



(43) International Publication Date
2 October 2014 (02.10.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/159052 A1

- (51) **International Patent Classification:**
A61K 39/12 (2006.01) *A61P 37/00* (2006.01)
A61K 39/02 (2006.01)
- (21) **International Application Number:**
PCT/US2014/021725
- (22) **International Filing Date:**
7 March 2014 (07.03.2014)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/779,040 13 March 2013 (13.03.2013) US
- (71) **Applicants:** THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY OF AGRICULTURE [US/US]; Washington, District of Columbia 20250 (US). BOARD OF SUPERVISORS, LOUISIANA STATE UNIVERSITY AND A&M COLLEGE [US/US]; LSU Agricultural Center, 104 J. Norman Efferon Hall, 110 LSU Union Square, Baton Rouge, Louisiana 70803-0106 (US).
- (72) **Inventors:** GUERRERO, Felicitio; 134 Oak Grove Drive, Boerne, Texas 78006 (US). PEREZ DE LEON, Alberto A.; 901 Golf Street, Kerrville, Texas 78028 (US). FOIL, Lane D.; LSU Agricultural Center, 104 J. Norman Efferon Hall, 110 LSU Union Square, Baton Rouge, Louisiana 70803-0106 (US).
- (74) **Agent:** GOLDBERG, Joshua B.; Nath, Goldberg & Meyer, 112 S. West Street, Alexandria, Virginia 22314 (US).
- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).
- Published:**
— with international search report (Art. 21(3))
— with sequence listing part of description (Rule 5.2(a))

(54) **Title:** VACCINATION OF COMPANION ANIMALS TO ELICIT A PROTECTIVE IMMUNE RESPONSE AGAINST TICK INFESTATIONS AND TICK-BORNE PATHOGEN TRANSMISSION

(57) **Abstract:** Compositions of either the aquaporin protein from the cattle tick, *Rhipicephalus microplus*, or a nucleic acid construct incorporating a nucleic acid sequence encoding this aquaporin protein, are effective for eliciting a protective immune response against other tick species in non-bovine animals. The *R. microplus* aquaporin protein is antigenic and can be administered as a protein vaccine, or in the alternative, the nucleic acid construct can be utilized as a DNA vaccine. Induction of the immune response significantly reduces or eliminates the infestation of treated, non-bovine animals with ticks other than the cattle tick, particularly the brown dog tick, *Rhipicephalus sanguineus*. Moreover, as ticks are vectors of a variety of pathogenic agents, the reduction in the incidence of tick infestation afforded by the vaccines may concurrently reduce the incidence of diseases caused by these pathogenic agents in susceptible animals.



WO 2014/159052 A1

VACCINATION OF COMPANION ANIMALS TO ELICIT A
PROTECTIVE IMMUNE RESPONSE AGAINST TICK
INFESTATIONS AND TICK-BORNE PATHOGEN TRANSMISSION

Background of the Invention

Field of the Invention

[0001] The invention relates methods to control and prevent tick infestations in treated non-bovine animals, including domestic dogs, which further protects the animals against the transmission of tick-borne pathogens. Vaccine compositions are prepared from the aquaporin protein from the cattle tick, *Rhipicephalus microplus*, or from a nucleic acid construct comprising a nucleic acid sequence encoding this aquaporin protein.

Description of the Prior Art

[0002] Ticks pose a significant risk to the health and welfare of warm-blooded animals as the vectors for a large number of pathogenic agents, including protozoan parasites, viruses and bacteria. For instance, the brown dog tick, *Rhipicephalus sanguineus*, is the primary reservoir and vector of canine ehrlichiosis, a lethal disease caused by the blood-borne intracellular bacterial pathogen, *Ehrlichia canis*, and canine babesiosis, caused by the intraerythrocytic protozoan parasites *Babesia canis* and *Babesia gibsoni*. Canine ehrlichiosis occurs throughout the world and is endemic in the United States, infecting dogs of all breeds and ages. Cats and humans may be infected with *E. canis* as well. The brown

dog tick can also transmit Rocky Mountain spotted fever to humans. Ticks are also carriers of a number of other common tick-borne infectious disease agents such as tick-borne encephalitis virus, Crimean-Congo hemorrhagic fever virus, Nairobi sheep virus, *Borrelia burgdorferi* (the agent of Lyme disease), *Theileria parva* (the agent of East Coast fever), and parasites of the genus *Babesia* (including the agents of babesiosis or cattle fever in bovine), as well as other injurious effects that have major impacts in human and veterinary medicine.

[0003] Presently, efforts to control these pests have primarily relied upon the use of pesticides. However, an increase in the resistance of ticks to approved acaricides and insecticides threatens efforts to control these pests in the U.S. and elsewhere.

[0004] As a result of the spread of pesticide-resistant strains of these and other ticks and flies, there is a growing need to develop improved tools for their control. Attempts have been made to use immunological means of control through vaccine technology. Some success has been met in identifying certain protective antigens of arthropod parasites as being potential vaccine candidates, but only a few have as yet come to commercial fruition, most notably the BM86 vaccine for the cattle tick *Rhipicephalus (Boophilus) microplus*. Despite these developments, there is nonetheless a continuing need for arthropod parasite vaccines and in particular for a vaccine which may be used against ticks, including the brown dog tick.

Summary of the Invention

[0005] We have discovered that compositions of either the aquaporin protein from the cattle tick, *Rhipicephalus*

microplus, or a nucleic acid construct incorporating a nucleic acid sequence encoding this aquaporin protein, are effective for eliciting a protective immune response against other tick species in non-bovine animals. The *R. microplus* aquaporin protein is antigenic and can be administered as a protein vaccine, or in the alternative, the nucleic acid construct can be utilized as a DNA vaccine. In this later embodiment, the nucleic acid constructs are administered to a subject animal such that the aquaporin protein is expressed *in vivo* within the cells of the vaccinated animal. Induction of the immune response significantly reduces or eliminates the infestation of treated, non-bovine animals with ticks other than the cattle tick, particularly the brown dog tick, *Rhipicephalus sanguineus*. Moreover, as ticks are vectors of a variety of pathogenic agents, the reduction in the incidence of tick infestation afforded by the vaccines may concurrently reduce the incidence of diseases caused by these pathogenic agents in susceptible animals.

[0006] In accordance with this discovery, it is an object of this invention to provide protective vaccines against ticks other than the cattle tick, *R. microplus*, in non-bovine animals.

[0007] Another object of the invention is to provide protective vaccines that control and prevent infestations with ticks of different species than the cattle tick, and in animals different from bovine.

[0008] A further object of the invention is to provide protective vaccines against the brown dog tick, *R. sanguineus*, in animals.

[0009] Yet another object of the invention is to provide protective vaccines against the brown dog tick, *R. sanguineus*,

in companion animals, including deer, horses, domestic dogs and cats.

[0010] Still another object of the invention is to provide protective vaccines that control and prevent animal infestations with ticks, and thereby reduce or eliminate the incidence of diseases caused by pathogenic agents carried by the ticks.

[0011] Still another object of the invention is to provide protective vaccines that control and prevent canine infestations with the brown dog tick, *R. sanguineus*, and thereby reduce or eliminate the incidence of canine ehrlichiosis caused by *Ehrlichia canis*, and canine babesiosis caused by the protozoan parasites *Babesia canis* and *Babesia gibsoni*.

[0012] Other objects and advantages of this invention will become readily apparent from the ensuing description.

Brief Description of the Drawings

[0013] Figure 1A shows the amino acid sequence (SEQ ID NO: 1) of the fragment of the aquaporin protein, Contig 12018, described herein which was isolated from *R. microplus*. Figure 1B shows the amino acid sequence (SEQ ID NO: 2) of the aquaporin protein fragment as cloned into and produced by *Pichia pastoris* expression vector pPICZalphaA as described in Guerrero et al. (U.S patent application serial no. 13/479,486, filed May 24, 2012). The amino acids of the tick aquaporin protein are underlined (amino acids 92-290 of SEQ ID NO: 2), while extra amino acids not of tick origin but from the vector are not underlined (amino acids 1-91 and 291-317 of SEQ ID NO: 2). Figure 1C shows the amino acid sequence (SEQ ID NO: 3) of

the aquaporin protein fragment as cloned into and produced by the DNA vaccine expression vector pcDNA4mycHis C as described in Guerrero *et al.* The amino acids of the tick aquaporin protein are underlined (amino acids 1 and 3-198 of SEQ ID NO: 3), while substituted/extra amino acids not of tick origin are not underlined (amino acids 2 and 199-226 of SEQ ID NO: 3). Although the substituted/extra amino acids are not of tick origin, they are present in the structure of the final protein product used in the vaccine in the Example.

[0014] Figure 2A shows the nucleotide sequence (SEQ ID NO: 5) of the cDNA from *R. microplus* encoding the isolated aquaporin protein fragment of Figure 1A and includes an untranslated 5' region. The translated region coding for the isolated aquaporin protein fragment of Figure 1A is underlined and corresponds to nucleotides 348-947 of the sequence. Figure 2B shows the aquaporin nucleotide sequence (SEQ ID NO: 6) as cloned into *P. pastoris* expression vector pPICZalphaA as described in Guerrero *et al.* The underlined sequence (nucleotides 274-870) corresponds to the nucleotide sequence of the coding region of Figure 2A above except that the three 3' terminal nucleotides have been removed. The nucleotide sequence shown includes extra, optional vector-provided nucleotides (nucleotides 1-273 and 871-954, not underlined) which code for the extra amino acids not of tick origin. Figure 2C shows the aquaporin nucleotide sequence (SEQ ID NO: 7) as cloned into the DNA expression vector pcDNA4mycHis C as described in Guerrero *et al.* The underlined sequence (nucleotides 4-598) corresponds to the nucleotide sequence of the coding region of Figure 2A above except that the second codon triplet has been changed from AAG to GAG, and the five 3' terminal nucleotides have been removed. The nucleotide sequence shown includes extra, optional DNA vaccine expression vector-provided nucleotides (1-3 and 599-684, not underlined)

of which the 5' terminal nucleotides code for the extra amino acids not of tick origin. The nucleotide sequence corresponding to nucleotides 4-598 of SEQ ID NO: 7 is also presented as SEQ ID NO: 8 (wherein the extra nucleotides from the DNA vaccine expression vector are not included).

[0015] Figure 3 shows a nucleotide sequence (SEQ ID NO: 9) encoding the isolated aquaporin protein fragment of Figure 1A wherein the translated or coding region of the nucleotide sequence of Figure 2A (nucleotides 348-942) has been optimized to enhance translation in *P. pastoris*.

[0016] Figure 4 shows the molting success of *R. sanguineus* ticks capillary fed pooled blood treatments as described in Example 1.

Definitions

The following terms are employed herein:

[0017] Cloning. The selection and propagation of (a) genetic material from a single individual, (b) a vector containing one gene or gene fragment, or (c) a single organism containing one such gene or gene fragment.

[0018] Cloning Vector. A plasmid, virus, retrovirus, bacteriophage or nucleic acid sequence which is able to replicate in a host cell, characterized by one or a small number of restriction endonuclease recognition sites at which the sequence may be cut in a predetermined fashion, and which contains a marker suitable for use in the identification of transformed cells, e.g., uracil utilization, tetracycline resistance, ampicillin resistance. A cloning vector may or may not possess the features necessary for it to operate as an expression vector.

[0019] Codon. A DNA sequence of three nucleotides (a triplet) which codes (through mRNA) for an amino acid, a translational start signal, or a translational termination signal. For example, the nucleotide triplets TTA, TTG, CTT, CTC, CTA, and CTG encode for the amino acid leucine, while TAG, TAA, and TGA are translational stop signals, and ATG is a translational start signal.

[0020] Complement or Complementary Sequence. The product of complementary base pairing in which purines bond with pyrimidines, as it occurs in the two polynucleotide chains of DNA (adenine with thymine, guanine with cytosine) and between DNA and messenger RNA nucleotides during transcription.

[0021] DNA Coding Sequence. A DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences and cDNA from eukaryotic mRNA. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0022] DNA Sequence. A linear series of nucleotides connected one to the other by phosphodiester bonds between the 3' and 5' carbons of adjacent pentoses.

[0023] Expression. The process undergone by a structural gene to produce a polypeptide. Expression requires both transcription of DNA and translation of RNA.

[0024] Expression Vector. A replicon such as a plasmid, virus, retrovirus, bacteriophage, or nucleic acid sequence which is able to replicate in a host cell, characterized by a

restriction endonuclease recognition site at which the sequence may be cut in a predetermined fashion for the insertion of a heterologous DNA sequence. An expression vector has a promoter positioned upstream of the site at which the sequence is cut for the insertion of the heterologous DNA sequence, the recognition site being selected so that the promoter will be operatively associated with the heterologous DNA sequence. A heterologous DNA sequence is "operatively associated" with the promoter in a cell when RNA polymerase, which binds the promoter sequence transcribes the coding sequence into mRNA which is then in turn translated into the protein encoded by the coding sequence.

[0025] Fusion Protein. A protein produced when two heterologous genes or fragments thereof coding for two different proteins not found fused together in nature are fused together in an expression vector. For the fusion protein to correspond to the separate proteins, the separate DNA sequences must be fused together in correct translational reading frame.

[0026] Gene. A segment of DNA which encodes a specific protein or polypeptide, or RNA.

[0027] Genome. The entire DNA of an organism. It includes, among other things, the structural genes encoding for the polypeptides of the substance, as well as operator, promoter and ribosome binding and interaction sequences.

[0028] Heterologous DNA. A DNA sequence inserted within or connected to another DNA sequence which codes for polypeptides not coded for in nature by the DNA sequence to which it is joined. Allelic variations or naturally occurring mutational events do not give rise to a heterologous DNA sequence as defined herein.

[0029] Hybridization. The pairing together or annealing of single stranded regions of nucleic acids to form double-stranded molecules.

[0030] Nucleotide. A monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via the glycosidic carbon (1' carbon of the pentose) and that combination of base and sugar is a nucleoside. The base characterizes the nucleotide. The four DNA bases are adenine ("A"), guanine ("G"), cytosine ("C"), and thymine ("T"). The four RNA bases are A, G, C, and uracil ("U").

[0031] Phage or Bacteriophage. Bacterial virus many of which include DNA sequences encapsidated in a protein envelope or coat ("capsid"). In a unicellular organism, a phage may be introduced by a process called transfection.

[0032] Plasmid. A non-chromosomal double-stranded DNA sequence comprising an intact "replicon" such that the plasmid is replicated in a host cell. When the plasmid is placed within a unicellular organism, the characteristics of that organism may be changed or transformed as a result of the DNA of the plasmid. A cell transformed by a plasmid is called a "transformant".

[0033] Polypeptide. A linear series of amino acids connected one to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent amino acids.

[0034] Promoter. A DNA sequence within a larger DNA sequence defining a site to which RNA polymerase may bind and initiate transcription.

[0035] Reading Frame. The grouping of codons during translation of mRNA into amino acid sequences. During translation the proper reading frame must be maintained. For example, the DNA sequence may be translated via mRNA into three reading frames, each of which affords a different amino acid sequence.

[0036] Recombinant DNA Molecule. A hybrid DNA sequence comprising at least two DNA sequences, the first sequence not normally being found together in nature with the second.

[0037] Ribosomal Binding Site. A nucleotide sequence of mRNA, coded for by a DNA sequence, to which ribosomes bind so that translation may be initiated. A ribosomal binding site is required for efficient translation to occur. The DNA sequence coding for a ribosomal binding site is positioned on a larger DNA sequence downstream of a promoter and upstream from a translational start sequence.

[0038] Start Codon. Also called the initiation codon, is the first mRNA triplet to be translated during protein or peptide synthesis and immediately precedes the structural gene being translated. The start codon is usually AUG, but may sometimes also be GUG.

[0039] Stringent Hybridization Conditions. The term "stringent conditions" or "stringent hybridization conditions" includes reference to conditions under which a probe will hybridize to its target sequence, to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will differ in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences can be identified which are 100% complementary to the probe (homologous probing).

Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, optionally less than 500 nucleotides in length. Typically, stringent hybridization conditions comprise hybridization in 50% formamide, 1 M NaCl, 1% SDS at 42°C, and a wash in 0.1xSSC at 60 to 65°C. It is also understood that due to the advances in DNA PCR and sequencing approaches that issues of gene identity and homology may be determined by sequence based rather than hybridization approaches.

[0040] Structural Gene. A DNA sequence which encodes through its template or messenger RNA (mRNA) a sequence of amino acids characteristic of a specific polypeptide.

[0041] Substantially Pure. The condition of a compound, such as a protein or a nucleotide, being cell free or being separated from other components that would interfere with or have a substantial qualitative effect on the activity of the compound or on a substrate on which the compound acts.

[0042] Transform. To change in a heritable manner the characteristics of a host cell in response to DNA foreign to that cell. An exogenous DNA has been introduced inside the cell wall or protoplast. Exogenous DNA may or may not be integrated (covalently linked) to chromosomal DNA making up the genome of the cell. In prokaryotes and some fungi, for example, the exogenous DNA may be maintained on an episomal element such as a plasmid. With respect to most eukaryotic cells, a stably transformed cell is one in which the exogenous DNA has been integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the

eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the exogenous DNA.

[0043] Transcription. The process of producing mRNA from a structural gene.

[0044] Translation. The process of producing a polypeptide from mRNA.

[0045] Vaccine. Vaccine is defined herein in its broad sense to refer to any type of biological agent in an administrable form capable of stimulating a protective immune response in an animal inoculated with the vaccine. For purposes of this invention, the vaccine may comprise either one or more of the immunogenic (antigenic) proteins or nucleic acid constructs encoding these proteins.

Detailed Description of the Invention

[0046] In the following description, the nomenclature used to define the proteins and peptides is that specified by Schroder and Lubke ["The Peptides," Academic Press (1965)] wherein, in accordance with conventional representation, the N-terminal appears to the left and the C-terminal to the right. Where the amino acid residue has isomeric forms, it is the L-form of the amino acid that is represented herein unless otherwise expressly indicated.

[0047] The immunogenic protein which is utilized herein is an aquaporin protein fragment from the cattle tick, *R. microplus*. This aquaporin protein fragment, as well as the nucleic acid sequences encoding this aquaporin protein, are described in Guerrero et al., U.S patent application serial no. 13/479,486, filed May 24, 2012, the contents of which are incorporated by reference herein. Guerrero et al. also

discloses that this aquaporin protein fragment, and nucleic acid constructs comprising the nucleic acid sequence encoding this aquaporin protein, may be used as vaccines to control and prevent infestations of *R. microplus* in treated livestock, including bovines. We have now discovered that the same aquaporin protein from *R. microplus*, and the nucleic acid constructs incorporating the nucleic acid sequence encoding this aquaporin protein, are not only effective for eliciting a protective immune response against the cattle tick in bovine animals, but also for eliciting a protective immune response against other tick species in other, non-bovine animals. In particular, we have discovered that the aquaporin protein or nucleic acid constructs are effective for eliciting a protective immune response against the brown dog tick, *R. sanguineus*, in a variety of animals, preferably deer (including white-tailed deer), horses, domestic dogs and cats. Moreover, without being limited thereto, it is envisioned that the aquaporin protein or nucleic acid constructs are effective for eliciting a protective immune response against other ticks, including *Ixodes holocyclus* (Australia paralysis tick), *I. ricinus*, *I. pacificus*, *I. hexagonus*, *I. canisuga*, *Rhipicephalus turanicus*, *Dermacentor variabilis* (American dog tick), *D. andersoni*, *D. reticulatus*, *Amblyomma americanum* (Lone star tick), *A. maculatum* and *Otobius megnini*.

[0048] The aquaporin protein fragment of the cattle tick, *R. microplus*, has been isolated, substantially free from other proteins or cell components which are normally present in the cells of the tick, such that the aquaporin protein is the only significant protein or peptide in the sample and may be used effectively as a vaccine. Moreover, the protein has been produced in recombinant form as described in Guerrero et al. and herein below. The term "isolated" encompasses not only proteins which have been recovered from naturally occurring

cells, but also recombinant proteins and synthesized proteins. The aquaporin protein fragments, including recombinants, are immunogenic, effective for eliciting a protective immune response against ticks other than the cattle tick, which response is mediated by humoral, i.e., antibodies, and/or cellular processes.

[0049] The isolated aquaporin protein fragment of *R. microplus* has been sequenced, and its amino acid sequence is shown in Figure 1A. The amino acid sequence of the isolated aquaporin protein fragment corresponds to SEQ ID NO: 1. The isolated aquaporin protein fragment has a calculated molecular weight of 21.2 kDa based on the amino acid sequence. Moreover, when utilizing recombinant proteins, the aquaporin protein fragment may be modified to assist cloning in the selected vector, and the expressed protein may further include optional, additional terminal amino acid sequences from the vector (not of tick origin). For example, Figure 1B shows the amino acid sequence (SEQ ID NO: 2) of the aquaporin protein fragment as cloned into and produced by the *Pichia pastoris* expression vector pPICZalphaA as described in Guerrero *et al.* The underlined sequence of Figure 1B corresponds to the amino acid sequence of Figure 1A, except that the C-terminal amino acid (L) of the isolated fragment has been removed to assist cloning of the aquaporin gene into the plasmid. The amino acid sequence also includes extra, optional amino acids from the cloning vector. The amino acids of the tick aquaporin protein are underlined (amino acids 92-290 of SEQ ID NO: 2), while the extra amino acids not of tick origin are not underlined (amino acids 1-91 and 291-317 of SEQ ID NO: 2). Figure 1C shows the amino acid sequence (SEQ ID NO: 3) of the aquaporin protein fragment as cloned into and produced by the DNA vaccine expression vector pcDNA4mycHis C also as described in Guerrero *et al.* The underlined sequence corresponds to the

amino acid sequence of Figure 1A above, except that amino acid 2 has been changed (from K to E) and the two C-terminal amino acids (PL) of the isolated fragment have been removed to assist cloning of the aquaporin gene into the plasmid. Thus, amino acids 3-198 of SEQ ID NO: 1 are common to each of the aquaporin sequences of Figures 1A, B and C. The amino acid sequence of Figure 1C also includes optional amino acids from the DNA vaccine expression vector. The amino acids of the tick aquaporin protein are underlined (amino acids 1 and 3-198 of SEQ ID NO: 3), while the changed/extra amino acids not of tick origin are not underlined (amino acids 2 and 199-226 of SEQ ID NO: 3). The amino acid sequence corresponding solely to amino acids 1-198 of SEQ ID NO: 3 is also presented as SEQ ID NO: 4 (the extra amino acids from the DNA vaccine expression vector are not included).

[0050] It is envisioned that the *R. microplus* aquaporin protein may be synthesized by any suitable method well known to those skilled in the art of peptide synthesis, such as exclusively solid-phase techniques, partial solid-phase techniques, fragment condensation, or classical solution addition. For example, without being limited thereto, suitable solution phase synthesis methods are described by Fien and Hoffman [In "Proteins," Vol. 2, 3rd Ed., H. Neurath and R. L. Hill (eds.), Academic Press, New York, pp. 105-253 (1976)], while solid phase synthesis methods are described by Barany and Merrifield [In "The Peptides," Vol. 2, E. Gross and J. Meienhofer (eds.), Academic Press, New York, pp. 3-284 (1979)], and stepwise solid phase synthesis methods are described by Merrifield [J. Am. Chem. Soc. 85: 2149-2154 (1963)], the contents of each of which are incorporated herein by reference. However, the protein is preferably produced by recombinant DNA techniques which are particularly suitable for large-scale use. Without being limited thereto, nucleotide

sequences encoding the protein which are preferred for use in recombinant DNA techniques are described in detail below. The synthetic protein may be obtained by transforming a microorganism using an expression vector including a promoter or operator, or both, together with the aquaporin structural gene and causing such transformed microorganisms to express the protein.

[0051] The gene encoding the aquaporin protein fragment of *R. microplus* has also been isolated and its cDNA nucleic acid sequences is shown in Figure 2A. The nucleic acid sequence of the translated region of the cDNA encoding the aquaporin protein fragment corresponds to nucleotides 348-947 of SEQ ID NO: 5. Figure 2A also includes an untranslated 5' region (not underlined). As used herein, isolated nucleic acid sequences refer to sequences which have been substantially separated from other nucleic acids or cell components which are normally present in the cells of the tick, such that the aquaporin encoding sequences are the only significant sequences in the sample that can be used to express or produce the protein in a host cell as described below. The term encompasses not only nucleic acid sequences which have been recovered from naturally occurring cells, but also recombinant or cloned nucleic acid sequences, and synthesized nucleic acid sequences. The nucleic acid sequences may be recovered from cells of *R. microplus*, for example, by constructing a genomic DNA or cDNA library and screening for the aquaporin protein nucleic acid using the disclosed sequences as probes. However, in a preferred embodiment, the sequences are synthesized using techniques established in the art for automated DNA synthesis or amplification. As used herein, the nucleic acid sequences of the aquaporin protein encompass either or both of the coding strand or its complement.

[0052] As noted above, the amino acid sequence of the aquaporin protein fragment may be modified to assist cloning in the selected vector, and may further include optional, additional terminal amino acid sequences from the vector (not of tick origin). Thus, the nucleic acid sequence may be modified to reflect these changes. For example, Figure 2B shows the aquaporin nucleotide sequence (SEQ ID NO: 6) as cloned into the *P. pastoris* expression vector pPICZalphaA as described in Guerrero et al. The underlined sequence of Figure 2B (nucleotides 274-870) corresponds to the nucleotide sequence of the coding region of Figure 2A above except that the three 3' terminal nucleotides (nucleotides 945-947 of Figure 2A) have been removed to assist cloning of the gene into the plasmid. The nucleotide sequence shown includes extra, optional vector-provided nucleotides (nucleotides 1-273 and 871-954, not underlined) which code for the extra amino acids not of tick origin (amino acids 1-91 and 291-317 of SEQ ID NO: 2). Figure 2C shows the aquaporin nucleotide sequence (SEQ ID NO: 7) as cloned into the DNA expression vector pcDNA4mycHis C as described in Guerrero et al. The underlined sequence (nucleotides 4-598) corresponds to the nucleotide sequence of the coding region of Figure 2A above except that the second codon triplet (nucleotides 351-353 of Figure 2A) has been changed from AAG to GAG, and the five 3' terminal nucleotides (nucleotides 943-947 of Figure 2A) have been removed, to assist cloning of the gene into the plasmid and enhance translation. Thus, nucleotides 354-942 of SEQ ID NO:5 are common to each of the aquaporin coding sequences of Figures 2A, B and C. The nucleotide sequence shown includes extra, optional DNA vaccine expression vector-provided nucleotides on both of the 3' and 5' ends (1-3 and 599-684, not underlined) of which the 3' terminal nucleotides code for the extra amino acids not of tick origin (amino acids 200-226

of SEQ ID NO: 2). The nucleotide sequence corresponding to nucleotides 4-598 of SEQ ID NO: 7 is also presented as SEQ ID NO: 8 (wherein the extra nucleotides from the DNA vaccine expression vector are not included). In addition, because of the degeneracy of the genetic code, there exists a finite set of nucleotide sequences which can code for a given amino acid sequence. Consequently, nucleic acids may be identical in sequence to the sequence which is naturally occurring or they may include alternative codons which encode the same amino acid as that which is found in the naturally occurring sequence. Furthermore, nucleic acids may include codons which represent conservative substitutions of amino acids as are well known in the art. Moreover, because of the degeneracy of the genetic code, different species can preferentially use different codons to code for the same amino acid and significant differences in tRNA abundance can exist. Translation of recombinant proteins can often be enhanced by optimizing codon usage to the preferred codons used by the expression species. For example, in the yeast *P. pastoris* the amino acid arginine is encoded by the nucleotide triplet of AGA approximately 10 times more frequently than by the nucleotide triplet of CGG. Substitution of CGG triplets with AGA in a *R. microplus* protein coding region used in a recombinant *P. pastoris* expression system would be expected to enhance recombinant protein expression levels. It is understood that all such equivalent sequences are operable variants of the disclosed sequences, since all give rise to the same aquaporin protein (i.e., the same amino acid sequence) during in vivo transcription and translation, and are hence encompassed herein. Without being limited thereto, examples of codon-optimized sequences which would be suitable for enhancing translation of the aquaporin protein fragment in *P. pastoris* are shown in Figure 3. The sequence of Figure 3

(SEQ ID NO: 9) corresponds to the translated or coding region of the nucleotide sequence of Figure 2A (nucleotides 348-942) which has been optimized to enhance translation in *P. pastoris*. DNA sequences which contain significant sequence similarity to the coding regions of the nucleotide sequence of SEQ ID NO: 5 are also encompassed by the invention. As defined herein, two DNA sequences contain significant sequence similarity when at least 85% (preferably at least 90% and most preferably 95%) of the nucleotides match over the defined length of the sequence. Sequences that are significantly similar can be identified in a Southern hybridization experiment under stringent hybridization conditions as is known in the art. See, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual* (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989, or *DNA Cloning: A Practical Approach*, Vol. I and II (Ed. D. N. Glover), IRL Press, Oxford, 1985.

[0053] Any one or combinations of the isolated cDNA nucleic acid sequences encoding the *R. microplus* aquaporin protein may be cloned into any suitable vector for subsequent use as either a DNA vaccine or for the production of recombinant *R. microplus* aquaporin protein. For use as a DNA vaccine, the nucleic acid constructs comprising the nucleic acid sequences encoding the *R. microplus* aquaporin protein are administered to a subject animal such that the protein is expressed *in vivo* within the cells of the vaccinated animal. Similarly, where the object is the production of recombinant protein, the nucleic acid constructs are used for the transformation of a microorganism and causing such transformed microorganism to express the protein *in vitro*.

[0054] A variety of vectors are suitable for use herein, and are selected to be operable as cloning vectors or

expression vectors in the selected host cell, although expression vectors are preferred. Numerous vectors are known to practitioners skilled in the art, and selection of an appropriate vector and host cell is a matter of choice. The vectors may, for example, be bacteriophage, plasmids (including linearized or circular plasmids), viruses or hybrids thereof, such as those described in Sambrook et al. (*ibid*) or Ausubel et al. (Current Protocols in Molecular Biology, John Wiley & Sons, Inc, 1995), the contents of each of which are herein incorporated by reference. Further, the vectors may be non-fusion vectors (i.e., those producing the proteins of the invention not fused to any heterologous polypeptide), or alternatively, fusion vectors (i.e., those producing the proteins fused to a vector encoded polypeptide). The fusion proteins would of course vary with the particular vector chosen. In accordance with a preferred embodiment, and particularly for applications as DNA vaccines, the vectors are eukaryotic expression vectors, most preferably plasmids. Particularly preferred plasmids for use herein include plasmids commercially available from Invitrogen Inc., Carlsbad, CA for both the DNA vaccine and recombinant protein vaccine protocols. The pcDNA 4/myc 5.1 kb vectors are designed for overproduction of recombinant proteins in mammalian cells. This plasmid contains a human cytomegalovirus immediate-early (CMV) promoter for high-level expression, a c-myc epitope and 6XHis metal-binding peptide tag for facilitating protein purification and verification, and a Zeocin antibiotic resistance marker gene coding region for selection purposes. The preferred plasmids used to produce recombinant protein are the pPICZ and pPICZ α from Invitrogen Inc. Both plasmids contain the AOX1 gene promoter for methanol-inducible high-level expression in *Pichia pastoris*, a c-myc epitope and 6XHis metal-binding peptide tag

for facilitating protein purification and verification, and a Zeocin antibiotic resistance marker gene coding region for selection purposes. The pPICZ α also contains a native *Saccharomyces cerevisiae* α -factor secretion signal.

[0055] Regardless of the specific vector utilized, various sites may be selected for insertion of the isolated nucleotide sequences. These sites are usually designated by the restriction enzyme or endonuclease that cuts them.

[0056] The particular site chosen for insertion of the selected nucleotide sequences into the vector to form a recombinant vector is determined by a variety of factors. These include size and structure of the protein to be expressed, susceptibility of the desired protein to enzymatic degradation by the host cell components and contamination by its proteins, expression characteristics such as the location of start and stop codons, and other factors recognized by those skilled in the art. None of these factors alone absolutely controls the choice of insertion site for a particular polypeptide. Rather, the site chosen reflects a balance of these factors, and not all sites may be equally effective for a given protein.

[0057] The nucleotide sequences comprising the *R. microplus* aquaporin protein fragment encoding gene may be inserted into the desired vector by known techniques. If, however, the vector is to serve as an expression vector, the vector should have a promoter effective for expression in the selected host cell, and the DNA sequences should be inserted in the vector downstream of the promoter and operationally associated therewith (that is, the promoter should be recognized by the RNA polymerase of the host cell). In addition, the vector should have a region which codes for a ribosome binding site positioned between the promoter and the site at which the DNA

sequence is inserted so as to be operatively associated with the DNA sequence of the invention once inserted (in correct translational reading frame therewith). The vector should be selected to provide a region which codes for a ribosomal binding site recognized by the ribosomes of the host cell into which the vector is to be inserted. The vector should contain a terminator with necessary 3' untranslated sequences for RNA termination, stability, and/or poly(A) tail addition (if eukaryotic). Alternatively, any or all of the above control sequences may be ligated to the coding sequence prior to insertion into the vector.

[0058] For use in animal vaccinations, the isolated *R. microplus* aquaporin protein fragment or the nucleic acid constructs comprising the nucleic acid sequences encoding this protein, will typically be formulated in conjunction with a suitable pharmaceutically acceptable carrier or diluent as is known in the art, including, but not limited to, physiological saline, mineral oil, vegetable oils, aqueous carboxymethyl cellulose or polyvinylpyrrolidone. The skilled practitioner will recognize that such carriers should of course be compatible with the protein or nucleic acid constructs. Phosphate buffered saline (PBS) is preferred. Because aquaporin may precipitate in the presence of aqueous buffers such as PBS, in a preferred embodiment the aquaporin is stored in a non-polar solvent or oil. A variety of non-polar solvents are suitable for use herein, although a preferred solvent is composed of 50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl, 2 mM beta-mercaptoethanol, 0.4% w/v OTG (octyl-beta-D-1-thioglucoopyranoside), 300 mM imidazole, and 50% glycerol. The concentration and amount of the protein or nucleic acid constructs in the final composition may vary depending upon the desired use and type of response needed, and the host animal. In any event, the protein or nucleic acid constructs

should be provided in an amount effective to induce the preferred response as determined by routine testing. Appropriate adjuvants as known in the art may also be included in the formulation. As described herein, adjuvants include agents (a compound or combination of compounds) capable of enhancing either or both of a humoral (antibody) immunity response or a cell-mediated immunity response in the treated animal against the target tick. Without being limited thereto, suitable adjuvants include but are not limited to one or more of mineral oil, vegetable oils, aluminum salts such as alum, water-in-oil adjuvants such as Freund's incomplete adjuvant, oil-in-water emulsions such as MF59 (Novartis, Switzerland), liposomes, virosomes, microparticles or nanoparticles or beads of biocompatible matrix materials such as (although not limited to) agar or polyacrylate, saponin-based adjuvants (such as QA-21 or QS-21 marketed by Antigenics, Lexington, MA), toll-like receptor (TLR) agonists such as 3-O-desacyl-4'-monophosphoryl lipid A (MPL) and immunostimulatory sequences (ISS) of microbial DNA, imidazoquinolines, immune stimulating complexes (ISCOMs and ISCOMATRIXs), and other agents such as described by Leroux-Roels (2010. Vaccine. 285:C25-C36, the contents of which are incorporated by reference herein). In accordance with an optional embodiment, other known immunogenic agents used in conventional vaccines for the animal of interest may also be included in the formulation. For example, additional immunogenic agents may be an attenuated or inactivated form of a pathogen, or subunits thereof. Without being limited thereto, these pathogens include, for example, one or more of the rabies virus, *Borrelia burgdorferi*, canine distemper virus, canine parvovirus, canine adenovirus, canine corona virus, canine herpesvirus, *Giardia* spp., *Leptospira*

interrogans, *Babesia canis*, *Hepatozoon canis*, *Dipylidium caninum* and *Isospora* spp.

[0059] The immunogenic *R. microplus* aquaporin protein fragment or the nucleic acid constructs comprising the nucleic acid sequences encoding this protein are administered in an amount effective to reduce or eliminate the incidence of infestation of the treated animal, with the specific target tick, particularly but not limited to the brown dog tick, *R. sanguineus*. As noted hereinabove, the administration of the *R. microplus* aquaporin protein, or the nucleic acid constructs comprising the nucleic acid sequences encoding this protein (such that the nucleic acid sequences are expressed and the encoded protein is produced *in vivo* in the cells of the vaccinated animal), stimulates an immune response in the animal. Thus, as used herein, an "effective amount" of *R. microplus* aquaporin protein or the nucleic acid constructs comprising the nucleic acid sequences encoding this protein, is preferably defined as that amount which will elicit a protective immune response against the target tick, which may be either or both of antibody production against the protein or a cell-mediated immune response against the tick, in a treated animal in comparison to an untreated control animal. In a preferred embodiment, an immune response may be demonstrated by production of antibodies against the *R. microplus* aquaporin protein, by a significant reduction in the percentage of animals infested with the target tick, by a significant reduction in the average number of target ticks on animals, or by a significant reduction in the number of viable eggs produced by the target ticks present on animals, all in vaccinated animals as compared to an unvaccinated control group (measured at a confidence level of at least 80%, preferably measured at a confidence level of 95%). The actual effective amount will of course vary with the specific vaccine

component (protein vaccine or DNA vaccine), the particular animal of interest and its age and size, and the route of administration, and may be readily determined empirically by the practitioner skilled in the art using an antigen dose response assay. By way of example and without being limited thereto, for vaccines administered to small animals (such as dogs and cats) by subcutaneous or intramuscular injection, or with a needle-less device, it is envisioned that typical doses of protein vaccine (*R. microplus* aquaporin protein), may be greater than 0.3 µg protein/animal/dose, preferably between about 0.3 to 1.75 µg protein/animal/dose, while typical doses of DNA vaccine (nucleic acid constructs) may be greater than 100 µg of DNA construct/animal/dose, preferably between about 300 to 800 µg DNA construct/animal/dose. For vaccines administered to large animals (such as deer and horses) by subcutaneous or intramuscular injection, or with a needle-less device, it is envisioned that typical doses of protein vaccine (*R. microplus* aquaporin protein), may be greater than 10 µg protein/animal/dose, preferably between about 50 to 150 µg protein/animal/dose, while typical doses of DNA vaccine (nucleic acid constructs) may be greater than 100 µg of DNA construct/animal/dose, preferably between about 300 to 800 µg DNA construct/animal/dose.

[0060] The vaccines (*R. microplus* aquaporin protein or the nucleic acid constructs comprising the nucleic acid sequences encoding this protein) may be used for the treatment of a broad spectrum of wild or domesticated animals, ranging from pets and companion animals to livestock and large domestic or wild animals. Without being limited thereto, the vaccines are preferably used for the treatment of Cervidae, equine, canines and felines, and particularly deer (including white-tailed deer), horses, domestic dogs and cats. The vaccines may be effectively administered any time after the animal attains

immunocompetence. The vaccines may be administered to the subject animal by any convenient route which enables an immune response. However, parenteral injection (e.g., subcutaneous, intravenous, or intramuscular) is preferred, with intradermal injection being particularly preferred for administration of the DNA vaccines and intramuscular injection being particularly preferred for administration of the protein vaccines. The vaccine products could also be administered using a needle-less device. The vaccine may be administered in a single dose or in a plurality of doses. Dependent upon rearing conditions, the vaccine may be administered in multiple doses, the timing of which may be readily determined by the skilled artisan.

[0061] Where the nucleic acid constructs are to be employed for the production of recombinant *R. microplus* aquaporin protein, a variety of vector-host cell expression systems may be employed. Strains of yeast, particularly *Pichia pastoris*, are preferred. However, the novel invention described here can be applied with numerous host cells that would be desirable. Host strains may be of bacterial, fungal, insect cell line, plant, or yeast origin. Ascertaining the most appropriate host-vector system is within the skill of the person in the art.

[0062] DNA constructs may be introduced into the appropriate host cell by numerous methods described in the technical and scientific literature. Transformation of bacteria or yeast may be performed using standard techniques described in Sambrook et al., (*ibid*). Techniques for transforming filamentous fungi may include those described by Goosen et al. [Handbook for Applied Mycology, Arora, Elander & Mukerji, eds. (1992) pp. 151-195] and May et al. [Applied Molecular Genetics of Filamentous Fungi, Kinghorn and Turner,

eds. (1992) pp. 1-27]. In general, linear or circular DNA constructs may be introduced into the host cell by techniques utilizing protoplast fusion, polyethylene glycol, liposomes, lithium acetate, electroporation, physical damage, biolistic bombardment, or *Agrobacterium* mediated transformation.

[0063] Successful transformants may be isolated by using markers, contained on the expression vectors, which confer a selectable trait to the transformed host cell. These may include nutritional selection related to substrate utilization (such as, growth on acetamide containing medium) or prototrophy of a required growth product (such as, arginine, leucine, or uracil). Dominant selectable markers [such as, resistance to ampicillin, G418, hygromycin, and phleomycin, and Zeocin (a composition of bleomycin and phleomycin, Invitrogen, Grand Island, NY)] are also useful in selecting transformants that have taken up the introduced DNA construct.

[0064] The DNA construct may be replicated autonomously or integrated into the genome of the host cell. Integration typically occurs by homologous recombination (for example, arginine selectable marker integrating in the chromosomal arginine gene) or at a chromosomal site unrelated to any genes on the DNA construct. Integration may occur by either a single or double cross-over event. It is also possible to have any number of these integration and replication types occurring in the same transformant.

[0065] The following example is intended only to further illustrate the invention and is not intended to limit the scope of the invention which is defined by the claims.

Example 1

[0066] Recombinant aquaporin protein fragment of *R. microplus* was prepared as described in Example 1 of Guerrero et al. (U.S. patent application serial no. 13/479,486, referred to above). Prior to use, the recombinant aquaporin protein was stored in a phosphate buffered solution composed of 50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl, 2 mM beta-mercaptoethanol, 0.4% w/v CTG (octyl-beta-D-1-thioglucopyranoside), 300 mM imidazole, and 50% glycerol. Recombinant Bm86Texas antigenic protein of *R. (Boophilus) microplus* was obtained from cloning of the protein antigen Bm86 from a Texas outbreak strain of *R. microplus* known as Deutsch. Recombinant aquaporin protein and Bm86Texas antigens were each adjuvated with Montanide ISA 61 VG (water-in-oil adjuvant, Seppic, Paris) into doses of 2 ml containing 100 ug of the recombinant protein.

[0067] Three groups of tick-naïve white-tailed deer (n=4 per group) received 1.0 mL of either the recombinant aquaporin protein vaccine, Bm86 vaccine, or a control vaccine at week 1. Deer were housed at the LSU Idelwild Research Station in Clinton, La. Each deer was vaccinated on day 1, and blood was collected from each animal at the intervals shown in Table 1. The blood from the Week 3 collections from the control, aquaporin and Bm86Texas groups was used to prepare serum and feed to ticks in the following in vitro study. Individual blood samples within treatment groups were pooled in the tick feeding trials.

[0068] Four groups of *R. sanguineus* ticks (n=10 nymphs per group) were partially-fed on rats for 2 days prior to the treatment feeding with blood from the blood samples collected from the deer. Experimental groups were fed from 50 µL glass microcapillary tubes containing the pooled blood treatments. The control tick group only fed from the rats. Nymphs were

subsequently fed to repletion on rats. Nymphs were placed in environmental chambers at 26°C at 92% humidity and monitored for molting success up to 21 days post feeding to repletion. Tick groups were less than n=10 at the time of chamber placement. Deaths due to handling techniques were experienced in each group and additional deaths also occurred within 48 hours of feeding the vaccinated blood. Groups were decreased to the following sizes: aquaporin (n=6), Bm86 (N=7), vaccinated control (n=9), and tick control (n=9).

[0069] The data for tick molting success was analyzed using an exact probability chi-square test; there were significant differences in the molting success of the four groups ($p=0.0255$) with the majority of the significance associated with the ARS-1 group having more dead than expected. The results are shown in Figure 4. Only 50% of the ticks fed with blood of deer vaccinated with aquaporin protein survived the molting process. This is compared to the Bm86 and blood control vaccinated groups which both had a molting/survival rate of 100%. The tick control group had a 90% molting/survival rate (Figure 4).

[0070] Blood from the deer was also sampled at the intervals shown in Table 1 and separated serum was collected for analysis by ELISA to determine antibody production in vaccinated deer to the aquaporin protein or the Bm86 antigens. For the ELISA, sera from each group were pooled according to the day of collection. Microtiter plates were coated with the aquaporin protein or the Bm86 antigen (50 μ L per well of a 1 μ g antigen/ ml solution in 20 mM sodium carbonate buffer, pH 9.6) and incubated overnight at 4°C. Blocking with 2% bovine serum albumin in PBS-T was followed by washing five times with PBS pH 7.4. The plates were incubated for 45 min at 37°C with 100 μ L per well of immunized bovine serum diluted to 1:100 in

PBS. After washing, 50 μ l of rabbit anti-bovine IgG peroxidase conjugate (Sigma, St. Louis, MO) diluted to 1:20,000 was added and the plate incubated for 30 min at room temperature. After incubation and washing 50 μ l of chromogenic substrate o-phenylenediamine (1.0 mM) was added and the reaction was stopped after 15 min by adding 50 μ l of NaOH (0.2 M). A microplate reader was used to assess the results with absorbance set at 490 nm. The results are shown in Table 1 and demonstrate antibody production specific to the antigens.

[0071] It is understood that the foregoing detailed description is given merely by way of illustration and that modifications and variations may be made therein without departing from the spirit and scope of the invention.

Table 1

Deer ID	PreVaccine	Week 3	Week 6	Week 10	Week 14	Week 18	Week 22
Unvaccinated Control Group Vaccinated on day 1, Week 3 and Week 6 with only adjuvant							
W-19	<50	<50	<50	<50	<50	<50	<50
55	50	<50	<50	<50	died	died	died
57	<50	<50	<50	<50	<50	100	<50
59	<50	<50	<50	<50	<50	<50	<50
Aquaporin Group Vaccinated on day 1, Week 3 and Week 6 with Aquaporin plus adjuvant.							
75	50	800	6400	3200	400	800	200
76	100	800	>6400	>6400	6400	6400	3200
78	<50	6400	1600	3200	400	1600	400
85	<50	800	6400	6400	1600	3200	3200
Bm86Texas antigen Group Vaccinated on day 1, Week 3 and Week 6 with Bm86Texas plus adjuvant.							
88	<50	6400	800	3200	1600	3200	1600
90	<50	200	3200	6400	1600	1200	400
91	<50	1600	1600	6400	1600	1200	400
W-94	<50	800	400	1600	100	200	200

We claim:

1. A method of reducing tick infestations in animals comprising administration of a vaccine composition to a non-bovine animal, wherein said vaccine composition comprises an immunogenic aquaporin protein of *Rhipicephalus microplus* and a pharmaceutically acceptable carrier, and wherein said aquaporin protein is in an amount effective to stimulate an immune response in said animal to said tick.

2. The method of claim 1 wherein said aquaporin protein comprises amino acids 3-198 of SEQ ID NO:1.

3. The method of claim 1 wherein said aquaporin protein comprises an amino acid sequence selected from the group consisting of amino acids 1-199 of SEQ ID NO: 1 and SEQ ID NO: 4.

4. The method of claim 1 wherein said animal is selected from the group consisting of canines, felines, equine and Cervidae.

5. The method of claim 4 wherein said animal is selected from the group consisting of domestic cats, domestic dogs and deer.

6. The method of claim 5 wherein said animal is a domestic dog.

7. The method of claim 1 wherein said tick is the brown dog tick, *Rhipicephalus sanguineus*.

8. The method of claim 5 wherein said tick is the brown dog tick, *Rhipicephalus sanguineus*.

9. The method of claim 6 wherein said tick is the brown dog tick, *Rhipicephalus sanguineus*.

10. The method of claim 1 wherein said vaccine composition further comprises an adjuvant.

11. A method of reducing the incidence of tick infestations in animals, comprising administering a vaccine composition to a non-bovine animal, wherein said vaccine composition comprises the nucleic acid construct and a pharmaceutically acceptable carrier, wherein said nucleic acid construct comprises a nucleic acid sequence encoding an aquaporin protein of *Rhipicephalus microplus*, operatively linked to one or more expression control sequences, and is administered in an amount effective to stimulate an immune response in said animal to said tick.

12. The method of claim 11 wherein said nucleic acid sequence encodes said aquaporin protein comprising amino acids 3-198 of SEQ ID NO:1.

13. The method of claim 12 wherein said nucleic acid sequence encodes said aquaporin protein comprising an amino acid sequence selected from the group consisting of amino acids 1-199 of SEQ ID NO: 1 and SEQ ID NO: 4.

14. The method of claim 11 wherein said animal is selected from the group consisting of canines, felines, equine and Cervidae.

15. The method of claim 14 wherein said animal is selected from the group consisting of domestic cats, domestic dogs and deer.

16. The method of claim 15 wherein said animal is a domestic dog.

17. The method of claim 11 wherein said tick is the brown dog tick, *Rhipicephalus sanguineus*.

18. The method of claim 15 wherein said tick is the brown dog tick, *Rhipicephalus sanguineus*.

19. The method of claim 16 wherein said tick is the brown dog tick, *Rhipicephalus sanguineus*.

20. The method of claim 11 wherein said vaccine composition further comprises an adjuvant.

Figure 1A

MKIENLLIRQLINEFLGTMILITIGDSIMAIHAGDNESLAACVGPLGWGVAIYVAVQISGGVS
SHLNPAVTLAQASVRKFPIAKVPLYFAAQYLGGFVGAALVFATYKDAIEHFDQGIRQVTGE
KATAGIFATYPRPHVSTLTCTFIDQVIATGIMMVCVEAIGDTRNFGGIPPHIHPICLGLMIMAI
IFSFAYNMCPL

Figure 1B

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPFSNSTNN
GLLFINTTIAAIAAKEEGVSLEKREAEAEFMKIENLLIRQLINEFLGTMILITIGDSIMAIHAG
DNESLAACVGPLGWGVAIYVAVOISGGVSSHLNPAVTLAQASVRKFPIAKVPLYFAAOYLG
GFVGAALVFATYKDAIEHFDQGIRQVTGEKATAGIFATYPRPHVSTLTCTFIDQVIATGIMM
VCVEAIGDTRNFGGIPPHIHPICLGLMIMAIIFSFAYNMCPAAASFLEQKLISEEDLNSAVD
HHHHHH*

Figure 1C

MEIENLLIRQLINEFLGTMILITIGDSIMAIHAGDNESLAACVGPLGWGVAIYVAVOISGGVS
SHLNPAVTLAQASVRKFPIAKVPLYFAAQYLGGFVGAALVFATYKDAIEHFDQGIRQVTGE
KATAGIFATYPRPHVSTLTCTFIDQVIATGIMMVCVEAIGDTRNFGGIPPHIHPICLGLMIMAI
IFSFAYNMCMLESRGPFQKLISEEDLNMHTGHHHHHH*

Figure 2A

AAGCAGTGGTATCAACGCAGAGTACGCGGGGGGGCTGGGAAAAGCTGCTAGCATCAACT
CGGCTTCTAGCTTGGGGTCTCGCACCGCGCCTCGAGCCCGACCAGCCTGCGGTGGCGCCGT
CTCGCTGAAAGGGGGAAAGAGGAAAGAGAAAGAAGAAAAGAAAATATCGCCGGCATCG
GCGACGAAGGCGGAGCAGCAATGCGATCGTCAGAGCACGCATTTGACGGTGAGATTGCG
AAGCTCGAAGGCGTCGCCGGCACTGCGAGAAAGCCGGTGAAGTACTTTGGGACCGCCGCG
TAGGCGTCTTGACAGTCCGCTCCCGAGGCAACGACGACACGCTCCAAGATGAAGATCGAG
AACCTGCTCATACGGCAGCTCATCAACGAGTTCCTCGGAACAATGATTCTAATTACTATC
GGCGACTCCATCATGGCCATCATCATCGCCGGTGACAACGAGTCTCTGGCTGCTTGCGTG
GGGCCCTTGGGATGGGGCGTCGCCATCTACGTGGCCGTGCAAATCTCCGGAGGAGTCTCG
TCCCACCTGAATCCTGCGGTGACGCTGGCCCAGGCGTCCGTGCGCAAGTTTCCGATCGCCA
AAGTGCCGCTATACTTCGCGGCTCAGTACCTGGGTGGCTTCGTGCGTGCGGCGCTCGTGT
TTGCCACCTACAAAGACGCTATTGAACACTTCGACCAGGGAATCCGCCAAGTGACGGGAG
AGAAGGCCACGGCTGGTATATTTGCAACTTACCCAGACCACAGTCTCCACTCTGACCT
GCTTCATTGATCAGGTCATCGCAACGGGCATAATGATGGTGTGCGTCGAGGCCATCGGCG
ACACTCGCAACTTCGGCGGCATTCCGCCGCACATTACCCCAATCTGCTTGGGTCTCATGA
TCATGGCTATTATCTTCAGTTTCGCCTACAACTGCATGTGCCCGCTC

Figure 2B

ATGAGATTTCTTCAATTTTTACTGCTGTTTTATTCGCAGCATCCTCCGCATTAGCTGCT
CCAGTCAACACTACAACAGAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTCATCGGT
TACTCAGATTTAGAAGGGGATTTGATGTTGCTGTTTTGCCATTTTCCAACAGCACAAAT
AACGGGTATTGTTTATAAATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGT
ATCTCTCGAGAAAAGAGAGGCTGAAGCTGAATTCATGAAGATCGAGAACCTGCTCATAC
GGCAGCTCATCAACGAGTTCCTCGGAACAATGATTCTAATTACTATCGGCGACTCCATCA
TGGCCATCATCATCGCCGGTGACAACGAGTCTCTGGCTGCTTGCGTGCGGGCCCTTGGGAT
GGGGCGTCGCCATCTACGTGGCCGTGCAAATCTCCGGAGGAGTCTCGTCCCACCTGAATC
CTGCCGTGACGCTGGCCCAGGCGTCCGTGCGCAAGTTTCCGATCGCCAAAGTGCCGCTAT
ACTTCGCGGCTCAGTACCTGGGTGGCTTCGTGCGTGCGGCGCTCGTGTTCGCCACCTACA
AAGACGCTATTGAACACTTCGACCAGGGAATCCGCCAAGTGACGGGAGAGAAGGCCACG
GCTGGTATATTTGCAACTTACCCAGACCACAGTCTCCACTCTGACCTGCTTCATTGAT
CAGGTCATCGCAACGGGCATAATGATGGTGTGCGTCGAGGCCATCGGCGACACTCGCAAC
TTCCGGCGGCATTCCGCCGCACATTCACCCAATCTGCTTGGGTCTCATGATCATGGCTATT
ATCTTCAGTTTCGCCTACAACTGCATGTGCCCGGCGGCGCCAGCTTTCTAGAACAAAAA
CTCATCTCAGAAGAGGATCTGAATAGCGCCGTCGACCATCATCATCATCATCATTGA

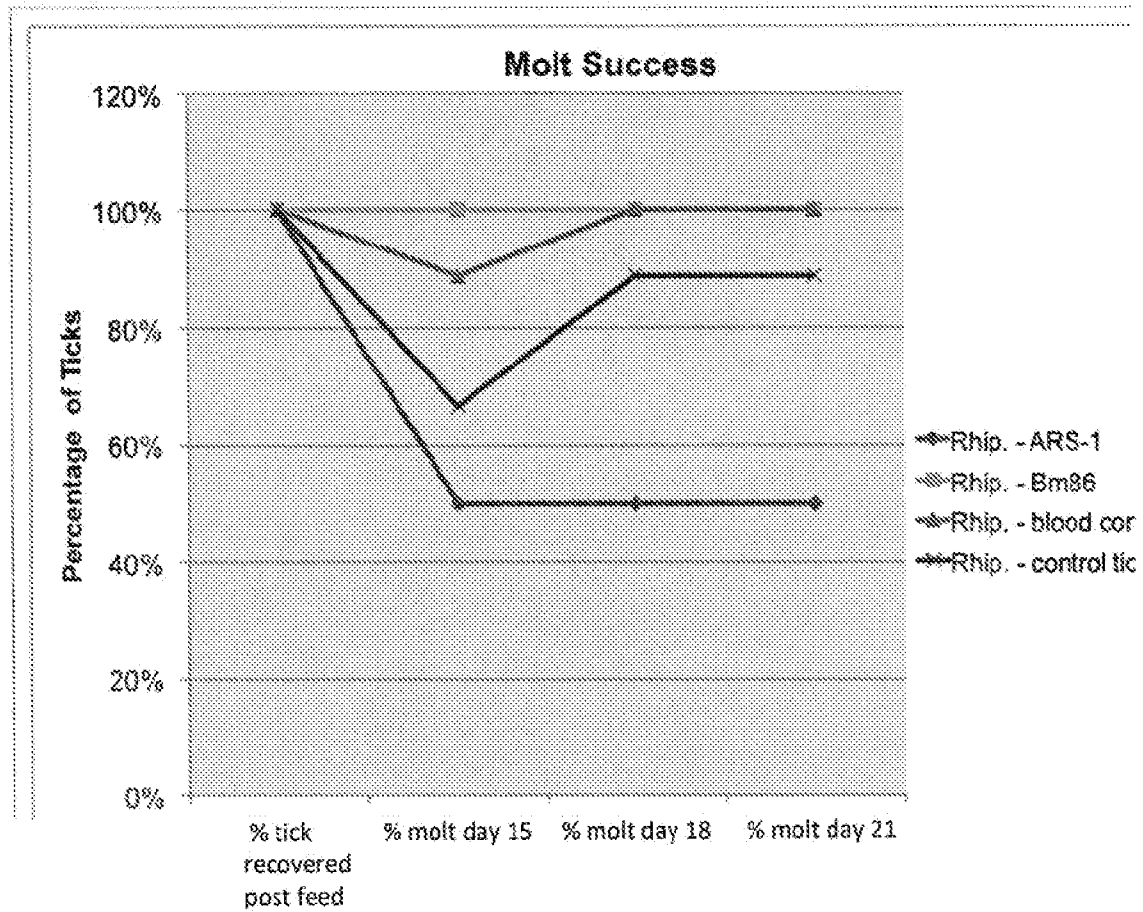
Figure 2C

AAGATGGAGATCGAGAACCTGCTCATACGGCAGCTCATCAACGAGTTCCTCGGAACAATG
ATTCTAATTACTATCGGCGACTCCATCATGGCCATCATCATCGCCGGTGACAACGAGTCT
CTGGCTGCTTGCGTGGGGCCCTTGGGATGGGGCGTCGCCATCTACGTGGCCGTGCAAATC
TCCGGAGGAGTCTCGTCCCACCTGAATCCTGCCGTGACGCTGGCCCAGGCGTCCGTGCGC
AAGTTTCCGATCGCCAAAGTGCCGCTATACTTCGCCGGCTCAGTACCTGGGTGGCTTCGTC
GGTGCGGCGCTCGTGTTTGCCACCTACAAAGACGCTATTGAACACTTCGACCAGGGAATC
CGCCAAGTGACGGGAGAGAAGGCCACGGCTGGTATATTTGCAACTTACCCCAGACCACAC
GTCTCCACTCTGACCTGCTTCATTGATCAGGTCATCGCAACGGGCATAATGATGGTGTGC
GTCGAGGCCATCGGCGACACTCGCAACTTCGGCGGCATTCCGCCGCACATTCACCCAATC
TGCTTGGGTCTCATGATCATGGCTATTATCTTCAGTTTCGCCTACAACTGCATGTGCCTC
GAGTCTAGAGGGCCCTTCGAACAAAACTCATCTCAGAAGAGGATCTGAATATGCATAC
CGGTCATCATCACCATCACCATTGA

Figure 3

ATGAAGATTGAGAACTTGTTGATTAGACAATTGATTAACGAGTTCTTGGGTACTATGAT
TTTGATTACTATTGGTGACTCTATTATGGCTATTATTATTGCTGGTGACAACGAGTCTTT
GGCTGCTTGCGTTGGTCCATTGGGTTGGGGTGTTGCTATTTACGTTGCTGTTCAAATTC
TGGTGGTGTTTCTTCTCACTTGAATCCAGCTGTTACTTTGGCTCAAGCTTCTGTTAGAAA
GTTTCCAATTGCTAAAGTTCCATTGTACTTCGCTGCTCAATACTTGGGTGGTTTCGTTGG
TGCTGCTTTGGTTTTTGCTACTTACAAAGACGCTATTGAACACTTCGACCAAGGTATTAG
ACAAGTTACTGGTGAGAAGGCTACTGCTGGTATTTTTGCTACTTACCCAAGACCACACGT
TTCTACTTTGACTTGCTTCATTGATCAAGTTATTGCTACTGGTATTATGATGGTTTGCGT
TGAGGCTATTGGTGACACTAGAACTTCGGTGGTATTCCACCACACATTCACCCAATTTG
CTTGGGTTTGATGATTATGGCTATTATTTCTCTTCGCTTACAACTGCATGTGCC

Figure 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/021725**A. CLASSIFICATION OF SUBJECT MATTER****A61K 39/12(2006.01)i, A61K 39/02(2006.01)i, A61P 37/00(2006.01)i**

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 39/12; A61K 39/395; A61K 039/00; A61K 39/00; C07K 16/18; C07K 014/435; A61B 5/00; A61K 39/02; A61P 37/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & keywords: vaccine, tick, aquaporin protein, Rhipicephalus microplus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BALL, A. et al., `Identification, functional characterization and expression patterns of a water-specific aquaporin in the brown dog tick, Rhipicephalus sanguineus`, Insect Biochemistry and Molecular Biology, 2009, Vol. 39, pages 105-112 See abstract.	1-20
A	SEIXAS, A. et al., `Rhipicephalus (Boophilus) microplus embryo proteins as target for tick vaccine`, Veterinary Immunology and Immunopathology, 2012, Vol. 148, pages 149-156 See abstract.	1-20
A	US 2004-0052807 A1 (SALCEDO, J. H. P. et al.) 18 March 2004 See abstract; and claims 1-3.	1-20
A	US 2010-0278752 A1 (KOTSYFAKIS, M. et al.) 4 November 2010 See abstract; and claims 72-80.	1-20
A	US 2007-0031411 A1 (TRIMNELL, A. R. et al.) 8 February 2007 See abstract; and claims 1-7.	1-20



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

26 June 2014 (26.06.2014)

Date of mailing of the international search report

26 June 2014 (26.06.2014)

Name and mailing address of the ISA/KR

International Application Division
Korean Intellectual Property Office
189 Cheongsu-ro, Seo-gu, Daejeon Metropolitan City, 302-701,
Republic of Korea

Facsimile No. +82-42-472-7140

Authorized officer

CHOI, Sung Hee

Telephone No. +82-42-481-8740



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2014/021725

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004-0052807 A1	18/03/2004	AT 496632 T AU 2001-54538 A1 AU 2001-54538 B2 AU 779537 B2 BR 0001717 A BR 0001717 B1 DE 60143940 D1 EP 1289545 A1 EP 1289545 B1 ES 2360430 T3 MX PA02010851 A PT 1289545 E US 2005-0281836 A1 US 8110202 B2 WO 01-82957 A1	15/02/2011 12/11/2001 27/01/2005 27/01/2005 19/03/2002 08/10/2013 10/03/2011 12/03/2003 26/01/2011 03/06/2011 20/05/2004 03/05/2011 22/12/2005 07/02/2012 08/11/2001
US 2010-0278752 A1	04/11/2010	WO 2009-017689 A2 WO 2009-017689 A3	05/02/2009 03/09/2009
US 2007-0031411 A1	08/02/2007	AU 2001-50512 A1 AU 2001-50512 B2 AU 781622 B2 BR 0110278 A CA 2405537 A1 CA 2405537 C CN 100558399 C CN 101406696 A CN 1438896 A EP 1283716 A1 EP 1283716 B1 JP 04925236 B2 JP 2003-531177 A MX PA02010417 A NZ 522301 A US 2003-0170257 A1 WO 01-80881 A1	07/11/2001 02/06/2005 02/06/2005 07/01/2003 01/11/2001 26/06/2012 11/11/2009 15/04/2009 27/08/2003 19/02/2003 28/03/2012 25/04/2012 21/10/2003 25/04/2003 26/11/2004 11/09/2003 01/11/2001