauuaa
gcaguacgaccccgaucggccuuga

Feline Leukemia Virus envelope glycoprotein (gp70) SEQ ID NO: 7
aatcctagtccacaccaaatatataatgtaacttgggtaataaccaatgtacaaactaacacc
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ggacctaagatgtggggattgcgactataccgtacaggatatgaccctatcgctttattcacg
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tacacacattttgccaaagctgtcaggttccgaaga

Feline Leukemia Virus envelope glycoprotein (gp70) SEQ ID NO: 8
NPSPHQIYNVTWVITNVQTNTQANATSMLGTLTDAYPTLHVDLCDLVGDTWEPIVLNPTNVKHGARYSSS
KYGCKTTDRKKQQQTYPFYVCPGHAPSLGPKGTHCGGAQDGFCAAWGCETTGEAWWKPTSSWDYITVKRG
SSQDNSCEGKCNPLVLQFTQKGRQASWDGPKMWGLRLYRTGYDPIALFTVSRQVSTITPPQAMGPNLVLP
DQKPPSRQSQTGSKVATQRPQTNESAPRSVAPTTMGPKRIGTGDRLINLVQGTYLALNATDPNKTKDCWL
CLVSRPPYYEGIAILGNYSNQTNPPPSCLSTPQHKLTISEVSGQGMCIGTVPKTHQALCNKTQQGHTGAH
YLAAPNGTYWACNTGLTPCISMAVLNWTSDFCVLIELWPRVTYHQPEYVYTHFAKAVRFRR

Feline Leukemia Virus envelope glycoprotein (gp70) SEQ ID NO: 15

aauccuaguccacaccaaauauauaauguaacuuggguaauaaccaauguacaaacuaacacc
caagcuaacgccaccucuauguuaggaaccuuaaccgaugccuacccuacccuacauguugac
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accuauugggccuguaacacuggacucaccccaugcauuuccauggcggugcucaauuggacc
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RABIES VIRUS G (SEQ ID NO: 9)

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atgcaccaacctgtcggggttctcctacatggaattgaaagtcggatacatcagtgccattaagatgaacgggttcact
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ttcctgatgacttgttggaggagagtcaatcggtcggagccaacacaacataatctcagaggaacaggaagggaggtgt
cagtcacaccccaaagcgggaagatcatttcgtcttgggagtcatacaagagcggaggtgaaaccggactgtga

RABIES VIRUS G (SEQ ID NO: 10)

MVPQALLFVPLLVFPLCFGKFPIYTIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSGF
SYMELKVGYISAIKMNGFTCTGVVTEAETYTNFVGYVTTTFKRKHFRPTPDACRAAYNWK
MAGDPRYEESLHNPYPDYHWLRTVKTTKESLVIISPSVADLDPYDRSLHSRVFPGGNCSG
VAVSSTYCSTNHDYTIWMPENPRLGMSCDIFTNSRGKRASKGSETCGFVDERGLYKSLKG
ACKLKLCGVLGLRLMDGTWVAMQTSNETKWCPPGQLVNLHDFRSDEIEHLVVEELVKKRE
ECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKSVRTWNEIIPS
KGCLRVGGRCHPHVNGVFFNGIILGPDGNVLIPEMQSSLLQQHMELLVSSVIPLMHPLAD
PSTVFKNGDEAEDFVEVHLPDVHERISGVDLGLPNWGKYVLLSAGALTALMLIIFLMTCW
RRVNRSEPTQHNLRGTGREVSVTPQSGKIISSWESYKSGGETGL*

RABIES VIRUS G (SEQ ID NO: 16)

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caauccguacccugacuaccacuggcuucggacugucaaaaccaccaaggagucacucgugaucauuaguccaagugug
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caccaauucucgggggaagagggcuuccaaagggucugaaacuugcggcuuugucgaugagcggggcuuguauaaguca
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cagucacaccccaaagcgggaagaucauuucgucuugggagucauacaagagcggaggugaaaccggacuguga

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention.

EXAMPLES

EXAMPLE 1

INCORPORATION OF THE CODING SEQUENCES FOR FCV CAPSID PROTEINS INTO THE ALPHAVIRUS RNA REPLICON PARTICLES

INTRODUCTION

RNA viruses have been used as vector-vehicles for introducing vaccine antigens, which have been genetically engineered into their genomes. However, their use to date has been limited primarily to incorporating viral antigens into the RNA virus and then introducing the virus into a recipient host. The result is the induction of protective antibodies against the incorporated viral antigens. Alphavirus RNA replicon particles have been used to encode pathogenic antigens. Such alphavirus replicon platforms have been developed from several different alphaviruses, including Venezuelan equine encephalitis virus (VEE) [Pushko *et al.*, Virology 239:389-401 (1997)], Sindbis (SIN) [Bredenbeek *et al.*, *Journal of Virology* 67:6439-6446 (1993) the contents of which are hereby incorporated herein in their entireties], and Semliki Forest virus (SFV) [Liljestrom and Garoff, *Biotechnology* (NY) 9:1356- 1361 (1991), the contents of which are hereby incorporated herein in their entireties]. Moreover, alphavirus RNA replicon particles are the basis for several USDA-licensed vaccines for swine and poultry. These include: Porcine Epidemic Diarrhea Vaccine, RNA Particle (Product Code 19U5.P1), Swine Influenza Vaccine, RNA (Product Code 19A5.D0), Avian Influenza Vaccine, RNA (Product Code 19O5.D0), and Prescription Product, RNA Particle (Product Code 9PP0.00).

ALPHAVIRUS RNA REPLICON PARTICLE CONSTRUCTION

Amino acid sequences for FCV capsid proteins were used to generate codon-optimized (feline codon usage) nucleotide sequences *in silico*. Optimized sequences were prepared as synthetic DNA by a commercial vendor (ATUM, Newark, CA). Accordingly, synthetic genes were designed based on the amino acid sequences of a VS-FCV capsid protein and an FCV F9-like capsid protein, respectively. The constructs that encode the amino acid sequence for the VS-FCV capsid protein [SEQ ID NO: 2], or for the FCV F9-like capsid protein [SEQ ID NO: 4], were codon-optimized for *feline*, with flanking sequence appropriate for cloning into the alphavirus replicon plasmid.

The VEE replicon vectors designed to express FCV capsid proteins were constructed as previously described [*see*, U.S. 9,441,247 B2; the contents of which are hereby incorporated herein by reference], with the following modifications. The TC-83-derived replicon vector “pVEK” [disclosed and described in U.S. 9,441,247 B2] was digested with restriction enzymes *AscI* and *PacI*. A DNA plasmid containing the codon-optimized open reading frame nucleotide sequence of one of the FCV capsid protein genes (either FCV F9-Like or VS-FCV), with 5' flanking sequence (5'-GGCGCGCCGCACC-3') [SEQ ID NO: 11] and 3' flanking sequence (5'-TTAATTAA-3'), were similarly digested with restriction enzymes *AscI* and *PacI*. The synthetic gene cassette was then ligated into the similarly digested pVEK vector, and the resulting clones were re-named "pVHV-F9" and "pVHV-Kalem", encoding the FCV F9-Like and the VS-FCV capsid proteins respectively. The "pVHV" vector nomenclature was chosen to refer to pVEK-derived replicon vectors containing transgene cassettes cloned *via* the *AscI* and *PacI* sites in the multiple cloning site of pVEK.

To create the dual construct, the pVHV vector region encoding the VEE subgenomic promoter and FCV Kalem (VS-FCV) capsid sequences was removed by PCR and ligated into the pVHV-F9 vector between the 3' end of the F9 FCV capsid sequence and the VEE 3' UTR sequence. The duplication of the subgenomic promoter sequence and confirmation of the FCV Kalem capsid sequence were achieved by sequencing of the final vector clone, termed "pVHV-F9-Kalem".

Production of TC-83 RNA replicon particles (RP) was conducted according to methods previously described [U.S. 9,441,247 B2 and U.S. 8,460,913 B2; the contents of which are hereby incorporated herein by reference in their entireties]. Briefly, pVHV replicon vector DNA and helper DNA plasmids were linearized with *NotI* restriction enzyme prior to *in vitro* transcription using MegaScript T7 RNA polymerase and cap analog (Promega, Madison, WI). Importantly, the helper RNAs used in the production lack the VEE subgenomic promoter sequence, as previously described [Kamrud *et al.*, *J Gen Virol.* 91(Pt 7):1723-1727 (2010)]. Purified RNA for the replicon and helper components were combined and mixed with a suspension of Vero cells, electroporated in 4 mm cuvettes, and returned to OptiPro® SFM cell culture media (Thermo Fisher, Waltham MA). Following overnight incubation, alphavirus RNA replicon particles were purified from the cells and media by passing the suspension through a ZetaPlus BioCap depth filter (3M, Maplewood, MN), washing with phosphate buffered saline containing 5% sucrose (w/v), and finally eluting the retained RP with 400 mM NaCl buffer. Eluted RP were formulated to a final 5% sucrose (w/v), passed through a 0.22 micron membrane filter, and dispensed into aliquots for storage. Titer of functional RP was determined by immunofluorescence assay on infected Vero cell monolayers.

EXAMPLE 2

EVALUATION OF EFFICACY AND SAFETY OF A DUAL CONSTRUCT FCV VACCINE IN CATS

A dual-construct vaccine comprising a propagation defective RNA particle (RP) encoding the capsid proteins from two different strains of FCV, a virulent systemic strain (VS-FCV) and a classical vaccine strain (FCV F9-Like) along with the capsid protein and glycoproteins of the avirulent TC-83 strain of Venezuelan Equine Encephalitis Virus (VEE) was formulated in 5% sucrose and stored frozen. This dual-construct vaccine was used to evaluate the effectiveness against challenge by two FCV strains, as shown in Table 1 below. Two groups of 10 cats each were vaccinated with the dual-construct FCV vaccine in a prime/ boost regimen at 13-14 weeks of age and then 21 days later. Two groups of control cats were vaccinated by the same regimen with a placebo vaccine consisting of cell culture media (Minimal Essential Media with Earle's salts, EMEM).

TABLE 1
VACCINATION PROTOCOL

Treatment Group	No. of Animals	Test Product	Vaccine Dose	Challenge Strain
1	9	Dual-construct RP-FCV	6.1×10^7	Classical FCV (Strain 255)
2	7	Placebo	NA	Classical FCV (Strain 255)
3	9	Dual-construct RP-FCV	6.1×10^7	Virulent Systemic FCV (Kalem# strain)
4	7	Placebo	NA	Virulent Systemic FCV

				(Kalem strain)

#Internal reference

Following the vaccinations the cats were observed for adverse reactions to the vaccines by observing the cats for any local or systemic reactions to the vaccines as well as clinical signs of FCV infection. No adverse reactions were observed for any of the vaccinated cats.

Three weeks following the booster vaccination, cats in Groups 1 and 2 were challenged intranasally with a virulent culture of FCV strain 255 (classical FCV challenge strain). Three weeks after booster vaccination cats in Groups 3 and 4 were challenged intranasally with a virulent culture of virulent systemic FCV challenge strain (FCV strain Kalem).

Cats were observed for clinical signs of FCV infection for 14 days following challenge as follows: cats were observed and scored daily for clinical signs including: death, depression/ lethargy, body temperature, nasal and oral ulcers, nasal and ocular discharge, lameness, dehydration and sneezing. Body weight was measured on four days spaced throughout the 14 day post-challenge period. Each of the clinical signs observed was given a weighted numerical score based on severity and the number of days it was observed. Each cat was then given a total, weighted score based on the sum of the daily weighted scores. A mean and median weighted score was then calculated for each treatment group. For the challenge to be considered valid, 80% of the control cats must show clinical signs of FCV infection (other than fever). The results of the challenge are summarized in Table 2 below:

TABLE 2
CHALLENGE RESULTS

Treatment Group	Test Product	Challenge Strain	Median Weighted Clinical Score	Mean Weighted Clinical Score
1	Dual-construct RP-FCV	Classical FCV (Strain 255)	5	6.3
2		Classical FCV		

	Placebo	(Strain 255)	16	14.3
3	Dual-construct RP-FCV	Virulent Systemic FCV (Kalem strain)	10	8.8
4	Placebo	Virulent Systemic FCV (Kalem strain)	38	36.9

The challenges for both strains were considered valid as 100% of placebo-vaccinated control cats exhibited clinical signs of FCV infection (other than fever). The dual-construct RP-FCV vaccine encoding a virulent systemic and classical vaccine strain of FCV protected cats against two distinct strains of FCV: a classical FCV strain as well as a virulent systemic FCV strain. The experimental vaccine was found safe in cats.

EXAMPLE 3

EVALUATION OF INTERFERENCE WITH ADMINISTRATION OF TWO DIFFERENT RNA PARTICLE VACCINES

The study was conducted to evaluate multiple aspects of the alphavirus RNA replicon particle FCV vaccine including serological response, efficacy against challenge, and interference. A RP-FCV construct vaccine encoding the capsid protein of a classical FCV vaccine strain (F9) was formulated in stabilizer consisting of gelatin, NZ-amine, and sucrose and lyophilized. Two groups of five cats were vaccinated with the RP-FCV vaccine at 17 weeks of age. Twenty-one days later, cats in Group 1 were administered a booster dose of the RP-FCV vaccine only, cats in Group 2 were administered a booster dose of the RP-FCV vaccine and administered a dose of an RP-Rabies vaccine at the same time as shown in Table 3 below. The RP-Rabies virus vaccine is a construct encoding the rabies virus glycoprotein (G) in the same TC-83 VEE alphavirus platform.

TABLE 3
VACCINATION PROTOCOL

Treatment Group	Test Product –Initial Vaccination (Day 0)	Test Product(s)-Booster Vaccination (Day 21)
1	RP-FCV (F9)	RP-FCV (F9)
2	RP-FCV (F9)	RP-FCV (F9)
		RP-Rabies virus

Following each vaccination the cats were observed for adverse reactions to the vaccines by observing the cats for any local or systemic reactions to the vaccines. No adverse reactions were observed for any of the vaccinated cats.

Cats were bled for serum collection on the day of initial vaccination (Study Day 0), the day of booster vaccination (Study Day 21) and six weeks after the initial vaccination (Study Day 42). The serum was tested for antibody titer to FCV F9 by a serum neutralization assay. Serum was also tested for antibody titer to rabies virus by the Rapid Fluorescent Focus Inhibition Test (RFFIT). RIFFT results are reported as International Units per milliliter (IU/ mL). Serology results are summarized in Tables 4 and 5 below.

TABLE 4
FCV F9 SEROLOGY RESULTS

Treatment Group	Vaccination Regimen (Day 0/ Day 21)	FCV (F9) Antibody Titer (Geometric Mean)		
		Day 0	Day 21	Day 42
1	RP-FCV (F9)/ RP-FCV (F9)	<2	3	34
2	RP-FCV (F9)/ RP-FCV (F9)+ RP-Rabies virus	<2	3	38

Based on comparison of FCV (F9) antibody titers (on serum samples collected post-booster) concurrent vaccination with an RP-Rabies virus vaccine does not interfere with the antibody response to an RP-FCV (F9) vaccine.

TABLE 5
RABIES VIRUS SEROLOGY RESULTS

Treatment Group	Vaccination Regimen (Day 0/ Day 21)	Rabies Antibody Titer (Geometric Mean IU/ mL)		
		Day 0	Day 21	Day 42
1	RP-FCV (F9)/ RP-FCV (F9)	2	1	Not Tested
2	RP-FCV (F9)/ RP-FCV (F9)+ RP-Rabies virus	1	<1	39

Although this study did not include a control group, which was vaccinated with only an RP-Rabies virus vaccine, for the purpose of comparing the post-booster rabies titer of Group 2, historical data from other studies is presented below in Table 6.

TABLE 6
RABIES VIRUS SEROLOGY RESULTS, MULTIPLE STUDIES

Treatment Group	Vaccination Regimen	RP-Rabies virus Potency	Rabies Antibody Titer (Geometric Mean IU/ mL)	
			Pre-vaccination	Approximately 1 month post-vaccination*
2	RP-FCV (F9)/ RP-FCV (F9)+ RP-Rabies virus	1.3×10^7	1	39
Study RUS-006	RP-Rabies virus alone Single Vaccination	2.7×10^7	<0.1	42.7
Study RUS-006	RP-Rabies virus alone Single Vaccination	2.6×10^6	<0.1	17.6

*Study RUS-006 cats were bled for serum 30 days after vaccination

Based on the potency of the RP-Rabies virus vaccines and the post-vaccination rabies antibody titers, a prior vaccination with an RP-FCV vaccine does not interfere with the antibody response to an RP-Rabies virus vaccine. Vector immunity is a concern with platform-based vaccines however, the results of this study suggest that multiple RP-based vaccines can be used in an animal without compromising efficacy.

In order to confirm the lack of interference by concurrent vaccination with RP-Rabies virus on RP-FCV (F9) efficacy the study was amended and continued. A group of five age-matched cats were added to the study to serve as non-vaccinated controls. All cats were challenged intranasally with a virulent, classical strain of FCV (FCV 255) 79 days after the initial vaccination of Groups 1 and 2.

For 14 days following challenge cats were observed and scored daily for the following clinical signs of FCV infection: death, depression/ lethargy, body temperature, nasal and oral ulcers, nasal and ocular discharge, lameness, dehydration, and sneezing. Body weight was measured on four days spaced throughout the 14-day post-challenge period. Each of the clinical signs observed was given a weighted numerical score based on severity and the number of days it was observed. Each cat was then given a total, weighted score based on the sum of the daily weighted scores. A mean and median weighted score was then calculated for each treatment group. For the challenge to be considered valid, 80% of the control cats must show clinical signs of FCV infection (other than fever). The results of the challenge are summarized in the table below:

TABLE 7
INTERFERENCE STUDY CHALLENGE RESULTS

Treatment Group	Vaccination Regimen (Day 0/ Day 21)	Mean Weighted Clinical Score	Median Weighted Clinical Score
1	RP-FCV (F9)/ RP-FCV (F9)	3	4.0
2	RP-FCV (F9)/ RP-FCV (F9)+ RP-Rabies virus	2	4.4
3	Non-Vaccinated	20	19.6

The challenge was considered valid as 100% of non-vaccinated control cats exhibited clinical signs of FCV infection (other than fever). Both vaccinate groups (Groups 1 and 2) were significantly protected from virulent FCV challenge (p values 0.012 for both groups).

Based on comparison of clinical scores, concurrent vaccination with an RP-Rabies virus vaccine does not interfere with the efficacy of an RP-FCV (F9) vaccine. The experimental vaccine was found safe in cats.

EXAMPLE 4

EVALUATION OF VACCINE EFFICACY IN CATS OF A RP CONSTRUCT ENCODING A SINGLE FCV F9-LIKE CAPSID PROTEIN

This study was conducted to further evaluate the efficacy of a feline vaccine comprising an alphavirus RNA replicon particle that encoded a single FCV F9-Like capsid protein (RP-FCV F9). The vaccine was compared to a placebo control group against a classical FCV challenge. Cats were inoculated with either this monovalent vaccine or a placebo and subsequently challenged with a classical FCV. The clinical scores are based on the typical signs of FCV infection, mainly oral and external ulcers and rhinitis, scored over a period of 14 days following the FCV challenge. The scoring system is the same as that described in Examples 2 and 3 above. Whereas, the mean clinical score for the placebo controls was 92, the mean clinical score for the RP-FCV F9 vaccine was only 2. Surprisingly the score for the RP-FCV F9 vaccine also was significantly lower than that obtained with two vaccines that individually comprised a single attenuated-live FCV F9-Like virus. This experiment further demonstrates that an RP-FCV vaccine encoding a classical vaccine strain of FCV protects cats against an FCV F9-Like virus.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

WE CLAIM:

1. An immunogenic composition comprising an alphavirus RNA replicon particle that encodes a feline calicivirus (FCV) antigen.

2. The immunogenic composition of Claim 1, wherein the FCV antigen is a capsid protein or an antigenic fragment thereof.

3. The immunogenic composition of Claim 1 or 2, wherein the alphavirus RNA replicon particle is a Venezuelan Equine Encephalitis (VEE) alphavirus RNA replicon particle.

4. The immunogenic composition of Claim 2 or 3, wherein the capsid protein is selected from the group consisting of an FCV F9-Like capsid protein, an antigenic fragment of the FCV F9-Like capsid protein, a virulent systemic FCV (VS-FCV) capsid protein, and an antigenic fragment of the VS-FCV capsid protein.

5. The immunogenic composition of Claim 4, wherein the capsid protein is a VS-FCV capsid protein or an antigenic fragment thereof.

6. The immunogenic composition of Claim 4, wherein the capsid protein is a FCV F9-Like capsid protein or an antigenic fragment thereof.

7. The immunogenic composition of Claim 1, 2, 3, 4, 5, or 6, that comprises an additional alphavirus RNA replicon particle that encodes a second FCV antigen, wherein the second FCV antigen originates from a different strain of FCV than the one from which the FCV antigen originates from.

8. The immunogenic composition of Claim 5, that comprises an additional alphavirus RNA replicon particle that encodes an FCV F9-Like capsid protein or an antigenic fragment thereof.

9. The immunogenic composition of Claim 7, or 8, wherein the additional alphavirus RNA replicon particle is an VEE alphavirus RNA replicon particle.

10. The immunogenic composition of Claim 5, wherein the alphavirus RNA replicon particle also encodes an FCV F9-Like capsid protein, or an antigenic fragment of the FCV F9-Like capsid protein.

11. The immunogenic composition of Claim 4, 5, 7, 8, 9, or 10, wherein the VS-FCV capsid protein comprises an amino acid sequence comprising at least 95% identity with the amino acid sequence of SEQ ID NO: 2.

12. The immunogenic composition of Claim 4, 6, 7, 8, 9, 10, or 11, wherein the FCV F9-Like capsid protein comprises an amino acid sequence comprising at least 95% identity with the amino acid sequence of SEQ ID NO: 4.

13. A vaccine to aid in the prevention of disease due to FCV comprising the immunogenic composition of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and a pharmaceutically acceptable carrier.

14. The vaccine composition of Claim 13, that further comprises at least one non-FCV antigen for eliciting protective immunity to a non-FCV feline pathogen.

15. The vaccine of Claim 14, wherein the non-FCV feline pathogen is selected from the group consisting of feline herpesvirus (FHV), feline leukemia virus (FeLV), feline pneumovirus (FPN), feline parvovirus (FPV), feline infectious peritonitis virus (FIPV), feline immunodeficiency virus, borna disease virus (BDV), feline influenza virus, feline panleukopenia virus (FPLV), feline coronavirus (FCoV), feline rhinotracheitis virus (FVR), *Chlamydophila felis*, and any combination thereof.

16. The vaccine of Claim 14, wherein the non-FCV antigen is a killed or attenuated non-FCV antigen selected from the group of killed or attenuated non-FCV antigens consisting of feline herpesvirus (FHV), feline leukemia virus (FeLV), feline pneumovirus (FPN), feline parvovirus (FPV), feline infectious peritonitis virus

(FIPV), feline immunodeficiency virus, borna disease virus (BDV), feline influenza virus, feline panleukopenia virus (FPLV), feline coronavirus (FCoV), feline rhinotracheitis virus (FVR), *Chlamydophila felis*, and any combination thereof.

17. The vaccine of Claim 16, wherein the attenuated non-FCV antigen is a modified live feline pathogen selected from the group consisting of a modified live *Chlamydophila felis*, a modified live feline rhinotracheitis Virus (FVR), a modified live feline leukemia virus (FeLV), a modified live feline panleukopenia virus (FPL), a modified live feline herpesvirus (FHV), a modified live feline pneumovirus (FPN), a modified live feline parvovirus (FPV), a modified live feline infectious peritonitis virus (FIPV), a modified live feline immunodeficiency virus, a modified live borna disease virus (BDV), a modified live feline coronavirus (FCoV), and a modified live feline influenza virus.

18. The vaccine composition of Claim 13, 14, 15, 16, or 17 that further comprises an alphavirus RNA replicon particle comprising a nucleotide sequence encoding at least one protein antigen or an antigenic fragment thereof that originates from a non-FCV antigen.

19. The vaccine of Claim 18, wherein the protein antigen or an antigenic fragment thereof that originates from a non-FCV feline pathogen selected from the group consisting of feline herpesvirus (FHV), feline leukemia virus (FeLV), feline pneumovirus (FPN), feline parvovirus (FPV), feline infectious peritonitis virus (FIPV), feline immunodeficiency virus, borna disease virus (BDV), feline influenza virus, feline panleukopenia virus (FPLV), feline coronavirus (FCoV), feline rhinotracheitis virus (FVR), *Chlamydophila felis*, and any combination thereof.

20. The vaccine composition of Claim 13, 14, 15, 16, 17, 18, or 19, that is a nonadjuvanted vaccine.

21. A method of immunizing a feline against a pathogenic FCV comprising administering to the feline an immunologically effective amount of the vaccine of Claim 13, 14, 15, 16, 17, 18, 19, or 20.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/080096

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

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

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

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

EPO-Internal, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
7 February 2019	19/02/2019

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

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