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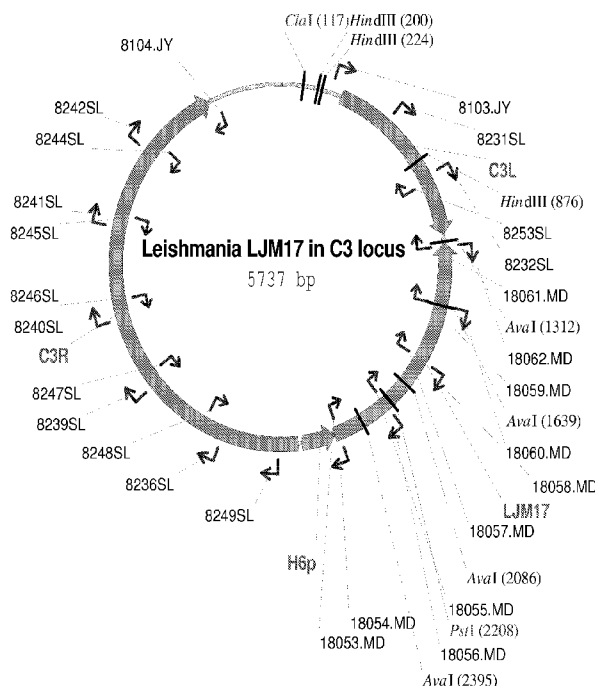
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(54) Title: LEISHMANIA VACCINE USING SAND FLY SALIVARY IMMUNOGEN

FIGURE 3



(57) Abstract: The present invention provides vectors that contain and express in vivo or in vitro sand fly Lu. longipalpis salivary antigens that elicit an immune response in animal or human against Leishmania, vaccine compositions comprising said vectors and/or Lu. longipalpis salivary polypeptides, methods of vaccination against Leishmania, and kits for use with such methods and compositions.



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LEISHMANIA VACCINE USING SAND FLY SALIVARY IMMUNOGEN**INCORPORATION BY REFERENCE**

This application claims benefit of US provisional application Serial No. 61/051,635 filed
5 May 8, 2008, and of US provisional application Serial No. 61/101,345 filed September 30, 2008,
which make reference to International Application No. PCT/US2003/034453 entitled *Lutzomyia*
Longipalpis Polypeptides and Methods of Use filed October 29, 2003, which claims priority to
provisional application No. 60/422,303 filed October 29, 2002.

All documents cited or referenced herein ("herein cited documents"), and all documents
10 cited or referenced in herein cited documents, together with any manufacturer's instructions,
descriptions, product specifications, and product sheets for any products mentioned herein or in
any document incorporated by reference herein, are hereby incorporated herein by reference, and
may be employed in the practice of the invention.

FIELD OF THE INVENTION

15 The present invention relates to formulations for combating *Leishmania* infections in
animals or humans. Specifically, the present invention provides vectors that contain and express
in vivo or *in vitro* sand fly *Lu. longipalpis* salivary antigens that elicit an immune response in
animal or human against *Leishmania*, including compositions comprising said vectors, methods
of vaccination against *Leishmania*, and kits for use with such methods and compositions.

BACKGROUND OF THE INVENTION

20 Leishmaniasis is a major and severe parasitic disease that affects humans, canines (dogs,
wolves, foxes, coyotes, jackals), and to a lesser degree, felines (lions, tigers, domestic cats, wild
cats, other big cats, and other felines including cheetahs and lynx).

Leishmania and Viannia subgenera are grouped into complexes of species and subspecies
25 based upon molecular, biochemical and immunological similarities. There are several forms of
the disease named by their clinical presentation including cutaneous, mucocutaneous or visceral
leishmaniasis. Each of these forms of disease is caused by different species of sand flies found in
different regions of the world. Cutaneous leishmaniasis of humans is associated with members of
L. aethiopica, *L. major*, and *L. tropica* complexes in the Old World and *L. mexicana* and *L.*
30 *braziliensis* complexes in the New World. Visceral leishmaniasis is caused by *L. donovani* and
L. infantum in Old World regions while *L. chagasi* is primarily responsible for visceral disease in

the New World. Because *L. infantum* is the primary agent associated with canine leishmaniasis, infections in dogs often are regarded as visceral even though they tend to cause both visceral and cutaneous disease.

The agent of visceral leishmaniasis is a protozoan parasite and belongs to the *leishmania*
5 *donovani* complex. This parasite is widely distributed in temperate and subtropical countries of Southern Europe, Africa, Asia, South America and Central America (Desjeux P. *et al.*). *Leishmania donovani infantum* (*L. infantum*) is responsible for the feline and canine disease in Southern Europe, Africa, and Asia. In South America and Central America, the agent is *Leishmania donovani chagasi* (*L. chagasi*), which is closely related to *L. infantum*. In humans,
10 the agent is *Leishmania donovani donovani* (*L. donovani*), which is also related to *L. infantum* and *L. chagasi*.

Sand flies of the genus *Phlebotomus* (Old World) and *Lutzomyia* (New World) are the primary vectors responsible for disease transmission. Currently these are the only known vectors capable of spread; fleas, ticks and other arthropods have not been shown to be competent
15 vectors. However, rare cases of leishmaniasis have been contracted through exchange of blood or body fluids, direct contact and at least one case of congenital transmission. The importance of native sand flies is yet undetermined but could be related to infectious dose of the organism. Still, in recent years, and in the absence of known vectors, there has been an overwhelming incidence of leishmaniasis in Foxhound kennels across the United States. It is still not known
20 how transmission of disease occurred or how this disease is maintained in these dogs because infected sand flies have not been reported in the United States. However, certain species of *Lutzomyia* (*L. shannoni*), found along the eastern United States and as far north as New Jersey, are considered a potentially competent vector for *L. mexicana*. *Phlebotomus ariasi* (*P. ariasi*) and *Phlebotomus perniciosus* (*P. perniciosus*), *Phlebotomus neglectus* (*P. neglectus*) are the
25 most common carriers in Southern Europe, Africa, and Asia, whereas *Lutzomyia longipalpis* (*Lu. longipalpis*) is the most common carrier in Southern and Central America.

Leishmaniasis is a slowly progressive disease that can take up to 7 years to become clinically apparent (McConkey SE *et al.*; Slappendel RJ *et al.*). Even then, signs are frequently nonspecific and a diagnosis of *Leishmania* is seldom considered. Dogs are most commonly
30 infected with *L. infantum* (*L. donovani* complex) which is responsible for viscerotropic disease in people. However, up to 90% of infected dogs present with both visceral and cutaneous lesions

(Slappendel RJ *et al.*). On the other hand, many dogs appear naturally resistant to this parasite and may remain asymptomatic despite known infection (Grosjean NL *et al.*). It is estimated that only 10% of dogs residing in endemic areas actually develop clinical disease (Lindsay DS *et al.*). This lower incidence of clinical disease is attributed to a genetic predisposition of certain dogs to mount a more protective cell-mediated immune response than a humoral response (Lindsay DS *et al.*, McConkey SE *et al.*, Slappendel RJ, *et al.*). Furthermore, it has been reported that up to 20% of infected dogs may mount an adequate immune response and spontaneously recover from clinical illness (McConkey SE *et al.*). In animals that mount a humoral response, IgG1 appears to correlate with clinical disease while asymptomatic dogs have higher IgG2 antibody levels (Lindsay *et al.*).

Some of the more frequently reported clinical signs of leishmaniasis include listlessness, fatigue and exercise intolerance coupled with anorexia and weight loss that eventually culminate as wasting disease (McConkey SE *et al.*). These signs may or may not be accompanied by fever, local or generalized lymphadenopathy (90%) and/or hepatosplenomegaly (Grosjean NL *et al.*, Lindsay DS *et al.*, McConkey SE *et al.*, Martínez-Subiela S *et al.*). Articular involvement is also fairly common and may present as lameness with swollen joints or simply as a stiff gait. Less common findings include ocular lesions (<5%), chronic diarrhea (30%) and long, deformed brittle nails (20%) referred to as onychogryphosis (Lindsay DS *et al.*, Slappendel RJ *et al.*). Cutaneous lesions are present in up to 89% of infected dogs, with or without overt signs of visceral involvement. Lesions of cutaneous leishmaniasis may occur anywhere on the body but the most common sites are those which are exposed to the environment and are therefore more susceptible to bites from the sand flies. The initial papule rapidly gives rise to an ulcer. Visceral leishmaniasis is invariably fatal if not treated promptly. Visceral leishmaniasis affects the internal body organs, specifically the spleen and the liver.

Dogs are considered the major reservoir of Leishmaniasis. The disease is characterized by chronic evolution of viscerocutaneous signs occurring in less than 50% of infected animals (Lanotte G. *et al.*). Both asymptomatic and symptomatic dogs with detectable antibodies may be infectious (Molina R. *et al.*; Courtenay O. *et al.*). Cats may also be carriers of the protozoan parasites and are thus considered secondary potential reservoirs.

Due to a number of factors, treatment options for leishmaniasis in dogs and response to therapy are limited at best. For some undefined reason, visceral leishmaniasis is more difficult to

treat in dogs than in humans. No treatment option is 100% effective in clearing parasitic infection and clinical disease often reappears with cessation of therapy (Lindsay DS *et al.*). In endemic areas, the most common treatment regimen has been a combination of allopurinol with a pentavalent antimonial such as meglumine antimonite or sodium stibogluconate (Lindsay DS *et al.*, Slappendel RJ *et al.*). However, in recent years this protocol has fallen out of favor due to increasing resistance of the parasite to the drug as well as adverse side effects associated with these compounds (Lindsay DS *et al.*). To further limit treatment options, Pentostam® (sodium stibogluconate) is the only available antimonial in the United States and its distribution is regulated by the Centers for Disease Control and Prevention (CDC) in Atlanta, GA (Lindsay DS *et al.*).

Other protocols have been tried but have proven no more efficacious at clearing parasitic infection or at preventing clinical relapse. In addition, each protocol is associated with potential adverse effects. Amphotericin B binds sterols and disrupts cell membrane permeability but is nephrotoxic (Lindsay DS *et al.*). When given parenterally, Paramomycin acts synergistically with antimonials causing higher levels of the antimonial for longer periods of time but is also nephrotoxic and is not currently recommended for clinical use (Lindsay DS *et al.*). Pentamidine isethionate is effective against leishmaniasis but requires at least 15 intramuscular injections and is quite painful (Lindsay DS *et al.*). Ketoconazole, miconazole, fluconazole and itraconazole are oral drugs that may be useful in containing the disease but are cost prohibitive and carry the risk of drug resistance when treating patients symptomatically. In summary, the various treatment regimens for leishmaniasis in dogs have been investigated but are not 100% efficacious; relapses are the rule rather than the exception. Ultimately, the veterinary practitioner is faced with the dilemma of treating symptomatic outbreaks of leishmaniasis in dogs at the risk of developing drug resistant strains of this parasite within the United States.

Mass detection of seropositive dogs followed by culling and/or drug treatment, or the mass application of deltamethrin-impregnated collars, was shown to have an impact in reducing human and canine Leishmaniasis prevalence in endemic areas of Southern Europe, Africa, and Asia (Maroli M. *et al.* Mazloumi Gavgani A.S. *et al.*), although the efficacy of eliminating seropositive canines has been debated (Dietze R. *et al.*; Moreira Jr. E.D. *et al.*). These control measures are either considered unacceptable, expensive or not effective (Gradoni L. *et al.*).

Mathematical models used to compare the effectiveness of various tools for controlling Leishmaniasis suggest that a canine vaccine may be the most practical and effective method (Dye C.). Therefore, the development of vaccines able to protect canines from Leishmaniasis and/or to prevent disease progression in infected animals is highly desirable for the
5 implementation of Leishmaniasis control programs as well for the veterinary community (Gradoni L. *et al.*).

Previous investigations have sought to identify methods of diagnosing and treating Leishmania through, for example, administration of antigenic polypeptides (see, for example, WO 2004/039958 which is hereby incorporated herein by reference in its entirety). However, to
10 date, no vaccine is available for the treatment of *Leishmania*. The vectors and vaccine formulations of the present invention fulfill this long-felt need in the art.

Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY OF THE INVENTION

15 An object of this invention can be any one or all of providing recombinant vectors or viruses as well as methods for making such viruses, and providing compositions and/or vaccines as well as methods for treatment and prophylaxis of infection by Leishmania.

The invention provides a recombinant vector, such as a recombinant virus, e.g., a recombinant poxvirus, that contains and expresses at least one exogenous nucleic acid molecule
20 and, the at least one exogenous nucleic acid molecule may comprise a nucleic acid molecule encoding an immunogen or epitope of interest from salivary proteins from sand fly vectors of *Lu. longipalpis*.

In particular, the present invention provides a recombinant vector, such as a recombinant virus, e.g., a recombinant poxvirus, that contains and expresses at least one exogenous nucleic
25 acid molecule and, the at least one exogenous nucleic acid molecule may comprise salivary *Lu. longipalpis* polypeptides and/or variants or fragments thereof.

These vaccines may prevent diffusion and/or replication of the parasite in a host.

The invention further provides immunological (or immunogenic), or vaccine compositions comprising such an expression vector or the expression product(s) of such an
30 expression vector.

The invention further provides methods for inducing an immunological (or

immunogenic) or protective response against Leishmania, as well as methods for preventing or treating Leishmania or disease state(s) caused by Leishmania, comprising administering the expression vector or an expression product of the expression vector, or a composition comprising the expression vector, or a composition comprising an expression product of the expression
5 vector.

The invention also relates to expression products from the virus as well as antibodies generated from the expression products or the expression thereof *in vivo* and uses for such products and antibodies, e.g., in diagnostic applications.

These and other embodiments are disclosed or are obvious from and encompassed by, the
10 following Detailed Description.

BRIEF DESCRIPTION OF DRAWINGS

The following detailed description, given by way of example, and which is not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying figures, incorporated herein by reference, in which:

15 Figure 1 shows the nucleic acid sequence of one strand of the plasmid pVR2001 LJM17 (SEQ ID NO:9), wherein the two BamHI restriction sites are in bold, the sequence encoding the tPA signal peptide is underlined and the sequence encoding the LJM17 is in bold capital letters.

Figure 2 shows the nucleic acid sequence of one strand of the plasmid pVR2001 LJL143 (SEQ ID NO:10), wherein the two BamHI restriction sites are in bold, the sequence encoding the
20 tPA signal peptide is underlined and the sequence encoding the LJL143 is in bold capital letters.

Figure 3 shows a map of the donor vector, pALVAC C3 H6p-LJM17, having 5737 base pairs.

Figure 4 illustrates the vCP2390 canarypox expression vector, for both forward and reverse complementary strands (SEQ ID NO:93 and SEQ ID NO:92). SEQ ID NO:93 represents
25 the vCP2390 vector strand which contains the codon-optimized LJM17 polynucleotide in the direction that encodes the LJM17 polypeptide (SEQ ID NO:5).

Figure 5 shows a map of the donor vector, pALVAC C3 H6p-LJL143, having 5400 base pairs.

Figure 6 illustrates the vCP2389 canarypox expression vector with its insert encoding
30 LJL143, and provides the nucleotide sequence of one strand of the expression vector (SEQ ID NO:94).

Figure 7 illustrates anti-IgG antibodies to both LJM17 and LJL143, absorbance measured at 405 nm, in vaccinated and control dogs, from one day before V1 administration to two weeks after V5 administration.

Figure 8 illustrates interferon-gamma secretion (pg/mL) from dogs PBMC corresponding to the 5 groups, 2 weeks after the 5th immunization (V5 administration). PBMC were stimulated by SGH (2 pairs), LJL143 (4 µg), LJM17 (4 µg), ConA (4 µg) or non-stimulated by medium (med).

Figure 9 shows a scheme of an *in vitro* killing assay including results, expressed in percentage of infected macrophages (NT: no treatment, LPS: lipopolysaccharide, conA: concavaline A).

Figure 10 shows biopsies of vaccinated and control dogs, at the sand flies bite sites, stained with hematoxylin/eosin (H & E), Luna's, Toluidine blue and immunohistochemical procedures for CD3 and macrophage/monocyte markers (Mac).

Figure 11 shows the nucleic acid sequence of one strand of the plasmid pNBO002 (SEQ ID NO:19), wherein the two BamHI restriction sites are in bold, the sequence encoding the tPA signal peptide is underlined and the sequence encoding the LJM17 is in bold capital letters.

Figure 12 shows a map of the pNBO002 plasmid vector with its insert encoding LJM17, having 6247 base pairs.

Figure 13 shows the nucleic acid sequence of one strand of the plasmid pNBO003 (SEQ ID NO:20), wherein the two BamHI restriction sites are in bold, the sequence encoding the tPA signal peptide is underlined and the sequence encoding the LJL143 is in bold capital letters.

Figure 14 shows a map of the pNBO003 plasmid vector with its insert encoding LJL143, having 5899 base pairs.

Figure 15 shows the protein sequences of LJL143 and LJM17 from *L. longipalpis*.

Figure 16 shows the DNA sequences of LJL143 and LJM17 from *L. longipalpis*.

Figure 17 shows the sequence identity tables.

Figure 18 shows the SEQ ID NO assigned to each DNA and polypeptide.

Figure 19 is a set of graphs and images demonstrating anti-saliva immunity in dogs exposed to the bites of the *Lu. longipalpis* sand fly. FIG. 19A is a set of graphs demonstrating the early kinetics of anti-*Lu. longipalpis* IgG, IgG2, and IgG1 antibody titers in exposed dogs. FIG. 19B is a graph of a delayed-type hypersensitivity reaction on a representative dog

throughout exposure experiments. FIG. 19C is a set of images of an H/E histological analysis performed on skin punch biopsies before exposure (E0), 47 h after first exposure (E1), 48 h after second exposure (E2) and 48 h after third exposure (E3) to sand fly bites. FIG. 19D is a set of images characterizing the inflammatory population at 48 h after third exposure (E3) to sand fly bites with immunohistochemistry for CD3+ T lymphocytes and macrophages and Luna's stain for eosinophils.

Figure 20 is a set of graphs and images demonstrating a cDNA reverse antigen screening assay in dogs.

Figure 21 is a set of graphs and images demonstrating a protein reverse antigen screening assay in dogs.

Figure 22 is a set of two graphs demonstrating interferon γ production of peripheral blood mononuclear cells (PBMCs) from vaccinated dogs after stimulation with recombinant salivary protein.

Figure 23 is a set of four bar graphs demonstrating an *in vitro* killing assay for *Leishmania chagasi* by PBMCs from immunized dogs (NT, no treatment; Ly, lymphocytes).

DETAILED DESCRIPTION

It is noted that in this disclosure and particularly in the claims, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, Genes V. published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8). The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicate

otherwise. The word “or” means any one member of a particular list and also includes any combination of members of that list.

It is noted in this disclosure and the appended claims and/or paragraphs, the term “salivary *Lu. longipalpis* polypeptide”, “*Lu. longipalpis* polypeptide”, or “salivary polypeptide”
5 is used interchangeably, the term “salivary *Lu. longipalpis* polynucleotide”, “*Lu. longipalpis* polynucleotide”, or “salivary polynucleotide” is used interchangeably.

The terms “polypeptide” and “protein” are used interchangeably herein to refer to a polymer of consecutive amino acid residues.

The term “nucleic acid”, “nucleotide”, and “polynucleotide” refers to RNA or DNA and
10 derivatives thereof, such as those containing modified backbones. It should be appreciated that the invention provides polynucleotides comprising sequences complementary to those described herein. Polynucleotides according to the invention can be prepared in different ways (e.g. by chemical synthesis, by gene cloning etc.) and can take various forms (e.g. linear or branched, single or double stranded, or a hybrid thereof, primers, probes etc.).

15 The term “gene” is used broadly to refer to any segment of polynucleotide associated with a biological function. Thus, genes or polynucleotides include introns and exons as in genomic sequence, or just the coding sequences as in cDNAs, such as an open reading frame (ORF), starting from the start codon (methionine codon) and ending with a termination signal (stop codon). Genes and polynucleotides can also include regions that regulate their expression,
20 such as transcription initiation, translation and transcription termination. Thus, also included are promoters and ribosome binding regions (in general these regulatory elements lie approximately between 60 and 250 nucleotides upstream of the start codon of the coding sequence or gene; Doree S M *et al.*; Pandher K *et al.*; Chung J Y *et al.*), transcription terminators (in general the terminator is located within approximately 50 nucleotides downstream of the stop codon of the
25 coding sequence or gene; Ward C K *et al.*). Gene or polynucleotide also refers to a nucleic acid fragment that expresses mRNA or functional RNA, or encodes a specific protein, and which includes regulatory sequences.

The term “immunogenic polypeptide” or “immunogenic fragment” as used herein refers to a polypeptide or a fragment of a polypeptide which comprises an allele-specific motif, an
30 epitope or other sequence such that the polypeptide or the fragment will bind an MHC molecule and induce a cytotoxic T lymphocyte (“CTL”) response, and/or a B cell response (for example,

antibody production), and/or T-helper lymphocyte response, and/or a delayed type hypersensitivity (DTH) response against the antigen from which the immunogenic polypeptide or the immunogenic fragment is derived. A DTH response is an immune reaction in which T cell-dependent macrophage activation and inflammation cause tissue injury. A DTH reaction to the subcutaneous injection of antigen is often used as an assay for cell-mediated immunity.

By definition, an epitope is an antigenic determinant that is immunologically active in the sense that once administered to the host, it is able to evoke an immune response of the humoral (B cells) and/or cellular type (T cells). These are particular chemical groups or peptide sequences on a molecule that are antigenic. An antibody specifically binds a particular antigenic epitope on a polypeptide. Specific, non-limiting examples of an epitope include a tetra- to penta-peptide sequence in a polypeptide, a tri- to penta-glycoside sequence in a polysaccharide. In the animal most antigens will present several or even many antigenic determinants simultaneously. Such a polypeptide may also be qualified as an immunogenic polypeptide and the epitope may be identified as described further.

An “isolated” biological component (such as a nucleic acid or protein or organelle) refers to a component that has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, for instance, other chromosomal and extra-chromosomal DNA and RNA, proteins, and organelles. Nucleic acids and proteins that have been “isolated” include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant technology as well as chemical synthesis.

The term “purified” as used herein does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified polypeptide preparation is one in which the polypeptide is more enriched than the polypeptide is in its natural environment. A polypeptide preparation is substantially purified such that the polypeptide represents several embodiments at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%, of the total polypeptide content of the preparation. The same applies to polynucleotides. The polypeptides disclosed herein can be purified by any of the means known in the art.

A recombinant polynucleotide is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more

commonly, by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques. In one embodiment, a recombinant polynucleotide encodes a fusion protein.

In one aspect, the present invention provides polypeptides from sand fly species *Lu. longipalpis*. In another aspect, the present invention provides a polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, or 87, and variant or fragment thereof.

Moreover, homologs of polypeptides from sand fly species *Lu. longipalpis* are intended to be within the scope of the present invention. As used herein, the term “homologs” includes orthologs, analogs and paralogs. The term “anologs” refers to two polynucleotides or polypeptides that have the same or similar function, but that have evolved separately in unrelated organisms. The term “orthologs” refers to two polynucleotides or polypeptides from different species, but that have evolved from a common ancestral gene by speciation. Normally, orthologs encode polypeptides having the same or similar functions. The term “paralogs” refers to two polynucleotides or polypeptides that are related by duplication within a genome. Paralogs usually have different functions, but these functions may be related. Analogs, orthologs, and paralogs of a wild-type salivary polypeptide can differ from the wild-type salivary polypeptide by post-translational modifications, by amino acid sequence differences, or by both. In particular, homologs of the invention will generally exhibit at least 80-85%, 85-90%, 90-95%, or 95%, 96%, 97%, 98%, 99% sequence identity, with all or part of the wild-type salivary polypeptide or polynucleotide sequences, and will exhibit a similar function.

In another aspect, the present invention provides a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, or 87.

In yet another aspect, the present invention provides fragments and variants of the *L. longipalpis* polypeptides identified above (SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81,

83, 85, or 87) which may readily be prepared by one of skill in the art using well-known molecular biology techniques.

Variants are homologous polypeptides having an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in
5 SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, or 87.

Variants include allelic variants. The term "allelic variant" refers to a polynucleotide or a polypeptide containing polymorphisms that lead to changes in the amino acid sequences of a protein and that exist within a natural population (e.g., a virus species or variety). Such natural
10 allelic variations can typically result in 1- 5% variance in a polynucleotide or a polypeptide. Allelic variants can be identified by sequencing the nucleic acid sequence of interest in a number of different species, which can be readily carried out by using hybridization probes to identify the same gene genetic locus in those species. Any and all such nucleic acid variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation
15 and that do not alter the functional activity of gene if interest, are intended to be within the scope of the invention.

A variant is any secreted polypeptide from *Lu. longipalpis* saliva, capable of inducing in animals, such as dogs, vaccinated with this polypeptide a specific cell-based immune response characterized by secretion of interferon gamma (IFN-gamma) upon stimulation by *Lu.*

20 *longipalpis* salivary gland extract compounds. Such IFN-gamma secretion may be demonstrated using *in vitro* methodology (i.e. Quantikine® immunoassay from R&D Systems Inc. (catalog number# CAIF00); Djoba Siawaya JF *et al.*).

As used herein, the term "derivative" or "variant" refers to a polypeptide, or a nucleic acid encoding a polypeptide, that has one or more conservative amino acid variations or other
25 minor modifications such that (1) the corresponding polypeptide has substantially equivalent function when compared to the wild type polypeptide or (2) an antibody raised against the polypeptide is immunoreactive with the wild-type polypeptide. These variants or derivatives include polypeptides having minor modifications of the *Lu. longipalpis* polypeptide primary amino acid sequences that may result in peptides which have substantially equivalent activity as
30 compared to the unmodified counterpart polypeptide. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. The term "variant" further contemplates

deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein.

The term "conservative variation" denotes the replacement of an amino acid residue by another biologically similar residue, or the replacement of a nucleotide in a nucleic acid

5 sequence such that the encoded amino acid residue does not change or is another biologically similar residue. In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic--aspartate and glutamate; (2) basic--lysine, arginine, histidine; (3) non-polar--alanine, valine, leucine, isoleucine, proline, 10 phenylalanine, methionine, tryptophan; and (4) uncharged polar--glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another hydrophobic residue, or the substitution of one polar residue for another polar residue, such as 15 the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like; or a similar conservative replacement of an amino acid with a structurally related amino acid that will not have a major effect on the biological activity.

Proteins having substantially the same amino acid sequence as the reference molecule but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of 20 the protein are, therefore, within the definition of the reference polypeptide. All of the polypeptides produced by these modifications are included herein. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

25 An immunogenic fragment of a *Lu. longipalpis* polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids, at least 21 amino acids, at least 23 amino acids, at least 25 amino acids, or at least 30 amino acids of a *L. longipalpis* polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, or 87, or variants thereof. In 30 another embodiment, a fragment of a *Lu. longipalpis* polypeptide includes a specific antigenic epitope found on a full-length *Lu. longipalpis* polypeptide.

Procedures to determine fragments of polypeptide and epitope such as, generating overlapping peptide libraries (Hemmer B. *et al.*), Pepscan (Geysen H. M. *et al.*, 1984; Geysen H. M. *et al.*, 1985; Van der Zee R. *et al.*; Geysen H. M.) and algorithms (De Groot A. *et al.*; Hoop T. *et al.*; Parker K. *et al.*), can be used in the practice of the invention, without undue experimentation. Generally, antibodies specifically bind a particular antigenic epitope. Specific, non-limiting examples of epitopes include a tetra- to penta- peptide sequence in a polypeptide, a tri- to penta glycoside sequence in a polysaccharide. In animals most antigens will present several or even many antigenic determinants simultaneously. Preferably wherein the epitope is a protein fragment of a larger molecule it will have substantially the same immunological activity as the total protein.

In another aspect, the present invention provides a polynucleotide encoding a polypeptide from *Lu. longipalpis* sand fly, such as a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, or 87. In yet another aspect, the present invention provides a polynucleotide encoding a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at east ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides.

In another aspect, the present invention provides a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 12, 14, 16, 18, 21, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 89, 90, or 91, or a variant thereof. In yet another aspect, the present invention provides a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 95%, 96%, 97%, 98% or 99% sequence identity to one of a polynucleotide having a sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 12, 14, 16, 18, 21, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 89, 90, or 91, or a variant thereof.

These polynucleotides may include DNA, cDNA, and RNA sequences that encode a *Lu. longipalpis* polypeptide. It is understood that all polynucleotides encoding a *Lu. longipalpis* polypeptide are also included herein, as long as they encode a polypeptide with the recognized activity, such as the binding to an antibody that recognizes the polypeptide, the induction of an immune response to the polypeptide, or an effect on survival of *Leishmania* when administered to a subject exposed to the parasite or who undergoes a decrease in a sign or a symptom of *Leishmania* infection.

The polynucleotides of the disclosure include sequences that are degenerate as a result of the genetic code, e.g., optimized codon usage for a specific host. As used herein, “optimized” refers to a polynucleotide that is genetically engineered to increase its expression in a given species. To provide optimized polynucleotides coding for salivary polypeptides, the DNA sequence of the salivary protein gene can be modified to 1) comprise codons preferred by highly expressed genes in a particular species; 2) comprise an A+T or G+C content in nucleotide base composition to that substantially found in said species; 3) form an initiation sequence of said species; or 4) eliminate sequences that cause destabilization, inappropriate polyadenylation, degradation and termination of RNA, or that form secondary structure hairpins or RNA splice sites. Increased expression of salivary protein in said species can be achieved by utilizing the distribution frequency of codon usage in eukaryotes and prokaryotes, or in a particular species. The term “frequency of preferred codon usage” refers to the preference exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the disclosure as long as the amino acid sequence of the *Lu. longipalpis* salivary polypeptide encoded by the nucleotide sequence is functionally unchanged.

The sequence identity between two amino acid sequences may be established by the NCBI (National Center for Biotechnology Information) pairwise blast and the blosum62 matrix, using the standard parameters (see, e.g., the BLAST or BLASTX algorithm available on the "National Center for Biotechnology Information" (NCBI, Bethesda, Md., USA) server, as well as in Altschul *et al.*; and thus, this document speaks of using the algorithm or the BLAST or BLASTX and BLOSUM62 matrix by the term “blasts”).

Sequence identity between two nucleotide sequences also may be determined using the “Align” program of Myers and Miller, (“Optimal Alignments in Linear Space”, CABIOS 4, 11-

17, 1988) and available at NCBI, as well as the same or other programs available via the Internet at sites thereon such as the NCBI site.

Alternatively or additionally, the term “identity”, for instance, with respect to a nucleotide or amino acid sequence, may indicate a quantitative measure of homology between
5 two sequences. The percent sequence homology may be calculated as:

$(N_{ref} - N_{dif}) * 100 / N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC ($N_{ref} = 8$; $N_{dif} = 2$).

10 Alternatively or additionally, “identity” with respect to sequences can refer to the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur and Lipman), for instance, using a window size of 20 nucleotides, a word length of 4 nucleotides, and a gap
15 penalty of 4, and computer-assisted analysis and interpretation of the sequence data including alignment can be conveniently performed using commercially available programs (e.g., Intelligenetics™ Suite, Intelligenetics Inc. CA). When RNA sequences are said to be similar, or have a degree of sequence identity or homology with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence. Thus, RNA sequences are
20 within the scope of the invention and can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences.

The sequence identity or sequence similarity of two amino acid sequences, or the sequence identity between two nucleotide sequences can be determined using Vector NTI software package (Invitrogen, 1600 Faraday Ave., Carlsbad, CA).

25 Advantageously, sequence identity or homology such as amino acid sequence identity or homology can be determined using the BlastP program (Altschul *et al.*) and available at NCBI, as well as the same or other programs available via the Internet at sites thereon such as the NCBI site.

The following documents provide algorithms for comparing the relative identity or
30 homology of sequences, and additionally or alternatively with respect to the foregoing, the teachings in these references can be used for determining percent homology or identity:

Needleman SB and Wunsch CD; Smith TF and Waterman MS; Smith TF, Waterman MS and Sadler JR; Feng DF and Dolittle RF; Higgins DG and Sharp PM; Thompson JD, Higgins DG and Gibson TJ; and, Devereux J, Haeblerle P and Smithies O. And, without undue experimentation, the skilled artisan can consult with many other programs or references for determining percent
5 homology.

The *Lu. longipalpis* polynucleotides may include a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (for example, a cDNA) independent of other sequences.

10 Recombinant vectors disclosed herein may include a polynucleotide encoding a polypeptide, a variant thereof or a fragment thereof. Recombinant vectors may include plasmids and viral vectors and may be used for *in vitro* or *in vivo* expression. Recombinant vectors may include further a signal peptide. Signal peptides are short peptide chain (3-60 amino acids long) that direct the post-translational transport of a protein (which are synthesized in the cytosol) to
15 certain organelles such as the nucleus, mitochondrial matrix, endoplasmic reticulum, chloroplast, apoplast and peroxisome. Typically, the naturally occurring salivary *Lu. longipalpis* polypeptides, such as LJM17 and LJL143 proteins, may be translated as precursors, having an N-terminal signal peptide sequence and a “mature” protein domain. The signal peptide may be cleaved off rapidly upon translation. The signal sequence may be the natural sequence from the
20 salivary protein or a peptide signal from a secreted protein e.g. the signal peptide from the tissue plasminogen activator protein (tPA), in particular the human tPA (S. Friezner Degen *et al.*; R. Rickles *et al.*; D. Berg. *et al.*), or the signal peptide from the Insulin-like growth factor 1 (IGF1), in particular the equine IGF1 (K. Otte *et al.*), the canine IGF1 (P. Delafontaine *et al.*), the feline IGF1 (WO03/022886), the bovine IGF1 (S. Lien *et al.*), the porcine IGF1 (M. Muller *et al.*), the
25 chicken IGF1 (Y. Kajimoto *et al.*), the turkey IGF1 (GenBank accession number AF074980). The signal peptide from IGF1 may be natural or optimized which may be achieved by removing cryptic splice sites and/or by adapting the codon usage. Upon translation, the unprocessed polypeptide may be cleaved at a cleavage site to lead to the mature polypeptide. The cleavage site may be predicted using the method of Von Heijne (1986).

30 A plasmid may include a DNA transcription unit, for instance a nucleic acid sequence that permits it to replicate in a host cell, such as an origin of replication (prokaryotic or

eukaryotic). A plasmid may also include one or more selectable marker genes and other genetic elements known in the art. Circular and linear forms of plasmids are encompassed in the present disclosure.

In a further aspect, the present invention relates to an *in vivo* expression vector
5 comprising a polynucleotide sequence, which contains and expresses *in vivo* in a host the salivary *Lu. longipalpis* polypeptides and/or variants or fragments thereof.

The *in vivo* expression vector may include any transcription unit containing a polynucleotide or a gene of interest and those essential elements for its *in vivo* expression. These expression vectors may be plasmids or recombinant viral vectors. For *in vivo* expression, the
10 promoter may be of viral or cellular origin. In one embodiment, the promoter may be the cytomegalovirus (CMV) early promoter (CMV-IE promoter), the SV40 virus early or late promoter or the Rous Sarcoma virus LTR promoter, a promoter of a cytoskeleton gene, such as the desmin promoter (Kwissa M. *et al.*), or the actin promoter (Miyazaki J. *et al.*). When several genes are present in the same plasmid, they may be provided in the same transcription unit or in
15 different units.

As used herein, the term "plasmid" may include any DNA transcription unit comprising a polynucleotide according to the invention and the elements necessary for its *in vivo* expression in a cell or cells of the desired host or target; and, in this regard, it is noted that a supercoiled or non-supercoiled, circular plasmid, as well as a linear form, are intended to be within the scope of
20 the invention. The plasmids may also comprise other transcription-regulating elements such as, for example, stabilizing sequences of the intron type. In several embodiments, the plasmids may include the first intron of CMV-IE (WO 89/01036), the intron II of the rabbit beta-globin gene (van Ooyen *et al.*), the signal sequence of the protein encoded by the tissue plasminogen activator (tPA; Montgomery *et al.*), and/or a polyadenylation signal (polyA), in particular the
25 polyA of the bovine growth hormone (bGH) gene (US 5,122,458) or the polyA of the rabbit beta-globin gene or of SV40 virus.

In a further aspect, the present invention relates to a vaccine composition comprising: a) an *in vivo* expression vector, wherein the vector comprises a polynucleotide encoding one or more polypeptide selected from the group consisting of a salivary *Lu. longipalpis* polypeptide, a
30 variant or fragment of the salivary *Lu. longipalpis* polypeptide, and a mixture thereof; and b) a pharmaceutically or veterinary acceptable vehicle, diluent or excipient.

In another aspect, the present invention relates to a vaccine composition comprising: a) a first *in vivo* expression vector, wherein the vector comprises a polynucleotide encoding one or more polypeptide selected from the group consisting of a salivary *Lu. longipalpis* polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, and a mixture thereof; b) a
5 second *in vivo* expression vector, wherein the vector comprises a polynucleotide encoding one or more polypeptide selected from the group consisting of a salivary *Lu. longipalpis* polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, and a mixture thereof; and c) a pharmaceutically or veterinary acceptable vehicle, diluent or excipient.

The term “vaccine composition” or “vaccine” comprises any composition, once it has
10 been injected to a host, including canines, felines and humans, that protects the host from cutaneous leishmaniasis and/or visceral leishmaniasis, and/or which may prevent implantation of the parasite, and/or which may prevent disease progression in infected subjects, and/or which may limit the diffusion of runaway parasites to internal organs, and/or which may avoid or limit parasite uptake by a sand fly during a blood meal on a vaccinated dog. This may be
15 accomplished upon vaccination according to the present invention through the induction of cytokine secretion, notably IFN-gamma secretion (as example of a method of measurement of IFN-gamma secretion, the Quantikine® immunoassay from R&D Systems Inc. (catalog number# CAIF00) could be used (Djoba Siawaya JF *et al.*)).

The pharmaceutically acceptable vehicles or excipients of use are conventional.
20 *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the polypeptides, plasmids, viral vectors herein disclosed. In general, the nature of the vehicle or excipient will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and
25 physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, freeze-dried pastille, powder, pill, tablet, or capsule forms), conventional non-toxic solid vehicles or excipients can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral vehicles or excipients, immunogenic
30 compositions to be administered can contain minor amounts of non-toxic auxiliary substances,

such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

The vaccines according to the instant invention may include vectors encoding any polynucleotide according to the present invention as described above.

5 Multiple insertions may be done in the same vector using different insertion sites or using the same insertion site. When the same insertion site is used, each polynucleotide insert, which may be any polynucleotide of the present invention aforementioned, may be inserted under the control of the same and/or different promoters. The insertion can be done tail-to-tail, head-to-head, tail-to-head, or head-to-tail. IRES elements (Internal Ribosome Entry Site, see EP
10 0803573) can also be used to separate and to express multiple inserts operably linked to the same and/or different promoters.

In one embodiment, the present invention relates to an expression vector comprising a polynucleotide aforementioned. The expression vector may be an *in vivo* expression vector, or an *in vitro* expression vector.

15 More generally, the present invention encompasses *in vivo* expression vectors including any plasmid (EP-A2-1001025; Chaudhuri P.) containing and expressing *in vivo* in a host the polynucleotide or gene of *Lu. longipalpis* salivary polypeptide, variant thereof or fragment thereof and elements necessary for its *in vivo* expression.

In a specific, non-limiting example, the pVR1020 or pVR1012 plasmid (VICAL Inc.;
20 Luke C. *et al.*; Hartikka J. *et al.*), pVR2001-TOPA (or pVR2001-TOPO) (Oliveira F. *et al.*) or pAB110 (US 6,852,705) can be utilized as a vector for the insertion of a polynucleotide sequence. The pVR1020 plasmid is derived from pVR1012 and contains the human tPA signal sequence. The pVR1020 is a plasmid backbone available from Vical, Inc., (San Diego, CA) which has been previously used, see, e.g., US Patent Nos. 6,451,769 and 7,078,507. As described
25 in Oliveira *et al.*, plasmid pVR2001-TOPO (or pVR2001-TOPA) is pVR1020 modified by the addition of topoisomerases flanking the cloning site and containing coding for and expressing a signal secretory peptide, for example, tissue plasminogen activator signal peptide (tPA), that increases the likelihood of producing a secreted protein, (see Figure 1 in Oliveira F. *et al.*).

Each plasmid may comprise or contain or consist essentially of, the polynucleotide
30 according to the present invention, operably linked to a promoter or under the control of a promoter or dependent upon a promoter, wherein the promoter may be advantageously adjacent

to the polynucleotide for which expression is desired. In general, it is advantageous to employ a strong promoter that is functional in eukaryotic cells. One example of a useful promoter may be the immediate early cytomegalovirus promoter (CMV-IE) of human or murine origin, or it may optionally have another origin such as from rat or guinea pig. The CMV-IE promoter may
5 comprise the actual promoter part, which may or may not be associated with the enhancer part. Reference can be made to EP 260 148, EP 323 597, US 5,168,062, 5,385,839, and 4,968,615, as well as to WO 87/03905. The CMV-IE promoter may advantageously be a human CMV-IE (Boshart M. *et al.*) or murine CMV-IE. In more general terms, the promoter may have either a viral or a cellular origin. A strong viral promoter other than CMV-IE that may be usefully
10 employed in the practice of the invention is the early/late promoter of the SV40 virus or the LTR promoter of the Rous sarcoma virus. A strong cellular promoter that may be usefully employed in the practice of the invention is the promoter of a gene of the cytoskeleton, such as the desmin promoter (Kwissa M. *et al.*), or the actin promoter (Miyazaki J. *et al.*). Functional sub fragments of these promoters, i.e., portions of these promoters that maintain adequate promoter activity, are
15 included within the present invention, e.g. truncated CMV-IE promoters according to WO 98/00166 or US 6,156,567 and may be used in the practice of the invention. A promoter useful in the practice of the invention consequently may include derivatives and/or sub fragments of a full-length promoter that maintain adequate promoter activity and hence function as a promoter, and which may advantageously have promoter activity that is substantially similar to that of the
20 actual or full-length promoter from which the derivative or sub fragment is derived, e.g., akin to the activity of the truncated CMV-IE promoters of US 6,156,567 in comparison to the activity of full-length CMV-IE promoters. Thus, a CMV-IE promoter in the practice of the invention may comprise or consist essentially of or consist of the promoter portion of the full-length promoter and/or the enhancer portion of the full-length promoter, as well as derivatives and/or sub
25 fragments thereof.

Advantageously, the plasmids comprise or consist essentially of other expression control elements. It is especially advantageous to incorporate stabilizing sequence(s), e.g., intron sequence(s), for example, the first intron of the hCMV-IE (WO 89/01036), the intron II of the rabbit β -globin gene (van Ooyen *et al.*). As to the polyadenylation signal (polyA) for the
30 plasmids and viral vectors other than poxviruses, use can be made of the poly(A) signal of the

bovine growth hormone (bGH) gene (*see* US 5,122,458), or the poly(A) signal of the rabbit β -globin gene or the poly(A) signal of the SV40 virus.

In one embodiment of the present invention, the plasmid vector is pVR2001 comprising LJM17 polynucleotide, as described in example 1 herein.

5 In another embodiment of the present invention, the plasmid vector is pVR2001 comprising LJM143 polynucleotide, as described in example 2 herein.

In another embodiment of the present invention, the plasmid vector is pNBO002, as described in example 9 herein.

10 In yet another embodiment of the present invention, the plasmid vector is pNBO003, as described in example 10 herein.

More generally, the present invention encompasses *in vivo* expression vectors including any recombinant viral vector containing a polynucleotide or gene encoding one or more salivary *Lu. longipalpis* immunogens and/or variants or fragments thereof, including any elements necessary for its *in vivo* expression.

15 Said recombinant viral vectors could be selected from, for example, the poxviruses, especially avipox viruses, such as fowlpox viruses or canarypox viruses. In one embodiment, the fowlpox virus is a TROVAC (*see* WO 96/40241). In another embodiment, the canarypox vector is an ALVAC. The use of these recombinant viral vectors and the insertion of polynucleotides or genes of interest are fully described in US 5,174,993; US 5,505,941 and US 5,766,599 for
20 fowlpox, and in US 5,756,103 for canarypox. More than one insertion site inside the viral genome could be used for the insertion of multiple genes of interest.

In one embodiment the viral vector is an adenovirus, such as a human adenovirus (HAV) or a canine adenovirus (CAV).

25 In another embodiment the viral vector is a human adenovirus, specifically a serotype 5 adenovirus, rendered incompetent for replication by a deletion in the E1 region of the viral genome, especially from about nucleotide 459 to about nucleotide 3510 by reference to the sequence of the hAd5 disclosed in Genbank under the accession number M73260 and in the referenced publication Chroboczek et al, 1992. The deleted adenovirus is propagated in E1-expressing 293 (Graham *et al.*, 1977) or PER cells, especially PER.C6 (Falloux *et al.*, 1998). The
30 human adenovirus can additionally or alternatively be deleted in the E3 region, especially from about nucleotide 28592 to about nucleotide 30470. The deletion in the E1 region can be done in

combination with a deletion in the E3 region (see, e.g. Shriver *et al.*; Graham *et al.*; Ilan *et al.*; U.S. Patent Nos. 6,133,028 and 6,692,956; Tripathy *et al.*; Tapnell; Danthinne *et al.*; Berkner; Berkner *et al.*; Chavier *et al.*). The insertion sites can be the E1 and/or E3 loci (region) eventually after a partial or complete deletion of the E1 and/or E3 regions. Advantageously, when the expression vector is an adenovirus, the polynucleotide to be expressed is inserted under the control of a promoter functional in eukaryotic cells, such as a strong promoter, advantageously a cytomegalovirus immediate-early gene promoter (CMV-IE promoter), especially the enhancer / promoter region from about nucleotide -734 to about nucleotide +7 in Boshart *et al.*, or the enhancer / promoter region from the pCI vector from Promega Corp. The CMV-IE promoter is advantageously of murine or human origin. The promoter of the elongation factor 1 α can also be used. A muscle specific promoter can also be used (Li *et al.*). Strong promoters are also discussed herein in relation to plasmid vectors. In one embodiment, a splicing sequence can be located downstream of the enhancer / promoter region. For example, the intron 1 isolated from the CMV-IE gene (Stenberg *et al.*), the intron isolated from the rabbit or human β -globin gene, especially the intron 2 from the β -globin gene, the intron isolated from the immunoglobulin gene, a splicing sequence from the SV40 early gene or the chimeric intron sequence isolated from the pCI vector from Promega Corp. A poly(A) sequence and terminator sequence can be inserted downstream the polynucleotide to be expressed, e.g. a bovine growth hormone gene, especially from about nucleotide 2339 to about nucleotide 2550 in Genbank under the accession number BOVGHRH, a rabbit β -globin gene or a SV40 late gene polyadenylation signal.

In another embodiment the viral vector is a canine adenovirus, especially a CAV-2 (see, e.g. Fischer *et al.*; U.S. Patent Nos. 5,529,780 and 5,688,920; WO 95/14102). For CAV, the insertion sites can be in the E3 region and /or in the region located between the E4 region and the right ITR region (see U.S. Patent Nos. 6,090,393 and 6,156,567). In one embodiment the insert is under the control of a promoter, such as a cytomegalovirus immediate-early gene promoter (CMV-IE promoter) or a promoter already described for a human adenovirus vector. A poly(A) sequence and terminator sequence can be inserted downstream the polynucleotide to be expressed, e.g. a bovine growth hormone gene or a rabbit β -globin gene polyadenylation signal.

In another embodiment, the viral vector is a herpesvirus such as a canine herpesvirus (CHV) or a feline herpesvirus (FHV). For CHV, the insertion sites may be in the thymidine

kinase gene, in the ORF3, or in the UL43 ORF (see US 6,159,477). In one embodiment the polynucleotide to be expressed is inserted under the control of a promoter functional in eukaryotic cells, advantageously a CMV-IE promoter (murine or human). A poly(A) sequence and terminator sequence can be inserted downstream the polynucleotide to be expressed, e.g. bovine growth hormone or a rabbit β -globin gene polyadenylation signal.

For recombinant vectors based on a poxvirus vector, a vaccinia virus or an attenuated vaccinia virus, (for instance, MVA, a modified Ankara strain obtained after more than 570 passages of the Ankara vaccine strain on chicken embryo fibroblasts; see Stickl & Hochstein-Mintzel; Sutter *et al.*; available as ATCC VR-1508; or NYVAC, see US 5,494,807, and U.S. Patent No. 5,494,807 which discuss the construction of NYVAC, as well as variations of NYVAC with additional ORFs deleted from the Copenhagen strain vaccinia virus genome, as well as the insertion of heterologous coding nucleic acid molecules into sites of this recombinant, and also, the use of matched promoters; see also WO 96/40241), an avipox virus or an attenuated avipox virus (e.g., canarypox, fowlpox, dovepox, pigeonpox, quailpox, ALVAC or TROVAC; see, e.g., U.S. Patent Nos. 5,505,941, 5,494,807) can be used. Attenuated canarypox viruses are described in US 5,756,103 (ALVAC) and WO 01/05934. Reference is also made to US 5,766,599 which pertains to the attenuated fowlpox strain TROVAC. Reference is made to the canarypox available from the ATCC under access number VR-111. Numerous fowlpox virus vaccination strains are also available, e.g. the DIFTOSECT strain marketed by Merial and the NOBILIS VARIOLE vaccine marketed by Intervet. For information on the method used to generate recombinants thereof and how to administer recombinants thereof, the skilled artisan can refer documents cited herein and to WO 90/12882, e.g., as to vaccinia virus, mention is made of U.S. Patents Nos. 4,769,330, 4,722,848, 4,603,112, 5,110,587, 5,494,807, and 5,762,938 *inter alia*; as to fowlpox, mention is made of U.S. Patents Nos. 5,174,993, 5,505,941 and 5,766,599 *inter alia*; as to canarypox, mention is made of U.S. Patent No. 5,756,103 *inter alia*. When the expression vector is a vaccinia virus, insertion site or sites for the polynucleotide or polynucleotides to be expressed are advantageously at the thymidine kinase (TK) gene or insertion site, the hemagglutinin (HA) gene or insertion site, the region encoding the inclusion body of the A type (ATI); see also documents cited herein, especially those pertaining to vaccinia virus. In the case of canarypox, advantageously the insertion site or sites are ORF(s) C3, C5 and/or C6; see also documents cited herein, especially those pertaining to canarypox virus. In the case of fowlpox,

advantageously the insertion site or sites are ORFs F7 and/or F8; see also documents cited herein, especially those pertaining to fowlpox virus. The insertion site or sites for MVA virus are advantageously as in various publications, including Carroll M. W. *et al.*; Stittelaar K. J. *et al.*; Sutter G. *et al.*; and, in this regard it is also noted that the complete MVA genome is
5 described in Antoine G., Virology, which enables the skilled artisan to use other insertion sites or other promoters. Advantageously, the polynucleotide to be expressed is inserted under the control of a specific poxvirus promoter, e.g., the vaccinia promoter 7.5 kDa (Cochran *et al.*), the vaccinia promoter I3L (Riviere *et al.*), the vaccinia promoter HA (Shida), the cowpox promoter ATI (Funahashi *et al.*), the vaccinia promoter H6 (Taylor J. *et al.*; Guo P. *et al.* J.; Perkus M. *et al.*), *inter alia*.
10

In a further embodiment, the recombinant viral vector is the recombinant ALVAC canarypox virus vCP2390-SEQ ID NO:6, expressing the *Lu. longipalpis* salivary LJM17 polypeptide, as described in example 3.

In a further embodiment, the recombinant viral vector is the recombinant ALVAC
15 canarypox virus vCP2390, expressing the *Lu. longipalpis* salivary LJM17 polypeptide, as described in example 11.

In a further embodiment, the recombinant viral vector is the recombinant ALVAC canarypox virus vCP2389-SEQ ID NO:2, expressing the *Lu. longipalpis* salivary LJL143 polypeptide, as described in example 4.

In a further embodiment, the recombinant viral vector is the recombinant ALVAC
20 canarypox virus vCP2389, expressing the *Lu. longipalpis* salivary LJL143 polypeptide, as described in the example 12.

In a further embodiment, the recombinant viral vector is the recombinant MVA virus MVA-LJM17, expressing the *Lu. longipalpis* salivary LJM17 polypeptide, as described in
25 example 5.

In a further embodiment, the recombinant viral vector is the recombinant MVA virus MVA-LJL143, expressing the *Lu. longipalpis* salivary LJL143 polypeptide, as described in the example 6.

Any of the polynucleotides disclosed here may be expressed *in vitro* by DNA transfer or
30 expression vectors into a suitable host cell. The host cell may be prokaryotic or eukaryotic. The term "host cell" also includes any progeny of the subject host cell. Methods of stable transfer,

meaning that the foreign polynucleotide is continuously maintained in the host cell, are known in the art. Host cells may include bacteria (for example, *Escherichia coli*), yeast, insect cells, and vertebrate cells. Methods of expressing DNA sequences in eukaryotic cells are well known in the art. As a method for *in vitro* expression, recombinant Baculovirus vectors (for example,

5 Autographa California Nuclear Polyhedrosis Virus (AcNPV)) may be used with the nucleic acids disclosed herein. For example, polyhedrin promoters may be utilized with insect cells (for example, *Spodoptera frugiperda* cells, like Sf9 cells available at the ATCC under the Accession number CRL 1711, or Sf21 cells) (see for example, Smith *et al.*; Pennock *et al.*; Vialard *et al.*; Verne A.; O'Reilly *et al.*; Kidd I. M. & Emery V.C.; EP 0370573; EP 0265785; US 4,745,051).

10 For expression, the BaculoGold Starter Package (Cat # 21001K) from Pharmingen (Becton Dickinson) may be used. As a method for *in vitro* expression, recombinant *E. coli* may be used with a vector. For example, when cloning in bacterial systems, inducible promoters such as arabinose promoter, pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter), and the like may be used. Transformation of a host cell with recombinant DNA may be carried out by

15 conventional techniques are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl₂ method using procedures well known in the art. Alternatively, MgCl₂ or RbCl can be used. Transformation can also be performed by electroporation. When the host is a eukaryote, such methods of

20 transduction of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells may also be cotransformed with *L. longipalpis* polynucleotide sequences, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector (see above), such as a herpes virus or adenovirus (for example, canine adenovirus 2), to transiently transduce eukaryotic cells and express the protein (Gluzman EA). In addition, a transfection agent can be utilized, such as dioleoyl-phosphatidyl-ethanolamine (DOPE).

25 Isolation and purification of recombinantly expressed polypeptide may be carried out by conventional means including preparative chromatography (for example, size exclusion, ion exchange, affinity), selective precipitation and ultra-filtration. Examples of state of the art techniques that can be used, but not limited to, may be found in "Protein Purification

Applications”, Second Edition, Edited by Simon Roe and available at Oxford University Press. Such a recombinantly expressed polypeptide is part of the present disclosure. The methods for production of any polypeptide according to the present invention as described above are also encompassed, in particular the use of a recombinant expression vector comprising a
5 polynucleotide according to the disclosure and of a host cell.

The vaccines containing recombinant viral vectors according to the invention may be freeze-dried, advantageously with a stabilizer. Freeze-drying can be done according to well-known standard freeze-drying procedures. The pharmaceutically or veterinary acceptable stabilizers may be carbohydrates (e.g. sorbitol, mannitol, lactose, sucrose, glucose, dextran,
10 trehalose), sodium glutamate (Tsvetkov T *et al.*; Israeli E *et al.*), proteins such as peptone, albumin, lactalbumin or casein, protein containing agents such as skimmed milk (Mills C K *et al.*; Wolff E *et al.*), and buffers (e.g. phosphate buffer, alkaline metal phosphate buffer). An adjuvant may be used to make soluble the freeze-dried preparations.

Any vaccine composition according to the invention can also advantageously contain one
15 or more adjuvant.

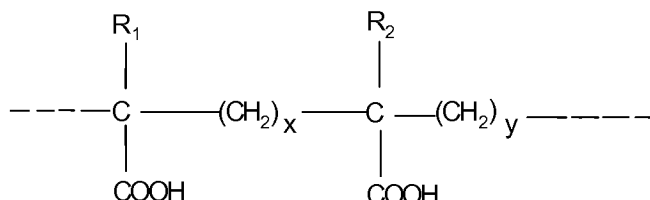
The plasmid-based vaccines may be formulated with cationic lipids, advantageously with DMRIE (N-(2-hydroxyéthyl)-N,N-diméthyl-2,3-bis(tétradécyloxy)-1-propanammonium ; WO96/34109), and advantageously in association with a neutral lipid, for example DOPE (dioleoyl-phosphatidyl-ethanolamine ; Behr J. P.), in order to form DMRIE-DOPE. In one
20 embodiment, the mixture is made extemporaneously, and before its administration it is advantageous to wait about 10 min to about 60 min, for example, about 30 min, for the appropriate complexation of the mixture. When DOPE is used, the molar ratio of DMRIE/DOPE can be from 95/5 to 5/95 and is advantageously 1/1. The weight ratio plasmid/DMRIE or DMRIE-DOPE adjuvant is, for example, from 50/1 to 1/10, from 10/1 to 1/5 or from 1/1 to 1/2.

25 Optionally a cytokine may be added to the composition, especially GM-CSF or cytokines inducing Th1 (e.g. IL12). These cytokines can be added to the composition as a plasmid encoding the cytokine protein. In one embodiment, the cytokines are from canine origin, e.g. canine GM-CSF which gene sequence has been deposited at the GenBank database (accession number S49738). This sequence can be used to create said plasmid in a manner similar to what
30 was made in WO 00/77210.

The recombinant viral vector-based vaccine may be combined with fMLP (N-formyl-

methionyl-leucyl-phenylalanine; US 6,017,537) and/or Carbomer adjuvant (Phameuropa Vol. 8, No. 2, June 1996). Persons skilled in the art can also refer to US 2,909,462, which describes such acrylic polymers cross-linked with a polyhydroxylated compound having at least 3 hydroxyl groups, advantageously not more than 8, the hydrogen atoms of at least three hydroxyls being replaced by unsaturated aliphatic radicals having at least 2 carbon atoms. For example, the radicals are those containing from 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals may themselves contain other substituents, such as methyl. The products sold under the name Carbopol® (BF Goodrich, Ohio, USA) are appropriate. The products are cross-linked with an allyl sucrose or with allyl pentaerythritol.

Among them, there may be advantageously mentioned Carbopol® 974P, 934P and 971P.



Among the copolymers of maleic anhydride and alkenyl derivative, the copolymers EMA® (Monsanto) which are copolymers of maleic anhydride and ethylene, linear or cross-linked, for example cross-linked with divinyl ether, are advantageous. Reference may be made to J. Fields *et al.*.

The polymers of acrylic or methacrylic acid and the copolymers EMA® are formed, for example, of basic units of the following formula in which:

- R₁ and R₂, which are identical or different, represent H or CH₃
- x = 0 or 1, preferably x = 1
- y = 1 or 2, with x + y = 2

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

The dissolution of these polymers in water leads to an acid solution, which is neutralized, advantageously to physiological pH, in order to provide the adjuvant solution into which the vaccine itself is incorporated. The carboxyl groups of the polymer are then partly in COO⁻ form.

In one embodiment, a solution of adjuvant, especially of carbomer (*Pharmeuropa*, vol. 8, No.2, June 1996), is prepared in distilled water, advantageously in the presence of sodium chloride, the solution obtained being at an acidic pH. This stock solution is diluted by adding it to the desired quantity (for obtaining the desired final concentration), or a substantial part thereof, of water charged with NaCl, advantageously physiological saline (NaCl 9 g/l) all at once in several portions with concomitant or subsequent neutralization (pH 7.3 to 7.4), advantageously with NaOH. This solution at physiological pH is used for mixing with the vaccine, which may be especially stored in freeze-dried, liquid or frozen form.

The polymer concentration in the final vaccine composition can be from 0.01% to 2% w/v, from 0.06 to 1% w/v, or from 0.1 to 0.6% w/v.

The sub-unit vaccine may be combined with adjuvants, like oil-in-water, water-in-oil-in-water emulsions based on mineral oil and/or vegetable oil and non ionic surfactants such as block copolymers, Tween®, Span®. Such emulsions are notably those described in page 147 of “Vaccine Design – The Subunit and Adjuvant Approach”, Pharmaceutical Biotechnology, 1995, or TS emulsions, notably the TS6 emulsion, and LF emulsions, notably LF2 emulsion (for both TS and LF emulsions, see WO 04/024027). Other suitable adjuvants are for example vitamin E, saponins, and Carbopol® (Noveon; see WO 99/51269; WO 99/44633), aluminium hydroxide or aluminium phosphate (“Vaccine Design, The subunit and adjuvant approach”, Pharmaceutical Biotechnology, vol. 6, 1995), biological adjuvants (i.e. C4b, notably murine C4b (Ogata R T *et al.*) or equine C4b, GM-CSF, notably equine GM-CSF (US 6,645,740)), toxins (i.e. cholera toxins CTA or CTB, *Escherichia coli* heat-labile toxins LTA or LTB (Olsen C W *et al.*; Fingerut E *et al.*; Zurbriggen R *et al.* Peppoloni S *et al.*), and CpG (i.e. CpG #2395 (see Jurk M *et al.*), CpG #2142 (see SEQ. ID. NO: 890 in EP 1,221,955)).

The vaccine may also may also contain or comprise one or more *Leishmania* antigens, for example, kinetoplastid membrane protein 11 (KMP11).

Leishmania KMP11 antigens are derived from, for example, *L. infantum* or *L. chagasi*. KMP11 is a highly conserved surface membrane protein present in all members of the family Kinetoplastidae, and is differentially expressed both in amastigote and promastigote forms of *Leishmania* (Jardim A. *et al.*; Jardim A. *et al.*; Berberich C. *et al.*). The nucleic acid sequence of the gene and the amino acid sequence of the protein KMP11 of *Leishmania* are available in public databases, notably as *L. infantum* in the GenBank database under the accession numbers

X95627 and X95626. The nucleic acid sequence of *L. donovani* is also available from the GenBank database, notably under the accession number S77039.

The state of the art regarding KMP11, vectors expressing KMP11 and vaccines are best summarized in patent application WO 08/064181. A plasmid-based vaccine comprising
5 pVR1020 KMP11 is described in example 1 and the canarypox virus vector-based vaccine comprising vCP2350 is described in example 3. WO 08/064181 also gives information regarding adjuvants, formulation, doses and route of administration.

KMP11 polypeptides and variants or fragments thereof may be produced, isolated and purified in the same manner set forth for *in vitro* expression of sand fly salivary polypeptides.

10 In one embodiment, the vaccine comprises *Lu. longipalpis* salivary polypeptides and/or variants or fragments thereof, and/or vectors comprising a polynucleotide encoding the *Lu. longipalpis* polypeptides and/or variants or fragments thereof, and/or vectors comprising the KMP11 polynucleotide encoding the KMP11 polypeptide and/or fragments or variants thereof from *Leishmania*. In one specific, non-limiting example of this embodiment, the *Lu. longipalpis*
15 salivary polypeptide includes a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, or 87. In another specific, non-limiting example of this embodiment, the polynucleotide encodes a
20 *Lu. longipalpis* salivary polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, or 87. In yet another specific, non-limiting example, the polynucleotide has at least 70%, at least 75%, at
25 least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% to a polynucleotide having a sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 12, 14, 16, 18, 21, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 89, 90, or 91.

In a particular embodiment, the vaccine comprises *Lu. longipalpis* salivary LJM17
30 polypeptides and/or variants or fragments thereof, and/or vectors comprising the polynucleotide encoding the *Lu. longipalpis* LJM17 polypeptide and/or variants or fragments thereof, and/or

vectors comprising the KMP11 polynucleotide encoding the KMP11 polypeptide and/or fragments or variants thereof from *Leishmania*. For example, the vectors for LJM17 may be selected from the group consisting of pVR2001 LJM17, pNBO002, vCP2390, vCP2390-SEQ ID NO:6 and MVA-LJM17. The vectors for KMP11 may be selected from the group consisting of
5 pVR1020 KMP11 and vCP2350. In one embodiment, the vaccine comprises plasmids pVR2001 LJM17 and pVR1020 KMP11. In another embodiment, the vaccine comprises vectors vCP2390 and vCP2350. In yet another embodiment, the vaccine comprises plasmids pNBO002 and pVR1020 KMP11. In yet another embodiment, the vaccine comprises vCP2390-SEQ ID NO:6 and vCP2350.

10 In another particular embodiment, the vaccine comprises *Lu. longipalpis* salivary LJL143 polypeptides and/or variants or fragments thereof, and/or vectors comprising the polynucleotide encoding the *Lu. longipalpis* LJL143 polypeptide and/or variants or fragments thereof, and/or vectors comprising the polynucleotide encoding *Leishmania* KMP11 polypeptides and/or variants or fragments thereof. For example, the vectors for LJL143 may be selected from the
15 group consisting of pVR2001 LJL143, pNBO003, vCP2389, vCP2389-SEQ ID NO:2 and MVA-LJL143. The vectors for KMP11 may be selected from the group consisting of pVR1020 KMP11 and vCP2350. In one embodiment, the vaccine comprises plasmids pVR2001 LJL143 and pVR1020 KMP11. In another embodiment, the vaccine comprises vectors vCP2389 and vCP2350. In yet another embodiment, the vaccine comprises plasmids pNBO003 and pVR1020
20 KMP11. In yet another embodiment, the vaccine comprises vectors vCP2389-SEQ ID NO:2 and vCP2350.

In another particular embodiment, the vaccine comprises *Lu. longipalpis* salivary LJL143 polypeptides, variants thereof, fragments thereof, and/or vectors comprising the polynucleotide encoding the *Lu. longipalpis* LJL143 polypeptide and/or variants or fragments thereof, and/or
25 *Lu. longipalpis* salivary LJM17 polypeptides and/or variants or fragments thereof, and/or vectors comprising the LJM17 polynucleotide encoding the *Lu. Longipalpis* LJM17 polypeptide and/or variants or fragments thereof, and/or *Leishmania* KMP11 polypeptides and/or variants or fragments thereof, and/or vectors comprising the KMP11 polynucleotide or gene. For example, the vectors for LJL143 may be selected from the group consisting of pVR2001 LJL143,
30 pNBO003, vCP2389, vCP2389-SEQ ID NO:2 and MVA-LJL143. The vectors for LJM17 may be selected from the group consisting of pVR2001 LJM17, pNBO002, vCP2390, vCP2390-SEQ

ID NO:6 and MVA-LJM17. The vectors for KMP11 may be selected from the group consisting of pVR1020 KMP11 and vCP2350. In one embodiment, the vaccine comprises plasmids pVR2001 LJM143, pVR2001 LJM17 and pVR1020 KMP11. In another embodiment, the vaccine comprises vectors vCP2389, vCP2390 and vCP2350. In yet another embodiment, the vaccine comprises plasmids pNBO003, pNBO002 and pVR1020 KMP11. In another embodiment, the vaccine comprises vCP2389-SEQ ID NO:2, vCP2390-SEQ ID NO:6 and vCP2350 vectors.

The vaccine may also be associated with at least one *Leishmania* antigen, for example inactivated *Leishmania*.

In a particular embodiment, the *Leishmania* strain may be *Leishmania infantum*, and/or *Leishmania braziliensis*. In a preferred embodiment, the *Leishmania* strain may be *Leishmania braziliensis*.

These strains of *Leishmania* may be inactivated by chemical or physical methods. The chemical methods are notably BPL, formaldehyde. The physical methods may notably be sonication. One method for inactivating *Leishmania* for use in a vaccine is described in R.

Cordeiro Giunchetti *et al.*, Vaccine, 2007. The promastigotes are cultivated in NNN/LIT medium for 6 to 14 days, but more preferably 10 days, until the differentiation between promastigote procyclic form into promastigote metacyclic form is achieved on the basis of a microscopic observation. The culture can then be harvested by centrifugation (2000Xg, 20minutes, 4°C). When applicable, the supernatant is discarded and the biomass is washed three times in saline buffer. Whether the culture is clarified or not, the promastigote suspension (i.e. crude culture or promastigote resuspended in saline buffer after centrifugation) is subsequently disrupted by ultrasound treatment using a power from 10 to 375W, but more preferably 40W, for 1 minute, at 0°C. The batch volume for this treatment is between 5 and 150 mL, preferably 30 mL. After treatment the lysate can be stored at -80°C.

The vaccine formulation may be prepared from the protein concentrate that is obtained following cell lysis. The cell lysate protein quantity that may be used for vaccination of a canine is from about 50 µg to about 2000 µg, preferably from about 50 µg to about 600 µg. Protein concentration is determined according to the method of Lowry.

The inactivated *Leishmania* vaccine may be combined with adjuvants, like those described previously for sub-unit vaccines.

In one embodiment, the vaccine comprises *Lu. longipalpis* salivary polypeptides and/or variants or fragments thereof, and/or vectors comprising the *Lu. longipalpis* salivary polynucleotide and/or variants or fragments thereof, and/or inactivated *Leishmania*. In one specific, non-limiting example of this embodiment, the *Lu. longipalpis* salivary polypeptide includes a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, or 87. In another specific, non-limiting example of this embodiment, the polynucleotide encodes a *Lu. longipalpis* salivary polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, or 87. In yet another specific, non-limiting example, the polynucleotide has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% to a polynucleotide having a sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 12, 14, 16, 18, 21, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 89, 90, or 91.

In a particular embodiment, the vaccine comprises *Lu. longipalpis* salivary LJM17 polypeptides and/or variants or fragments thereof, and/or vectors comprising the LJM17 polynucleotide and/or variants or fragments thereof, and/or inactivated *Leishmania*. For example, the vectors for LJM17 may be selected among the group consisting of pVR2001 LJM17, pNBO002, vCP2390, vCP2390-SEQ ID NO:6 and MVA-LJM17, and may be combined with inactivated *Leishmania* selected from the group consisting of sonicated inactivated *Leishmania infantum*, and/or *Leishmania braziliensis*. For example, in one embodiment, the vaccine comprises the vCP2390 vector and sonicated inactivated *Leishmania braziliensis*. In another embodiment, the vaccine comprises the vCP2390-SEQ ID NO:6 vector and sonicated inactivated *Leishmania braziliensis*.

In another particular embodiment, the vaccine comprises *Lu. longipalpis* salivary LJL143 polypeptides and/or variants or fragments thereof, and/or vectors comprising the LJL143 polynucleotide and/or variants or fragments thereof, and/or inactivated *Leishmania*. For example,

the vectors for LJL143 may be selected from the group consisting of pVR2001 LJL143, pNBO003, vCP2389, vCP2389-SEQ ID NO:2 and MVA-LJL143. The inactivated *Leishmania* may be selected from the group consisting of sonicated inactivated *Leishmania infantum*, and/or *Leishmania braziliensis*. For example, in one embodiment, the vaccine comprises the vCP2389
5 vector and sonicated inactivated *Leishmania braziliensis*. In another embodiment, the vaccine comprises the vCP2389-SEQ ID NO:2 vector and sonicated inactivated *Leishmania braziliensis*.

In another particular embodiment, the vaccine comprises *Lu. longipalpis* salivary LJL143 polypeptides and/or variants or fragments thereof, and/or vectors comprising the LJL143 polynucleotide and/or variants or fragments thereof, and/or *Lu. longipalpis* salivary LJM17
10 polypeptides and/or variants or fragments thereof, and/or vectors comprising the LJM17 polynucleotide and/or variants or fragments thereof, and/or inactivated *Leishmania*. For example, the vectors could be selected for LJL143 from the group consisting of pVR2001 LJL143, pNBO003, vCP2389, vCP2389-SEQ ID NO:2 and MVA-LJL143. For LJM17, the vectors may be selected from the group consisting of pVR2001 LJM17, pNBO002, vCP2390, vCP2390-SEQ
15 ID NO:6 and MVA-LJM17. The inactivated *Leishmania* may be selected from the group consisting of sonicated inactivated *Leishmania infantum*, and/or *Leishmania braziliensis*. In one embodiment, the vaccine comprises vCP2389 and vCP2390 vectors and sonicated inactivated *Leishmania braziliensis*. In another embodiment, the vaccine comprises vCP2389-SEQ ID NO:2 and vCP2390-SEQ ID NO:6 vectors and sonicated inactivated *Leishmania braziliensis*.

20 Another aspect of the present invention relates to methods of vaccinating a host against *Leishmania* using the vaccine compositions disclosed herein.

The host may be any one or all of humans, felines (for example, domesticated cats, kittens, big cats and wild cats) and canines (for example, dogs, bitches, puppies, foxes, jackals, and wolves). In one embodiment, the host is a canine.

25 The routes of administration may be, for example, intramuscular (IM) or intradermal (ID) or transdermal (TD) or subcutaneous (SC). The means of administration may be, for example, a syringe with a needle, or needle free apparatus, or a syringe with a needle coupled to electrotransfer (ET) treatment, or needle free apparatus coupled to ET treatment.

Another aspect of the invention relates to the use of a plasmid-based vaccine according to
30 the present invention for administration to *Leishmania*, a host, wherein this administration is coupled to ET treatment. The administration of a plasmid-based vaccine is advantageously

intramuscular. The means of administration is, for example, a syringe and a needle. One or several injections may be administered successively. In the case of several injections, they may be carried out 2 to 6 weeks apart, for example, about 3 weeks apart. In one embodiment, a semi-annual booster or an annual booster is further administered.

5 For plasmid-based vaccines, advantageous routes of administration may be ID or IM. This administration may be through use of a syringe with a needle or with a needle free apparatus like Dermojet or Biojector (Bioject, Oregon, USA) or Vetjet™ (Merial) or Vitajet™ (Bioject Inc.), see US 2006/0034867. The dosage may be from 50 µg to 500 µg per plasmid. When DMRIE-DOPE is added, 100 µg per plasmid may be utilized. When canine GM-CSF or
10 other cytokines are used, the plasmid encoding this protein may be present at a dosage of from about 200 µg to about 500 µg and may advantageously be 200 µg. The volume of doses can be between 0.01 ml and 0.5 ml, for example, 0.25 ml. Administration may be provided with multiple points of injection.

Alternatively, plasmid-based vaccines may be administered via the IM route coupled to
15 electrotransfer (ET) treatment. The ET treatment may be performed using an apparatus for electrotransfer and the specifications of the manufacturer (i.e. Sphergen G250 generator (Sphergen SARL, Evry Genopole, France); MedPulser® DNA electroporation system (Innovio Biomedical Corporation, San Diego, California, USA)). In one embodiment, the apparatus for electrotransfer has a unipolar field. The field intensity may be from about 50 to about 250 V/cm,
20 from about 50 to about 200 V/cm, or from about 50 to about 175 V/cm. The pulse duration may be from about 1 to about 50 msec, or from about 15 to about 25 msec. The frequency may be from about 1 to about 50 Hz, or from about 5 to about 15 Hz. The interpulse interval may be from about 1 to 1000 msec, or from about 1 to about 200 msec. The number of pulses may be from 1 to 20, or from 5 to 10. The intra tissular intensity may advantageously be up to about 2 A.
25 The distance between electrodes may be from about 0.2 to about 1 cm, or from about 0.2 to about 0.5 cm.

For recombinant viral vector-based vaccines, the routes of administration may advantageously be SC or IM or TD or ID. This administration may be made by a syringe with a needle or with a needle free apparatus like Dermojet or Biojector (Bioject, Oregon, USA) or
30 Vetjet™ (Merial) or Vitajet™ (Bioject Inc.). The dosage may be from about 10³ pfu to about 10⁹ pfu per recombinant poxvirus vector. When the vector is a canarypox virus, the dosage may be,

for example, from about 10^5 pfu to about 10^9 pfu, or from about 10^6 pfu to about 10^8 pfu. The volume of doses may be from about 0.01 ml to 0.2 ml, and is advantageously 0.1 ml.

Administration may comprise multiple points of injection.

For the IM route the volume of the vaccine provided may be from 0.2 to 2 ml, in particular from about 0.5 to 1 ml. The same dosages are utilized for any of the vectors of the present invention.

For sub-unit vaccines, the route of administration may advantageously be via SC or IM or TD or ID. This administration may be made by a syringe with a needle or with a needle free apparatus like Dermojet or Biojector (Bioject, Oregon, USA) or Vetjet™ (Merial) or Vitajet™ (Bioject Inc.). The dosage may be from about 50 to about 500 µg, in particular from about 50 to about 150 µg, and more particularly from about 50 to about 100 µg. The volume of the sub-unit vaccine provided is from 0.2 to 2 ml, in particular from about 0.5 to 1 ml.

In another aspect, the present invention relates to a vaccine strategy, which is based on a prime-boost administration regimen, where the primo-administration and the boost administration(s) utilize a composition comprising a pharmaceutically or veterinary acceptable excipient, diluent or vehicle and an *in vivo* expression vector comprising a polynucleotide sequence, that contains and expresses the *Lu. longipalpis* salivary polypeptide and/or variants or fragments thereof.

The present invention relates to the use of *in vivo* expression vectors in a prime-boost administration regimen, comprising a primo-administration of a vaccine comprising a pharmaceutically or veterinary acceptable vehicle, diluent or excipient, an *in vivo* expression vector containing a polynucleotide sequence for expressing, *in vivo*, *Lu. longipalpis* salivary polypeptides and/or variants or fragments thereof, followed by a boost administration of a vaccine comprising a pharmaceutically or veterinary acceptable vehicle or excipient, an *in vivo* expression vector containing a polynucleotide sequence for expressing, *in vivo*, sand fly *Lu. longipalpis* polypeptides and/or variants or fragments thereof as described above, to protect a host from leishmaniasis and/or to prevent disease progression in infected hosts.

A prime-boost regimen comprises at least one primo-administration and at least one boost administration using at least one common polypeptide and/or variants or fragments thereof. The vaccine used in primo-administration may be different in nature from those used as a later

booster vaccine. The primo-administration may comprise one or more administrations. Similarly, the boost administration may comprise one or more administrations.

The routes of administration, doses and volumes are as previously disclosed herein.

The prime-boost administrations may be advantageously carried out 2 to 6 weeks apart, for example, about 3 weeks apart. According to one embodiment, a semi-annual booster or an annual booster, advantageously using the viral vector-based vaccine, is also envisaged. The animals are advantageously at least 6 to 8 weeks old at the time of the first administration.

In a particular embodiment, the prime-boost administration regimen comprises at least one primo-administration of a plasmid-based vaccine according to the present invention and at least one boost-administration of a recombinant viral vector-based vaccine according to the present invention.

In another particular embodiment, the prime-boost administration regimen comprises at least one primo-administration of a recombinant viral vector-based vaccine according to the present invention and at least one boost-administration of a sub-unit vaccine according to the present invention.

In another particular embodiment, the prime-boost administration regimen comprises at least one primo-administration of a recombinant viral vector-based vaccine according to the present invention and at least one boost-administration of a plasmid-based vaccine according to the present invention.

In one embodiment, the present invention relates to a method of vaccinating a subject susceptible to *Leishmania* comprising a prime-boost administration regimen wherein said regiment comprises a primo-administration of a vaccine comprising, in a pharmaceutically or veterinary acceptable vehicle, diluent or excipient, a plasmid containing a polynucleotide for expressing, *in vivo*, a salivary *Lu. longipalpis* polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, or a mixture thereof, followed by a boost administration of a vaccine comprising, in a pharmaceutically or veterinary acceptable vehicle or excipient, a recombinant viral vector comprising a polynucleotide for expressing, *in vivo*, the same salivary *Lu. longipalpis* polypeptide(s), variant thereof, fragment thereof, to protect the subject from leishmaniasis and/or to prevent disease progression in infected subject.

In another embodiment, the present invention relates to a method vaccinating a subject susceptible to *Leishmania* comprising a prime-boost administration regimen wherein said

regiment comprises a primo-administration of a vaccine comprising, in a pharmaceutically or veterinary acceptable vehicle, diluent or excipient, a recombinant viral vector comprising a polynucleotide for expressing, *in vivo*, a salivary *Lu. longipalpis* polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, or a mixture thereof, followed by a boost
5 administration of a vaccine comprising, in a pharmaceutically or veterinary acceptable vehicle or excipient, a plasmid containing a polynucleotide for expressing, *in vivo*, the same salivary *Lu. longipalpis* polypeptide(s), variant thereof, fragment thereof, to protect the subject from leishmaniasis and/or to prevent disease progression in infected subject.

In yet another embodiment, the present invention related to a method of vaccinating a
10 subject susceptible to *Leishmania* comprising a prime-boost administration regimen wherein said regiment comprises a primo-administration of a vaccine comprising, in a pharmaceutically or veterinary acceptable vehicle, diluent or excipient, a recombinant viral vector comprising a polynucleotide for expressing, *in vivo*, a salivary *Lu. longipalpis* polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, or a mixture thereof, followed by a boost
15 administration of a vaccine comprising, in a pharmaceutically or veterinary acceptable vehicle or excipient, the same salivary *Lu. longipalpis* polypeptide(s), variant thereof, fragment thereof, to protect the subject from leishmaniasis and/or to prevent disease progression in infected subject.

In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a pVR2001 LJM17 or pNBO002 plasmid-based vaccine, and at least
20 one boost-administration of a vCP2390 or vCP2390-SEQ ID NO:6 vector-based vaccine.

In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a pVR2001 LJL143 or pNBO003 plasmid-based vaccine, and at least one boost-administration of a vCP2389 or vCP2389-SEQ ID NO:2 vector-based vaccine.

In another embodiment, the prime-boost administration regimen comprises at least one
25 primo-administration of a pVR2001 LJL143 and pVR2001 LJM17 plasmid-based vaccine, and at least one boost-administration of a vCP2389 and vCP2390 vector-based vaccine.

In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a pNBO003 and pNBO002 plasmid-based vaccine, and at least one boost-administration of a vCP2389-SEQ ID NO:2 and vCP2390-SEQ ID NO:6 vector-based
30 vaccine.

In yet another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a pVR2001 LJM17 or pNBO002 plasmid-based vaccine, and at least one boost-administration of a MVA-LJM17 vector-based vaccine.

5 In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a pVR2001 LJL143 or pNBO003 plasmid-based vaccine, and at least one boost-administration of a MVA-LJL143 vector-based vaccine.

In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a pVR2001 LJL143 and pVR2001 LJM17 plasmid-based vaccine, and at least one boost-administration of a MVA-LJL143 and MVA-LJM17 vector-based vaccine.

10 In yet another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a pNBO003 and pNBO002 plasmid-based vaccine, and at least one boost-administration of a MVA-LJL143 and MVA-LJM17 vector-based vaccine.

15 In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a vCP2390 or vCP2390-SEQ ID NO:6 vector-based vaccine, and at least one boost-administration of a LJM17 polypeptide sub-unit vaccine.

In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a vCP2389 or vCP2389-SEQ ID NO:2 vector-based vaccine, and at least one boost-administration of a LJL143 polypeptide sub-unit vaccine.

20 In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a vCP2389 or vCP2389-SEQ ID NO:2 and vCP2390 or vCP2390-SEQ ID NO:6 vector-based vaccine, and at least one boost-administration of a LJL143 polypeptide and LJM17 polypeptide sub-unit vaccine.

25 In yet another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a MVA-LJM17 vector-based vaccine, and at least one boost-administration of a LJM17 polypeptide sub-unit vaccine.

In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a MVA-LJL143 vector-based vaccine, and at least one boost-administration of a LJL143 polypeptide sub-unit vaccine.

30 In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a MVA-LJL143 and MVA-LJM17 vector-based vaccine, and at least one boost-administration of a LJL143 polypeptide and LJM17 polypeptide sub-unit vaccine.

In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a vCP2390 or vCP2390-SEQ ID NO:6 vector-based vaccine, and at least one boost-administration of a pVR2001 LJM17 or pNBO002 plasmid-based vaccine.

5 In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a vCP2389 or vCP2389-SEQ ID NO:2 vector-based vaccine, and at least one boost-administration of a pVR2001 LJM143 or pNBO003 plasmid-based vaccine.

In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a vCP2389 and vCP2390 vector-based vaccine, and at least one boost-administration of a pVR2001 LJM143 and pVR2001 LJM17 plasmid-based vaccine.

10 In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a vCP2389-SEQ ID NO:2 and vCP2390-SEQ ID NO:6 vector-based vaccine, and at least one boost-administration of a pNBO003 and pNBO002 plasmid-based vaccine.

15 In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a MVA-LJM17 vector-based vaccine, and at least one boost-administration of a pVR2001 LJM17 or pNBO002 plasmid-based vaccine.

In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a MVA-LJM143 vector-based vaccine, and at least one boost-administration of a pVR2001 LJM143 or pNBO003 plasmid-based vaccine.

20 In another embodiment, the prime-boost administration regimen comprises at least one primo-administration of a MVA-LJM143 and MVA-LJM17 vector-based vaccine, and at least one boost-administration of a pNBO003 and pNBO002 plasmid-based vaccine.

25 Another aspect of the present invention relates to a kit for prime-boost vaccination according to the present invention. The kit may comprise at least two vials: a first vial containing a vaccine for the primo-vaccination according to the present invention, and a second vial containing a vaccine for the boost-vaccination according to the present invention. The kit may advantageously contain additional first or second vials for additional primo-vaccinations or additional boost-vaccinations.

30 In one embodiment, the kit may comprise two vials, one containing a plasmid-based vaccine for the primo-vaccination according to the present invention, the other vial containing a

recombinant viral vector-based vaccine for the boost-vaccination according to the present invention.

In another embodiment, the kit may comprise two vials, one containing a recombinant viral vector-based vaccine for the primo-vaccination according to the present invention, the other
5 vial containing a sub-unit vaccine for the boost-vaccination according to the present invention.

In another embodiment, the kit may comprise two vials, one containing a recombinant viral vector-based vaccine for the primo-vaccination according to the present invention, the other vial containing a plasmid-based vaccine for the boost-vaccination according to the present invention.

10 It is disclosed herein that individuals who experience an anti-*Leishmania* DTH response conversion also have an increase in antibodies against *Lu. longipalpis* salivary proteins. Thus, the presence or absence of antibodies to *Lu. longipalpis* salivary proteins can be used to ascertain if a subject has a *Leishmania* infection.

A method is disclosed herein for diagnosing infection with *Leishmania* by detecting the
15 presence of antibodies that specifically bind one or more polypeptides having an amino acid sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, or 87, or a polypeptide having at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these polypeptides, a conservative variant, a homolog or an immunogenic fragment of one of
20 these polypeptides. The method can utilize a single *Lu. longipalpis* polypeptide or a combination of these polypeptides. In certain examples, the method of diagnosis detects antibodies that specifically bind at least 3, 6, or 10 of these polypeptides, or immunogenic fragments thereof.

In one embodiment, one or more *Lu. longipalpis* polypeptide can be bound to a solid substrate. For example, the *Lu. longipalpis* polypeptide having an amino acid sequence as set
25 forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, or 87 can be bound to the substrate. One of more of these polypeptides can be bound to the substrate, for example at least 3, 6, or 10 of these polypeptides, or an immunogenic fragment thereof. In one example, one or more polypeptides having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, 25,
30 33, 39, 47, or 77 can be bound to the substrate. In another example, one or more *Lu. longipalpis* polypeptides having a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, 17, or 57 can be

bound to the substrate. In one specific, non-limiting example, at least six *Lu. longipalpis* polypeptides are bound to a solid substrate, wherein each of the polypeptides comprises an amino acid sequence as set forth in SEQ ID NO: 25, SEQ ID NO: 1 (or 3, or 11, or 13), SEQ ID NO: 5 (or 7, or 15, or 17), SEQ ID NO: 47, SEQ ID NO: 73, or SEQ ID NO: 77, or an immunogenic fragment thereof. In another specific, non-limiting example, at least three *Lu. Longipalpis* polypeptides are bound to a solid substrate, wherein each of the polypeptides comprises an amino acid sequence as set forth in SEQ ID NO: 1 (or 3, or 11, or 13), SEQ ID NO: 5 (or 7, or 15, or 17), or SEQ ID NO: 57, or an immunogenic fragment thereof.

In one embodiment, two or more (for example at least 3, 6, or 10) *Lu. longipalpis* polypeptides (or immunogenic fragments thereof) are applied to a solid substrate, for example as a series of "dots," such as in a "dot blot" assay. In another embodiment, two or more *Lu. longipalpis* polypeptides are applied to a substrate such as in a linear array. In a further embodiment, *Lu. longipalpis* polypeptides are applied to a membrane in a two-dimensional array. In this manner, the presence of antibodies to more than one *Lu. longipalpis* polypeptide is assessed. Each *Lu. longipalpis* polypeptide can be applied directly to the surface of a membrane in a single location or in a combination of locations.

The solid substrate can be a polystyrene bead, a membrane, a chip or a plate. A plastic or glass substrate can be utilized. In other embodiments, a membrane is utilized that is composed of porous materials such as nylon, nitrocellulose, cellulose acetate, glass fibers, and other porous polymers. The surface of a solid support may be activated by chemical processes that cause covalent linkage of polypeptide to the support. However, any other suitable method may be used for immobilizing a polypeptide to a solid support including, without limitation, ionic interactions, hydrophobic interactions, covalent interactions and the like. Once the polypeptide is applied to the substrate, the substrate can be contacted with a substance, such as protein-containing solution, which non-specifically saturates the binding sites thereon. Specific, non-limiting examples of a protein-containing solution include a solution made from powdered milk or serum albumin, such as bovine serum albumin.

A specimen (for example, sera, blood, plasma, urine, semen, saliva, sputum, lacrimal fluid, lymph fluid) is then added to the substrate, and the combined specimen and substrate are incubated for a sufficient time to allow specific binding. Specific binding of antibodies to the *Lu. longipalpis* polypeptides disclosed herein, are then detected using any means known to one

of skill in the art. In one embodiment, a labeled secondary antibody is used to detect the antibodies that specifically bind the *Lu. longipalpis* polypeptides. The label can be a radiolabel (for example, ¹²⁵I), an enzymatic label (for example, alkaline phosphatase or horseradish peroxidase), or a fluorescent label (for example, fluorescein isothiocyanate). Detection systems
5 for these labels are known to one of skill in the art. Binding of the specimen, or a component of the specimen, to the *Lu. longipalpis* polypeptide, as indicated by the presence of the marker, indicates infection with *Leishmania*.

In another embodiment, the specimen is adsorbed onto a solid substrate containing binding sites for polypeptides, such as antibody molecules. In one embodiment, the solid
10 substrate is a polystyrene bead, a chip, a membrane or a plate. The substrate is thereafter contacted with a substance, such as a protein-containing solution that non-specifically saturates the binding sites thereon. The substrate is then washed with a buffer. A solution of one or more *Lu. longipalpis* polypeptides is then added to the bound specimens. In one embodiment, the *Lu. longipalpis* polypeptide is directly labeled. The labeling of the *Lu. longipalpis* polypeptide can
15 be brought about by use of any marker, such as by incorporation of a radioactive isotope or group, or by coupling this component to an enzyme, a dyestuff, for example a chromophoric moiety or a fluorescent group. The enzymes of use are those which can be colorimetrically, spectrophotometrically, or fluorimetrically determined. Non-limiting examples of enzymes for use in the present invention include enzymes from the group of oxidoreductases, such as
20 catalase, peroxidase, glucose oxidase, beta-glucuronidase, beta-D-glucosidase, beta-D-galactosidase, urease and galactose oxidase. After the labeled *Lu. longipalpis* polypeptide is incubated with the solid substrate, any unbound labeled *Lu. longipalpis* polypeptide is removed by washing. Bound labeled *Lu. longipalpis* polypeptide is then detected by an appropriate assay. Binding of the labeled *Lu. longipalpis* polypeptide to the specimen, or to a component of the
25 specimen, is indicative of infection with *Leishmania*.

In general, the incubation steps utilized in carrying out the procedures can be performed in a known manner, such as by incubating at temperatures between about 4° C and about 25° C, for about 30 minutes to about 48 hours. Washings can be included with an aqueous solution such as a buffer, wherein the buffer is from about pH 6 to about pH 8, such as by using an
30 isotonic saline solution of a pH of about 7.

Competitive binding assays are also of use in detecting infection with *Leishmania*. One of skill in the art, given the *Lu. longipalpis* polypeptides disclosed herein, will readily be able to design additional assays, such as competitive binding assays, of use in detecting *Leishmania* infection.

5 In another embodiment, the *Lu. longipalpis* polypeptides disclosed herein can be included in a diagnostic test kit. For example, a diagnostic test kit for detecting a *Leishmania* infection includes a solid substrate having applied thereon one or more *Lu. longipalpis* polypeptide disclosed herein. In other embodiments, the kit includes written instructions and/or a container including a specified amount of labeled antibodies to immunoglobulins, such as IgG or IgM, or
10 labeled secondary antibodies that bind antibodies from a species of interest. For example labeled antibodies can be provided that specifically detect dog or human immunoglobulins. The labeled antibodies can be fluorescently labeled, enzymatically labeled, or radiolabeled. Labeled antibodies used in the above-described test kits can be packaged in either solution or lyophilized form suitable for reconstitution.

15 In another embodiment the test kit includes a specified amount of one or more *Lu. longipalpis* polypeptide described herein in a container, and written instructions. In one example, the *Lu. longipalpis* polypeptide is directly labeled. In another example, the one or more *Lu. longipalpis* polypeptide is unlabeled. If the *Lu. longipalpis* polypeptide is unlabeled, a container can also be included with a detection reagent that specifically binds the *Lu. longipalpis*
20 polypeptide, such as a labeled monoclonal antibody. The kit can also optionally include a solid substrate for binding the specimen.

The above described process and test kit for detection of antibodies to the *Lu. longipalpis* polypeptides disclosed herein can be utilized in many applications, including, but not limited to detecting *Leishmania* infection in a subject using the methods disclosed herein. The tests and
25 kits disclosed herein can be used to detect the efficacy of a therapeutic treatment in a subject. In yet another embodiment, the tests and kits disclosed herein can also be used to assess a primary infection with *Leishmania* or to predict recovery from *Leishmania* infection by taking a body fluid from an infected subject, for example at various times following infection, and applying the above described detection procedures.

30 ***

The invention will now be further described by way of the following non-limiting examples.

EXAMPLES

Without further elaboration, it is believed that one skilled in the art can, using the preceding descriptions, practice the present invention to its fullest extent. The following detailed examples are to be construed as merely illustrative, and not limitations of the preceding disclosure in any way whatsoever. Those skilled in the art will promptly recognize appropriate variations from the procedures both as to reactants and as to reaction conditions and techniques.

Construction of DNA inserts, plasmids and recombinant viral vectors was carried out using the standard molecular biology techniques described by J. Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989). All the restriction fragments used for the present invention were isolated using the "GeneClean" kit (BIO 101 Inc., La Jolla, Calif.).

EXAMPLE 1: Construction of the pVR2001 LJM17 plasmid expressing the *Lu. longipalpis* salivary LJM17 polypeptide

The polynucleotide encoding the *Lu. longipalpis* LJM17 polypeptide is synthesized and has the sequence described in SEQ ID NO: 8 which contains poly(A) tail. The LJM17 fragment is amplified by PCR and cloned into the TOPO cloning site of the pVR2001-TOPA donor plasmid (also referred to as pVR2001-TOPO).

The resultant plasmid, pVR2001 LJM17 therefore contains and expresses a nucleotide encoding a promoter capable of driving expression in a mammalian cell, a leader peptide for facilitating secretion/release of a prokaryotic protein sequence from a mammalian cell, LJM17, topoisomerases flanking the DNA encoding LJM17, as well as a termination sequence.

The nucleic acid sequence of one strand of the plasmid pVR2001 LJM17 is described in SEQ ID NO: 9 and in Figure 1, wherein BamHI sites are in positions [4-9] and [5051-5056], the nucleotide sequence encoding the tPA signal peptide is in position [4976-5062] and the nucleotide sequence encoding LJM17 is in position [5063-6247].

EXAMPLE 2: Construction of the pVR2001 LJJ143 plasmid expressing the *Lu. longipalpis* salivary LJJ143 polypeptide

The polynucleotide encoding the *Lu. longipalpis* LJJ143 polypeptide is synthesized and has the sequence described in SEQ ID NO: 4 which contains poly(A) tail. The LJJ143 fragment

is amplified by PCR and cloned into the TOPO cloning site of the pVR2001-TOPA plasmid as described in example 1, to generate the plasmid pVR2001 LJM143.

The nucleic acid sequence of one strand of the plasmid pVR2001 LJM143 is described in SEQ ID NO: 10 and in Figure 2, wherein BamHI sites are in positions [4-9] and [5051-5056], the
5 nucleotide sequence encoding the tPA signal peptide is in position [4976-5062] and the nucleotide sequence encoding LJM143 is in position [5063-5899].

EXAMPLE 3: Construction of an ALVAC canarypox virus vector expressing the *Lu. longipalpis* salivary LJM17 polypeptide

For a discussion and examples of the plasmid pALVAC, and the C3 locus, see e.g., U.S.
10 Patent Nos. 5,756,103; 5,833,975; and 6,780,407. The sequence of the vaccinia virus H6 promoter has been previously described (see e.g., Taylor *et al.*; Taylor *et al.*; Guo *et al.*).

The polynucleotide encoding the *Lu. longipalpis* LJM17 polypeptide is synthesized and has the sequence described in SEQ ID NO:6. This polynucleotide is then ligated to the pALVAC C3 H6p donor plasmid resulting in pALVAC C3 H6p-LJM17 containing 5737 base pairs (Figure
15 3).

To generate vCP2390-SEQ ID NO:6, the pALVAC C3 H6p-LJM17 plasmid is linearized with NotI restriction enzyme. The linearized fragments were individually transfected into ALVAC-infected primary CEF cells using the calcium phosphate precipitation method (see, Panicali *et al.*; Piccini *et al.*). After 24h, the transfected cells are harvested, sonicated and used
20 for recombinant virus screening.

Recombinant plaques are screened based on the plaque lift hybridization method using a *Lu. longipalpis* LJM17-specific probe which is labeled with horse radish peroxidase according to the manufacturer's protocol (Amersham Cat# RPN-3001). After three sequential rounds of plaque purification, the recombinants are generated by hybridization confirmation as 100%
25 positive for the *Lu. longipalpis* LJM17 insert and 100% negative for the C3 ORF.

A single plaque is selected from the third round of plaque purification and expanded to obtain P1 (60 mm), P2 (T75 flasks), and P3 (roller bottles) stocks to amplify vCP2390-SEQ ID NO:6. The infected cell culture fluid from the roller bottles is harvested and concentrated to produce virus stock.

The construct is sequenced to confirm the sequences of the *Lutzomyia longipalpis* LJM17 insert and the C3 left and right arms around the *Lutzomyia longipalpis* LJM17 insert in vCP2390-SEQ ID NO:6.

EXAMPLE 4: Construction of an ALVAC canarypox virus vector expressing the *Lu. longipalpis* salivary LJL143 polypeptide

The polynucleotide encoding the *Lu. longipalpis* LJL143 polypeptide is synthesized and has the sequence described in SEQ ID NO:2. This sequence is then ligated to a pALVAC C3 H6p donor plasmid. The resulting plasmid, pALVAC C3 H6p-LJL143 comprises 5400 base pairs (Figure 5), and is sequenced to confirm the nucleic acid sequence (SEQ ID NO: 2) of the LJL143 gene.

To generate vCP2389-SEQ ID NO:2, the pALVAC C3 H6p-LJL143 plasmid is linearized with NotI restriction enzyme. The linearized fragments are individually transfected into ALVAC-infected primary CEF cells by using the calcium phosphate precipitation method (see, Panicali *et al.*; Piccini *et al.*). After 24h, the transfected cells are harvested, sonicated and used for recombinant virus screening.

Recombinant plaques are screened based on the plaque lift hybridization method using a *Lu. longipalpis* LJL143-specific probe which is labeled with horse radish peroxidase according to the manufacturer's protocol (Amersham Cat# RPN-3001). After three sequential rounds of plaque purification, the recombinants are generated by hybridization confirmation as 100% positive for the *Lu. longipalpis* LJL143 insert and 100% negative for the C3 ORF.

A single plaque is selected from the third round of plaque purification and expanded to obtain P1 (60 mm), P2 (T75 flasks), P3 (roller bottles) stocks to amplify vCP2389-SEQ ID NO:2. The infected cell culture fluid from the roller bottles are harvested and concentrated to produce virus stock.

The construct is sequenced to confirm the sequences of the *Lutzomyia longipalpis* LJL143 insert and the C3 left and right arms around the *Lutzomyia longipalpis* LJL143 insert in vCP2389-SEQ ID NO:2.

EXAMPLE 5: Construction of a MVA vector expressing the *Lu. longipalpis* salivary LJM17 polypeptide

MVA is a modified Ankara strain obtained after more than 570 passages of the Ankara vaccine strain on chicken embryo fibroblasts (see Stickl & Hochstein-Mintzel; Sutter *et al.*)

available as ATCC VR-1508. Its adaptation to these cells caused the excision of 6 regions which are nonessential for its development and its infectious cycle on this type of cells (disappearance of about 15% of the viral genome; Meyer *et al.*). Exogenous genetic material may be inserted into any of these excision regions. In the context of the present invention, foreign genetic material is inserted into excisions II and III which are located using the HindIII restriction fragments N and A respectively (Altenburger *et al.*).

Engineering of the recombinant MVA virus expressing LJM17 is performed as previously described in Staib C. *et al.*, with the exception that the 723-base pairs DNA fragment containing the gfp ORF is replaced by the 1239 base pairs DNA fragment encoding LJM17 antigen (SEQ ID NO: 6), generating MVA-LJM17.

EXAMPLE 6: Construction of a MVA vector expressing the *Lu. longipalpis* salivary LJL143 polypeptide

Construction of the recombinant MVA virus expressing LJL143 is performed as previously described in Staib C. *et al.*, with the exception that the 723-base pairs DNA fragment containing the gfp ORF is replaced by the 906 base pairs DNA fragment encoding LJL143 antigen (SEQ ID NO: 2), generating MVA-LJL143.

EXAMPLE 7: Expression of *Lu. longipalpis* salivary protein *in vitro*

Expression plasmids containing His-tagged *Lu. longipalpis* salivary antigens encoded by cDNA derived from the plasmids pVR2001 LJM17 (see example 1) and pVR2001 LJL143 (see example 2) are constructed and transfected in HEK-293F cells. Supernatants collected at 72 h are analyzed by HPLC chromatography using Nickel column and imidazole gradient. HPLC fractions positive for the recombinant salivary protein with 6x His motif at their C-terminus are tested with polyclonal sera of mice previously immunized with the corresponding original cDNA plasmids. Recombinant proteins are tested by SDS-PAGE and Western blotting, aliquoted in PBS and stored at -70° C.

The sub-unit vaccine comprising LJM17 is referred to herein as rLJM17. The vaccine is prepared by combining 100 µg of purified recombinant protein LJM17 with 300 µg of the adjuvant CpG #2142 in 20% Emulsigen® (MVP laboratories) (for CpG #2142, see SEQ. ID. NO: 890 in EP-B1-1,221,955).

The sub-unit vaccine comprising LJL143 is referred to herein as rLJL143. The vaccine is prepared by combining 100 µg of purified recombinant protein LJL143 with 300 µg of the adjuvant CpG #2142 in 20% Emulsigen®.

EXAMPLE 8: Vaccination of dogs against leishmaniasis

25 dogs (1-2 years old female beagles) are randomly divided into 5 groups of 5 dogs each. All dogs from groups 1 to 4 are vaccinated at D0 (V1) by the intradermal (ID) route into the ear pinna using a syringe and a needle with 500 µg of purified pVR2001 LJM17 plasmid (example 1) expressing LJM17 (groups 1 and 2) or pVR2001 LJL143 plasmid (example 2) expressing LJL143 (groups 3 and 4).

Dogs from group 1 and group 3 are boosted at D14 (V2) and D28 (V3) with 500 µg of the same plasmids used for V1 by the transdermal (TD) route in the inner upper part of both hind legs using the VetJet™ (Merial) needle-free delivery device. Dogs from group 1 and group 3 were further boosted at D42 (V4) with 500 µg of the same plasmids by the intramuscular route coupled to electroporation (ET/IM) in the external side of both thighs using the Sphergen device and technology (parameters: 88V, T1=20, T2=80, N=10).

Dogs from groups 2 and 4 are boosted at D14 (V2) and D28 (V3) with 500 µg of the same plasmids used for V1 by the IM route coupled to electroporation (ET/IM) as described above. Dogs from groups 2 and 4 are further boosted at D42 (V4) by the ID route into the ear pinna as described above with sub-unit vaccines (see example 7) rLJM17 (group 2) or rLJL143 (group 4), respectively.

All dogs from groups 1 and 2 receive a final vaccine booster at D192 (V5) by the IM route in the left quadriceps using 10⁸ pfu of a recombinant canarypox virus vCP2390 (example 3) expressing LJM17. All dogs from groups 3 and 4 receive a final vaccine booster at D192 (V5) by the IM route in the left quadriceps using 10⁸ pfu of a recombinant canarypox virus vCP2389 (example 4) expressing LJL143.

Dogs from group 5 are vaccinated with 500 µg of the purified parental plasmid pVR2001 which expresses no antigen at D0 by ID, D14 by TD, D28 by TD and D42 by ET/IM and receive a final booster at D192 by the IM route using 10⁸ pfu of a control recombinant canarypox virus (Purevax™).

EXAMPLE 9: Construction of the pNBO002 plasmid expressing the *Lu. longipalpis* salivary LJM17 polypeptide

The nucleic acid sequence encoding the *Lu. longipalpis* LJM17 polypeptide was synthesized and has the sequence described in SEQ ID NO: 18 which contains poly(A) tail. The LJM17 fragment was amplified by PCR and cloned into the TOPO cloning site of the pVR2001-TOPA donor plasmid as described in example 1, to generate the plasmid pNBO002.

5 The nucleic acid sequence of one strand of the plasmid pNBO002 is described in SEQ ID NO: 19 and in Figure 11, wherein BamHI sites are in positions [1819-1824] and [3019-3024], the nucleotide sequence encoding the tPA signal peptide is in position [1744-1830] and the nucleotide sequence encoding LJM17 is in position [1831-3015]. The map of the pNBO002 plasmid is shown in Figure 12.

10 pNBO002 is analogous to pVR2001 LJM17. In this case, this construct and the construct of example 1 are identifiable by the nucleic acid sequence of their inserts, SEQ ID NO: 18 and SEQ ID NO: 8, respectively.

EXAMPLE 10: Construction of the pNBO003 plasmid expressing the *Lu. longipalpis* salivary LJJ143 polypeptide

15 The nucleic acid sequence encoding the *Lu. longipalpis* LJJ143 was synthesized and has the sequence described in SEQ ID NO: 14, which contains poly(A) tail. The LJJ143 fragment was amplified by PCR and cloned into the TOPO cloning site of the pVR2001-TOPA plasmid, as described in example 1, to generate the plasmid pNBO003.

20 The nucleic acid sequence of one strand of the plasmid pNBO003 is described in SEQ ID NO: 20 and in Figure 13, wherein BamHI sites are in positions [1819-1824] and [2671-2676], the nucleotide sequence encoding the tPA signal peptide is in position [1744-1830] and the nucleotide sequence encoding LJJ143 is in position [1831-2667]. The map of the pNBO003 plasmid is shown in Figure 14.

25 pNBO003 is analogous to pVR2001 LJJ143. In this case, this construct and the construct of example 2 are identifiable by the nucleic acid sequence of their inserts, SEQ ID NO: 14 and SEQ ID NO: 4, respectively.

EXAMPLE 11: Construction of an ALVAC canarypox virus vector expressing the *Lu. longipalpis* salivary LJM17 polypeptide

30 For discussion and examples of the plasmid pALVAC and the C3 locus, see e.g., U.S. Patent Nos. 5,756,103; 5,833,975; and 6,780,407. The sequence of the vaccinia virus H6 promoter has been previously described (see e.g., Taylor *et al.*; Taylor *et al.*; Guo *et al.*).

The nucleic acid sequence encoding the *Lu. longipalpis* LJM17 polypeptide was synthesized and has the sequence described in SEQ ID NO:6. This sequence was codon-optimized for mammalian expression by Geneart GmbH (Regensburg, Germany), resulting in the sequence described in SEQ ID NO:91, which encodes the LJM15 polypeptide (SEQ ID NO:5).

5 This codon-optimized sequence was then ligated to the pALVAC C3 H6p donor plasmid to generate pALVAC C3 H6p-LJM17 containing 5737 base pairs (Figure 3). The resulting plasmid, pALVAC C3 H6p-LJM17, was sequenced and confirmed to contain the nucleic acid sequence (SEQ ID NO:91) of the LJM17 gene.

10 To generate vCP2390, the pALVAC C3 H6p-LJM17 plasmid was linearized with NotI restriction enzyme. The linearized fragments were individually transfected into ALVAC-infected primary CEF cells by using the calcium phosphate precipitation method described previously (Panicali *et al.*; Piccini *et al.*). After 24h, the transfected cells were harvested, sonicated and used for recombinant virus screening.

15 Recombinant plaques were screened based on the plaque lift hybridization method using a *Lu. longipalpis* synthetic LJM17-specific probe which was labeled with horse radish peroxidase according to the manufacturer's protocol (Amersham Cat# RPN-3001). After three sequential rounds of plaque purification, the recombinants were generated and confirmed by hybridization as 100% positive for the synthetic LJM17 insert and 100% negative for the C3 ORF.

20 A single plaque was selected from the third round of plaque purification and expanded to obtain P1 (60 mm), P2 (T75 flasks), P3 (roller bottles) stocks to amplify vCP2390. The infected cell culture fluid from the roller bottles was harvested and concentrated to produce virus stock.

The construct was sequenced to confirm the sequences of the condon-optimized LJM17 insert and the C3 left and right arms around the codon-optimized LJM17 insert in vCP2390.

25 The vCP2390 vector is illustrated in Figure 4. The nucleic acid sequences for both vCP2390 strands are described in Figure 4.

EXAMPLE 12: Construction of an ALVAC canarypox virus vector expressing the *Lu. longipalpis* salivary LJL143 polypeptide

30 The nucleic acid sequence encoding the *Lu. longipalpis* LJL143 was synthesized and has the sequence described in SEQ ID NO:2. This sequence was codon-optimized for mammalian

expression by Geneart GmbH (Regensburg, Germany), resulting in the sequence described in SEQ ID NO:22, which encodes the LJL143 protein (SEQ ID NO:1).

This codon-optimized sequence was then ligated to the pALVAC C3 H6p donor plasmid resulting in pALVAC C3 H6p-LJL143 containing 5400 base pairs (Figure 5), which was
5 sequenced and confirmed to contain the nucleic acid sequence (SEQ ID NO:22) of the LJL143 gene.

To generate vCP2389, the plasmid pALVAC C3 H6p-LJL143 plasmid was linearized with NotI restriction enzyme. The linearized fragments were individually transfected into ALVAC-infected primary CEF cells by using the calcium phosphate precipitation method (see,
10 Panicali *et al.*; Piccini *et al.*). After 24h, the transfected cells were harvested, sonicated and used for recombinant virus screening.

Recombinant plaques were screened based on the plaque lift hybridization method using a *Lu. longipalpis* synthetic LJL143-specific probe which was labeled with horse radish peroxidase according to the manufacturer's protocol (Amersham Cat# RPN-3001). After three
15 sequential rounds of plaque purification, the recombinants were generated and confirmed by hybridization as 100% positive for the synthetic LJL143 insert and 100% negative for the C3 ORF.

A single plaque was selected from the third round of plaque purification and expanded to obtain P1 (60 mm), P2 (T75 flasks), P3 (roller bottles) stocks to amplify vCP2389. The infected
20 cell culture fluid from the roller bottles was harvested and concentrated to produce virus stock.

After sequencing, the results showed that the sequence of the synthetic LJL143 insert and the C3 left and right arms around the synthetic LJL143 insert in vCP2389 were correct.

The vCP2389 vector is illustrated in Figure 6.

EXAMPLE 13: Expression of *Lu. longipalpis* salivary protein *in vitro*

25 Expression plasmids containing His-tagged *Lu. longipalpis* salivary antigens encoded by cDNA derived from plasmids pNBO002 (see example 9) and pNBO003 (see example 10) were constructed and transfected into HEK-293F cells. Supernatants collected at 72 h were analyzed by HPLC chromatography using Nickel column and imidazole gradient. HPLC fractions positive for the recombinant salivary protein with 6x His motif at their C-terminus were tested with
30 polyclonal sera of mice previously immunized with the corresponding original cDNA plasmids.

Recombinant proteins were tested by SDS-PAGE and Western blotting, aliquoted in PBS and stored at -70 °C.

The sub-unit vaccine comprising LJM17 is referred to herein as rLJM17. The vaccine is prepared by combining 100 µg of purified recombinant protein LJM17 with 300 µg of the adjuvant CpG #2142 in 20% Emulsigen® (MVP laboratories) (for CpG #2142, see SEQ. ID. NO: 890 in EP 1,221,955).

The sub-unit vaccine comprising LJM143 is referred to herein as rLJM143. The vaccine is prepared by combining 100 µg of purified recombinant protein LJM143 with 300 µg of the adjuvant CpG #2142 in 20% Emulsigen®.

EXAMPLE 14: Vaccination of dogs against leishmaniasis

25 dogs (1-2 years old female beagles) were randomly divided into 5 groups of 5 dogs each. All dogs from groups 1 to 4 were vaccinated at D0 (V1) by the intradermal (ID) route into the ear pinna using a syringe and a needle with 500 µg of purified pNBO002 plasmid (example 9) expressing LJM17 (groups 1 and 2) or pNBO003 plasmid (example 10) expressing LJM143 (groups 3 and 4) antigens, respectively.

Dogs from group 1 and group 3 were boosted at D14 (V2) and D28 (V3) with 500 µg of the same plasmids used for V1 by the transdermal (TD) route in the inner upper part of both hind legs using the VetJet™ (Merial) needle-free delivery device. Dogs from group 1 and group 3 were further boosted at D42 (V4) with 500 µg of the same plasmids by the intramuscular route coupled to electroporation (ET/IM) in the external side of both thighs using the Sphergen device and technology (parameters: 88V, T1=20, T2=80, N=10).

Dogs from groups 2 and 4 were boosted at D14 (V2) and D28 (V3) with 500 µg of the same plasmids used for V1 by the IM route coupled to electroporation (ET/IM) as described above. Dogs from groups 2 and 4 were further boosted at D42 (V4) by the ID route into the ear pinna as described above with sub-unit vaccines (see example 13) rLJM17 (group 2) or rLJM143 (group 4), respectively.

All dogs from groups 1 and 2 received a final vaccine booster at D192 (V5) by the IM route in the left quadriceps using 10⁸ pfu of a recombinant canarypox virus vCP2390 (example 11, having SEQ ID NO: 21 as insert) expressing LJM17. All dogs from groups 3 and 4 received a final vaccine booster at D192 (V5) by the IM route in the left quadriceps using 10⁸ pfu of a

recombinant canarypox virus vCP2389 (example 12, having SEQ ID NO: 22 as insert) expressing LJM17.

Dogs from group 5 were vaccinated with 500 µg of the purified parental plasmid VR2001 which expresses no antigen at D0 by ID, D14 by TD, D28 by TD and D42 by ET/IM and received a final booster at D192 by the IM route using 10⁸ pfu of a control recombinant canarypox virus (PurevaxTM).

Specific and significant humoral immunity to both LJM17 and LJM143 was evidenced by ELISA in vaccinated dogs (see Figure 7). 96-well plates (MaxisorpTM, Nunc) were coated overnight at 4° C with rLJM17 protein (2 µg/mL) or rLJM143 (2 µg/mL). Dog serum was successively added to the plates in triplicate at a dilution of 1/50 with alkaline phosphatase-conjugated AffiniPure rabbit anti-dog IgG (Jackson ImmunoResearch) at 1/5000 and p-nitrophenylphosphate (Sigma). Absorbance was measured at 405 nm using a Spectramax Plus (Molecular Devices).

Significant antibody titers persisted up to 6 months after V4 administration. The vCP booster (V5) recalled specific antibody responses in vaccinated dogs efficiently. Post vCP anamnestic responses established the expression of LJM143 and LJM17 from vCP2389 and vCP2390 respectively, and the ability of the vectors to boost humoral immune responses *in vivo*.

PBMC from dogs taken 2 weeks after the V5 administration were stimulated by 2 pairs of salivary gland homogenate (SGH), LJM143 (4 µg), and LJM17 (4 µg), or by ConA (4 µg). PBMCs that were non-stimulated by medium (med) served as controls. IFN-gamma production was evaluated by measuring the levels of IFN-gamma secretion in the medium at 72 hours (Quantikine ELISA; R&D Systems).

The results are illustrated in Figure 8. Addition of purified recombinant LJM17 or LJM143 proteins caused PBMCs from LJM17- or LJM143-vaccinated dogs to increase secretion of IFN-gamma. No increased secretion of IFN-gamma was evidenced when PBMCs were in the presence of control medium (med).

The addition of ConA or SGH to PBMCs from LJM17- or LJM143-vaccinated dogs also caused an increased secretion of IFN-gamma. Notably, the LJM17 antigen caused very strong INF-gamma responses in animals of group 2 (1800 pg/mL), and also caused a strong response in animals of group 1 (200 pg/mL). The LJM143 antigen also caused very strong INF-gamma

responses in animals of groups 3 and 4, with more than 2000 pg/mL, which were comparable to results seen when PBMCs were stimulated by ConA.

2 weeks after the V5 administration, PBMCs were isolated from two dogs that had been vaccinated with LJM17 and LJL143. Autologous T cells lymphocytes ($5 \cdot 10^6$ cells) were stimulated with either 25 μ g of recombinant LJM17, 25 μ g of recombinant LJL143, or 4 μ g of ConA. Cells were then incubated in the presence of macrophages infected by *Leishmania chagasi* amastigotes (infected at a 5:1 ratio). Lipopolysaccharides (LPS) and non-stimulated T cells (NT, no treatment) served as controls. Cells were evaluated for efficiency of killing which was measured by a significant reduction in the percent of infected macrophages (Figure 9).

Lymphocytes stimulated with recombinant LJM17 or recombinant LJL143 were as cytotoxic to macrophages as lymphocytes that had been stimulated by the non-specific mitogen ConA.

LJL143- and LJM17-vaccinated and control dogs were fitted with a Velcro collar device. The Velcro collar device was prepared by disposing twenty uninfected 6 day old female *Lu. longipalpis* were in a 10 mm-thin Plexiglas device which contained a secured screw and a nylon mesh on one of its sides so that sand flies were able to probe through the mesh. The device was kept at 25° C before exposure to limit condensation and was then firmly attached to a Velcro collar for 10 minutes on the shaved belly of LJL143- and LJM17-vaccinated and control dogs. Dogs were unrestrained throughout the time of exposure. 4 mm or 6 mm-skin punches biopsies (Acuderm) were taken from the belly of anesthetized dogs 48 hours after the sand fly bites.

Biopsies were stored in neutral-buffered formaldehyde (10% formalin) and were then routinely processed for staining with hematoxylin/eosin (H & E), Luna's, Toluidine blue and immunohistochemical procedures for CD3 and macrophage/monocyte markers (Mac). Figure 10 provides the immunohistochemical results of sand fly bite sites. Specific immune cellular infiltration (darker spots on the pictures) is observed on vaccinated dogs whereas no infiltration was observed on control dogs.

EXAMPLE 15: Vaccination of dogs with LJL143 and LJM17 salivary proteins produce a protective immune response that killed *Leishmania chagasi* amastigotes *in vitro*.

In this study, *Lu. longipalpis* salivary proteins were tested to determine if they are immunogenic in dogs. In addition, *Lu. longipalpis* salivary proteins were identified that can produce a protective cellular immune response and kill *Leishmania infantum* in an *in vitro* assay.

To identify these salivary components, an *in vivo* high-throughput DNA-based reverse antigen-screening assay was developed. This screening identified two strong DTH-inducing antigens out of the 35 most abundant proteins in the salivary glands of *Lu. longipalpis*. These data were further validated with recombinant proteins, resulting in the confirmation of DTH-inducing potential for the *Lu. longipalpis* salivary proteins LJM17 (SEQ ID NO:5) and LJM143 (SEQ ID NO:1). Furthermore, peripheral blood mononuclear cells of dogs vaccinated with LJM143 and LJM17 produced interferon (IFN)- γ upon stimulation with the salivary recombinant proteins and, more importantly, stimulation of these cells with the recombinant proteins resulted in the killing of *Leishmania infantum* *in vitro*. These molecules represent therefore strong candidates to be used as a vaccine to control *Leishmania infantum* in dogs.

Material and Methods

Dogs: 1-2 year old female beagles (Marshall Farms) were housed at the animal facility of NIH, Bethesda, USA, following the Animal Care and User Committee guidelines. They were well-fed animals under constant scrutiny for health problems by a veterinarian and had all received routine vaccinations. No ectoparasitic treatments were administered during the last four months before sand fly exposure experiments.

Sand flies: *Lutzomyia longipalpis*, Jacobina strain, were reared using as larval food a mixture of fermented rabbit feces and rabbit food. Adult sand flies were offered a cotton swab containing 20% sucrose but were starved from sugar 24 hours before exposure experiments. Some females were used for dissection of salivary glands at 4–7 days following emergence and salivary gland homogenates (SGH) were prepared as described above.

Exposure to sand fly bites: Six day old female *Lu. longipalpis* were placed in a 10 mm-thin Plexiglas device. The device contained a secured screw and a nylon mesh on one of its sides for sand fly probing through it. The device was kept at 25°C before exposure to limit condensation and was firmly attached with a Velcro collar on the shaved neck of dogs for 20 minutes. Dogs were unrestrained throughout the time of exposure.

Reverse Antigen-screening (RAS): Dogs pre-exposed to sand fly bites were anesthetized and injected intradermally with DNA plasmids or recombinant proteins. Injection sites were separated from each other by 15mm. For DNA plasmids, the molecules were coded and randomly regrouped for each dog prior to injection on the belly. The code was broken only after induration and erythema measurements were completed. For DNA-RAS, 38 samples were

injected in a total volume of 40 μ L, including PBS, 1 pair of *Lu. longipalpis* SGH diluted in PBS, and 20 μ g of control vector and the 35 recombinant DNA plasmids, diluted in PBS, encoding *Lu. longipalpis* individual salivary proteins. For protein-RAS, 5 samples were injected in duplicates (40 μ L) including PBS, 1 pair of *Lu. longipalpis* SGH diluted in PBS, and 3 *Lu.*
5 *longipalpis* salivary recombinant proteins (300 ng). Measure of induration and erythema diameters were performed 48 hours after intradermal injection of the samples.

Histology and real-time PCR on skin punch biopsies: 4mm or 6mm-skin punches biopsies (Acuderm) were taken from the neck or belly of anesthetized dogs and split into two equal halves. One half was stored in neutral-buffered formaldehyde (10% formalin) then
10 routinely processed for staining with hematoxylin/eosin, Luna's, Toluidine blue and immunohistochemical procedures for CD3 and macrophage/monocyte markers. The other half, stored in RNeasy (Qiagen), was used for RNA extraction (RNeasyTM Tissue, Beckman Coulter). RNA were reverse-transcribed (Transcriptor first strand cDNA synthesis, Roche) and used for Real-time PCR using the LightCycler 480 (Roche), primer set (0.2 μ M final
15 concentration) and FAM/TAMRA dual-labeled probes to a total of 15 μ l per reaction in triplicates. Primers and probes for canine IL4, IL12, TGF- β , IFN- γ and GAPDH were described previously (Breathnach *et al.*). Amplification conditions, acquisition, melting curve analysis and standard curve were performed as described previously (Breathnach *et al.*). Expression levels of the interested gene were normalized to endogenous GAPDH levels to control for RNA quantity.

Recombinant cDNA: From a cDNA library (see above), the 35 most abundant molecules from *Lu. longipalpis* salivary glands were selected and their cDNA cloned in the pVR2001-TOPO vector by standard cloning techniques. Plasmids were prepared using GenEluteTM endotoxin-Free Plasmid Megaprep (Sigma), cleaned with ultrapure water using Centricon[®] Plus-20 (Millipore) and eluted in PBS. Quality control of the 35 purified plasmids included endotoxin
20 measurements, restriction profile analyses and sequencing. Purified salivary DNA plasmids were sterilely filtered and stored at -70 $^{\circ}$ C.

Recombinant proteins: Expression plasmids containing His-tagged *Lu. longipalpis* salivary antigens encoding cDNA derived from the parental salivary DNA plasmids were constructed as described (Oliveira *et al.*) and transfected in HEK-293F cells. Supernatants
30 collected at 72 h were submitted to HPLC chromatography using Nickel column and imidazole gradient. HPLC fractions positive for the recombinant salivary protein with 6x His motif at their

C-terminus were tested with polyclonal sera of mice previously immunized with the corresponding original cDNA plasmids. Recombinant proteins were tested by SDS-PAGE and Western blotting, aliquoted in PBS and stored at -70 °C.

Recombinant canarypox viruses: Two canarypox viruses derived from ALVAC vectors expressing respectively the LJL143 (vCP2389) or LJM17 (vCP2390) antigens were generated using standard methods and validated by sequencing of viral DNA, RT-PCR on mRNA of infected cells and Western Blotting of supernatants of infected cells. PurevaxTM ferret distemper vaccine (Meriel) was used as control of canarypox virus injections.

Immunization of dogs with salivary vaccines: 25 dogs were randomly divided in 5 groups of 5 dogs each. All dogs from groups 1 to 4 were vaccinated at D0 (V1) by the intradermal (ID) route into the ear pinna using a syringe and a needle with 500µg of purified plasmid expressing LJM17 (groups 1 and 2) or LJL143 (groups 3 and 4) antigens, respectively. Dogs from group 1 and group 3 were boosted at D14 (V2) and D28 (V3) with 500µg of the same plasmids used for V1 by the transdermal (TD) route in the inner upper part of both hind legs using the VetJetTM (Meriel) needle-free delivery device. Dogs from group 1 and group 3 were further boosted at D42 (V4) with 500µg of the same plasmids by the intramuscular (IM) route coupled to electroporation in the external side of both thighs using the Sphergen devise and technology (parameters: 88V, T1=20, T2=80, N=10). Dogs from groups 2 and 4 were boosted at D14 (V2) and D28 (V3) with 500µg of the same plasmids used for V1 by the IM route coupled to electroporation as described above. Dogs from groups 2 and 4 were further boosted at D42 (V4) with 100µg of aforementioned purified recombinant protein LJM17 (rLJM17) (group 2) or LJL143 (rLJL143) (group 4) in association with 300 µg CpG ODN in 20% Emulsigen® (MVP laboratories) by the ID route into the ear pinna as described above. All dogs from groups 1 to 4 received a final vaccine booster at D192 (V5) by the IM route in the left quadriceps using 10⁸ pfu of a recombinant canarypoxvirus vCP2390 and vCP2389 expressing respectively LJM17 (groups 1 and 2) or LJL143 (groups 3 and 4) antigens. Dogs from group 5 were vaccinated at D0, D14, D28 and D42 with 500µg of the purified parental plasmid VR2001 which expresses no antigen and received a final booster at D192 by the IM route using 10⁸ pfu of a control recombinant canarypoxvirus (PurevaxTM).

ELISA: 96-well plates (MaxisorpTM, Nunc) were coated overnight at 4 °C with *Lu. longipalpis* SGH (5 pairs/ml), rLJM17 protein (2µg/mL) or rLJL143 (2µg/mL). Dog serum in

triplicate at 1/50 dilution, alkaline phosphatase-conjugated AffiniPure rabbit anti-dog IgG (Jackson ImmunoResearch) at 1/5000 and p-nitrophenylphosphate (Sigma) were successively added to the plates. 405nm absorbance was measured using a Spectramax Plus (Molecular Devices). IFN- γ production in cell supernatants was measured after 72 h (Quantikine ELISA; R&D Systems) after stimulation with SGH (1 or 2 pairs), conA (4 μ g), rLJM17 (2 or 10 μ g) or rLJL143 (2 or 10 μ g).

Anti-leishmanial activity: Canine monocyte-derived macrophages were prepared as using standard methods. Autologous T cells (5×10^6 cells) were taken from the culture after 1 week, stimulated with *Lu. longipalpis* SGH (2 pairs), conA (4 μ g), rLJM17 (25 μ g) or rLJL143 (25 μ g), and put back in presence of macrophages infected by *L. infantum* infected at a 5:1 ratio. Anti-leishmanial activity was assessed by changes in the percentages of infected cells and number of amastigotes per macrophage after microscopic examination of Giemsa-stained preparations.

Bites of Lutzomyia longipalpis sand flies induce a strong delayed type hypersensitivity response in dogs

In rodent models, cellular immunity characterized by a Th1 delayed type hypersensitivity (DTH) response to sand fly salivary proteins, protect animals from cutaneous and visceral leishmaniasis. There is no information pertaining to the presence and nature of cellular immunity to sand fly saliva in dogs, the main reservoirs of visceral leishmaniasis caused by *Leishmania infantum* (*chagasi*) in Europe and Latin America. Thus, the early kinetics of anti-saliva immunity in dogs following exposure to bites of *Lutzomyia longipalpis*, the vector of *Leishmania infantum chagasi* in Latin America, was investigated. Seven of nine beagles showed specific anti-saliva antibodies one week after the third exposure to bites (FIG. 19A). Apart from a single dog, these animals showed a strong IgG2 antibody response in the absence of IgG1 (FIG. 19A). One dog showed a mixed IgG2/IgG1 antibody response. To investigate whether dogs exposed to sand fly bites develop a DTH response, the skin induration at the bite site up to 96 hours following each exposure was measured. Following the second exposure to sand fly bites, a small induration was observed in the 7 dogs that produced significant levels of *Lu. longipalpis* IgG antibodies (FIG. 19B). This was characterized by a localized erythema, swelling and eventually thickening of the skin. The intensity and duration of the observed induration was significantly increased following the third exposure lasting up to 96 hours following sand fly

bites (FIG. 19B). This induration was not observed after the first exposure in naive animals (FIG. 19B). Histological analyses of the induration site show minimal inflammation characterized by scattered perivascular lymphocytes and rare neutrophils within the superficial dermis 48 hours following the first and second exposure (FIG. 19C). A dramatic increase in the cellular infiltrate was noted 48 hours following the third exposure. This was characterized by a prominent thickening of the epidermis and the presence of multifocal infiltrates of inflammatory cells consisting of lymphocytes, macrophages and eosinophils (FIG. 19D). Based on the timing of the reaction as well as the nature of the infiltrate, it was concluded that sand fly saliva induces a delayed-type hypersensitivity reaction in the skin of dogs after repeated exposures.

***Lutzomyia longipalpis* salivary proteins that induce a DTH in dogs**

Salivary molecules of *Lu. longipalpis* which are responsible for the generation of a DTH response in dogs was investigated. Transcripts coding for the 35 most abundant secreted proteins from the salivary glands of this species were identified above. Identifying candidates capable of inducing a cellular immune response from a large pool of antigens in large animals such as dogs is prohibitive in terms of cost and space. To overcome this obstacle, a reverse antigen screening approach was developed that consisted of exposing a minimal number of dogs (five) to sand fly bites and injecting each animal with up to 38 samples (35 DNA plasmids encoding for salivary proteins and three controls) (FIG. 20A). Out of the 35 injected DNA plasmids only 4 (LJM17, LJM11, LJJ143 and LJJ138) induced a significant erythema in more than 3 dogs, 48 hours after challenge (FIG. 20B) and only 2 DNA plasmids (LJJ143 and LJM17) produced a strong induration in 3 dogs, 48 hours after challenge (FIG. 20B). The majority of the injected plasmids did not produce a significant erythema or induration (FIG. 20B) and the injection of PBS and empty vector control induced minimal erythema at the site of injection compared to LJM17 and LJJ143 (FIG. 20C). The specificity and reactivity of injected DNA plasmids is shown in FIG. 20D. Based upon these results LJM17 and LJJ143 were chosen for further analysis. LJJ143 and LJM17 induced the production of cytokines indicative of a Th1 environment at the injection site, including IL-12 and IFN- γ (FIG. 20E). Histological sections taken 48 hours after challenge show that LJJ143 and LJM17 recruit lymphocytes and macrophages at the site of injection indicative of a classical delayed type hypersensitivity (DTH) response (FIG. 20F). To validate the specificity of this approach soluble highly purified LJJ143, LJM17 and LJM111, among several other salivary recombinant proteins, was prepared (FIG.

21A). LJL143 and LJM17 recombinant proteins reproduced the DTH response observed upon injection of their respective DNA plasmids (FIG. 21B and 21C) including the recruitment of lymphocytes and macrophages to the site of injection (FIG. 21D).

Dogs immunized with LJL143 and LJM17 produce IFN- γ specific to recombinant salivary proteins.

Dogs (5 per group) were immunized with DNA plasmids coding for LJL143, LJM17 or control DNA plasmid, a prime boost with the respective recombinant salivary proteins and a final immunization with canarypox virus expressing the respective salivary proteins (Table 1). PBMCs from immunized dogs were stimulated with up to 4 ug of their respective recombinant protein, ConA and 1 pair of salivary gland homogenate (SGH). LJL143 vaccinated dogs produced significant levels of IFN- γ five weeks post-fourth vaccination and before canarypox injection (FIG. 22A). More importantly, the native LJL143 protein present in 1 pair of salivary gland homogenate was able to generate a similar response in LJL143 vaccinated dogs (FIG. 22A). LJM17 vaccinated dogs produced a considerable lower levels of IFN- γ compared to LJL143 vaccinated dogs (FIG. 22A). A similar profile was observed two weeks after canarypox vaccination, where PBMCs from dogs vaccinated with LJL143 produced two fold higher IFN- γ compared to their pre-pox status (FIG. 22B).

Table 1

Group	Antigen	D0 (V1)	D14 (V2)	D28 (V3)	D42 (V4)	D192 (V5)
1	LJM17	ID-cDNA	TD-cDNA	TD-cDNA	IM/ET-cDNA	IM-vCP
2	LJM17	ID-cDNA	IM/ET-cDNA	IM/ET-cDNA	ID-protein	IM-vCP
3	LJL143	ID-cDNA	TD-cDNA	TD-cDNA	IM/ET-cDNA	IM-vCP
4	LJL143	ID-cDNA	IM/ET-cDNA	IM/ET-cDNA	ID-protein	IM-vCP
5	Control	ID-cDNA	TD-cDNA	TD-cDNA	IM/ET-cDNA	IM-vCP

Vaccination with LJL143 and LJM17 generates a protective immune response that kills *Leishmania chagasi* amastigotes *in vitro*.

Infected macrophages from PBMCs of two dogs vaccinated with LJM17 and LJL143 efficiently killed *Leishmania chagasi* amastigotes following the addition of autologous lymphocytes stimulated with LJM17 and LJL143 recombinant proteins respectively (FIG. 23). The efficiency of killing was measured by a significant reduction in the percent of infected macrophages (FIG. 23A) as well as in the number of amastigotes per macrophages (FIG. 23B).

This killing effect was comparable to that observed upon the addition of the non-specific mitogen ConA (FIG. 23).

EXAMPLE 16: Production of an Immune Response in Dogs

Twelve dogs approximately three years old with natural immunity against Leishmaniasis are injected via an intradermal route (ID) in the back after shaving, with 100 µg of each individual plasmid suspended in 100 µl of PBS. Each plasmid is injected at a different point. The points are separated by at least 3 cm to avoid interference between DTH responses. The negative control (100 µl of buffer) is also inoculated by ID route.

The DTH response is assessed 72 hours after injection by measuring the larger diameter of the skin tumefaction area. The results are expressed as the mean value of the tumefaction area for all the dogs and as a percentage of dogs having a positive DTH response. A positive DTH is a tumefaction area diameter greater than or equal to 4 mm at 72 hours after injection.

In a second study, 10 naïve dogs 4 to 6 months old are immunized by ID injection in 10 points (100 µl per point) in the right ear with a pool of the plasmids encoding a *Lu. longipalpis* polypeptide, 100 µg for each one suspended in 1000 µl of PBS. On day 21, dogs are injected in 10 points (100 µl per point) in the left ear and in 10 points (100 µl per point) in the belly with a pool of the plasmids, 100 µg for each one suspended in 2000 µl of PBS. All dogs are challenged on day 35 by inoculation by ID route in the back (after shaving), with 100 µg of each individual plasmid suspended in 100 µl of PBS. Each plasmid is injected at a different point. The points are separated by at least 3 cm to avoid interference. As a negative control, 100 µl of buffer is inoculated intradermally. The DTH response is assessed 72 hours after challenge, by measuring the larger diameter of the skin tumefaction area. The results are expressed as the mean value of the tumefaction area for all the dogs and as a percentage of dogs having a positive DTH response. A positive DTH is a tumefaction area diameter higher or equal of 4 mm at 72 hours after injection.

The results of this study show that plasmids can induce a cellular immunity in dogs after injection, a cellular immunity revealed by a DTH response. The variation of the DTH response level can be by the variation of the expression of the insert.

It will be apparent that the precise details of the methods described may be varied or modified without departing from the spirit of the described disclosure. We claim all such

modifications and variations that fall within the scope and spirit of the claims below.

References

1. Adler and Theodor, *Ann. Trop. Med. Parasitol.* 20:109, 1926
2. Altenburger *et al.*, 1989, *Arch. Virol.* 105, 15-27
3. Altschul *et al.* *J. Mol. Biol.* 1990. 215. 403-410
- 5 4. Altschul *et al.*, *Nucl. Acids Res.* 25, 3389-3402
5. Antoine G., *Virology*, 1998, 244, 365-396
6. Bairoch, *Nucleic Acids Res.* 19 (Suppl.):2241, 1991
7. Barral *et al.*, *Am J Trop Med Hyg* 62:740-5, 200
8. Behr J. P., *Bioconjugate Chemistry*, 1994: 5: 382-389
- 10 9. Berberich C. *et al.*, *Biochim. Biophys. Acta*, 1998, 1442: 230-7
10. Boshart M. *et al.*, *Cell*, 1985, 41, 521-530
11. Boshart M. *et al.*, *Cen* 41:521-530, 1985
12. Breathnach *et al.*, *Vet Dermatol* 2006, 17:313-21
13. Carroll M. W. *et al.*, *Vaccine*, 1997, 15 (4), 387-394
- 15 14. Charlab *et al.*, *Proc Natl Acad Sci USA* 96:15155-60, 1999
15. Chaudhuri P *Res. Vet. Sci.* 2001, 70(3), 255-6
16. Chung J Y *et al.*, *FEMS Microbiol letters* 1998, 166: 289-296
17. Cochran *et al.*, *J. Virology*, 1985, 54, 30-35
18. D. Berg. *et al* *Biochem. Biophys. Res. Commun.* 1991, 179, 1289-1296
- 20 19. De Groot A. *et al.*, *Nature Biotechnology*, 1999, 17, 533-561
20. Desjeux P., *Trans. R. Soc. Trop. Med. Hyg.*, 2001, 95: 239-43
21. Devereux J, Haeberlie P and Smithies O, "A comprehensive set of sequence analysis program for the VAX," *Nucl. Acids Res.*, 12: 387-395 (1984)
22. Dietze R. *et al.*, *Clin. Infect. Dis.*, 1997, 25: 1240-2
- 25 23. Djoba Siawaya JF *et al.*, *PLoS ONE*, 2008, 3(7), e2535
24. Doree S M *et al.*, *J. Bacteriol.* 2001, 183(6): 1983-9
25. Dye C., *Am. J. Trop. Med. Hyg.*, 1996, 55: 125-30
26. Feng DF and Dolittle RF, "Progressive sequence alignment as a prerequisite to correct phylogenetic trees," *J. of Molec. Evol.*, 25:351-360 (1987)
- 30 27. Fingerut E *et al.*, *Vaccine*, 2005, 23(38): 4685-4696
28. Funahashi *et al.*, *J. Gen. Virol.*, 1988, 69, 35-47

29. Geysen H. M. *et al.*, Proc. Nat. Acad. Sci. USA, 1984, 81 (13), 3998-4002
30. Geysen H. M. *et al.*, Proc. Nat. Acad. Sci. USA, 1985, 82 (1), 178-182
31. Geysen H. M., Southeast Asian J. Trop. Med. Public Health, 1990, 21 (4), 523-533
32. Gradoni L. *et al.*, Vaccine, 2005, 23: 5245-51
- 5 33. Grosjean NL *et al.*, Lindsay DS *et al.*, McConkey SE *et al.*, Martínez-Subiela S, Tecles F, Eckersall PD, Cerón JJ: Serum concentrations of acute phase proteins in dogs with leishmaniasis. Vet Rec 150:241-244, 2002
34. Grosjean NL, Vrable RA, Murphy AJ, Mansfield LS: Seroprevalence of antibodies against *Leishmania* spp among dogs in the United States. J Am Vet Med Assoc 10 222:603-606, 2003
35. Guo P. *et al.* J. Virol., 1989, 63, 4189-4198
36. Hartikka J. *et al.*, Human Gene Therapy, 1996, 7, 1205-1217
37. Hemmer B. *et al.*, Immunology Today, 1998, 19 (4), 163-168
38. Henikoff *et al.*, *Bioinformatics* 15:471, 1999
- 15 39. Higgins DG and Sharp PM, "Fast and sensitive multiple sequence alignment on a microcomputer," CABIOS, 5: 151-153 (1989)
40. Hoop T. *et al.*, Mol. Immunol. 1983, 20(4), 483-489
41. Immunogenicity of a killed *Leishmania* vaccine with Saponin adjuvant in dogs", R. Cordeiro Giunchetti *et al.*, Vaccine, 2007, 25: 7674-7686
- 20 42. Israeli E *et al.*, Cryobiology 1993, 30(5): 519-23
43. J. Fields *et al.*, Nature, 186: 778-780, 4 June 1960
44. J. Mol. Biol. 48:444-453 (1970)
45. J. Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989
- 25 46. Jardim A. *et al.*, Biochem. J., 1995, 305: 307-13
47. Jardim A. *et al.*, Biochem. J., 1995, 305: 315-20
48. Jeanmougin *et al.*, *Trends Biochem. Sci.* 23:403, 1998
49. Jurk M *et al.*, Immunobiology 2004, 209(1-2): 141-154
50. K. Otte *et al.* Gen. Comp. Endocrinol. 1996, 102(1), 11-15
- 30 51. Kidd I. M. & Emery V.C., "The use of baculoviruses as expression; vectors," Applied Biochemistry and Biotechnology 42:37-159, 1993

52. Kwissa M. *et al.*, Vaccine, 2000, 18, 2337-2344
53. Lanotte G. *et al.*, Ann. Parasitol. Hum. Comp., 1979, 54: 277-95
54. Lindsay DS, Zajac AM, Barr SC: Leishmaniasis in American Foxhounds: An Emerging Zoonosis? Compend Cont Educ Pract Vet 24:304-312, 2002
- 5 55. Luke C. *et al.*, Journal of Infectious Diseases, 1997, 175, 91-97
56. Muller M. *et al.* Nucleic Acids Res. 1990, 18(2), 364
57. Maniatis *et al.*, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory, 1982
58. Maroli M. *et al.*, Med. Vet. Entomol., 2001, 15: 358-63
- 10 59. Martínez-Subiela S, Tecles F, Eckersall PD, Cerón JJ: Serum concentrations of acute phase proteins in dogs with leishmaniasis. Vet Rec 150:241-244, 2002
60. Mazloumi Gavgani A.S. *et al.*, Lancet, 2002, 360: 374-9
61. McConkey SE, López A, Shaw D, Calder J: Leishmanial polyarthritis in a dog. Canine Vet J 43:607-609, 2002
- 15 62. Melanby, *Nature*. 158, 554-555.13, 1946
63. Meyer *et al.*, 1991, J. Gen. Virol. 72, 1031-1038
64. Mills C K *et al.*, Cryobiology 1988, 25(2): 148-52
65. Miyazaki J. *et al.*, Gene, 1989, 79, 269-277
66. Molina R. *et al.*, Trans. R. Soc. Trop. Med. Hyg., 1994, 88: 491-3; Courtenay O. *et al.*,
20 J. Infect. Dis., 2002, 186: 1314-20
67. Montgomery *et al.*, Cell. Mol. Biol. 43:285-292, 1997
68. Moreira Jr. E.D. *et al.*, Vet. Parasitol., 2004, 122: 245-52
69. Nielsen *et al.*, *Protein Eng.* 10:1, 1997
70. Ogata R T *et al.*, J. Biol. Chem. 1989, 264(28): 16565-16572
- 25 71. Oliveira F. *et al.* Vaccine (2006) 24: 374-90
72. Olsen C W *et al.*, Vaccine, 1997, 15(10): 1149-1156
73. Optimal Alignments in Linear Space", CABIOS 4, 11-17, 1988
74. O'Reilly *et al.*, "Baculovirus expression vectors, A laboratory manual," New York Oxford, Oxford University Press, 1994
- 30 75. P. Delafontaine *et al.* Gene 1993, 130, 305-306
76. Pandher K *et al.*, Infect. Imm. 1998, 66(12): 5613-9

77. Panicali *et al.*, Proc. Natl Acad Sci USA, 1982, 79: 4927-4931
78. Parker K. *et al.*, Immunol. Res. 1995, 14(1), 34-57
79. Piccini *et al.*, Methods Enzymol., 1987, 153: 545-563
80. Pasleau *et al.*, Gene 38:227-232, 1985
- 5 81. Pennock *et al.*, Mol. Cell Biol. 4: 399- 406, 1994
82. Peppoloni S *et al.*, Expert Rev Vaccines, 2003, 2(2): 285-293
83. Perkus M. *et al.*, J. Virol., 1989, 63, 3829-3836
84. Phameuropa Vol. 8, No. 2, June 1996
85. Pharmaceutical Biotechnology, 1995, volume 6, edited by Michael F. Powell and
10 Mark J. Newman, Plenum Press, New York and London
86. Piccini *et al.*, Methods Enzymol., 1987, 153: 545-563
87. Ribeiro *et al.*, *Insect Biochem.* 19:409-412, 1989
88. Rickles R. et al J. Biol. Chem. 1988, 263, 1563-1569
89. Riviere *et al.*, J. Virology, 1992, 66, 3424-3434
- 15 90. S. Friezner Degen et al J. Biol. Chem. 1996, 261, 6972-6985
91. S. Lien *et al.* Mamm. Genome 2000, 11(10), 877-882
92. Shida, Virology, 1986, 150, 451-457
93. Slappendel RJ, Ferrer L. In: Greene CE: Infectious Diseases of the Dog and Cat. WB
Saunders Co, Philadelphia, 1998, pp. 450-458
- 20 94. Smith *et al.*, Mol. Cell Biol. 3:2156-2165, 1983
95. Smith TF and Waterman MS, "Comparison of Bio-sequences," Advances in Applied
Mathematics 2:482-489 (1981)
96. Smith TF, Waterman MS and Sadler JR, "Statistical characterization of nucleic acid
sequence functional domains," Nucleic Acids Res., 11:2205-2220 (1983)
- 25 97. Soares *et al.*, *J. Immunol.* 160:1811-6, 1998
98. Staib C. *et al.*, Biotechniques, 2000, 28(6): 1137-42, 1144-6, 1148
99. Stickl & Hochstein-Mintzel, Munch. Med. Wschr., 1971, 113, 1149-1153
100. Stittelaar K. J. *et al.*, J. Virol., 2000, 74 (9), 4236-4243;
101. Sutter *et al.*, Proc. Natl. Acad. Sci. U.S.A., 1992, 89, 10847-10851
- 30 102. Sutter G. *et al.*, 1994, Vaccine, 12 (11), 1032-1040
103. Taylor *et al.* Vaccine. 6: 497-503, 1988a

104. Taylor *et al.* Vaccine. 6: 504-508, 1988b
105. Taylor J. *et al.*, Vaccine, 1988, 6, 504-508
106. Thompson JD, Higgins DG and Gibson TJ, "ClusterW: improving the sensitivity of
progressive multiple sequence alignment through sequence weighing, positions-
specific gap penalties and weight matrix choice," Nucleic Acid Res., 22:4673-480,
1994
107. Titus and Ribeiro, *Parasitol Today* 6:157-159, 1990
108. Tsvetkov T *et al.*, Cryobiology 1983, 20(3): 318-23
109. Vaccine Design, The subunit and adjuvant approach", Pharmaceutical Biotechnology,
vol. 6, Edited by Michael F. Powell and Mark J. Newman, 1995, Plenum Press New
York
110. Valenzuela *et al.*, *J. Exp. Med.* 194:331-42, 2001
111. Van der Zee R. *et al.*, Eur. J. Immunol., 1989, 19 (1), 43-47
112. van Ooyen *et al.*, Science, 1979, 206, 337-344
113. Verne A., Virology- 167:56 71, 1988
114. Vialard *et al.*, J. Virol. 64:37-50, 1990
115. VICAL Inc.; Luke C. *et al.*, Journal of Infectious Diseases, 1997, 175, 91-97
116. Von Heijne (1986), Nucleic Acid Research, 14; 4683-4691
117. Ward C K *et al.*, Infect. Imm. 1998, 66(7): 3326-36
118. Wilbur and Lipman, 1983 PNAS USA 80:726
119. Wolff E *et al.*, Cryobiology 1990, 27(5): 569-75
120. Y. Kajimoto *et al.* Mol. Endocrinol. 1989, 3(12), 1907-1913
121. Zurbriggen R *et al.*, Expert Rev Vaccines, 2003, 2(2): 295-304

What we claim is:

1. A vaccine composition comprising:
 - a) an expression vector, wherein the vector comprises a polynucleotide encoding one or more polypeptide selected from the group consisting of a salivary *Lu. longipalpis* polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, and a mixture thereof; and
 - b) a pharmaceutically or veterinary acceptable vehicle, diluent or excipient.
2. The vaccine of claim 1, wherein the expression vector is selected from the group consisting of pVR2001-TOPO, pVR2001-TOPA, pVR1020, pVR1012, pAB110, ALVAC, TROVAC, MVA, and baculovirus vector.
3. The vaccine of claim 1, wherein the polynucleotide encodes a polypeptide having at least 80% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17.
4. The vaccine of claim 1, wherein the polynucleotide has at least 70% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17.
5. The vaccine of claim 1, wherein the polynucleotide has at least 70% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 12, 14, 16, 18, 21, 22, 89, 90, or 91.
6. The vaccine of claim 1, wherein the vector is selected from the group consisting of pVR2001 LJM17 (SEQ ID NO:9), pVR2001 LJL143 (SEQ ID NO:10), vCP2390 (SEQ ID NO:93 or SEQ ID NO:92), vCP2389 (SEQ ID NO:94), MVA-LJM17, MVA-LJL143, pNBO002 (SEQ ID NO:19), pNBO003 (SEQ ID NO:20), vCP2390-SEQ ID NO:6, vCP2389-SEQ ID NO:2, and mixture thereof.
7. A vaccine composition comprising:
 - a) a first expression vector, wherein the vector comprises a polynucleotide encoding one or more polypeptide selected from the group consisting of a salivary *Lu. longipalpis* polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, and a mixture thereof;
 - b) a second expression vector, wherein the vector comprises a polynucleotide encoding one or more polypeptide selected from the group consisting of a salivary *Lu. longipalpis*

polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, and a mixture thereof; and

c) a pharmaceutically or veterinary acceptable vehicle, diluent or excipient.

8. The vaccine of claim 7, wherein the expression vector is selected from the group consisting of pVR2001-TOPO, pVR2001-TOPA, pVR1020, pVR1012, pAB110, ALVAC, TROVAC, MVA, and baculovirus vector.

9. The vaccine of claim 7, wherein the polynucleotide encodes a polypeptide having at least 80% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17.

10. The vaccine of claim 7, wherein the polynucleotide has at least 70% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17.

11. The vaccine of claim 7, wherein the polynucleotide has at least 70% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO: SEQ ID NO: 2, 4, 6, 8, 12, 14, 16, 18, 21, 22, 89, 90, or 91.

12. The vaccine of claim 7, wherein the first or second expression vector is selected from the group consisting of pVR2001 LJM17 (SEQ ID NO:9), pVR2001 LJL143 (SEQ ID NO:10), vCP2390 (SEQ ID NO:93 or SEQ ID NO:92), vCP2389 (SEQ ID NO:94), MVA-LJM17, MVA-LJL143, pNBO002 (SEQ ID NO:19), pNBO003 (SEQ ID NO:20), vCP2390-SEQ ID NO:6, vCP2389-SEQ ID NO:2, and mixture thereof.

13. A vaccine composition comprising:

a) a salivary *Lu. longipalpis* polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, or a mixture thereof; and

c) a pharmaceutically or veterinary acceptable vehicle, diluent or excipient.

14. The vaccine of claim 13, wherein the *Lu. longipalpis* polypeptide comprises an amino acid sequence having at least 80% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17.

15. The vaccine of any of claims 1-14, wherein the vaccine further comprises at least one *Leishmania* antigen.

16. The vaccine of claim 15, wherein the *Leishmania* antigen is KMP11.

17. The vaccine of claim 15, wherein the *Leishmania* antigen is inactivated *Leishmania*.

18. A vector comprising one or more polynucleotide selected from the group consisting of:

a) a polynucleotide encoding a polypeptide having at least 80% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17;

b) a polynucleotide has at least 70% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17; and

b) a polynucleotide having at least 70% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 12, 14, 16, 18, 21, 22, 89, 90, or 91;

wherein the vector is an *in vivo* expression vector or an *in vitro* expression vector.

19. The vector of claim 18, wherein the vector is a baculovirus vector.

20. The vector of claim 18, wherein the vector is a plasmid.

21. The vector of claim 18, wherein the vector is a viral vector.

22. A host cell transformed with the vector of claim 18.

23. A method of vaccinating a subject susceptible to *Leishmania* comprising at least one administration of the vaccine according to any one of claims 1 to 17.

24. The method of claim 23, wherein the subject is human, canine, or feline.

25. A method of vaccinating a subject susceptible to *Leishmania* comprising a prime-boost administration regimen, followed by a boost administration of a vaccine.

26. The method of claim 25, wherein said regiment comprises a primo-administration of a vaccine comprising, in a pharmaceutically or veterinary acceptable vehicle, diluent or excipient, a plasmid containing a polynucleotide for expressing, *in vivo*, a salivary *Lu. longipalpis* polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, or a mixture thereof, and wherein said boost administration of a vaccine comprising comprises, in a pharmaceutically or veterinary acceptable vehicle or excipient, a recombinant viral vector containing a polynucleotide for expressing, *in vivo*, the same salivary *Lu. longipalpis* polypeptide(s), variant thereof, fragment thereof, to protect the subject from leishmaniasis and/or to prevent disease progression in infected subject.

27. The method of claim 25, wherein said regiment comprises a primo-administration of a vaccine comprising, in a pharmaceutically or veterinary acceptable vehicle, diluent or excipient, a recombinant viral vector containing a polynucleotide for expressing, *in vivo*, a salivary *Lu. longipalpis* polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, or a mixture thereof, and wherein said boost administration of a vaccine

comprising, in a pharmaceutically or veterinary acceptable vehicle or excipient, a plasmid containing a polynucleotide for expressing, *in vivo*, the same salivary *Lu. longipalpis* polypeptide(s), variant thereof, fragment thereof, to protect the subject from leishmaniasis and/or to prevent disease progression in infected subject.

- 5 28. The method of claim 25, wherein said regiment comprises a primo-administration of a vaccine comprising, in a pharmaceutically or veterinary acceptable vehicle, diluent or excipient, a recombinant viral vector containing a polynucleotide for expressing, *in vivo*, a salivary *Lu. longipalpis* polypeptide, a variant or fragment of the salivary *Lu. longipalpis* polypeptide, or a mixture thereof, and wherein said boost administration of a vaccine
- 10 comprising, in a pharmaceutically or veterinary acceptable vehicle or excipient, the same salivary *Lu. longipalpis* polypeptide(s), variant thereof, fragment thereof, to protect the subject from leishmaniasis and/or to prevent disease progression in infected subject.
29. The method of any one of claims 23-28, wherein the salivary *Lu. longipalpis* polypeptide is a LJL143 polypeptide or a LJM17 polypeptide.
- 15 30. A method of diagnosing *Leishmania* infection in a subject, comprising:
- a) contacting a solid substrate comprising at least three, six, or ten polypeptides, wherein each of the polypeptides comprises one amino acid sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17, or an immunogenic fragment thereof;
 - b) contacting the solid substrate with a sample obtained from the subject; and
 - 20 c) detecting binding of a component of the sample to at least one polypeptide on the solid substrate, wherein detection of binding of the component to the substrate indicates that the subject is infected with *Leishmania*, thereby diagnosing *Leishmania* infection in the subject.
31. An isolated polynucleotide, wherein the polynucleotide comprises:
- a) a nucleotide sequence having at least 70% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 12, 14, 16, 18, 21, 22, 89, 90, or 91;
 - 25 b) a nucleotide sequence encoding a polypeptide having at least 80% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17; or
 - c) a polynucleotide has at least 70% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17.

32. The polynucleotide of claim 31, wherein the polynucleotide comprises a nucleotide sequence having at least 70% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO: 2, 4, 6, 8, 12, 14, 16, 18, 21, 22, 89, 90, or 91.

33. The polynucleotide of claim 31, wherein the polynucleotide comprises a nucleotide sequence encoding a polypeptide having at least 90% sequence identity to a polypeptide having the sequence as set forth in 1, 3, 5, 7, 11, 13, 15, or 17.

34. A substantially purified salivary *Lu. longipalpis* polypeptide, wherein the polypeptide comprises:

a) an amino acid sequence having at least 80% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17;

b) a conservative variant of the amino acid sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17;

c) an immunogenic fragment comprising at least eight consecutive amino acids of the amino acid sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17, that specifically binds to an antibody that specifically binds to the amino acid sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17; or

d) an amino acid sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17, and wherein administration of the polypeptide to a subject produces an immune response to *Lu. longipalpis*.

35. The polypeptide of claim 34, wherein the polypeptide comprises an amino acid sequence having at least 90% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17.

36. The polypeptide of claim 34, wherein the polypeptide comprises an immunogenic fragment comprising at least eight consecutive amino acids of the amino acid sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17, that specifically binds to an antibody that specifically binds to a polypeptide having the amino acid sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 11, 13, 15, or 17.

37. An antibody that specifically binds the polypeptide of claim 34.

FIGURE 1 (1/2)

(pVR2001 LJM17: SEQ ID NO:9)

aagggatccagatctgctgtgccttctagttgccagccatctgttggttggccctccccctgccttcttgaccct
ggaaggtgccactcccactgtcctttcctaataaaatgaggaaattgcatcgcatgtctgagtaggtgtcatttcta
ttctggggggtggggtggggcagcacagcaagggggaggattgggaagacaatagcaggcatgctgggatgcggtg
ggctctatgggtaccaggtgctgaagaattgacccgggttctcctggggcagaaagaagcaggcacatccccttct
ctgtgacacacccctgtccacgcccctgggttcttagttccagccccactcataggacactcatagctcaggagggtc
cgccttcaatcccacccgctaaagtacttggagcgggtctctccctccctcatcagcccaccaaaccacacctagcct
ccaagagtgggaagaaattaaagcaagataggctattaagtgcagagggagagaaaatgcctccaacatgtgaggaa
gtaatgagagaaatcatagaatttcttccgcttctcctcgtcactgactcgtgcgctcggctcgttcggctgcggcga
gcggtatcagctcactcaaaggcggtaatacgggttatccacagaatcaggggataacgcaggaaagaacatgtgagc
aaaaggccagcaaaaggccaggaaaccgtaaaaaggcgcggttgcgtggcgtttttccataggctccgccccctgacg
agcatcacaaaaatcgacgctcaagtgcagaggtggcgaaaccgcagaggactataaagataccaggcgtttccccct
ggaagctccctcgtgcgctctcctgttccgacccctgccgcttaccggataacctgtccgcctttctcccttcgggaag
cgtggcgctttctcaatgctcacgctgtaggatctcagttcgggtgtaggctcgttcgctccaagctgggtgtgtgc
acgaaccccccttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtcacacccggttaagacacgac
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FIGURE 1 (2/2)

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FIGURE 2 (1/2)

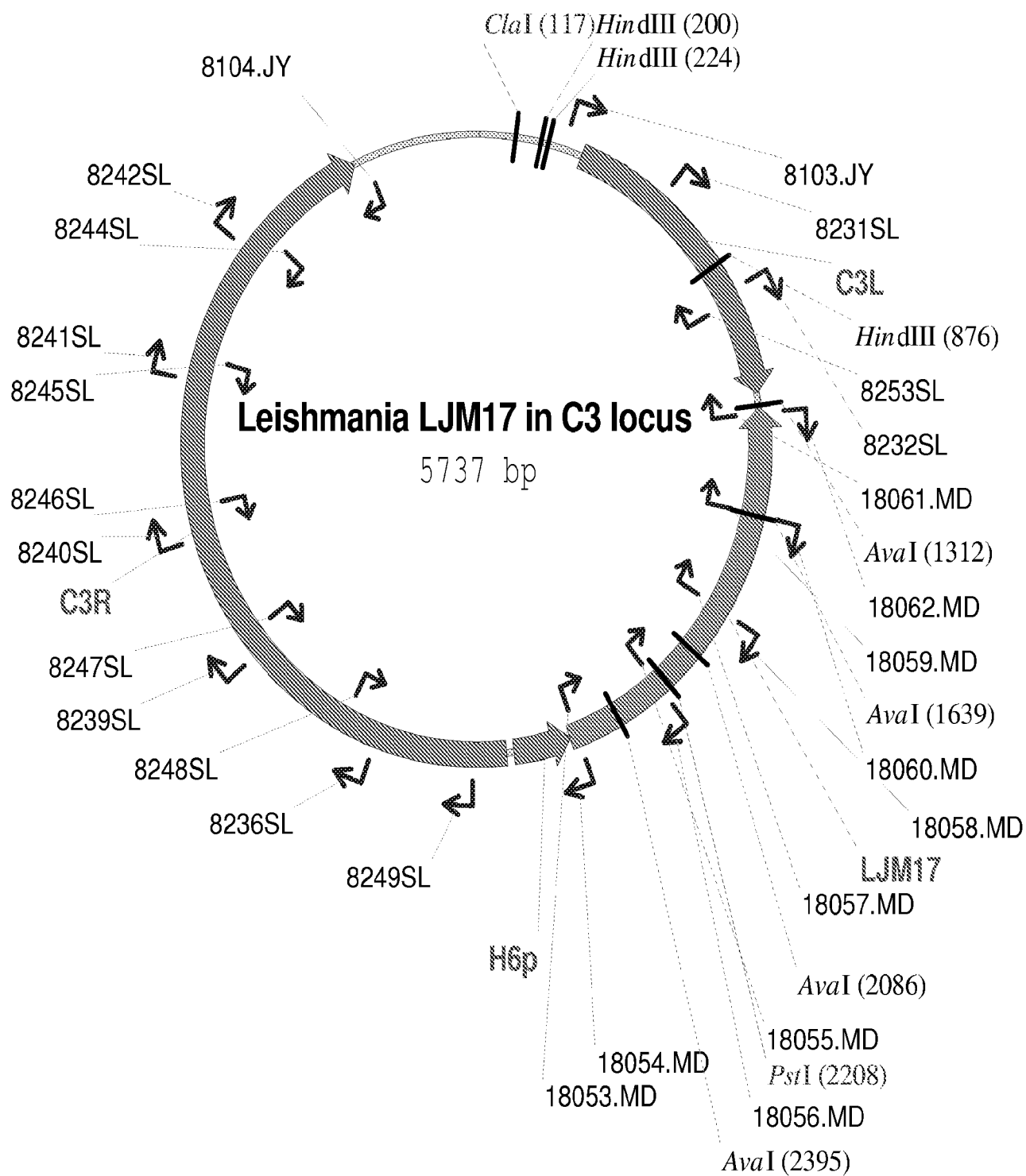
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FIGURE 2 (2/2)

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FIGURE 3



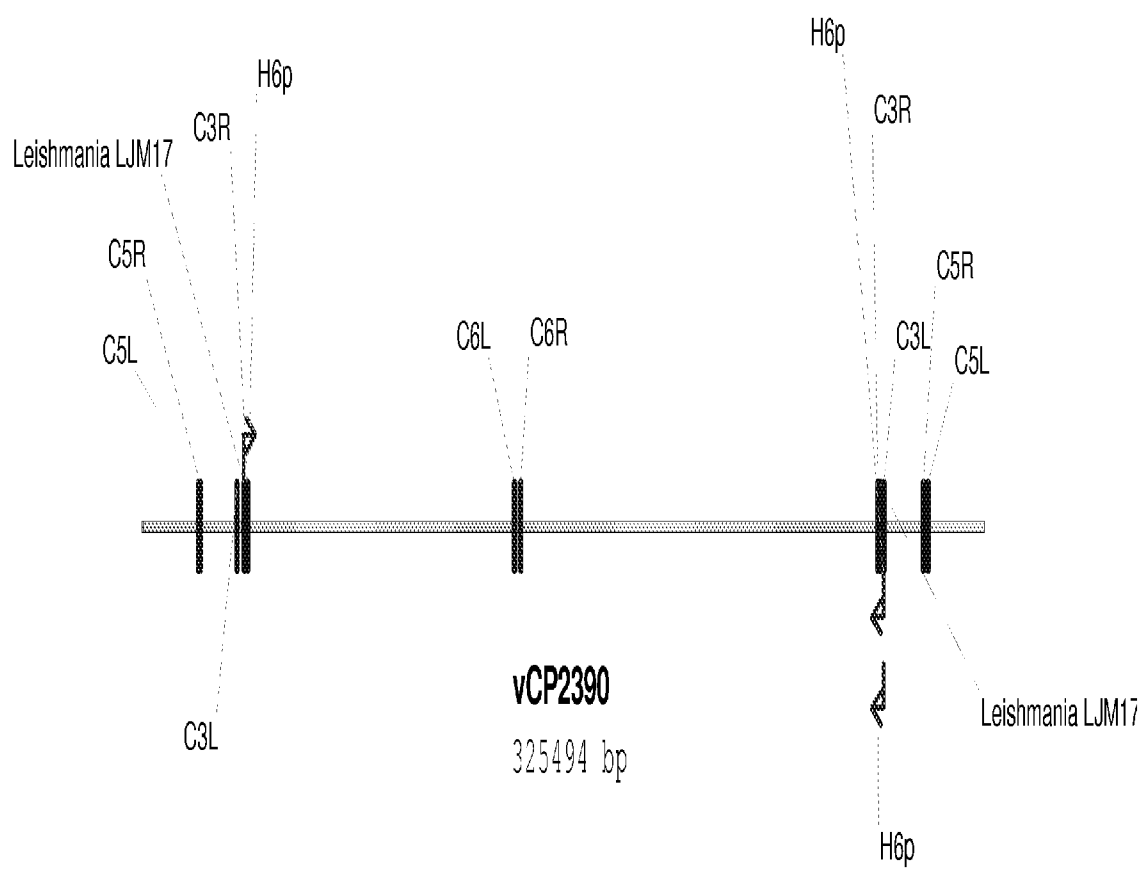


FIGURE 4 (1/5)

FIGURE 4 (2/5)

vCP2390 (ALVAC C3 H6p-synthetic Leishmania LJM17) (SEQ ID NO:92)

[1-940]: C3 left arm

[2224-2409]: H6 promoter

[982-2223]: Leishmania LJM17

[2433-4995]: C3 right arm

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1   TCAGATATATTAGATGCATTGTTAGTTCTGTAGATCAGTAACTATAGCATACGAGTATA
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181 GTTACGATAGTATTTCTAAAGTAAAGAGCAGGAATCCCTAGTATAATAGAAATAATCCAT
241 ATGAAAAATATAGTAATGTACATATTTCTAATGTTAACATATTTATAGGTAAATCCAGGA
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361 ATGTTAGAAAGTAAAAAAGAAAGAACTAATTTTACAAAGTGCCTTACCAAAATGCCAATGG
421 AAATTACTTAGTATGTATATAATGTATAAAGGTATGAATATCACAACAGCAAAATCGGGCT
481 ATTCCCAAGTTGAGAAAACGGTATAAATAGATATATTTCTAGATACCAATTAATTAACCTTATA
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1861 CAGCCGCTGCACTGCTCGATCACGGGCTGGTACACGTTCAACAGGTCTTCTTTGCCCTC
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1981 CATCACCATGTTTCACTCGGCCACGGTGTAGGGCACCTTGGGCTTCCGCTTGGGATGGC
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2581 ATCTGTAACTTTTACGACGTTAGGTTAGATACTGATGTTACAGATTATAAATATGTTAC
2641 AATAAAATACATGACAGGATGTGATATTTTCTCATATAACTCTTGGAAATAGCAAAATAT
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FIGURE 4 (3/5)

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4021 CGATATCGAAACAACAAAGACACTTTTGAAGAGAGGATCTAATGTTAATGTGGTTAATAA
4081 TCATATAGATACCGTTCTAAATATAGCTGTTGCATCTAAAAACAAAACATATAGTAAACTT
4141 ATTACTGAAGTACGGTACTCATACAAAGTTGGTAGGATTAGATAAACATGTTATTACAT
4201 AGCTATAGAAATGAAAGATATTAATATACTGAATGCGATCTTATTATATGGTTTCTATGT
4261 AAACGTCTATAATCATAAAGGTTTTCACTCCTCTATACATGGCAGTTAGTTCATGAAAAC
4321 AGAATTTGTTAAACTCTTACTTGACCACGGTGCTTACGTAAATGCTAAAGCTAAGTTATC
4381 TGGAAATACTCCTTTACATAAAGCTATGTTATCTAATAGTTTTTAATAATATAAAATTACT
4441 TTTATCTTATAACGCCGACTATAATTCTCTAAATAATCACGGTAATACGCCCTCTAACTTG
4501 TGTAGCTTTTTAGATGACAAGATAGCTATTATGATAATATCTAAATGATGTTAGAAAT
4561 ATCTAAAAATCCTGAAATAGCTAATTCAGAAGGTTTTATAGTAAACATGGAACATATAAA
4621 CAGTAATAAAAGACTACTATCTATAAAAGAATCATGCGAAAAAGAACTAGATGTTATAAC
4681 ACATATAAAAGTTAAATTTCTATATATTCTTTTAAATATCTTTCTTGACAAATACATAGATCT
4741 TATGGTAAAGTTTCGTAATAATCCTAGAGTTAATAAGATACCTGCATGTATACGTATATA
4801 TAGGGAAATTAATACGGAAAAATAAATCATTAGCTTTTCAATAGACATCAGCTAATAGTTAA
4861 AGCTGTAAAAGAGAGTAAGAATCTAGGAATATAGGTAGGTTACCTATAGATATCAAACA
4921 TATAATAATGGAATATTAAGTAATAATGATTTACATTCTGTTATCACCAGCTGTTGTAA
4981 CCCAGTAGTATAAAG

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FIGURE 4 (4/5)

vCP2390 (ALVAC C3 H6p-synthetic (coding) *Leishmania* LJM17) (SEQ ID NO:93)

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1  ctttatacta ctgggttaca acagctggtg ataacagaat gtaaatcatt attacttaat
61  agttccatta ttatatgttt gatattctata ggtaacctac ctattattcc tagattctta
121 ctctctttta cagctttaac tattagctga tgtctatgaa aagctaataga tttatTTTTc
181 cgtatttaatt ccctatatat acgtatacat gcaggatatct tattaactct aggattagtt
241 acgaacttta ccataagatc tatgttattg tcaagaaaga tattaanaaga atatatagaa
301 tttaacttta tatgtgttat aacatctagt tctttttcgc atgattcttt tatagatagt
361 agtcttttat tactgtttat atgttccatg tttactataa aaccttctga attagctatt
421 tcaggatttt tagatatttc taacatcatt ttagatatta tcataatagc tatcttgtca
481 tctaaaaagc taacacaagt tagaggcgta ttaccgtgat tatttagaga attatagtcg
541 gcgttataag ataaaagtaa ttttatatta ttaaaactat tagataacat agctttatgt
601 aaaggagtat ttccagataa cttagcttta gcattttacgt aagcaccgtg gtcaagtaag
661 agtttaacaa attctgtttt catagaacta actgccatgt atagaggagt gaaaccttta
721 tgattataga cgtttacata gcaaccatat aataagatcg cattcagtat attaatatct
781 ttcattttcta tagctatgtg aataacatgt ttatctaate ctaccaactt tgtatcagta
841 ccgtacttca gtaataagtt tactatagtt ttgtttttag atgcaacagc tatattttaga
901 acggtatcta tatgattatt aaccacatta acattagatc ctctttctaa aagtgtcttt
961 gttgtttcga tatcgttacg tgaacacagc taatgtaagg gactgcccac acagtcacat
1021 attacgttta tatcagctcc tagatttaac agaagtgtcg ttacatcttt tcttctatta
1081 attaccgaat gatgtaatgg ggttttacct aaatcatctt gttegtttat aggcaactcg
1141 tgatttataa gtaacgctat tatatcgtaa ctacaattat ttttaagtgc ctttatgaga
1201 tactgtttat gcaaaaataa acttttatct attttaatac tattatctaa caatatccta
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1681 gatagtatat ttacgttatt gtatttgcac tttttaagta tataccttac taaatttata
1741 tctctataacc ttatagcttt atgcagttca tttataagtc ttccattact catttctggt
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1861 catgttattt attatttgtt ataattatac tatttaataa attataccaa atacttagat
1921 acttattaat accatcctag aacttgtatt tcttgcccc taaacttggc catgcactcc
1981 attaggcggt tcttgttttc gacatcgtec tcttaacat atctactgt tatgtgagga
2041 ttccacggat tatctactgt gatataacca aacacgtcct tcgaacaggg taccgcattc
2101 agcagaacat ttcttagggc tctaagttca tcagatacct ccagtttcat aactacagcg
2161 catcctttcg ctcccaactg tttagaggcg ttactctgag gaaaacacat ctcttcttta
2221 cagactatag aaatagtctg taaatcttga tcagttattt gctttttgaa attttcaaat
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2401 taaaagttaa cagatgcca gttactataa tccaaggaa ccttaacatc taatcccatt
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2521 attcctgcca ttttgtaacg atagegaagc aatagcttgt atgcttttta tttgattaac
2581 taggggttaa ttaattagtc atcaggcagg gcgagaacga gactatctgc tcgttaatta
2641 attagagctt ctttattcta tacttaaaaa gtgaaaataa atacaaaggt tcttgagggt
2701 tgtgttaaat tgaaagcgag aaataatcat aaattatttc attatcgga tatccgttaa
2761 gtttgtatcg taatgcggtt cttcttcgtg ttcttgcca tcgtgtgttt ccagggcacc
2821 cacggcgctt acgtggagat cggctacagc ctgagggaaca tcaccttcca cggcctggac
2881 accgacgact acaaccccaa gttcaacatc cccaccggcc tgccgtgga ccccgagggc
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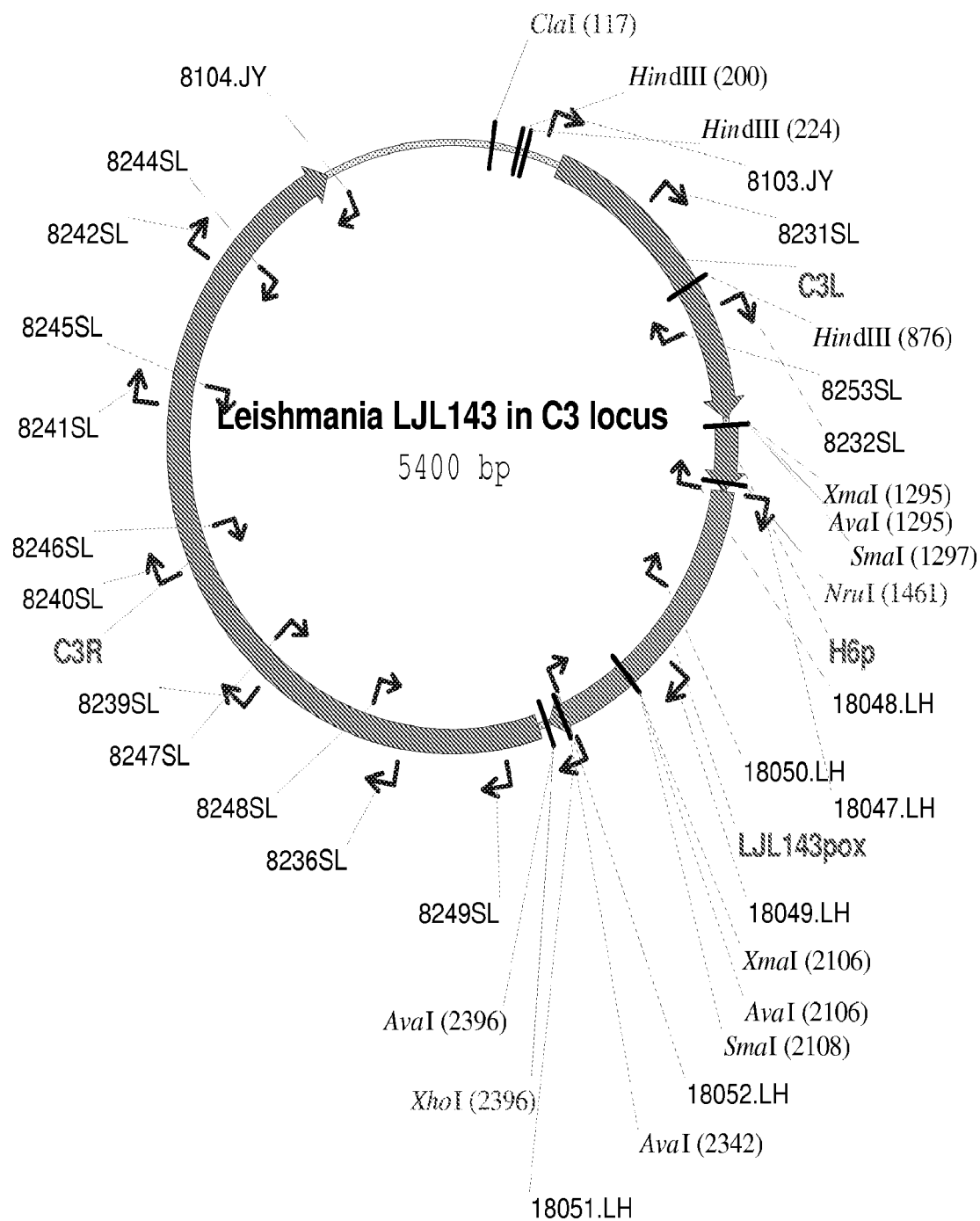
FIGURE 4 (5/5)

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3121 gactgcaggc ggctgtgggt gctggacatc ggcaaggtgg agtacacagg cggcgacgcc
3181 gaccagtacc ccaagggcaa gccaccctg atcgctacg acctgaagaa ggaccacacc
3241 cccgagatcc accggttcga gatccccgac gacctgtaca gcagccaggt ggagttcggc
3301 ggctttgccg tggacgtggg gaacaccaag ggcgactgca ccgagagctt cgtgtacctg
3361 accaacttca aggacaacag cctgatcgtg tacgacgaga cccagaagaa ggcttggaa
3421 ttcaccgaca agaccttcga ggccgacaaa gagagcacct tcagctacag cggcgaggaa
3481 cagatgaagt acaaagtggg cctgttcggc atcgccctgg gcgaccggga cgagatgggc
3541 cacaggcccg cctgctacat cgccggcagc agcaccaagg tgtacagcgt gaataccaaa
3601 gagctgaaaa ccgagaacgg ccagctgaac cccagctgc acggcgaccg gggaagtac
3661 accgacgcca ttgccctggc ctacgacccc gagcacaagg tctgtactt cggcgagagc
3721 gacagccggc aggtgtcctg ctggaacgtg aacatggaac tgaagccga caacaccgac
3781 gtgatcttca gcagcgcccg gttcaccttc ggaccgaca tctgtgtgga cagcaagggc
3841 atgtgttgga tcatggccaa cggccacccc cccgtggagg accaggaaaa gatctggaag
3901 atgcggttcg tgaaccggaa gatccggatc atgaaggtgg acaccgagcg ggtgttcaag
3961 tacagccggt gcaaccccaa ctacaagccc cccaaagaaa tcgaagtgtg atgactcgag
4021 tttttattga ctaggggttt ttatagctaa ttagtcaa atgtagtta attagtatac
4081 tacattacta atttattaca tattcattta tatcaatcta gtagcattta gcttttataa
4141 aacaatataa ctgaatagta catactttac taataagtta taaataagag atacatatat
4201 atagtatttt actttctaca ctgaatataa taatataatt atacaaatat aattttta at
4261 actatatagt atataactga aataaaat acagtgaata tagttattat acatttatac
4321 cacatcaaag atgagttata acatcagtg cactgttagc aacagtagtt atacgatgag
4381 tagttactct cgtatggcgt tagtatgtat gtatcttcta gttttcttag taggcattat
4441 aggaaacgct aagcttataa ggttattaat ggtatctaga aatatactta ttataccggt
4501 tctcaacttg ggaatagccg atttgctgtt tgtgatattc atacctttat acattatata
4561 catactaagt aatttccatt ggcatttttg taaagcactt tgtaaaatta gttctttctt
4621 ttttacttct aacatgtttg caagtatatt tttaataact gtaataagcg tatatagata
4681 tgtaaaaatt acccttcctg gatttaccta taaatatgtt aacattagaa atatgtacat
4741 tactatatat ttcatatgga ttatttctat tatactaggg attcctgctc ttacttttag
4801 aaatactatc gtaacaaaaa ataacgacac gctgtgtatt aatcattatc atgataatag
4861 agaaattgct gaattgattt acaaagttat tatctgtatc agatttattt taggatacct
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4981 atctaataata tctga

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FIGURE 5



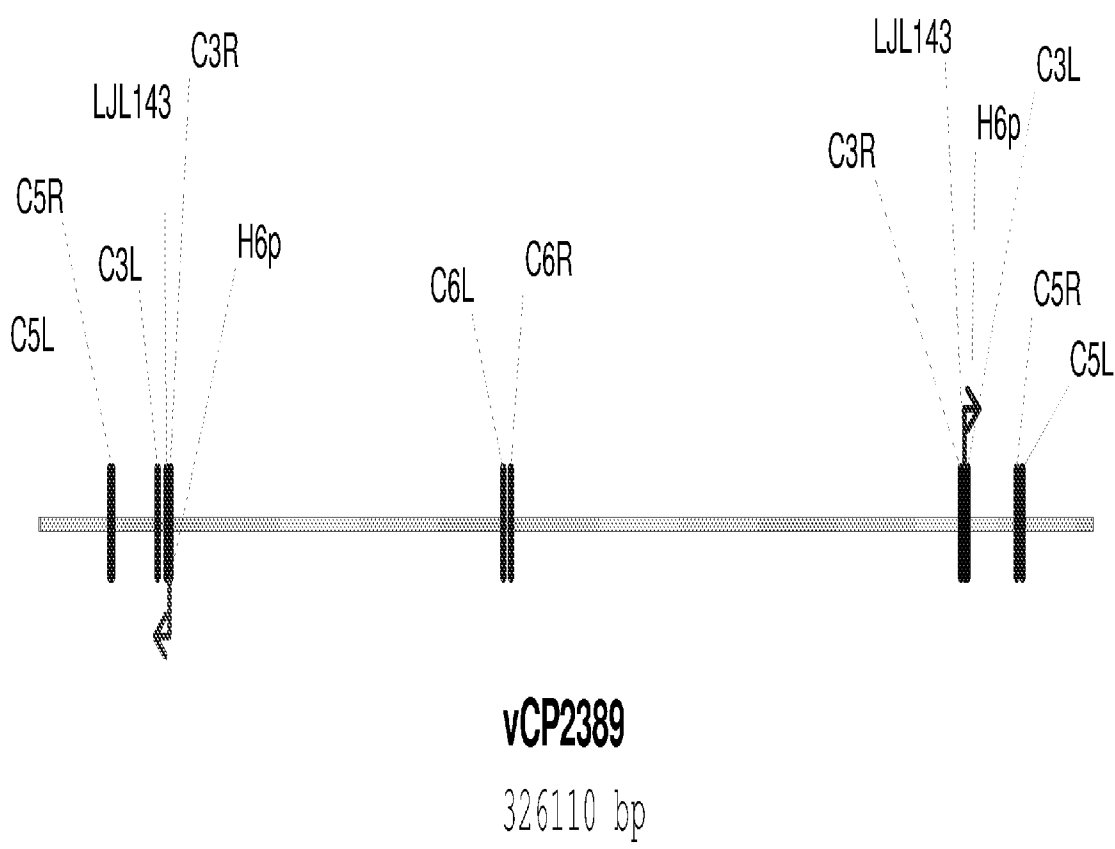


FIGURE 6 (1/3)

FIGURE 6 (2/3)

vCP2389 (ALVAC C3 H6p-synthetic Leishmania LJL143) (SEQ ID NO:94)

[336-1275]: C3 left arm
 [1300-1485]: H6 promoter
 [1486-2388]: Leishmania LJL143
 [2422-4993]: C3 right arm

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1   CGAGTCCTTTCTAACACTGTGGTTTTATTGGCTGGAATAAAAGGATAAAGACACCTATACTG
61  ATTCATTTTTTCATCTGTCAACGTTTCTCTAAGAGATTTCATAGGTATTATTATTACATCGAT
121 CTAGAAGTCTAATAAAGTCTAAGTATATTATTGGATTTAACGCGCTATAAACGCATCCAA
181 AACCTACAAATATAGGAGAAGCTTCTCTTATGAAACTTCTTAAAGCTTTACTCTTACTAT
241 TACTACTCAAAAGAGATATTACATTAATTATGTGATGAGGCATCCAACATATAAAGAAGA
301 CTAAGCTGTAGAAGCTGTTATGAAGAATATCTTATCAGATATATTAGATGCATTTGTTAG
361 TTCTGTAGATCAGTAACGTATAGCATACGAGTATAATTAATCGTAGGTAGTACGTATCCTA
421 AAATAAATCTGATACAGATAATAACTTTGTAATCAATTCAGCAATTTCTCTATTATCAT
481 GATAATGATTAAATACACAGCGTGTCTGTTATTTTTTTGTTACGATAGTATTTCTAAAGTAAA
541 GAGCAGGAATCCCTAGTATAATAGAAAAAATCCATATGAAAAAATAGTAATGTACATAAT
601 TTCTAATGTTAAACATATTTATAGGTAAATCCAGGAAGGGTAAATTTTACATATCTATATA
661 CGCTTATTACAGTTATTAAAAATATACCTTGCAAAACATGTTAGAAATAAAAAGAAAGAAC
721 TAATTTTACAAAGTGCTTTACCAAAATGCCAATGGAAATTAATAGTATGTATATAATGT
781 ATAAAGGTATGAATATCACAAACAGCAAAATCGGCTATTCCCAAGTTGAGAAACGGTATAA
841 TAGATATATTTCTAGATACCATTAATAACCTTATAAGCTTGACCTTCTCTATAATGCCCTA
901 CTAAGAAAACTAGAAAGATACATACATACTAACGCCATACGAGAGTAACCTACTCATCGTAT
961 AACTACTGTTCTAACAGTGCACACTGATGTTATAACTCATCTTTGATGTTGCTATAAATGT
1021 ATAACTACTATATTACACTGGTATTTTTATTTTCAGTTATATACATATAGTATTTAAAAATF
1081 ATATTTGTATAATTATATTATATTATATTTCAGTGTAGAAAGTAAATACTATAAATATGTAT
1141 CTCTTATTTATAACTTATTAAGTAAAGTAAGTACTAATTCAGTTAATTTGTTTATAAAAGC
1201 TAAATGCTACTAGATTGAATAAATGAATATGTAATTAATTAAGTAAATGTAATTAATTA
1261 ATTAACCTCACATTTGACTAATTAGCTATAAAAACCCGGGTTAAATTAATTAAGTAAATTA
1321 AGGGGAGAAACGAGACTATCTGCTCGTTAATTAATTAAGTAAATTAATTAAGTAAATTA
1381 AAAGTGAAATTAATTAACAAAGGTTCTTCAGGGGTTGTTGTTAAATTAAGTAAATTAAGT
1441 CATAAATTAATTTCAATTAATTCAGGATATTCAGTTAAGTTTGTATCGTAAATGAACAGCATCAAC
1501 TTTCTGAGCATCGTGGGCTGATCAGCTTCGGCTTCATCGTGGGCTGAGAGTGGGAGCGGC
1561 GAGGAGTACTTCATCGGCAAGTACAAAGAGAGAGGACGAGACCTGTTCTTCGGCAGCTAC
1621 GGGCTGAAGCGGGACCCCTGCCAGATCGTGTCTGGGCTACAAAGTGCAGCAACCAACGAGCC
1681 CACTTCGTGCTGAACCTTCAAGACCAACAAGAAGAGCTGCATCAGCGCCATCAAGCTGACC
1741 AGCTACCCCCAAGATCAACCAGAACAGCGACCTGACCAAGAACCTGTAATGCCAGACCGGC
1801 GGCATCGGCACCGACAACCTGCAAGCTGGTGTTCAGGAAGCGGAAGCGGCAGATCGCGGCC
1861 AACATCGAGATCTACGGCATCCCCGCCAAGAAGTGCAGCTTCAGGAGCCGTTACATCGGC
1921 GCGGACCCCCCTGCACGTGGACTCTACGGGCTGCCCTACCAGTTGAGCCAGGAACACGGGC
1981 TGGAACGTCGAGCGGTACAACATCTTCAAGGACACCCGGTTTCAGCACCGAGGTGTTCTAC
2041 CACAAGAACGGCCCTGTTCAACACCCAGATCACCTACCTGGCCGAAGAGGACAGCTTCAGC
2101 GAGGCCCCGGGAGATCACCGCCAAGGACATCAAGAAGAGTTTCAGCATCATCTCTGCCAAC
2161 GAGGAATACAAGCGGATCAGCTTCTGGACGTGTACTGGTTCCAGGAACCATGCGGAAG
2221 AAGCCCCAAGTACCCCTACATCCACTACAACGGCGAGTGTCTCCAAAGGAGACAAAGACCTGC
2281 GAACCTGGTGTTCGACACCGACGAGCTGATGACCTACGCCCTGGTGAAGGTGTTACCAAC
2341 CCGGAGAGCGACCGGACCGCGCTGAAAGAAGAGGACCTGGGCGAGGGCTGATGACTCGAG
2401 TTTTTATTGACTAGTTAATCAATAAAAAAGCATACAAGCTATTGCTTCGCTATCGTTACA
2461 AAATGGCAGGAATTTTGTGTAACTAAGCCACATACCTGCCAATGAAAAAATAGTAGAA
2521 AGGATACTATTTTAATGGGATTAGATGTTAAGGTTCTCTGGGATTATAGTAACCTGGGCAT
2581 CTGTTAACTTTTACGACGTTAGGTTAGATACTGATGTTACAGATTATTAATAATGTTACAA
2641 TAAATATCATGACAGGATGTGATATTTTTCTCATATAACTCTTGGAAATAGCAAAATATGG
2701 ATCAATGTGATAGATTTGAAAAATTTCAAAAAGCAAAATACTGATCAAGATTTACAGACTA
2761 TTTCTATAGTCTGTAAAGAAGAGATGTGTTTTCTCAGAGTAACGCTCTAAACAGTTGG

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FIGURE 6 (3/3)

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2821 GAGCGAAAGGATGCGCTGTAGTTATGAAACTGGAGGTATCTGATGAACTTAGAGCCCTAA
2881 GAAATGTTCTGCTGAATGCGGTACCCCTGTTTGAAGGACGTGTTTGGTGATATCACAGTAG
2941 ATAATCCGTGGAATCCTCACATAACAGTAGGATATGTTAAGGAGGACGATGTCGAAAAACA
3001 AGAAACCCCTAATGGAGTGCATGTCCAAGTTTAGGGGGCAAGAAATACAACTTCTAGGAT
3061 GGTATTAATAAGTATCTAAGTATTTGGTATAATTTATTAAATAGTATAATTATAACAAAT
3121 AATAAATAACATGATAACGGTTTTTATTAGAATAAAATAGAGATAATATCATAATGATAT
3181 ATAATACTTCATTACCAGAAATGAGTAATGGAAGACTTATAAATGAACTGCATAAAGCTA
3241 TAAAGTATAGAGATATAAATTTAGTAAGGTATATACTTAAAAAATGCATAATACAATAACG
3301 TAAATATACTATCAACGTCTTTGTATTTAGCCGTAAAGTATTTCTGATATAGAAATGGTAA
3361 AATTATTACTAGAACACGGTGCCGATATTTTAAAAATGTAAAAATCCCTCCCTTCATAAAG
3421 CTGCTAGTTTAGATAAATACAGAAATTGCTAAACTACTAATAGATTTCTGGCGCTGACATAG
3481 AACAGATACATTCTGGAAATAGTCCGTTATATATTTCTGTATATAGAAACAATAAGTCAT
3541 TAACTAGATATTTATTAAAAAAGGTGTTAATTGTAATAGATTTCTTCTAAATTATTACG
3601 ATGTACTGTATGATAAGATATCTGATGATATGTATAAAATATTTATAGATTTTAAATATTG
3661 ATCTTAATATACAACTAGAAATTTTGAAGTCCGTTACATTACCGCTATAAAGTATAAGA
3721 ATATAGATTTAATTAGGATATTGTTAGATAATAGTATTAAAAATAGATAAAAGTTTATTTT
3781 TGCATAAACAGTATCTCATAAAGGCACCTTAAAAATAATTGTAGTTACGATATAATAGCGT
3841 TACTTATAAATCACGGAGTGCCCTATRAACGAACRAGATGATTTAGGTAAAAACCCATTAC
3901 ATCATTCCGTAATTAATAGAAGAAAAGATGTAAACAGCACCTTCTGTTAAATCTAGGAGCTG
3961 ATATAAACGTAATAGATGACTGTATGGGCAGTCCCTTACATTACCGCTGTTTCACGTAACG
4021 ATATCGAAACAACARAAGACACTTTTAGAAAGAGGATCTAATGTTAATGTGGTTAATAATC
4081 ATATAGATACCGTTCTAAATATAGCTGTTGCATCTAAAAACAAAACATATAGTAAACTTAT
4141 TACTGAAGTACGGTACTGATACAAAGTTGGTAGGATTAGATAAACATGTTATTCACATAG
4201 CTATAGAAATGAARGATATTAATATACTGATGCGATCTTATTAATATGGTTGCTATGTAA
4261 ACGTCTATAATCATAAAGGTTTCACTCCTCTATACATGGCAGTTAGTTCTATGAAAAACAG
4321 AATTTGTTAAACTCTTACTTGACCACGGTGCTTACGTAAATGCTAAAGCTAAGTTATCTG
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4501 TTAGCTTTTTAGATGACAAGATAGCTATTATGATAATATCTAAATGATGTTAGAAATAT
4561 CTAAAAATCCTGAAATAGCTAATTCAGAAGGTTTTATAGTAAACATGGAACATATAAACA
4621 GTAATAAAAGACTACTATCTATAAAGAATCATGCGAAAAAGAACTAGATGTTATAACAC
4681 ATATAAAGTTAAATTCTATATATTCTTTTAATATCTTTCTTGACAATAACATAGATCTTA
4741 TGGTAAAGTTTCGTAACATAATCCTAGAGTTAATAAGATACCTGCATGTATACGTATATATA
4801 GCGAATTAATACGGAAAAAATAATCAATTAGCTTTTCATAGACATCAGCTAATAGTTAAAG
4861 CTGTAAAAGAGAGTAAGAATCTAGGAATAATAGGTAGGTTACCTATAGATATCAAAACATA
4921 TAATAATGGAACTATTAAAGTAATAATGATTTACATTCTGTTATCACCAGCTGTTGTAAAC
4981 CAGTAGTATAAAGTGATTTTATTCAATTACGAAGATAAACATTAAATTTGTTAAACAGATA

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FIGURE 7

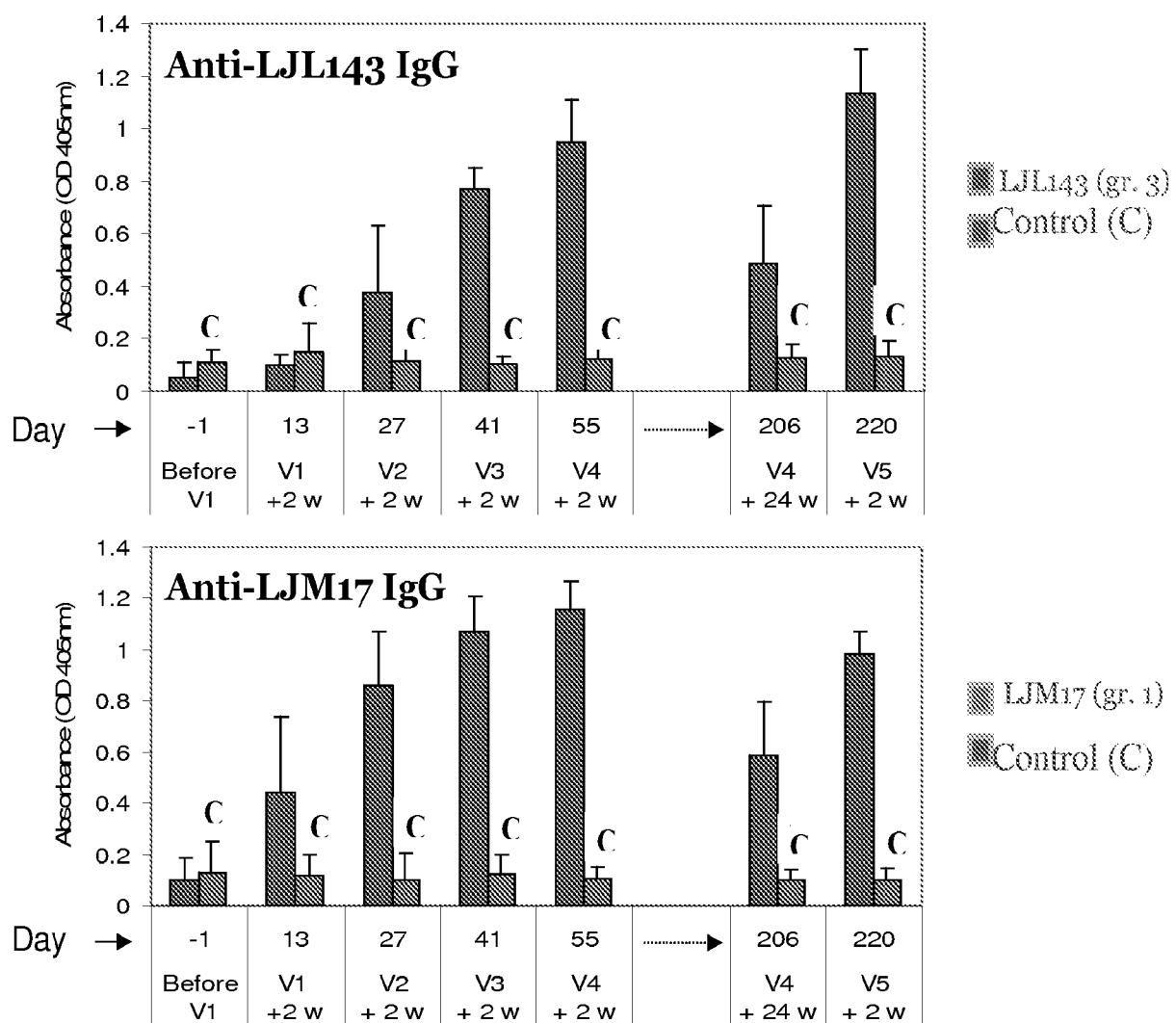


FIGURE 8

Measurement of IFN-gamma secreted at 72h – PBMC from dogs 2 week after 5th immunization, stimulated by SGH (2 pairs) / LJL143 (4 µg) / LJM17 (4 µg) / ConA (4 µg), or non-stimulated by medium (med)

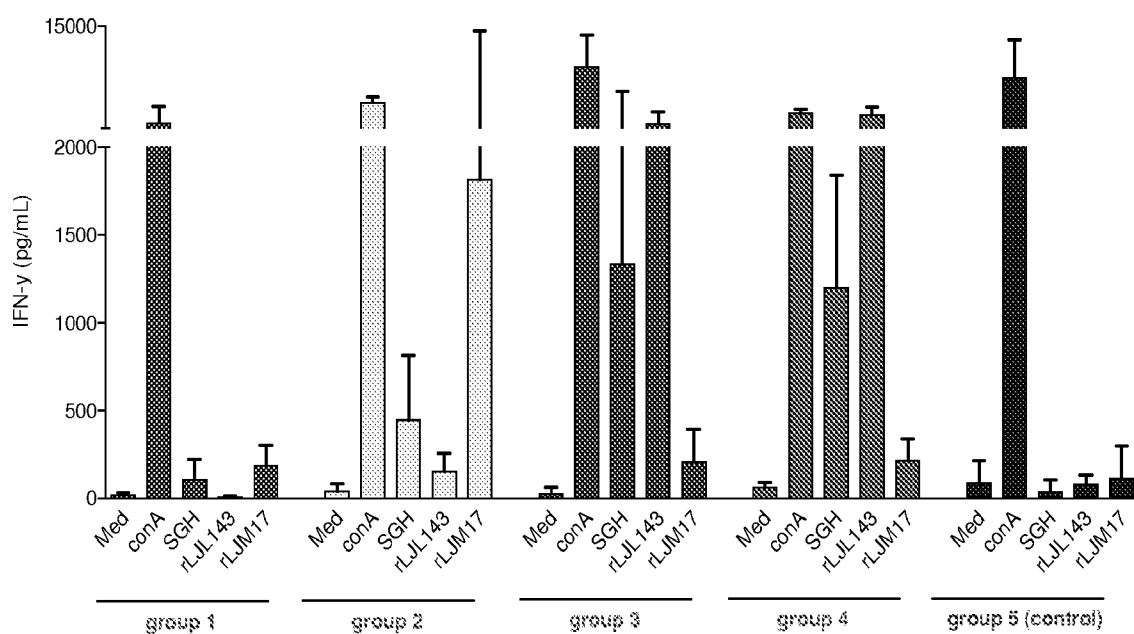


FIGURE 9

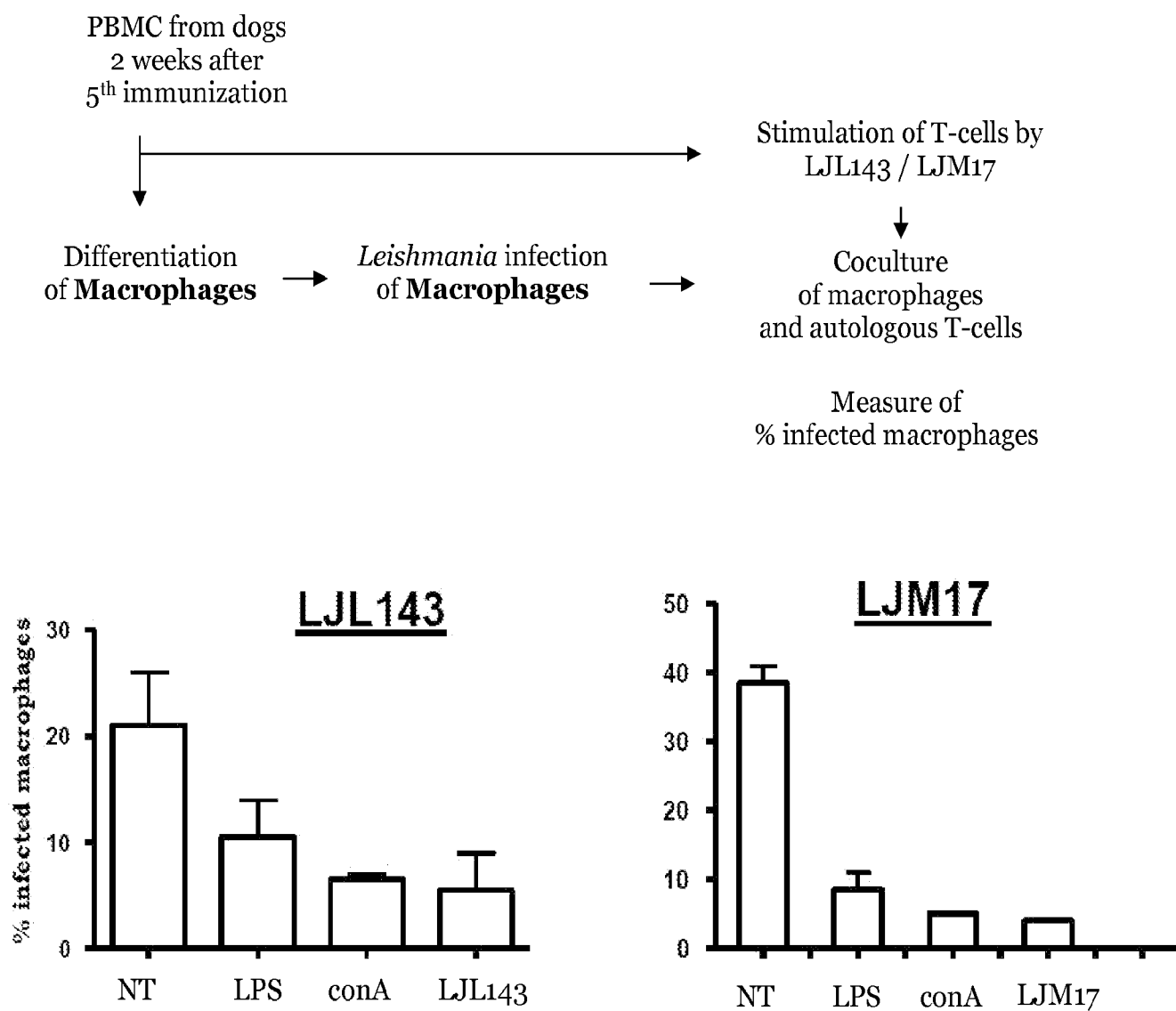


FIGURE 10

20 sand flies, 10 min on bellies of immunized dogs

→ Measure of DTH at 48h

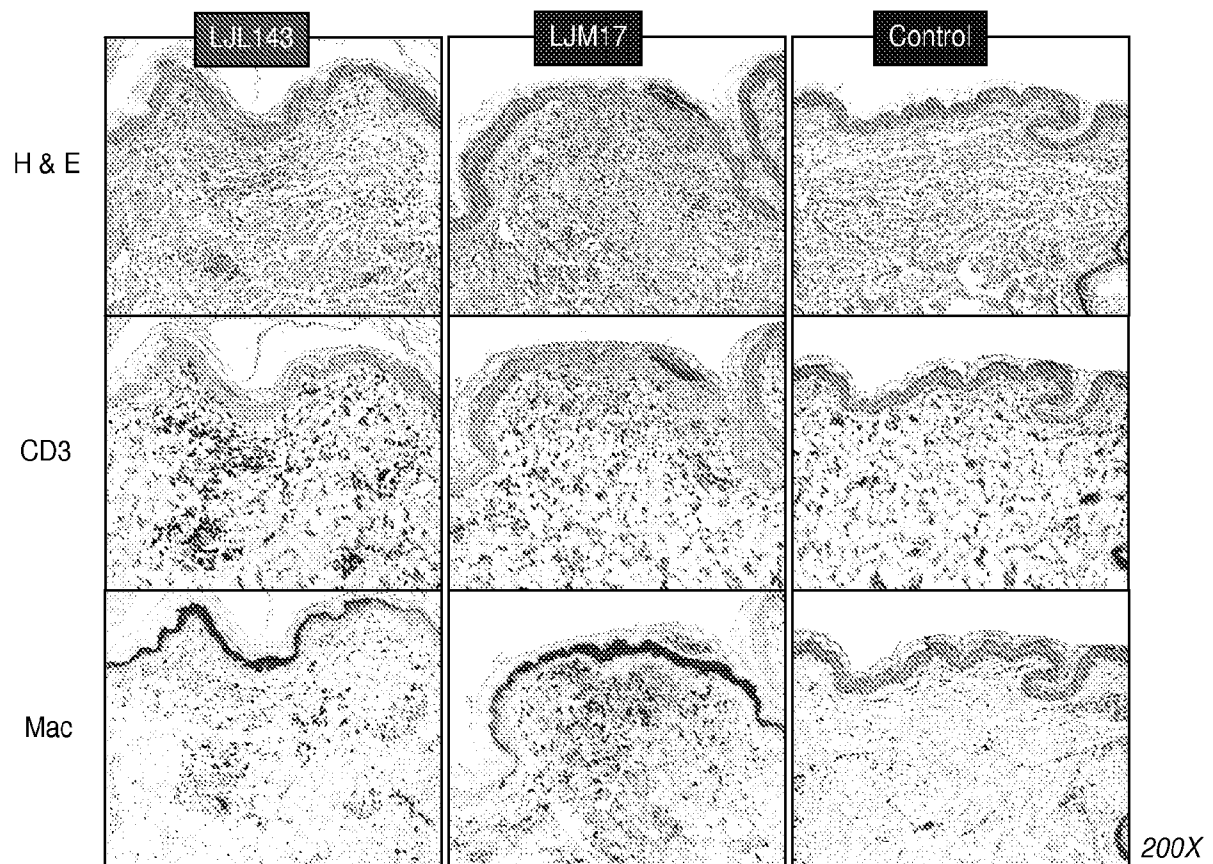


FIGURE 11 (1/2)
(pNBO002 : SEQ ID NO :19)

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AGGATCATACTCCGGAATTCATCGATTTGAAATTCAGACGATCTCTATAGCTCACAAGTTGAATTTGGTGGATTT
GCCGTTGATGTTGTTAACACGAAAGGAGACTGTACGGAGTCATTTGTCTACCTGACCAATTTCAAGGATAACTCTCT
AATTGTCTACGATGAGACACAAAAGAAAGCTTGGAAATTTACAGATAAAACATTTGAAGCTGATAAGGAATCCACGT
TCTCCTACTCGGGAGAGGAACAAATGAAGTACAAAGTTGGTCTTTTTGGGATAGCTCTGGGTGATAGGGATGAAATG
GGGCATCGTCTGCTACTATATCGCTGGGAGTAGCACCAAAGTCTACAGTGTTAAACACTAAAGAACTCAAAACAGA
GAATGGTCAGTTAAATCCTCAGCTTCACGGTGATCGTGGAAGTACACGGATGCAATTGCCCTAGCCCACGATCCTG
AGCATAAAGTCCTCTACTTTGCTGAATCCGACAGCAGGCAGGTGTCTGTTGGAATGTAGATATGGAGCTAAACCA
GACAATACGGATGTGATCTTCTAGTGCCCCGTTTTACTTTTGAACGGATATTTTGGTTGATAGCAAGGGAATGCT
GTGGATAATGGCTAATGGACATCCACCAGTAGAGGATCAAGAGAAGATTTGGAAGATGAGATTTCGTAACCGGAAGA
TCAGTATTATGAAAGTGGATACGGAACGTGTATTCAAATATTACGCTGCAATCCAAATTATAAGCCCCCGAAAGAA
ATTGAAGTTTGAaagggatccagatctgctgtgcttctagttgccagccatctgttggttggccctcccccggtgcc
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ggggatgcggtgggtctatgggtaccaggtgctgaagaattgacccggttctcctggggcagaaagaagcaggc
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tcaggaggggtccgccttcaatcccacccgctaaagtacttggagcgggtctctcctcctcatcagcccaccaaac
caaacctagcctccaagagtgggaagaaattaaagcaagataggctattaagtgcagagggagagaaaatgcctcca
acatgtgaggaagtaatgagagaaatcatagaatttcttccgcttctcctcgtcactgactcgtgcgctcggtcggt
cggtgcggcgagcgggtatcagctcactcaaaggcggtaatacgggttatccacagaatcaggggataacgcaggaaa
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gccccctgacgagcatcacaaaaatcgacgctcaagtgcagaggtggcgaaaccgacaggactataaagataccag
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FIGURE 11 (2/2)

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ccgcatcaga

FIGURE 12

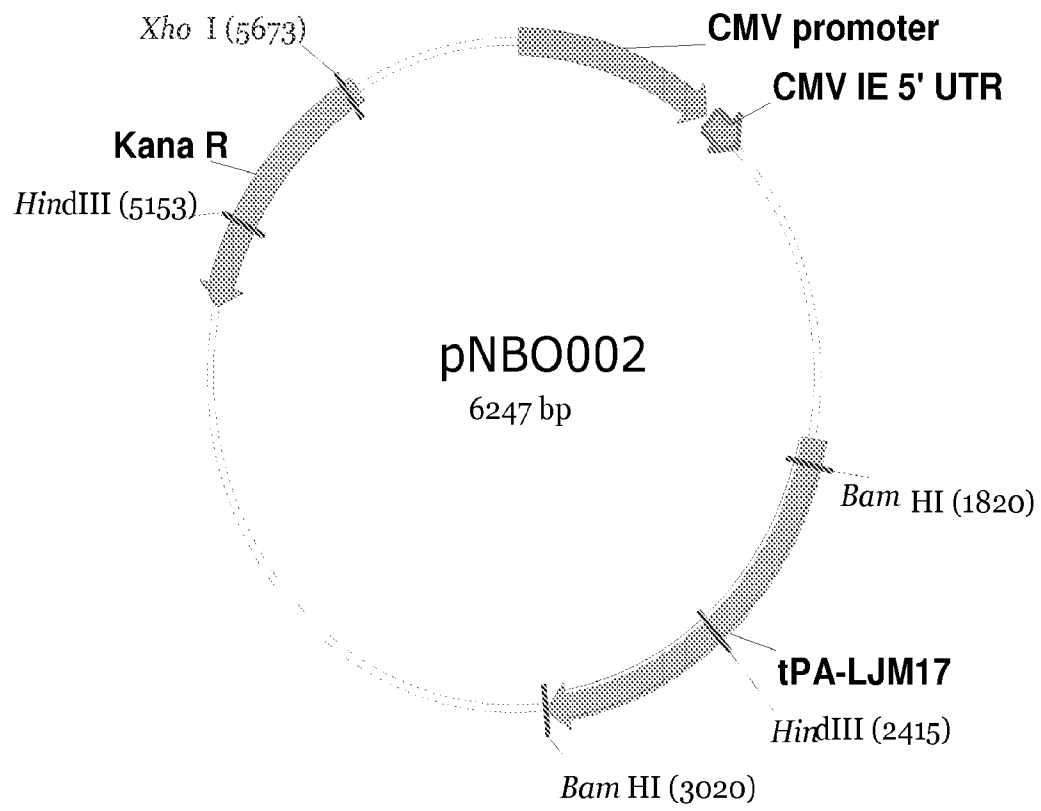


FIGURE 13 (1/2)
(pNBO003: SEQ ID NO:20)

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FIGURE 13 (2/2)

acaaaccaccgctggtagcgggtggttttttgtttgcaagcagcagattacgcgcagaaaaaaggatctcaagaag
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FIGURE 14

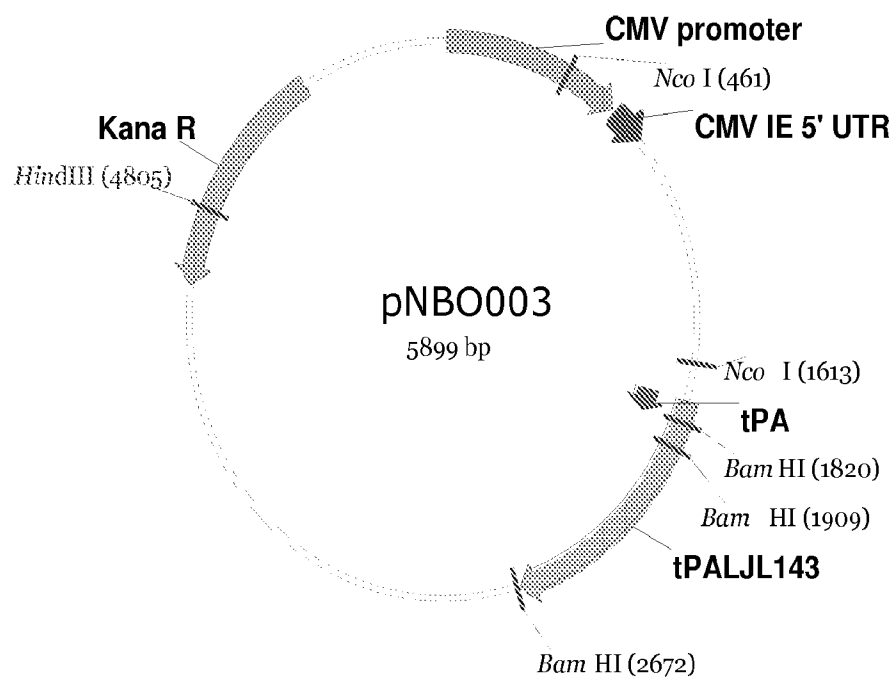


FIGURE 15 (1/2)**Unprocessed protein LJJ143 (SEQ ID NO: 1)**

MNSINFLSIVGLISFGFIVAVKCDGDEYFIGYKEKDETLFFASYGLKRDPCQIVLGYKCSNNQTHFVLNFKTNKKS
CISAIKLTSPKINQNSDLTKNLYCQTGGIGTDNCKLVFKRKRQIAANIEIYGIPAKKCSFKDRYIGADPLHVDSY
GLPYQFDQEHGWNVERYNIFKDRFSTEVFYHKNGLFNTQITYLAEEDSFSEAREITAKDIKKKFSIILPNEEYKRI
SFLDVYWFQETMRKKPKYPYIHYNGECSNENKTCELVFDTDLMTYALVKVFTNPESDGSRLKEEDLGRG

Mature protein LJJ143 (SEQ ID NO: 3)

DGDEYFIGYKEKDETLFFASYGLKRDPCQIVLGYKCSNNQTHFVLNFKTNKKS
CISAIKLTSPKINQNSDLTKNLYCQTGGIGTDNCKLVFKRKRQIAANIEIYGIPAKKCSFKDRYIGADPLHVDSY
GLPYQFDQEHGWNVERYNIFKDRFSTEVFYHKNGLFNTQITYLAEEDSFSEAREITAKDIKKKFSIILPNEEYKRI
SFLDVYWFQETMRKKPKYPYIHYNGECSNENKTCELVFDTDLMTYALVKVFTNPESDGSRLKEEDLGRG

Unprocessed protein LJM17 (SEQ ID NO: 5)

MRFFVFLAIVLFQGIHGAYVEIGYSLRNITFDGLDIDDYNNPKFNPTGLAVDPEGYRLFIAIPRRKPKVPYTV
AELNMVMNPGFPVERAPSFEEKFKFNNGEGKKDLNVVYQPVDDCRRWVLDIGKVEYTGADADQYPKGKPTLI
AYDLKKDHTPEIHRFEIPDDLYSSQVEFGGFAVDVVNTKGDCTESFVYLTNFKDNLIVYDETQKKAWKFTD
KTFEADKESTFSYSGEEQMKYKVGLFGIALGDRDEMGRHPACYIAGSSTKVYSVNTKELKTENGQLNPQLH
GDRGKYTDAIALAYDPEHKVLYFAESDSRQVSCWNVNMEKPDNTDVIFSSARFTFGTDILVDSKGMLWIM
ANGHPPVEDQEKIWKMRVNRKIRIMKVDTERVFKYSRCNPYKPPKEIEV

Mature protein LJM17 (SEQ ID NO: 7)

AYVEIGYSLRNITFDGLDIDDYNNPKFNPTGLAVDPEGYRLFIAIPRRKPKVPYTV
AELNMVMNPGFPVERAPSFEEKFKFNNGEGKKDLNVVYQPVDDCRRWVLDIGKVEYTGADADQYPKGKPTLI
AYDLKKDHTPEIHRFEIPDDLYSSQVEFGGFAVDVVNTKGDCTESFVYLTNFKDNLIVYDETQKKAWKFTD
KTFEADKESTFSYSGEEQMKYKVGLFGIALGDRDEMGRHPACYIAGSSTKVYSVNTKELKTENGQLNPQLH
GDRGKYTDAIALAYDPEHKVLYFAESDSRQVSCWNVNMEKPDNTDVIFSSARFTFGTDILVDSKGMLWIM
ANGHPPVEDQEKIWKMRVNRKIRIMKVDTERVFKYSRCNPYKPPKEIEV

Unprocessed protein LJJ143 (SEQ ID NO: 11)

MNSINFLSIVGLISFGFIVAVKCDGDEYFIGYKEKDETLFFASYGLKRDPCQIVLGYKCSNNQTHFVLNFKTNKKS
CISAIKLTSPKINQNSDLTRNLYCQTGGIGTDNCKLVFKRKRQIAANIEIYGIPAKKCSFKDRYIGADPLHVDSY
GLSYQFDQEHGWNLERNNIFKDRFSTEVFYHKNGLFNTQITYLAEEDSFSEAREITAKDIKKKFSIILPNEEYKRI
SFLDVYWFQETMRKKPKYPYIHYNGECSNENKTCELVFDTDLMTYALVKVFTNPESDGSRLKEEDLGRG

FIGURE 15 (2/2)**Mature protein LJJ143 (SEQ ID NO: 13)**

DGDEYFIGKYKEKDETLFFASYGLKRDPQIVLGYKCSNNQTHFVLNFKTNKKSCISAIKLTSPKINQNSDLTRNL
YCQTGGIGTDNCKLVFKKRKRQIAANIEIYGIPAKKCSFKDRYIGADPLHVDSYGLSYQFDQEHGWNLERNNIFKDT
RFSTEVFYHKNGLFNTQITYLAEEDSFSEAREITAKD_LKKFSIILPNEEYKRISFLDVYWFQETMRKKPKYPYIH
NGECSNENKTCELVFDTDELMTYALVKVFTNPESDGSRLKEED_LGRG

Unprocessed protein LJM17 (SEQ ID NO: 15)

MRFFVFLAIVLFQGIHGAYVEIGYSLRNITFDGLDITDDYNPKFNIPTGLAVDPEGYRLFIAIPRRKPKVPYTV
AELNMVMNPGFPVERAPSFEKFKKFNNGEGKKDLNVVYQPVDDCRRWVLDIGKVEYTTGGADQYPKGKPTLIAYDLKKD
HTPEIHRFEIPDDLYSSQVEFGGFAVDVVNTKGDCTESFVYLTNFKDNSLIVYDETQKKAWKFTDKTFEADKESTFS
YSGEEQMKYKVGLFGIALGDRDEMGRPAYYIAGSSTKVYSVNTKELKTENGQLNPQLHGDRGKYTDAIALAHDPEH
KVLYFAESDSRQVSCWNVDMELKPDNTDVIFSSARFTFGTDILVDSKGMLWIMANGHPPVEDQEKIWKMRVNRKIS
IMKVDTERVFKYSRCNPNYKPPKEIEV

Mature protein LJM17 (SEQ ID NO: 17)

AYVEIGYSLRNITFDGLDITDDYNPKFNIPTGLAVDPEGYRLFIAIPRRKPKVPYTV
AELNMVMNPGFPVERAPSFEKFKKFNNGEGKKDLNVVYQPVDDCRRWVLDIGKVEYTTGGADQYPKGKPTLIAYDLKKDHTPEIHRFEIPDDLYSSQ
VEFGGFAVDVVNTKGDCTESFVYLTNFKDNSLIVYDETQKKAWKFTDKTFEADKESTFSYSGEEQMKYKVGLFGIAL
GDRDEMGRPAYYIAGSSTKVYSVNTKELKTENGQLNPQLHGDRGKYTDAIALAHDPEHKVLYFAESDSRQVSCWNV
DMELKPDNTDVIFSSARFTFGTDILVDSKGMLWIMANGHPPVEDQEKIWKMRVNRKISIMKVDTERVFKYSRCNPN
YKPPKEIEV

FIGURE 16 (1/5)**Polynucleotide encoding unprocessed protein LJL143 (SEQ ID NO: 2)**

ATGAATTCGATTAATTTCCCTAACAATAGTTGGTTTTAAACAGTTTTGGATTTCATTGTTGCAGTAAAGTGTGATGGTGAT
GAATATTTTCATTGGAAAATACAAAGAAAAAGATGAGACACTGTTTTTTGCAAGCTACGGCCTAAAGAGGGATCCTTGC
CAAATTGCTTTAGGCTACAAATGCTCAAACAATCAAACCCACTTTGTGCTTAATTTTAAACCAATAAGAAATCCTGC
ATATCAGCAATTAAGCTGACTTCTTACCCAAAAATCAATCAAACTCGGATTTAACTAAAAATCTCTACTGCCAACT
GGAGGAATAGGAACAGATAACTGCAAACCTTGCTTTCAAGAAACGTAAAAGACAAATAGCAGCTAATATTGAAATCTAC
GGCATTCCAGCGAAGAAATGTTCTTCAAGGATCGTTACATTGGAGCTGATCCACTCCACGTCGATTCCCTATGGGCTT
CCGTATCAGTTTGATCAGGAACATGGATGGAATGTGGAACGATATAACATTTTCAAAGACACAAGATTTTCCACAGAA
GTTTTCTACCACAAAAATGGTTTATTTAACACCCAAAATACTTATTTGGCTGAAGAAGATTCTTCTCTGAAGCTCGA
GAGATTACTGCGAAGGATATTAAGAAGAAGTTTTCAAATATTTTGCCCAATGAAGAGTATAAGAGGATTAGTTTCTTG
GACGTTTATTGGTTCCAGGAGACTATGCGAAAAAGCCTAAATATCCCTACATTCACTACAATGGAGAATGCAGCAAT
GAGAATAAACTTGTGAACCTGTCTTTGACACCGATGAACTAATGACCTACGCCCTTGTTAAAGTCTTTACTAATCCT
GAGAGTGATGGATCTAGGCTCAAAGAAGAGGATTTGGGAAGAGGATAA

Polynucleotide encoding mature protein LJL143 (SEQ ID NO: 4)

GATGGTGATGAATATTTTCATTGGAAAATACAAAGAAAAAGATGAGACACTGTTTTTTGCAAGCTACGGCCTAAAGAG
GGATCCTTGCCAAATTGCTTTAGGCTACAAATGCTCAAACAATCAAACCCACTTTGTGCTTAATTTTAAACCAATA
AGAAATCCTGCATATCAGCAATTAAGCTGACTTCTTACCCAAAAATCAATCAAACTCGGATTTAACTAAAAATCTC
TACTGCCAACTGGAGGAATAGGAACAGATAACTGCAAACCTTGCTTTCAAGAAACGTAAAAGACAAATAGCAGCTAA
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AGATTTTCCACAGAAGTTTTCTACCACAAAAATGGTTTATTTAACACCCAAATAACTTATTTGGCTGAAGAAGATTCT
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AGAGGATTAGTTTCTTGACGTTTATTGGTTCCAGGAGACTATGCGAAAAAGCCTAAATATCCCTACATTCACTAC
AATGGAGAATGCAGCAATGAGAATAAACTTGTGAACCTGTCTTTGACACCGATGAACTAATGACCTACGCCCTTGT
TAAAGTCTTTACTAATCCTGAGAGTGATGGATCTAGGCTCAAAGAAGAGGATTTGGGAAGAGGATAA

FIGURE 16 (2/5)**Polynucleotide encoding unprocessed protein LJM17 (SEQ ID NO: 6)**

ATGAGGTTCTTCTTTGTTTTCTTGCCATCGTCCTTTTCAAGGGATCCACGGAGCTTATGTGGAAATAGGATATTC
TCTGAGAAATATTACATTCGATGGATTGGATACAGATGACTACAATCCAAAGTTCAACATTCACCGGGTTTGGCAG
TTGATCCCGAAGGATATAGGCTCTTCATAGCCATCCCAAGGAGAAAGCCAAAGTTCCCTACACTGTGGCTGAACTG
AATATGGTCATGAATCCCGGATTTCCCGTCGAGAGAGCTCCGAGCTTTGAGAAATTCAAAAAATTCAATGGCGAGGG
CAAAAAGGATCTTGTTAATGTGTATCAGCCAGTCATTGATGATTGTCGTCGTCCTTTGGGTGCTTGACATTGGGAAGG
TGGAATACACCGGTGGTGATGCTGATCAATATCCCAAAGGAAAGCCTACCCTAATTGCCTACGACCTCAAGAAGGAT
CATACTCCGGAAATTCATCGATTTGAAATTCCAGACGATCTCTATAGCTCACAAGTTGAATTTGGTGGATTTGCCGT
TGATGTTGTTAACACGAAAGGAGACTGTACGGAGTCAATTGTCTACCTGACCAATTTCAAGGATAACTCTCTAATTG
TCTACGATGAGACACAAAAGAAAGCTTGGAATTCACAGATAAAACATTTGAAGCTGATAAGGAATCCACGTTCTCC
TACTCGGGAGAGGAACAAATGAAGTACAAAGTCGGTCTTTTTGGGATAGCTCTGGGTGATAGGGATGAAATGGGGCA
TCGTCCTGCCTGCTACATCGCTGGGAGTAGCACCAAAGTCTACAGTGTTAACTAAAGAACTCAAAACAGAGAATG
GTCAGTTAAATCCTCAGCTTCACGGTGATCGTGAAAGTACACAGATGCAATTGCCCTAGCCTACGATCCTGAGCAT
AAAGTCCTCTACTTTGCTGAAATCCGACAGCAGGCAGGTGTCCTGTTGGAATGTAAATATGGAGCTAAAACAGACAA
TACGGATGTGATCTTCTCTAGTGCCCGTTTTACTTTTGAACGGATATTTTGGTTGATAGCAAGGGAATGCTGTGGA
TAATGGCTAATGGACATCCACCAGTAGAGGATCAAGAGAAGATTTGGAAGATGAGATTCGTAAACCGGAAGATCCGT
ATTATGAAAGTGGATACGGAACGTGTTTTCAAATATTCACGCTGCAATCCAAATTATAAGCCCCCAAAGGAAATTGA
AGTTTGA

Polynucleotide encoding mature protein LJM17 (SEQ ID NO: 8)

GCTTATGTGGAAATAGGATATTTCTCTGAGAAATATTACATTCGATGGATTGGATACAGATGACTACAATCCAAAGTT
CAACATTCACCGGGTTTGGCAGTTGATCCCGAAGGATATAGGCTCTTCATAGCCATCCCAAGGAGAAAGCCAAAGG
TTCCCTACACTGTGGCTGAACGAATATGGTCATGAATCCCGGATTTCCCGTCGAGAGAGCTCCGAGCTTTGAGAAA
TTCAAAAAATTCAATGGCGAGGGCAAAAAGGATCTTGTTAATGTGTATCAGCCAGTCATTGATGATTGTCGTCGTCCT
TTGGGTGCTTGACATTGGGAAGGTGGAATACACCGGTGGTGATGCTGATCAATATCCCAAAGGAAAGCCTACCCTAA
TTGCCTACGACCTCAAGAAGGATCATACTCCGGAAATTCATCGATTTGAAATTCCAGACGATCTCTATAGCTCACAA
GTTGAATTTGGTGGATTTGCCGTTGATGTTGTTAACACGAAAGGAGACTGTACGGAGTCAATTTGTCTACCTGACCAA
TTTCAAGGATAACTCTCTAATTGTCTACGATGAGACACAAAAGAAAGCTTGGAATTCACAGATAAAACATTTGAAG
CTGATAAGGAATCCACGTTCTCCTACTCGGGAGAGGAACAAATGAAGTACAAAGTCGGTCTTTTTGGGATAGCTCTG
GGTGATAGGGATGAAATGGGGCATCGTCCTGCCTGCTACATCGCTGGGAGTAGCACCAAAGTCTACAGTGTTAACAC
TAAAGAACTCAAAACAGAGAAATGGTCAGTTAAATCCTCAGCTTCACGGTGATCGTGAAAGTACACAGATGCAATTG
CCCTAGCCTACGATCCTGAGCATAAAGTCCTCTACTTTGCTGAATCCGACAGCAGGCAGGTGTCCTGTTGGAATGTA
AATATGGAGCTAAAACAGACAATACGGATGTGATCTTCTCTAGTGCCCGTTTTACTTTTGAACGGATATTTTGGT
TGATAGCAAGGGAATGCTGTGGATAATGGCTAATGGACATCCACCAGTAGAGGATCAAGAGAAGATTTGGAAGATGA
GATTCGTAAACCGGAAGATCCGTATTATGAAAGTGGATACGGAACGTGTTTTCAAATATTCACGCTGCAATCCAAAT
TATAAGCCCCCAAAGGAAATTGAAGTTTGA

FIGURE 16 (3/5)**Polynucleotide encoding unprocessed protein LJL143 (SEQ ID NO: 12)**

ATGAATTCGATTAAATTCCTATCAATAGTTGGTTTAAACAGTTTTGGATTCAATTGTTGCAGTAAAGTGTGATGGTGA
TGAATATTTTCATTGGAAAATACAAAGAAAAAGATGAGACACTGTTTTTTGCAAGCTACGGCCTAAAGAGGGATCCTT
GCCAGATTGTCTTAGGCTACAAATGCTCAAACAATCAAACCCACTTTGTGCTTAATTTTAAACCAATAAGAAATCC
TGCATATCAGCAATTAAGCTGACTTCTTACCCAAAAATCAATCAAAACTCGGATTTAACTAGAAATCTCTACTGCCA
AACTGGAGGAATAGGAACAGATAAAGCTGCAAACTTGTCTTCAAGAAACGTAAAAGACAAATAGCAGCTAATATTGAAA
TCTACGGCATTCCAGCGAAGAAATGTTCTTCAAGGAACGTTACATTGGAGCTGATCCACTCCACGTCGATTCTCTAT
GGGCTTTTCGTATCAGTTTGATCAGGAACATGGATGGAATTTGGAACGAAATAACATTTTCAAAGACACAAGATTTTC
CACAGAAGTTTTCTACCACAAAAATGGTTTATTTAACACCCAAATAACTTATTTGGCTGAAGAAGATTCTCTCTG
AAGCTCGAGAGATTACTGCGAAGGATATTAAGAAGAAGTTTTCAATTATTTGCCCAATGAAGAGTATAAGAGGATT
AGTTTCTTGACGTTTATTGGTTCCAGGAGACTATGCGAAAAAGCCTAAATATCCCTACATTCACTACAATGGAGA
ATGCAGCAATGAGAATAAAACCTGTGAACCTGTCTTTGACACCGATGAACTAATGACCTACGCCCTTGTTAAAGTCT
TTACTAATCCTGAGAGTGATGGATCTAGGCTCAAAGAAGAGGATTTGGGAAGAGGATAA

Polynucleotide encoding mature protein LJL143 (SEQ ID NO: 14)

GATGGTGATGAATATTTTCATTGGAAAATACAAAGAAAAAGATGAGACACTGTTTTTTGCAAGCTACGGCCTAAAGAG
GGATCCTTGCCAGATTGTCTTAGGCTACAAATGCTCAAACAATCAAACCCACTTTGTGCTTAATTTTAAACCAATA
AGAAATCCTGCATATCAGCAATTAAGCTGACTTCTTACCCAAAAATCAATCAAAACTCGGATTTAACTAGAAATCTC
TACTGCCAAACTGGAGGAATAGGAACAGATAAAGCTGCAAACTTGTCTTCAAGAAACGTAAAAGACAAATAGCAGCTAA
TATTGAAATCTACGGCATTCCAGCGAAGAAATGTTCTTCAAGGATCGTTACATTGGAGCTGATCCACTCCACGTCG
ATTCTATGGGCTTTTCGTATCAGTTTGATCAGGAACATGGATGGAATTTGGAACGAAATAACATTTTCAAAGACACA
AGATTTTCCACAGAAGTTTTCTACCACAAAAATGGTTTATTTAACACCCAAATAACTTATTTGGCTGAAGAAGATTCT
CTTCTCTGAAGCTCGAGAGATTACTGCGAAGGATATTAAGAAGAAGTTTTCAATTATTTGCCCAATGAAGAGTATA
AGAGGATTAGTTTCTTGACGTTTATTGGTTCCAGGAGACTATGCGAAAAAGCCTAAATATCCCTACATTCACTAC
AATGGAGAATGCAGCAATGAGAATAAAACCTGTGAACCTGTCTTTGACACCGATGAACTAATGACCTACGCCCTTG
TAAAGTCTTTACTAATCCTGAGAGTGATGGATCTAGGCTCAAAGAAGAGGATTTGGGAAGAGGATAA

FIGURE 16 (4/5)**Polynucleotide encoding unprocessed protein LJM17 (SEQ ID NO: 16)**

ATGAGGTTCTTCTTTGTTTTCTTGCCATCGTCCTTTTCAAGGGATCCACGGAGCTTATGTGGAAATAGGATATTC
TCTGAGAAATATTACATTCGATGGATTGGATACAGATGACTACAATCCAAAGTTCAACATTCACCGGGTTTGGCAG
TTGATCCCGAAGGATATAGGCTCTTCATAGCCATCCCAAGGAGAAAGCCAAAGTTCCCTACACTGTGGCTGAACTG
AATATGGTCATGAATCCCGGATTTCCCGTCGAGAGAGCTCCGAGCTTTGAGAAATTCAAAAAATTCAATGGCGAGGG
CAAAAAGGATCTTGTTAATGTGTATCAGCCAGTCATTGATGATTGTCGTCGTCCTTTGGGTGCTTGACATTGGGAAGG
TGGAATACACCGGTGGTGATGCTGATCAATATCCCAAAGGAAAGCCTACCCTAATTGCCTACGACCTCAAGAAGGAT
CATACTCCGGAAATTCATCGATTTGAAATTCCAGACGATCTCTATAGCTCACAAGTTGAATTTGGTGGATTTGCCGT
TGATGTTGTTAACACGAAAGGAGACTGTACGGAGTCAATTGTCTACCTGACCAATTTCAAGGATAACTCTCTAATTG
TCTACGATGAGACACAAAAGAAAGCTTGGAATTTACAGATAAAACATTTGAAGCTGATAAGGAATCCACGTTCTCC
TACTCGGGAGAGGAACAAATGAAGTACAAAGTTGGTCTTTTTGGGATAGCTCTGGGTGATAGGGATGAAATGGGGCA
TCGTCCTGCCTACTATATCGCTGGGAGTAGCACCAAAGTCTACAGTGTTAACTAAAGAACTCAAAACAGAGAATG
GTCAGTTAAATCCTCAGCTTCACGGTGATCGTGAAAGTACACGGATGCAATTGCCCTAGCCCACGATCCTGAGCAT
AAAGTCCTCTACTTTGCTGAAATCCGACAGCAGGCAGGTGTCCTGTTGGAATGTAGATATGGAGCTAAAACAGACAA
TACGGATGTGATCTTCTCTAGTGCCCGTTTTACTTTTGAACGGATATTTTGGTTGATAGCAAGGGAATGCTGTGGA
TAATGGCTAATGGACATCCACCAGTAGAGGATCAAGAGAAGATTTGGAAGATGAGATTCGTAAACCGGAAGATCAGT
ATTATGAAAGTGGATACGGAACGTGTATTCAAATATTCACGCTGCAATCCAAATTATAAGCCCCCGAAAGAAATTGA
AGTTTGA

Polynucleotide encoding mature protein LJM17 (SEQ ID NO: 18)

GCTTATGTGGAAATAGGATATTTCTCTGAGAAATATTACATTCGATGGATTGGATACAGATGACTACAATCCAAAGTT
CAACATTCACCGGGTTTGGCAGTTGATCCCGAAGGATATAGGCTCTTCATAGCCATCCCAAGGAGAAAGCCAAAGG
TTCCCTACACTGTGGCTGAACGAAATATGGTCATGAAATCCCGGATTTCCCGTCGAGAGAGCTCCGAGCTTTGAGAAA
TTCAAAAAATTCAATGGCGAGGGCAAAAAGGATCTTGTTAATGTGTATCAGCCAGTCATTGATGATTGTCGTCGTCCT
TTGGGTGCTTGACATTGGGAAGGTGGAATACACCGGTGGTGATGCTGATCAATATCCCAAAGGAAAGCCTACCCTAA
TTGCCTACGACCTCAAGAAGGATCATACTCCGGAAATTCATCGATTTGAAATTCCAGACGATCTCTATAGCTCACAA
GTTGAATTTGGTGGATTTGCCGTTGATGTTGTTAACACGAAAGGAGACTGTACGGAGTCAATTTGTCTACCTGACCAA
TTTCAAGGATAACTCTCTAATTGTCTACGATGAGACACAAAAGAAAGCTTGGAATTTACAGATAAAACATTTGAAG
CTGATAAGGAATCCACGTTCTCCTACTCGGGAGAGGAACAAATGAAGTACAAAGTTGGTCTTTTTGGGATAGCTCTG
GGTGATAGGGATGAAATGGGGCATCGTCCTGCCTACTATATCGCTGGGAGTAGCACCAAAGTCTACAGTGTTAACAC
TAAAGAACTCAAAACAGAGAAATGGTCAGTTAAATCCTCAGCTTCACGGTGATCGTGAAAGTACACGGATGCAATTG
CCCTAGCCCACGATCCTGAGCATAAAGTCCTCTACTTTGCTGAATCCGACAGCAGGCAGGTGTCCTGTTGGAATGTA
GATATGGAGCTAAAACAGACAATACGGATGTGATCTTCTCTAGTGCCCGTTTTACTTTTGAACGGATATTTTGGT
TGATAGCAAGGGAATGCTGTGGATAATGGCTAATGGACATCCACCAGTAGAGGATCAAGAGAAGATTTGGAAGATGA
GATTCGTAAACCGGAAGATCAGTATTATGAAAGTGGATACGGAACGTGTATTCAAATATTCACGCTGCAATCCAAAT
TATAAGCCCCCGAAAGAAATTGAAGTTTGA

FIGURE 16 (5/5)

Codon optimzed unprocessed LJM17 DNA sequence (SEQ ID NO :91)

atgcggttcttcttcgtgttcctggccatcgtgctgttccagggcatccacggcgcctacgtggagatcggtacag
cctgcggaacatcaccttcgacggcctggacaccgacgactacaaccccaagttcaacatccccaccggcctggccg
tggacccccgagggctaccggtgttcatcgccatccccagggcggaagcccaaggtgcctacaccgtggccgagctg
aacatggtgatgaaccccggttccccgtggagagggccccagcttcgagaagttcaagaagtttaacggcgaggg
caagaaagacctggtgaacgtgtaccagcccgtgatcgacgactgcaggcggctgtgggtgctggacatcggcaagg
tggagtacacaggcggcgacgccgaccagtaaccccaagggcaagcccaccctgatcgccctacgacctgaagaaggac
cacacccccgagatccaccggttcgagatccccgaacacctgtacagcagccaggtggagttcggcggctttgccgt
ggacgtggtgaacaccaagggcgactgcaccgagagcttcgtgtacctgaccaacttcaaggacaacagcctgatcg
tgtacgacgagacccagaagaaggcctggaagttcacccgacaagaccttcgaggccgacaaagagagcaccttcagc
tacagcggcgaggaacagatgaagtacaaagtgggcctgttcggcatcgccctgggcgacccgggacgagatgggcca
cagggcccgctgtacatcgccggcagcagcaccaaggtgtacagcgtgaataccaaagagctgaaaaccgagaacg
gccagctgaacccccagctgcacggcgacccgggcaagtaacccgacgccattgcctggcctacgaccccgagcac
aaggtgctgtacttcgccgagagcgacagccggcaggtgtcctgctggaacgtgaacatggaactgaagcccgacaa
caccgacgtgatcttcagcagcgcgccggttcaccttcggcacccgacatcctggtggacagcaagggcatgctgtgga
tcatggccaacggccaccccccccggtggaggaccaggaaaagatctggaagatgcggttcgtgaacccggaagatccgg
atcatgaaggtggacaccgagcgggtgttcaagtaacagccggtgcaaccccaactacaagccccccaaagaaatcga
agtgtga

FIGURE 17

Table 1. Global amino acid sequence identity percentage between mature LJL143 and LJM17

	SEQ ID NO:3 (LJL143)	SEQ ID NO:7 (LJM17)	SEQ ID NO:13 (LJL143)	SEQ ID NO:17 (LJM17)
SEQ ID NO:3	100	20	98	20
SEQ ID NO:7		100	20	99
SEQ ID NO:13			100	20
SEQ ID NO:17				100

Table 2. Global amino acid sequence identity percentage between unprocessed LJL143 and LJM17

	SEQ ID NO:1 (LJL143)	SEQ ID NO:5 (LJM17)	SEQ ID NO:11 (LJL143)	SEQ ID NO:15 (LJM17)
SEQ ID NO:1	100	19	98	19
SEQ ID NO:5		100	19	99
SEQ ID NO:11			100	19
SEQ ID NO:15				100

Table 3. Global nucleic acid sequence identity percentage between polynucleotides encoding mature LJL143 and LJM17

SEQ ID NO:	4 (LJL143)	8 (LJM17)	14 (LJL143)	18 (LJM17)	22 (LJL143)	91 (LJM17)
4	100	36	99	47	74	43
8		100	47	99	44	76
14			100	47	78	43
18				100	44	75
22					100	51
91						100

Table 4. Global nucleic acid sequence identity percentage between polynucleotides encoding unprocessed LJL143 and LJM17

SEQ ID NO:	2 (LJL143)	6 (LJM17)	12 (LJL143)	16 (LJM17)	89 (LJL143)	90 (LJM17)	91 (codon optimized LJM17)	22 (codon optimized LJL143)
2	100	46	99	46	100	47	43	73
6		100	47	99	45	100	76	46
12			100	46	99	48	43	73
16				100	45	99	76	45
89					100	47	43	73
90						100	76	46
21							100	48
22								100

The percent sequence identity between two nucleic acid or polypeptide sequences is determined using Vector NTI 11.0 (PC) software package (Invitrogen, 1600 Faraday Ave., Carlsbad, CA). A gap opening penalty of 15 and a gap extension penalty of 6.66 are used for determining the percent identity of two nucleic acids. A gap opening penalty of 10 and a gap extension penalty of 0.1 are used for determining the percent identity of two polypeptides. The percent identity was calculated based on the shorter sequence.

FIGURE 18 (1/2)

SEQ ID NO	type	name	Corresponding SEQ ID NO in 61/101,345	Corresponding SEQ ID NO in 61/051,635
1	PRT	Unprocessed LJJ143 protein	1	15
2	DNA	Unprocessed LJJ143 DNA	2	
3	PRT	Mature LJJ143 protein	3	
4	DNA	Mature LJJ143 DNA	4	
5	PRT	Unprocessed LJM17 protein	5	23
6	DNA	Unprocessed LJM17 DNA	6	
7	PRT	Mature LJM17 protein	7	
8	DNA	Mature LJM17 DNA	8	
9	DNA	Plasmid pVR2001 LJM17	9	
10	DNA	Plasmid pVR2001 LJJ143	10	
11	PRT	Unprocessed LJJ143 protein	11	
12	DNA	Unprocessed LJJ143 DNA	12	
13	PRT	Mature LJJ143 protein	13	
14	DNA	Mature LJJ143 DNA	14	
15	PRT	Unprocessed LJM17 protein	15	
16	DNA	Unprocessed LJM17 DNA	16	
17	PRT	Mature LJM17 protein	17	
18	DNA	Mature LJM17 DNA	18	
19	DNA	Plasmid pNBO002	19	
20	DNA	Plasmid pNBO003	20	
21	DNA	Codon-optimized unprocessed LJM17 DNA (reverse complementary strand)	21	
22	DNA	Codon-optimized unprocessed LJJ143 DNA	22	
23	PRT	LJJ34 protein		1
24	DNA	LJJ34 DNA		2
25	PRT	LJJ18 protein		3
26	DNA	LJJ18 DNA		4
27	PRT	LJS193 protein		5
28	DNA	LJS193 DNA		6
29	PRT	LJS201 protein		7
30	DNA	LJS201 DNA		8
31	PRT	LJJ13 protein		9
32	DNA	LJJ13 DNA		10
33	PRT	LJJ23 protein		11
34	DNA	LJJ23 DNA		12
35	PRT	LJM10 protein		13
36	DNA	LJM10 DNA		14
37	PRT	LJS142 protein		17
38	DNA	LJS142 DNA		18
39	PRT	LJJ17 protein		19
40	DNA	LJJ17 DNA		20
41	PRT	LJM06 protein		21
42	DNA	LJM06 DNA		22
43	PRT	LJJ04 protein		25
44	DNA	LJJ04 DNA		26
45	PRT	LJM114 protein		27
46	DNA	LJM114 DNA		28
47	PRT	LJM111 protein		29
48	DNA	LJM111 DNA		30
49	PRT	LJM78 protein		31

Figure 18 (2/2)

50	DNA	LJM78 DNA		32
51	PRT	LJS238 protein		33
52	DNA	LJS238 DNA		34
53	PRT	LJS169 protein		35
54	DNA	LJS169 DNA		36
55	PRT	LJL11 protein		37
56	DNA	LJL11 DNA		38
57	PRT	LJL08 protein		39
58	DNA	LJL08 DNA		40
59	PRT	LJS105 protein		41
60	DNA	LJS105 DNA		42
61	PRT	LJL09 protein		43
62	DNA	LJL09 DNA		44
63	PRT	LJL38 protein		45
64	DNA	LJL38 DNA		46
65	PRT	LJM04 protein		47
66	DNA	LJM04 DNA		48
67	PRT	LJM26 protein		49
68	DNA	LJM26 DNA		50
69	PRT	LJS03 protein		51
70	DNA	LJS03 DNA		52
71	PRT	LJS192 protein		53
72	DNA	LJS192 DNA		54
73	PRT	LJM19 protein		55
74	DNA	LJM19 DNA		56
75	PRT	LJL138 protein		57
76	DNA	LJL138 DNA		58
77	PRT	LJL15 protein		59
78	DNA	LJL15 DNA		60
79	PRT	LJL91 protein		61
80	DNA	LJL91 DNA		62
81	PRT	LJM11 protein		63
82	DNA	LJM11 DNA		64
83	PRT	LJS138 protein		65
84	DNA	LJS138 DNA		66
85	PRT	LJL124 protein		67
86	DNA	LJL124 DNA		68
87	PRT	LJL35 protein		69
88	DNA	LJL35 DNA		70
89	DNA	LJL143 DNA		16
90	DNA	LJM17 DNA		24
91	DNA	Codon-optimized unprocessed LJM17 DNA		
92	DNA	vCP2390		
93	DNA	vCP2390 (containing LJM17 in coding direction)		
94	DNA	vCP2389		

FIGURE 19

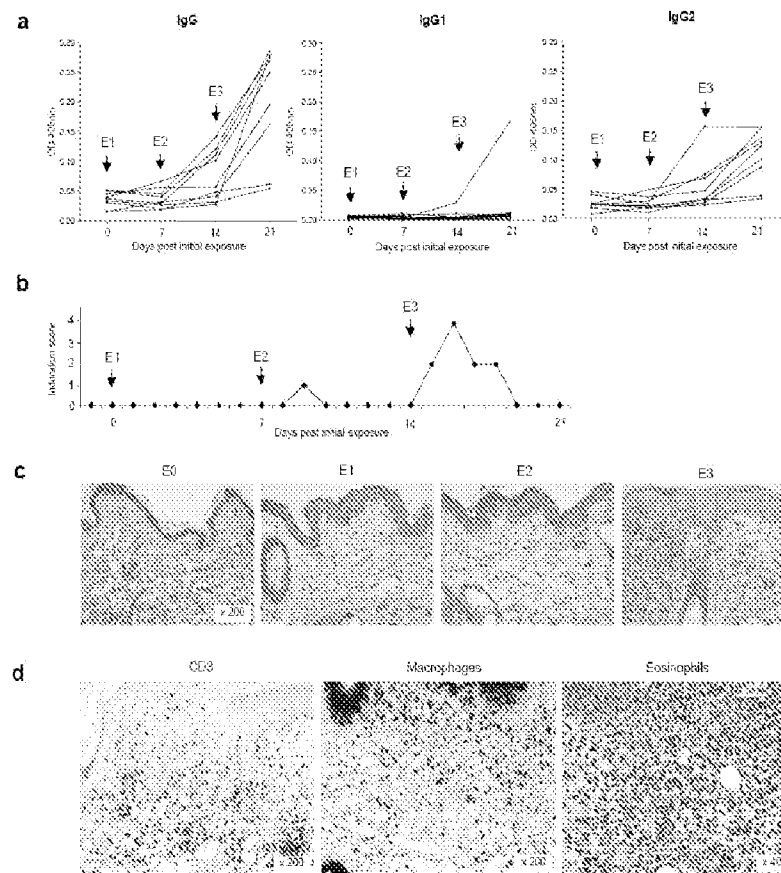


FIGURE 20

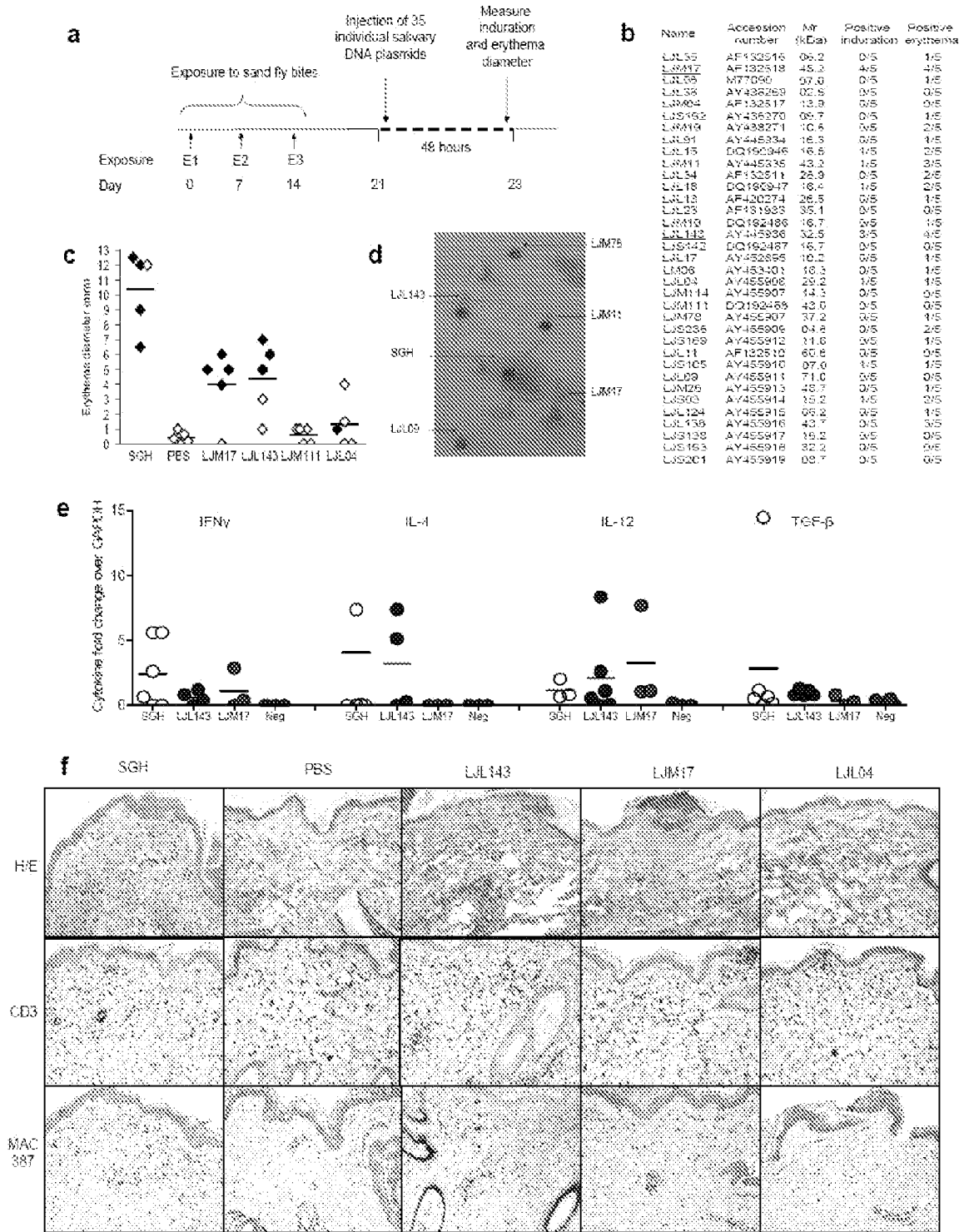


FIGURE 21

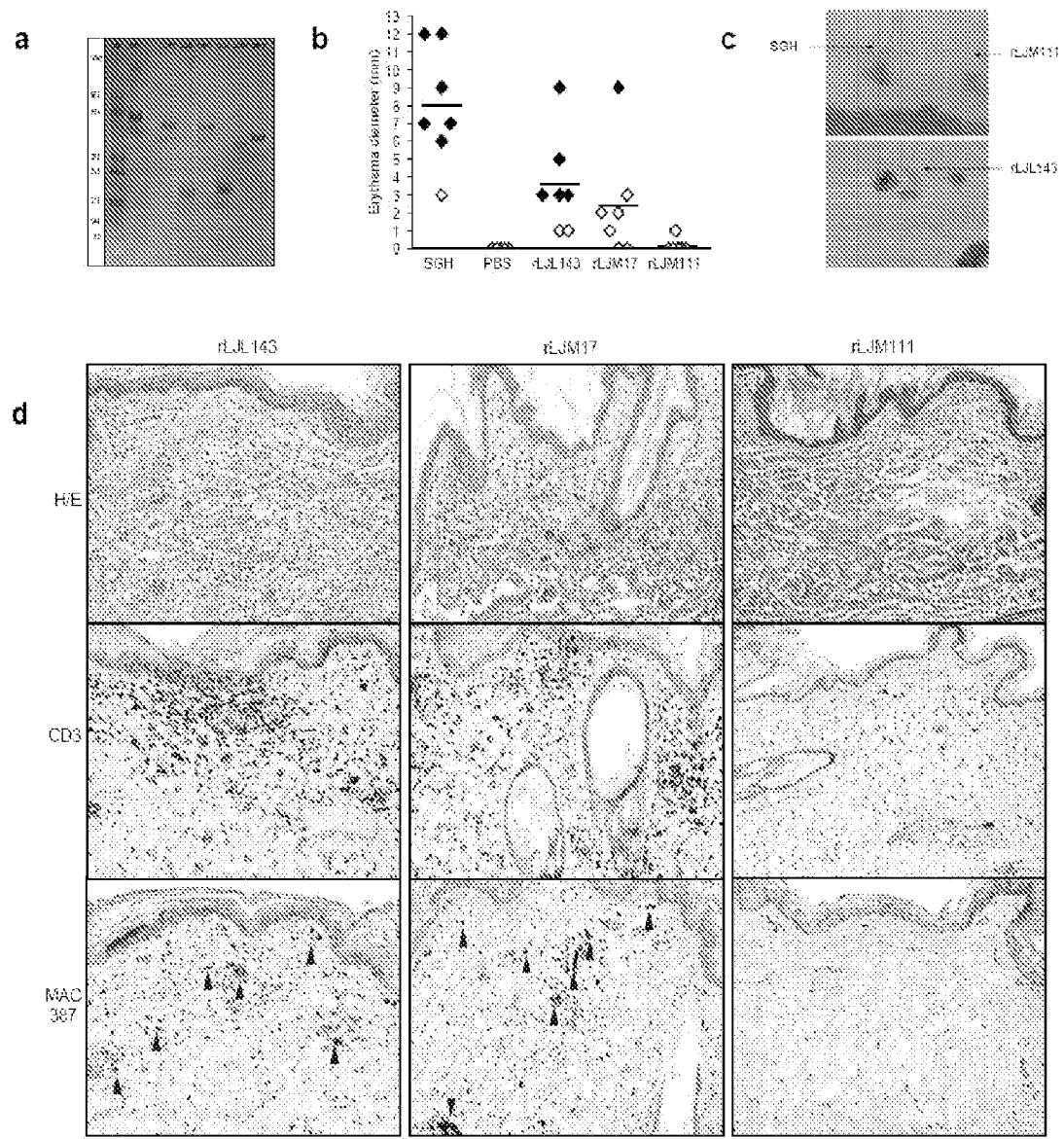


FIGURE 22

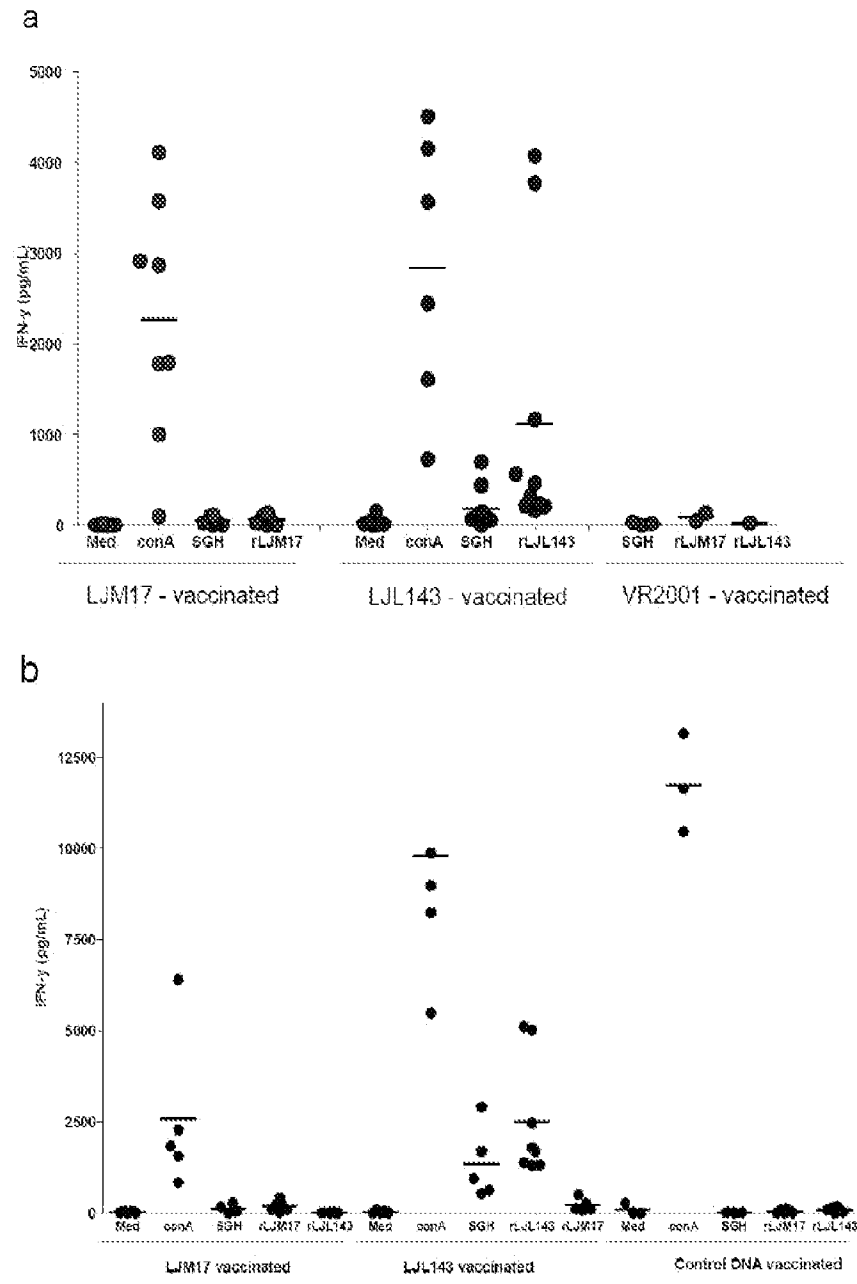


FIGURE 23

