

SUMMARY OF THE INVENTION

[0003] The present invention, in some aspects, overcomes limitations in the prior art by providing new compositions and methods that may be used to generate an immune response against *Ehrlichia canis*. In some examples, the composition contains at least two *E. canis* proteins or immunogenic peptides and an adjuvant, as described herein. Related methods for generating an immune response against *E. canis* are also provided. In some examples, an immune response can be induced in a mammalian subject (*e.g.*, a dog) by administering to the subject: (i) an *E. canis* bacterin, (ii) an adjuvant (*e.g.*, comprising Quil A, cholesterol, and an immunostimulatory oligonucleotide), and (iii) one or more TRP proteins or peptides, and these components may be administered in a single pharmaceutical composition (comprising the bacterin, the adjuvant, and the one or more TRP protein or peptides) or in multiple pharmaceutical compositions. For example, the subject may be administered both a first pharmaceutical composition (comprising the TRP protein(s) and the adjuvant), and a second pharmaceutical composition (comprising the bacterin and the adjuvant), wherein the first pharmaceutical composition and the second pharmaceutical composition are administered at substantially the same time or at different times.

[0004] In some examples, the present disclosure refers to an immunogenic composition comprising: (i) at least 1, 2, 3, 4, 5, 6, or all of TRP153, TRP36, TRP140, TRP28, TRP95, TRP 19, and/or TRP120 (*e.g.*, TRP140, TRP36, and/or TRP19); and at least 1, 2, 3, 4, 5, or more peptides comprising or consisting of a peptide of any one of SEQ ID NOs:1-16; (ii) at least 2, 3, 4, 5, 6, or all of TRP153, TRP36, TRP140, TRP28, TRP95, TRP19 and/or TRP120 (*e.g.*, TRP140, TRP36, and/or TRP19); (iii) an *E. canis* bacterin and at least 1, 2, 3, 4, 5, 6, or all of TRP153, TRP36, TRP140, TRP28, TRP95, TRP19, or TRP120 (*e.g.*, TRP140, TRP36, and/or TRP19); (iv) an *E. canis* bacterin and at least 1, 2, 3, 4, 5, or more peptides comprising or consisting of a peptide of any one of SEQ ID NOs:1-16; or (v) an *E. canis* bacterin, at least 1, 2, 3, 4, 5, 6, or all of TRP153, TRP36, TRP140, TRP28, TRP95, TRP19, TRP120 (*e.g.*, TRP140, TRP36, and/or TRP19) and at least 1, 2, 3, 4, 5, or more peptides comprising or consisting of a peptide of any one of SEQ ID NOs:1-16; and a pharmaceutically acceptable excipient. Optionally, the immunogenic composition may comprise 1, 2, 3, all of Ank200, Ank153, OMP-1 and/or P30/28. In some examples, the immunogenic composition does not include TRP120. In some examples, the pharmaceutically acceptable excipient comprises or consists of an adjuvant. In some examples, the adjuvant comprises a

triterpenoid saponin (*e.g.*, Quil A), a sterol (*e.g.*, cholesterol), and an immunostimulatory oligonucleotide (*e.g.*, a CpG-containing ODN). In some examples, the triterpenoid saponin is Quil A, the sterol is cholesterol, and the immunostimulatory oligonucleotide is a CpG-containing ODN. The CpG-containing ODN may be 5' JU*C-G*T*C*G*A*C*G*A*T*C*G*G*C*G*G*C*C*G*C*C* G*T 3' (SEQ ID NO:17), wherein “*” refers to a phosphorothioate bond, “-” refers to a phosphodiester bond, and “JU” refers to 5'-Iodo-2'-deoxyuridine. The composition may comprise at least one, at least two, or all of TRP140, TRP36, and/or TRP19. The composition may comprise a chimeric protein comprising 1, 2, or all of TRP140, TRP36, and/or TRP19. The composition may comprise a polypeptide comprising 1, 2, or all of SEQ ID NO:1, SEQ ID NO:3, and/or SEQ ID NO:14. The adjuvant may be an emulsion or liposomes, or the adjuvant may comprise a lipid. In some examples, the emulsion is an oil-in-water (O/W) emulsion or a water-in-oil (W/O) emulsion. The adjuvant may comprise a triterpenoid, a sterol, an immunomodulator, a polymer, and/or an immunostimulatory oligonucleotide. In some examples, the polymer is diethyl-aminoethyl (DEAE)-dextran, polyethylene glycol, or polyacrylic acid. The immunostimulatory oligonucleotide may be a CpG containing ODN. In some examples, the adjuvant comprises DEAE Dextran, an immunostimulatory oligonucleotide, and oil such as mineral oil, wherein the immunostimulatory oligonucleotide is a CpG containing ODN, and wherein the adjuvant formulation is a water-in-oil (W/O) emulsion. The adjuvant may comprise a saponin, a sterol, a quaternary ammonium compound, a polymer, and an ORN/ODN. In some examples, the saponin is Quil A or a purified fraction thereof, the sterol is cholesterol, the quaternary ammonium compound is dimethyl dioctadecyl ammonium bromide (DDA), the polymer is polyacrylic acid, and the ORN/ODN is a CpG-containing oligonucleotide. The saponin may be present in an amount of about 1 µg to about 5,000 µg per dose, the sterol may be present in an amount of about 1 µg to about 5,000 µg per dose, the quaternary ammonium compound may be present in an amount of about 1 µg to about 5,000 µg per dose, and the polymer may be present in an amount of about 0.0001% v/v to about 75% v/v. The adjuvant may further comprise a glycolipid such as, *e.g.*, N-(2-deoxy-2-L-leucylamino-β-D-glucopyranosyl)-N-octadecyldodecanamide acetate. In some examples, the adjuvant comprises a triterpenoid saponin, a sterol, a quaternary ammonium compound, and a polyacrylic acid polymer. In some examples, the saponin is Quil A or a purified fraction thereof, the sterol is cholesterol, and the quaternary ammonium compound is dimethyl dioctadecyl ammonium bromide (DDA). In some examples, wherein the saponin is present in an amount of about 1 mg to about 5,000 mg per dose, the sterol is present in an amount of

about 1 mg to about 5,000 mg per dose, the quaternary ammonium compound is present in an amount of about 1 mg to about 5,000 mg per dose, and the polyacrylic acid polymer is present in an amount of about 0.0001% v/v to about 75% v/v. In some examples, the adjuvant comprises or is a water-in-oil emulsion. The water-in-oil emulsion may comprise an oily phase and an aqueous phase, a polycationic carrier (*e.g.*, DEAE dextran), and a CpG-containing immunostimulatory oligonucleotide. The composition may further comprise an aluminum hydroxide gel. In some examples, the polycationic carrier is DEAE dextran. The composition may comprise or be an emulsion or an oil-in-water (O/W) emulsion. In some examples, the emulsion comprises an aqueous phase that comprises an alkyl-polyacrylic acid (alkyl-PAA) or both an acrylic polymer and dimethyl dioctadecyl ammonium bromide (DDA). In some examples, the aqueous phase of the oil-in-water emulsion comprises dimethyl dioctadecyl ammonium bromide (DDA) and an alkyl-polyacrylic acid (alkyl-PAA). In some embodiments, the alkyl-PAA is decyl-PAA, octyl-PAA, butyl-PAA, or methyl-PA. In some examples, the acrylic polymer is a polymer of acrylic acid crosslinked with polyallyl sucrose. The composition may comprise or be a water-in-oil (W/O) emulsion comprising a non-mineral oil and a emulsifier. In some examples, the emulsifier is a mannide mono-oleate emulsifier. In some examples, the adjuvant is MF59, AS01, AS02, AS03, AS04, Virosomes, CAF01, CAF04, CAF05, an acrylic polymer / DDA emulsion, a CpG/DEAE emulsion, a saponin/ cholesterol/ DDA adjuvant, or a polyacrylic acid polymer emulsion.

[0005] In some examples, the immunogenic composition comprises at least 2, 3, 4, 5, or all of TRP153, TRP36, TRP140, TRP28, TRP95, and TRP19. In some examples, the composition comprises (TRP153 and TRP36), (TRP153 and TRP140), (TRP153 and TRP28), (TRP153 and TRP95), (TRP36 and TRP140), (TRP36 and TRP28), (TRP36 and TRP95), (TRP140 and TRP28), (TRP140 and TRP95), (TRP28 and TRP95), (TRP19 and TRP153), (TRP19 and TRP36), (TRP19 and TRP140), (TRP19 and TRP28), (TRP19 and TRP95), (TRP120 and TRP153), (TRP120 and TRP36), (TRP120 and TRP140), (TRP120 and TRP28), (TRP120 and TRP95), or (TRP120 and TRP19). In some examples, the composition comprises TRP120, TRP140, and TRP36. In some examples, the composition comprises TRP140, TRP36, and TRP19. The composition may further comprise an *E. canis* bacterin. The *E. canis* bacterin may be a heat-inactivated or chemically-inactivated bacterin. In some examples, the chemically-inactivated bacterin was inactivated with formaldehyde, formalin, bi-ethylene amine, radiation, ultraviolet light, beta-propiolactone treatment, or formaldehyde. In some examples, the composition comprises: (SEQ ID NO:2 and at least

one of (SEQ ID NOs:3-11 or 16)), (SEQ ID NO:2 and at least one of SEQ ID NOs:12-13), (SEQ ID NO:2 and SEQ ID NO:14), (at least one of (SEQ ID NOs:3-11 or 16) and at least one of SEQ ID NOs:12-13), (at least one of (SEQ ID NOs:3-11 or 16) and SEQ ID NO:14), (at least one of SEQ ID NOs:12-13 and SEQ ID NO:14), (SEQ ID NO:1 and SEQ ID NO:2), (SEQ ID NO:1 and at least one of (SEQ ID NOs:3-11 or 16)), (SEQ ID NO:1 and at least one of SEQ ID NOs:12-13), (SEQ ID NO:1 and SEQ ID NO: 14), (SEQ ID NO: 15 and SEQ ID NO:2), (SEQ ID NO: 15 and at least one of (SEQ ID NOs:3-11 or 16)), (SEQ ID NO: 15 and at least one of SEQ ID NOs:12-13), (SEQ ID NO: 15 and SEQ ID NO:14), or (SEQ ID NO: 15 and SEQ ID NO: 1). In some examples, the composition comprises: SEQ ID NO:15, SEQ ID NO:14, and (any one of SEQ ID NOs:3-11 or 16). In some examples, the composition comprises SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:1. The composition may further comprise an *E. canis* bacterin. The *E. canis* bacterin may be a heat-inactivated or chemically-inactivated bacterin. In some examples, the chemically-inactivated bacterin was inactivated with formaldehyde, formalin, bi-ethylene amine, radiation, ultraviolet light, beta-propiolactone treatment, or formaldehyde. In some examples, the composition comprises an *E. canis* bacterin. The *E. canis* bacterin may be a heat-inactivated or chemically-inactivated bacterin. In some examples, the chemically-inactivated bacterin was inactivated with formaldehyde, formalin, bi-ethylene amine, radiation, ultraviolet light, beta-propiolactone treatment, or formaldehyde.

[0006] Another example of the present disclosure refers to a method of diagnosing exposure to or infection by *Ehrlichia* or *E. canis*, comprising: a) obtaining a biological sample from a mammalian subject, and b) testing the biological sample for immunoreactivity to TRP120, TRP140, and/or TRP36, or a peptide thereof, such as any of SEQ ID NOs: 3-11, 14, 15, or 16; wherein immunoreactivity to TRP120, TRP140, and/or TRP36 indicates that subject has been exposed to or infected by *Ehrlichia* or *E. canis*. In some examples, the mammalian subject is a dog. In some examples, the method comprises testing the biological sample for immunoreactivity to TRP120 and TRP36, or an immunoreactive peptide thereof, such as any of SEQ ID NOs: 3-11, 14, or 16. In some examples, the method comprises testing the biological sample for immunoreactivity to TRP120, TRP140, and TRP36, or an immunoreactive peptide thereof, such as any of SEQ ID NOs: 3-11, 14, 15, or 16. In some examples, the method further comprises a method of treating the mammalian subject, and wherein the mammalian subject is administered a pharmacologically relevant or therapeutically relevant amount of an antibiotic such as, *e.g.*, doxycycline.

[0007] Yet another example of the present disclosure refers to a method of inducing an immune response in a mammalian subject comprising administering to the subject a pharmaceutically relevant amount of an immunogenic composition described above or herein. The immunogenic composition may comprise an adjuvant. In some examples, the adjuvant comprises a triterpenoid saponin (*e.g.*, Quil A), a sterol (*e.g.*, cholesterol), and an immunostimulatory oligonucleotide (*e.g.*, a CpG-containing ODN). In some examples, the triterpenoid saponin is Quil A, the sterol is cholesterol, and the immunostimulatory oligonucleotide is a CpG-containing ODN. In some examples, the CpG-containing ODN is 5' JU*C-G*T*C*G*A*C*G*A*T*C*G*G*C*G*G*C*C*G*C*C* G*T 3' (SEQ ID NO:17), wherein “*” refers to a phosphorothioate bond, “-” refers to a phosphodiester bond, and “JU” refers to 5'-Iodo-2'-deoxyuridine. In some examples, the immunogenic composition comprises at least one, at least two, or all of TRP140, TRP36, and/or TRP19. The immunogenic composition may comprise a chimeric protein comprising 1, 2, or all of TRP140, TRP36, and/or TRP19. In some examples, the immunogenic composition comprises a polypeptide comprising 1, 2, or all of SEQ ID NO:1, SEQ ID NO:3, and/or SEQ ID NO:14. The immunogenic composition may comprise an adjuvant and 1, 2, or all of TRP140, TRP36, and/or TRP19. The method may further comprises administering a second immunogenic composition to the subject, wherein the second immunogenic composition comprises an *Ehrlichia* bacterin (*e.g.*, an *E. canis* bacterin). The second immunogenic composition may comprises an adjuvant, preferably wherein the adjuvant comprises a triterpenoid saponin (*e.g.*, Quil A), a sterol (*e.g.*, cholesterol), and an immunostimulatory oligonucleotide (a CpG-containing ODN). In some examples, the adjuvant in the second immunogenic composition comprises Quil A, cholesterol, and a CpG-containing ODN. In some examples, the CpG-containing ODN is 5' JU*C-G*T*C*G*A*C*G*A*T*C*G*G*C*G*G*C*C*G*C*C* G*T 3' (SEQ ID NO:17), wherein “*” refers to a phosphorothioate bond, “-” refers to a phosphodiester bond, and “JU” refers to 5'-Iodo-2'-deoxyuridine. In some examples, the mammalian subject is a dog.

[0008] In some examples, the adjuvant may comprise or consist of an immunologically active saponin fraction from the bark of *Quillaja saponaria*. The saponin may be, for example, Quil A, or another purified or partially-purified saponin preparation, which can be obtained commercially. Quil A is commercially available, *e.g.*, from E.M. Sergeant Pulp & Chemical Company (Clifton, NJ, USA). The adjuvant may comprise or consist of QS-7, QS-17, QS-18, and QS-21. QS-7, QS-17, QS-18, and QS-21 are also commercially

available, *e.g.*, from Antigenics (Framingham, Massachusetts, USA). The saponin extracts can be used as mixtures in the adjuvant, or purified individual components such as QS-7, QS-17, QS-18, and QS-21 can be included in the adjuvant. In some embodiments, the Quil A is at least about 85% pure. In other examples, the Quil A is at least about 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or about 99% pure.

[0009] The adjuvant may advantageously include a P-class immunostimulatory oligonucleotide, more preferably a modified P-class immunostimulatory oligonucleotides. P-class immunostimulatory oligonucleotides are CpG oligonucleotides that include at least one palindrome, typically 6-20 nucleotides in length. In some preferred examples, the P-Class oligonucleotide can spontaneously self-assemble into a concatamer, either *in vitro* and/or *in vivo*. P-Class oligonucleotides are individually single-stranded, but the presence of palindromes allows for formation of concatamers or stem-and-loop structures between multiple P-Class oligonucleotides (*e.g.*, multiple P-Class oligonucleotides having the same nucleotide sequence). In some examples, P-class immunostimulatory oligonucleotides is between 19 and 100 nucleotides in length, *e.g.*, 19-30 nucleotides, 30-40 nucleotides, 40-50 nucleotides, 50-60 nucleotides, 60-70 nucleotides, 70-80 nucleotides, 80-90 nucleotides, 90-100 nucleotides, or any range derivable therein.

[0010] In some preferred examples, the immunostimulatory oligonucleotide contains a 5' TLR activation domain and at least two palindromic regions, *e.g.*: a first palindromic region that is a 5' palindromic region of at least 6 nucleotides in length, and connected to second palindromic region that is a 3' palindromic region of at least 8 nucleotides in length, wherein the first palindromic region and the second palindromic regions are connected directly or indirectly (*e.g.*, via a spacer). TLR-9 activating motifs are known and include, without limitation, TCG, TTCG, TTTCG, TYpR, TTYpR, TTTYpR, UCG, UUCG, UUUCG, TTT, and TTTT. The 5' TLR area may be completely or partially included into the 5' palindromic region, or may be upstream of the 5' palindromic region. . The 3' palindrome or the 3' complementary area is, in certain examples, at least 8 bases long and is generally rich in C and G.

[0011] The P-class immunostimulatory oligonucleotide(s) may be modified according to techniques known in the art. For example, J-modification can be used to generate iodo-modified nucleotides. E-modification can be used to generate ethyl-modified nucleotide(s). In some examples, the immunostimulatory oligonucleotide is an E-modified P-class

immunostimulatory oligonucleotides. E-modified P-class immunostimulatory oligonucleotides are P-class immunostimulatory oligonucleotides that include at least one nucleotide (preferably the 5' nucleotide) that is ethylated. Additional modifications that can be used include attachment of 6-nitro-benzimidazol, O-methylation, modification with proynyl-dU, inosine modification, and/or 2-bromovinyl attachment (preferably to a uridine).

[0012] The P-class immunostimulatory oligonucleotides may also contain a modified internucleotide linkage, including, without limitation, phosphodiester linkages and phosphorothioate linkages. The oligonucleotides described herein may be synthesized, produced recombinantly, or obtained from a commercial source.

[0013] Sterols suitable for inclusion in the adjuvant include β -sitosterol, stigmasterol, ergosterol, ergocalciferol, and/or cholesterol. These sterols are well-known in the art and can be purchased commercially. For example, cholesterol is disclosed in the Merck Index, 12th Ed., p. 369. The amount of sterol(s) included in the adjuvant compositions can depend upon the nature of the sterol used. In some examples, the sterol is included in the adjuvant in an amount of about 1 μ g to about 5,000 μ g per ml. They also are used in an amount of about 1 μ g to about 4,000 μ g per ml; about 1 μ g to about 3,000 μ g per ml; about 1 μ g to about 2,000 μ g per ml; and about 1 pg to about 1,000 μ g per ml. They are also used in an amount of about 5 μ g to about 750 μ g per ml; about 5 μ g to about 500 μ g per ml; about 5 μ g to about 200 μ g per ml; about 5 μ g to about 100 μ g per ml; about 15 μ g to about 100 μ g per ml; and about 30 μ g to about 75 μ g per ml.

[0014] In certain examples, the adjuvant can comprise or consist of a sterol (*e.g.*, cholesterol), a saponin (*e.g.*, Quil-A), and an immunostimulatory oligonucleotide (*e.g.*, a CpG oligonucleotide, and/or a P-class immunostimulatory oligonucleotide). In various examples, it is anticipated that the adjuvant may comprise: (a sterol and a saponin), (a saponin and an immunostimulatory oligonucleotide), or (a sterol and an immunostimulatory oligonucleotide). The preparation of the adjuvant containing the saponin and the sterol according to the instant invention is within the ordinary skill in the art. For example, an aqueous mixture can be prepared that comprises an antigenic protein or peptide as described herein (*e.g.*, one or more antigenic peptides from TRP153, TRP36, TRP140, TRP28, TRP95, TRP 19, and/or TRP120; optionally further comprising an *E. canis* bacterin or an *E. chaffeensis* bacterin), the P-class immunostimulatory oligonucleotide, and the saponin. The sterol can then be gradually or dropwise added to the mixture.

VII. Examples

[0064] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Vaccine Efficacy Evaluation of the *Ehrlichia Canis* Bacterin and Recombinant TRP Antigens in Beagles

[0065] The objective of the study was to evaluate the efficacy of an *Ehrlichia canis* bacterin vaccine, produced via different processes, in a novel adjuvant formulation consisting of Quil A, cholesterol, and CpG ODN of SEQ ID NO:17 (5' JU*C-G*T*C*G*A*C*G*A*T*C*G*G*C*G*G*C*C*G*C*C* G*T 3', wherein "*" refers to a phosphorothioate bond, "-" refers to a phosphodiester bond, and "JU" refers to 5'-Iodo-2'-deoxyuridine) (QCT; each component present at 50µg/dose) against a heterologous *Ehrlichia canis* (*E. canis*) Ebony strain challenge in dogs approximately 12 weeks of age at first vaccination. Additionally, the efficacy of vaccines containing recombinant Tandem Repeat Proteins (TRP), 19, 36 & 120 kDa in size, of *E. canis* were evaluated either as a 3-way combination vaccine, or as a (bacterin with QCT) + (TRP antigens with QCT) co-administration combination vaccine.

[0066] Seventy-two (72) male beagles, approximately 12 weeks of age, were randomly assigned to one of six treatment groups (12 animals/treatment group) using a randomized complete block design. Treatment groups were defined as T01 – Saline controls; T02 – *E. canis* Sheba Whole Cell Silversen bacterin, BEI (binary ethyleneimine) inactivation + QCT; T03 – *E. canis* Sheba Whole Cell Silversen bacterin, BEI inactivation + QCT; T04 – [*E. canis* Sheba Whole Cell Silversen bacterin, BEI inactivation + QCT] and [TRP 140, 36, 19 + QCT] (co-administered); T05 – Formalin-inactivated Bacterin + QCT; and T06 – TRP 140, 36, 19 + QCT (Table 5).

[0067] All dogs were healthy and negative for *E. canis* by IDEXX SNAP 4Dx Plus prior to vaccination on Day 0. All animals were subcutaneously vaccinated with either placebo (saline) or Investigational Veterinary Product (IVP) on Days 0 and 28. Animals were challenged via the intravenous route with *E. canis* Ebony strain six weeks after second vaccination. Tympanic temperatures were collected daily, and all animals were observed daily for clinical signs of *E. canis* infection through the end of study (Day 134). Blood samples were collected for Complete Blood Chemistry (CBC) testing every three days post-challenge, as well as for qPCR testing, starting seven days post-challenge. Additional blood samples were collected for IDEXX SNAP® 4Dx Plus testing on Days 0, 28, 49, and 134; ELISA testing on Days 0, 28, 49, 68-70, and 134; and CMI analysis on Days 0, 35, 83, and 104.

Table 5. Study Design

TRT	# Animals	Treatment	Vaccination				Challenge
			Days*	Dose	Dose Units	Route	
T01	12	Saline	0, 28	1.0	mL	SQ	Day 70
T02	12	<i>E. canis</i> Sheba Whole Cell Silversen bacterin (BEI inactivation) + QCT		1.0			
T03	12	<i>E. canis</i> Sheba Whole Cell Silversen bacterin (media-free; BEI inactivation) + QCT		1.0			
T04	12	[<i>E. canis</i> Sheba Whole Cell Silversen bacterin (BEI inactivation) + QCT] and [TRP 140, 36, 19 + QCT]		1.0 of bacterin + 1.0 of TRP			
T05	12	<i>E. canis</i> Sheba Whole Cell Silversen bacterin		1.0			

		(Formalin inactivation) + QCT					
T06	12	TRP 140, 36, 19 + QCT		1.0			

*Vaccination on Day 0 was performed over the right shoulder region. Vaccination on Day 28 was performed over the left shoulder region.

[0068] Efficacy in this study was determined based on the reduction of thrombocytopenia (platelet counts $<143 \times 10^3/\mu\text{L}$ and $>50\%$ reduction from baseline) and clinical disease (mainly fever) post-challenge. A vaccine effect would be confirmed if the T02, T03, T04, T05, and/or T06 treatment groups had significantly less thrombocytopenia or clinical disease after challenge compared to T01 controls.

Results

[0069] For this study, qPCR was performed on bulk antigens to standardize vaccine formulations based on inactivated bacterin genomes. Target vaccines for this study were blended at 400 μg of inactivated bacterin.

[0070] The presence of thrombocytopenia (platelet count $<143 \times 10^3/\mu\text{L}$) post-challenge was significantly reduced in treatment groups T02 (67%, $P = 0.0497$), T03 (67%, $P = 0.0497$), and T04 (58%, $P = 0.0141$) when compared to treatment group T01 (100%). There was no significant reduction in thrombocytopenia in treatment groups T05 (75%) and T06 (83%) when compared to treatment group T01 (controls) (Tables 6 and 3).

Table 6. Ever-Present Thrombocytopenia by Treatment Group

Treatment	Result				Total
	NO		YES		
	n	(%)	n	(%)	
T01	0	0	12	100	12
T02	4	33	8	67	12
T03	4	33	8	67	12
T04	5	42	7	58	12
T05	3	25	9	75	12
T06	2	17	10	83	12

T01 – Saline control

WHAT IS CLAIMED IS:

1. An immunogenic composition comprising:

(i) an *E. canis* bacterin and at least 1, 2, 3, 4, 5, 6, or all of Tandem Repeat Protein 153 (TRP153), Tandem Repeat Protein 36 (TRP36), Tandem Repeat Protein 140 (TRP140), Tandem Repeat Protein 28 (TRP28), Tandem Repeat Protein 95 (TRP95), Tandem Repeat Protein 19 (TRP19), or Tandem Repeat Protein 120 (TRP120); or

(ii) an *E. canis* bacterin, at least 1, 2, 3, 4, 5, 6, or all of TRP153, TRP36, TRP140, TRP28, TRP95, TRP19, TRP120 and at least 1, 2, 3, 4, 5, or more peptides comprising or consisting of a peptide of any one of SEQ ID NOs:1-16;

and a pharmaceutically acceptable excipient.

2. The immunogenic composition of claim 1, wherein the pharmaceutically acceptable excipient comprises or consists of an adjuvant.

3. The immunogenic composition of claim 2, wherein the adjuvant comprises a triterpenoid saponin, a sterol, and an immunostimulatory oligonucleotide.

4. The immunogenic composition of claim 3, wherein the triterpenoid saponin is Quil A.

5. The immunogenic composition of any one of claims 3-4, wherein the sterol is cholesterol.

6. The immunogenic composition of any one of claims 3-5, wherein the immunostimulatory oligonucleotide is a CpG-containing ODN.

7. The immunogenic composition of claim 3, wherein the triterpenoid saponin is Quil A, the sterol is cholesterol, and the immunostimulatory oligonucleotide is a CpG-containing ODN.

8. The immunogenic composition of any one of claims 6-7, wherein the CpG-containing ODN is 5' JU*C-G*T*C*G*A*C*G*A*T*C*G*G*C*G*G*C*C*G*C*C* G*T 3' (SEQ ID NO:17), wherein "*" refers to a phosphorothioate bond, "-" refers to a phosphodiester bond, and "JU" refers to 5'-Iodo-2'-deoxyuridine.

9. The immunogenic composition of claim 7, wherein the composition comprises at least one of TRP140, TRP36, and/or TRP19.

10. The immunogenic composition of claim 9, wherein the composition comprises at least two of TRP140, TRP36, and/or TRP19.
11. The immunogenic composition of claim 10, wherein the composition comprises TRP140, TRP36, and TRP19.
12. The immunogenic composition of claim 7, wherein the composition comprises a chimeric protein comprising 1, 2, or all of TRP140, TRP36, and/or TRP19.
13. The immunogenic composition of claim 7, wherein the composition comprises a polypeptide comprising 1, 2, or all of SEQ ID NO:1, SEQ ID NO:3, and/or SEQ ID NO:14.
14. The immunogenic composition of any of claims 2-13, wherein the adjuvant is an emulsion or liposomes, or wherein the adjuvant comprises a lipid.
15. The immunogenic composition of claim 14, wherein the emulsion is an oil-in-water (O/W) emulsion or a water-in-oil (W/O) emulsion.
16. The immunogenic composition of claim 2, wherein the adjuvant comprises a triterpenoid, a sterol, an immunomodulator, a polymer, and/or an immunostimulatory oligonucleotide.
17. The immunogenic composition of claim 16, wherein the polymer is diethyl-aminoethyl (DEAE)-dextran, polyethelyne glycol, or polyacrylic acid.
18. The immunogenic composition of any of claims 16-17, wherein the immunostimulatory oligonucleotide is a CpG containing ODN.
19. The immunogenic composition of any of claims 16-18, wherein the adjuvant comprises DEAE Dextran, an immunostimulatory oligonucleotide, and oil such as mineral oil, wherein the immunostimulatory oligonucleotide is a CpG containing ODN, and wherein the adjuvant formulation is a water-in-oil (W/O) emulsion.
20. The immunogenic composition of claim 2, wherein the adjuvant comprises a saponin, a sterol, a quaternary ammonium compound, a polymer, and an ORN/ODN.
21. The immunogenic composition of claim 20, wherein the saponin is Quil A or a purified fraction thereof, the sterol is cholesterol, the quaternary ammonium compound is dimethyl

dioctadecyl ammonium bromide (DDA), the polymer is polyacrylic acid, and the ORN/ODN is a CpG.

22. The immunogenic composition of claim 21, wherein the saponin is present in an amount of 1 µg to 5,000 µg per dose, the sterol is present in an amount of 1 µg to 5,000 µg per dose, the quaternary ammonium compound is present in an amount of 1 µg to 5,000 µg per dose, and the polymer is present in an amount of 0.0001% v/v to 75% v/v.

23. The immunogenic composition of any one of claims 21-22, wherein the adjuvant further comprises a glycolipid.

24. The immunogenic composition of claim 23, wherein the glycolipid is N-(2-deoxy-2-L-leucylamino-β-D-glucopyranosyl)-N-octadecyldodecanamide acetate.

25. The immunogenic composition of claim 2, wherein the adjuvant comprises a triterpenoid saponin, a sterol, a quaternary ammonium compound, and a polyacrylic acid polymer.

26. The immunogenic composition of claim 25, wherein the saponin is Quil A or a purified fraction thereof, the sterol is cholesterol, and the quaternary ammonium compound is dimethyl dioctadecyl ammonium bromide (DDA).

27. The immunogenic composition of any one of claims 25-26, wherein the saponin is present in an amount of 1 mg to 5,000 mg per dose, the sterol is present in an amount of 1 mg to 5,000 mg per dose, the quaternary ammonium compound is present in an amount of 1 mg to 5,000 mg per dose, and the polyacrylic acid polymer is present in an amount of 0.0001% v/v to 75% v/v.

28. The immunogenic composition of claim 2, wherein the adjuvant comprises a water-in-oil emulsion.

29. The immunogenic composition of claim 28, wherein the water-in-oil emulsion comprises an oily phase and an aqueous phase, a polycationic carrier (*e.g.*, DEAE dextran), and a CpG containing immunostimulatory oligonucleotide.

30. The immunogenic composition of claim 29, wherein the composition further comprises an aluminum hydroxide gel.

31. The immunogenic composition of any one of claims 29-30, wherein the polycationic carrier is DEAE dextran.
32. The immunogenic composition of claim 2, wherein the composition comprises an emulsion or an oil-in-water (O/W) emulsion.
33. The immunogenic composition of claim 32, wherein the emulsion comprises an aqueous phase that comprises an alkyl-polyacrylic acid (alkyl-PAA) or both an acrylic polymer and dimethyl dioctadecyl ammonium bromide (DDA).
34. The immunogenic composition of claim 33, wherein the aqueous phase of the oil-in-water emulsion comprises dimethyl dioctadecyl ammonium bromide (DDA) and an alkyl-polyacrylic acid (alkyl-PAA).
35. The immunogenic composition of claim 34, wherein the alkyl-PAA is decyl-PAA, octyl-PAA, butyl-PAA, or methyl-PA.
36. The immunogenic composition of any of claims 33-35, wherein the acrylic polymer is a polymer of acrylic acid crosslinked with polyallyl sucrose.
37. The immunogenic composition of claim 2, wherein the composition comprises a water-in-oil (W/O) emulsion comprising a non-mineral oil and an emulsifier.
38. The immunogenic composition of claim 37, wherein the emulsifier is a mannide mono-oleate emulsifier.
39. The immunogenic composition of claim 2, wherein the adjuvant is MF59, AS01, AS02, AS03, AS04, Virosomes, CAF01, CAF04, CAF05, an acrylic polymer / DDA emulsion, a CpG/DEAE emulsion, a saponin/ cholesterol/ DDA adjuvant, or a polyacrylic acid polymer emulsion.
40. The immunogenic composition of any one of claims 1-39, comprising at least 2, 3, 4, or all of TRP153, TRP36, TRP140, TRP28, TRP95.
41. The immunogenic composition of any one of claims 1-39, wherein the composition comprises (TRP153 and TRP36), (TRP153 and TRP140), (TRP153 and TRP28), (TRP153 and TRP95), (TRP36 and TRP140), (TRP36 and TRP28), (TRP36 and TRP95), (TRP140 and TRP28), (TRP140 and TRP95), (TRP28 and TRP95), (TRP19 and TRP153), (TRP19 and

TRP36), (TRP19 and TRP140), (TRP19 and TRP28), (TRP19 and TRP95), (TRP120 and TRP153), (TRP120 and TRP36), (TRP120 and TRP140), (TRP120 and TRP28), (TRP120 and TRP95), or (TRP120 and TRP19).

42. The immunogenic composition of claim 41, wherein the composition comprises TRP120, TRP140, and TRP36.

43. The immunogenic composition of claim 41, wherein the composition comprises TRP140, TRP36, and TRP19.

44. The immunogenic composition of any one of claims 1-39, wherein the composition comprises: (SEQ ID NO:2 and at least one of (SEQ ID NOs:3-11 or 16)), (SEQ ID NO:2 and at least one of SEQ ID NOs:12-13), (SEQ ID NO:2 and SEQ ID NO:14), (at least one of (SEQ ID NOs:3-11 or 16) and at least one of SEQ ID NOs:12-13), (at least one of (SEQ ID NOs:3-11 or 16) and SEQ ID NO:14), (at least one of SEQ ID NOs:12-13 and SEQ ID NO:14), (SEQ ID NO:1 and SEQ ID NO:2), (SEQ ID NO:1 and at least one of (SEQ ID NOs:3-11 or 16)), (SEQ ID NO:1 and at least one of SEQ ID NOs:12-13), (SEQ ID NO:1 and SEQ ID NO: 14), (SEQ ID NO: 15 and SEQ ID NO:2), (SEQ ID NO: 15 and at least one of (SEQ ID NOs:3-11 or 16)), (SEQ ID NO: 15 and at least one of SEQ ID NOs:12-13), (SEQ ID NO: 15 and SEQ ID NO:14), or (SEQ ID NO: 15 and SEQ ID NO: 1).

45. The immunogenic composition of claim 46, wherein the composition comprises: SEQ ID NO:15, SEQ ID NO:14, and (any one of SEQ ID NOs:3-11 or 16).

46. The immunogenic composition of claim 46, wherein the composition comprises SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:1.

47. The immunogenic composition of any one of claims 1 to 39, wherein the *E. canis* bacterin is a heat-inactivated or chemically-inactivated bacterin.

48. The immunogenic composition of claim 47, wherein the chemically-inactivated bacterin was inactivated with formaldehyde, formalin, bi-ethylene amine, radiation, ultraviolet light, beta-propiolactone treatment, or formaldehyde.

49. Use of an immunogenic composition of any one of claims 1-48 in the manufacture of a medicament for inducing a protective immune response in a mammalian subject.

50. The use of claim 49, wherein the immunogenic composition comprises an adjuvant, and wherein the adjuvant comprises a triterpenoid saponin, a sterol, and an immunostimulatory oligonucleotide.
51. The use of claim 50, wherein the triterpenoid saponin is Quil A.
52. The use of any one of claims 50-51, wherein the sterol is cholesterol.
53. The use of any one of claims 50-52, wherein the immunostimulatory oligonucleotide is a CpG-containing ODN.
54. The use of claim 50, wherein the triterpenoid saponin is Quil A, the sterol is cholesterol, and the immunostimulatory oligonucleotide is a CpG-containing ODN.
55. The use of any one of claims 53-54, wherein the CpG-containing ODN is 5' JU*C-G*T*C*G*A*C*G*A*T*C*G*G*C*G*G*C*G*G*C*G*G*C*G*G*T 3' (SEQ ID NO:17), wherein “*” refers to a phosphorothioate bond, “-” refers to a phosphodiester bond, and “JU” refers to 5'-Iodo-2'-deoxyuridine.
56. The use of claim 49, wherein the immunogenic composition comprises at least one of TRP140, TRP36, and/or TRP19.
57. The use of claim 56, wherein the immunogenic composition comprises at least two of TRP140, TRP36, and/or TRP19.
58. The use of claim 57, wherein the immunogenic composition comprises TRP140, TRP36, and TRP19.
59. The use of claim 49, wherein the immunogenic composition comprises a chimeric protein comprising 1, 2, or all of TRP140, TRP36, and/or TRP19.
60. The use of claim 49, wherein the immunogenic composition comprises a polypeptide comprising 1, 2, or all of SEQ ID NO:1, SEQ ID NO:3, and/or SEQ ID NO:14.
61. The use of claim 49, wherein the immunogenic composition comprises an adjuvant and 1, 2, or all of TRP140, TRP36, and/or TRP19.

62. The use of any one of claims 49-62, further comprising a second immunogenic composition to be administered to the subject, wherein the second immunogenic composition comprises an *Ehrlichia* bacterin.

63. The use of claim 62, wherein the *Ehrlichia* bacterin is an *E. canis* bacterin.

64. The use of any of claims 62-63, wherein the second immunogenic composition comprises an adjuvant, preferably wherein the adjuvant comprises a triterpenoid saponin, a sterol, and an immunostimulatory oligonucleotide.

65. The use of claim 63, wherein the adjuvant in the second immunogenic composition comprises Quil A, cholesterol, and a CpG-containing ODN.

66. The use of claim 65, wherein the CpG-containing ODN is 5' JU*C-G*T*C*G*A*C*G*A*T*C*G*G*C*G*G*C*C*G*C*C* G*T 3' (SEQ ID NO:17), wherein “*” refers to a phosphorothioate bond, “-” refers to a phosphodiester bond, and “JU” refers to 5'-Iodo-2'-deoxyuridine.

67. The use of any one of claims 49-66, wherein the mammalian subject is a dog.