Title: ANTHELMINTIC COMBINATIONS AND METHODS OF USE THEREOF
Abstract: The present invention relates to a veterinary composition comprising an effective amount of at least one cyclic depsipeptide and at least one macrocyclic lactone; and a pharmaceutically acceptable carrier, for the treatment or prophylaxis of parasitic infection in a mammal wherein the parasite shows resistance to treatment or prophylaxis with the macrocyclic lactone alone.
ANTHELMINTIC COMBINATIONS AND METHODS OF USE THEREOF

RELATED APPLICATIONS/INCORPORATION BY REFERENCE

This application claims the benefit of priority to U.S. provisional application no. 62/142304 filed April 2, 2015, which is incorporated herein by reference in its entirety.

Any foregoing applications and all documents cited therein or during their prosecution ("application cited documents") and all documents cited or referenced in the application cited documents, and all documents cited or referenced herein ("herein cited documents"), and all documents 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. 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.

FIELD OF THE INVENTION

This invention relates to anthelmintic combinations which comprise at least one macrocyclic lactone and at least one cyclic depsipeptide to treat parasitic resistant worms or helminth infections. This invention also relates to uses of the compounds to make medicaments and treatments comprising the administration of the compounds to mammals in need of the treatments. Moreover this invention relates to pharmaceutical compositions and kits comprising the compounds. This invention also provides for an improved method for eradicating, controlling, and preventing parasite infestation in mammals.

BACKGROUND OF THE INVENTION

Animals, such as mammals and birds, are often susceptible to parasite infestations. These parasites may be ectoparasites, such as insects, and endoparasites such as filariae and worms. Animals and humans also suffer from endoparasitical infections including, for example, helminthiasis which is most frequently caused by a group of parasitic worms described as nematodes or roundworms. These parasites cause severe economic losses in pigs, sheep, horses, and cattle as well as affecting domestic animals and poultry. Other parasites which occur in the gastrointestinal tract of animals and humans include Ancylostoma, Necator, Ascaris, Strongyloides, Trichinella, Capillaria, Toxocara, Toxascaris, Trichiris, Enterobius and parasites which are found in the blood or other tissues and organs such as filarial worms and the extra intestinal stages of Strogyloides, Toxocara and Trichinella.

One type of endoparasite which seriously harms mammals is *Dirofilaria immitis*, also known as heartworm. The most common hosts are dogs and cats but other mammals such as ferrets and raccoons may also be infected. Heartworms go throughout several life stages before they become adults infecting the pulmonary artery of the host mammal. The worms require the mosquito as an intermediate stage to complete their life cycles. The period between the initial infection when the dog
is bitten by a mosquito and the maturation of the worms into adults living in the heart takes six to seven months in dogs and is known as the "prepatent period". L3 larvae migrate during blood feeding of the mosquito to the tip of the mosquito's mouth parts (labium), leave the mosquito and are deposited on the skin of the dog where they then migrate through the bite wound into the host. Most L3 larvae molt to fourth-stage larvae (L4s) in canine subcutaneous tissues within 1-3 days after infection. Then, they migrate to the muscles of the chest and abdomen, and 45 to 60 days after infection, molt to the fifth stage (L5, immature adult). Between 75 and 120 days after infection, these immature heartworms then enter the bloodstream and are carried through the heart to reside in the pulmonary artery. Around seven months after infection, Dirofilaria immitis adults reach maturity and sexually reproduce in the pulmonary arteries and right ventricle. Adult males are around 15cm in length, and females are around 25cm in length and their normal life span as adults is calculated to be about 5 years. After mating, female worms release larvae known as microfilariae (or LI) into the circulation. The microfilariae circulate in the bloodstream for as long as two years, waiting for the next stage in their life cycles in the gut of a bloodsucking mosquito. When ingested by a mosquito, the microfilariae undergo a series of molts to the infective third larval stage, and then migrate to the salivary glands of the mosquito, where they wait to infect another host.

Heartworm infection is a severe and life-threatening disease. Canine heartworm infection is preventable and prophylaxis treatment is a priority in heartworm endemic areas. Treatment of mature heartworm infection with an adulticide (e.g. melarsomine dihydrochloride) is costly and can cause serious adverse side effects, thus prevention by monthly administration of drugs that interrupt larvae development is widely used. The goal of heartworm preventive therapy in dogs has been to stop infection by Dirofilaria immitis by killing the stage that is deposited by the mosquito and first enters the dog, the third-stage larva (L3), as well as the young and maturing fourth-stage larva (L4). Macrocyclic lactones (MLs) can be used monthly for uninfected dogs to suppress reproduction in adult worms and remove microfilariae, thereby reducing transmission and gradually causing the attrition of adult worms (Veterinary Parasitology 2005 Oct 24 133(2-3) 197-206).

The macrocyclic lactones (e.g. ivermectin, milbemycin oxime, moxidectin, and selamectin) are the most commonly used chemoprophylaxis agents and are administered at monthly or six-month intervals. These drugs have been effective against Dirofilaria immitis third-stage larvae (L3) and L4, which have developed within the previous 30 days, and thus prevent disease caused by adult worms.

However, in recent years an increased number of lack of efficacy (LOE) cases have been reported, in which dogs develop mature heartworm infections despite receiving monthly prophylactic doses of macrocyclic lactones drugs. For example, Atkins et al, (Veterinary Parasitology 206 (2014) 106-113) recently reported that an increasing number of cases of dogs that tested heartworm antigen positive while receiving heartworm preventive medication which speculates that Dirofilaria immitis has developed selectional resistance to heartworm preventives (American Heartworm Society, 2010. Heartworm Preventive Resistance. Is it Possible, vol. 37. Bulletin of the American Heartworm
In recently reported studies, isolates of *D. immitis* with, in particular the JYD-34 *Dirofilaria immitis* strain, shows less than 100% susceptibility to heartworm preventive products in an induced heartworm infection model have been identified (Blagburn et al, Comparative efficacy of four commercially available heartworm preventive products against the JYD-34 laboratory strain of *Dirofilaria immitis*. In: Proceedings of the Triennial Heartworm Symposium, vol.14, p. 39 (abstract); and Blagburn et al, Evidence of genetic selection following treatment of a heartworm-infected, microfilaric dog with increasing dosages of ivermectin [abstract]. *Proc. Am. Assoc. Vet. Parasitol.* 58, 31.).

A number of studies have shown some resistance of *D. immitis* larvae to macrocyclic lactones e.g. ivermectin, and milbemycin oxime. *(J. Vet. Intern. Med. 2011; 25:61-64 and Can. Vet. J. 2011 Dec; 52(12): 1279-1280).* It has recently been reported that a high frequency of a genotype marker has been correlated with potential macrocyclic resistance, for example some *D. immitis* strains having a single nucleotide polymorphism at sites 11 and 618 (GG-GG) of a gene encoding for "-glycoprotein have shown some resistance to ivermectin *(Topics in Companion Animal Medicine Volume 26, Issue 4, November 2011, Pages 186-192; Veterinary Parasitology 176 (2011) 374-381; and Parasites & Vectors 2014, 7:494).* Also, recent reports indicate incomplete efficacy with normal prophylaxis regimens for treating MP3 strain of *D. immitis* with ivermectin, milbemycin oxime, or selamectin [abstract]. These reports suggest that the efficacy of most macrocyclic lactones may no longer be 100% against all *D. immitis* strains.

US 2011/0263489, US 8709440, US 2014/0163056, US 2012/0295931, AU 2010249226 and AU2010101389 describes anthelmintic compositions in the form of a micellar solution, comprising at least two anthelmintic agents, wherein the anthelmintic agents for use treating by parasites resistant to one or more antiparasitic compounds.

US6, 159,932 describes mixtures of avermectins, ivermectins and milbemycins in combination with cyclic depsipeptides, optionally in the presence of praziquantan or etsiprantel, for increasing the endoparasiticidal action in endoparasiticidical compositions.

US 2011/0046072 describes a delayed release solid pharmaceutical preparation comprising a least one pharmaceutically active ingredient which can include a depsipeptide and/or macrocyclic lactone and polyvinylpyrrolidone or a derivative thereof.

Notwithstanding the compositions comprising emodepside or macrocyclic lactones alone or in combination with other active agents described in the documents above, there is a need for veterinary compositions and methods with improved efficacy and spectrum of coverage to protect mammals against the constantly evolving resistance of parasites to present day treatments.

SUMMARY OF THE INVENTION

The present invention is directed to a combination of an effective amount of at least one cyclic depsipeptide and at least one macrocyclic lactone for the treatment or prophylaxis of
parasites of mammals, in particular, cats, dogs, and humans with the aim of ridding these hosts of all the parasites commonly encountered, in particular parasites resistant to at least one anthelmintic macrocyclic lactone.

In certain embodiments, the invention also provides for effective and long lasting destruction of endoparasites, nematodes, such as filariae, hookworms, whipworms and roundworms of the digestive tract of mammals.

In an embodiment, the invention provides compositions and methods for the prevention of heartworm disease caused by a Dirofilaria immitis strain that is resistant to macrocyclic lactones. In a particular embodiment, the invention provides compositions and methods for the prevention of heartworm disease caused by a resistant strain of Dirofilaria immitis.

In particular, this invention provides for a combination of at least one macrocyclic lactone derivative and at least one cyclic depsipeptide which exhibit additive or synergistic activity against parasites when compared to formulations which contain only macrocyclic lactone. The invention also provides for an easy method of treating parasitic infestations or for the prophylaxis of parasite infestations in mammals which may comprise administering to said mammal an effective amount of a combination composition according to the present invention.

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

DETAILED DESCRIPTION OF THE INVENTION

Terms used herein will have their customary meaning in the art unless specified otherwise.

It is noted that the invention does not intend to encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO ((35 U.S.C. ) 112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer 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.

It is further noted that in this disclosure and particularly in the claims and/or paragraphs, 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.
Unless otherwise specifically noted or apparent by context, "active agent" or "active ingredient" or "therapeutic agent" as used in this specification, means an anthelmintic compound and/or cyclic depsipeptide of the invention.

Also, use of "a" or "an" are employed to describe elements and components of the invention. This is done merely for convenience and to give a general sense of the invention. This description should be read to include one or at least one and the singular also includes the plural unless it is obvious that it is meant otherwise.

The term "sequentially" or "sequential" as used herein refers to separate administration of each active agent in a sequential manner in either order, for example at an interval or intervals of minutes, hours, days or weeks, and if appropriate the active agents may be administered in a regular repeating cycle. If there is sequential administration, the delay in administering one of the active agents should not be such as to lose the benefit of the efficacious effect of the combination of the active agents. In all cases of "sequential" administration, the route of administration may be the same or different.

The term "concomitant" or "concomitantly" as used herein refer to the administration of at least two active agents to a mammal simultaneously. In all cases of "concomitant" administration, the route of administration may be the same or different.

The term "mammal" as used herein include, but are not limited to, cats, dogs and humans. It also includes an individual mammal in all stages of development, including embryonic and fetal stages.

The term "effective amount" as used herein means a concentration of the active agents in the composition sufficient to elicit the desired biological response to the target parasite(s) after administration of the composition to the animal, as measured by methods known in the art and/or described in the examples herein. In some embodiments, an "effective amount" of the active agents in the composition will provide an efficacy of at least 80%, or at least 85% compared to untreated controls. More typically, "an effective amount" of the active agents will provide an efficacy of at least 90%, at least 93%, at least 95% or at least 97% against the target parasite. In certain embodiments, including the prevention of heartworm disease caused by a resistant strain of Dirofilaria immitis, the term "effective amount" may provide efficacy as high as 100%.

The term "treatment", "treating", and the like, as used herein, unless otherwise indicated, refers to eliminating, or ameliorating the parasitic infection, infestation, or condition. It also includes reducing the period of infection or incidence of symptoms of the parasitic infection, as well as references to "control" (e.g., kill, repel, expel, incapacitate, eliminate, alleviate, minimize, and eradicate).

The term "treatment or prophylaxis with the macrocyclic lactone alone" as used herein refers to treatment or prophylaxis with the macrocyclic lactone without treatment or prophylaxis with a cyclic depsipeptide.
The term "prophylaxis" or "prophylactic" or "preventative therapy", "prevention" or "protecting against" as referred to herein includes keeping the parasitic infection, or infestation, from occurring or to hinder, defend or protect from the occurrence of a disease caused by the parasitic infection, as used herein, these terms also encompass, depending on the condition of the mammal, preventing the onset of a disorder or condition, or of symptoms associated with a disorder or condition, prior to affliction with said infection or infestation. For example, administration of the composition of the invention to a mammal so as to prevent heartworm disease caused by a resistant strain of *Dirofilaria immitis* by killing the third-stage larva (L3), as well as the young and maturing fourth-stage larva (L4) in the mammal, so that they do not mature into adult worms. Thus, these terms can refer to administration of the compounds of the present invention to a mammal that is not at the time of administration afflicted with the infection or infestation. As used herein, these terms also encompass preventing the recurrence of an infection or infestation or of symptoms associated therewith.

The terms "resistance", "resistant" and the like, as used herein, unless otherwise indicated, refers to the ability of a parasite to display a delayed, lessened and/or null response to treatment or prophylaxis with a macrocyclic lactone alone (i.e. without treatment with a cyclic depsipeptide) at the therapeutically recommended dosages, which would normally treat or protect against said parasites of the same species and stage. For example, after treatment with a macrocyclic lactone alone (i.e. without treatment with a cyclic depsipeptide), the parasitic load of a mammal infected with a macrocyclic lactone-resistant parasite (e.g. resistant *Dirofilaria immitis* strain) may be reduced to a lesser degree compared to the amount in parasitic load reduction exhibited by a mammal infected with a non-resistant parasitic strain. The term is used to include such separately identifiable forms of resistance as "full resistance", "immunity", "partial resistance", "hypersensitivity" and "tolerance". The term also includes parasitic-infected mammals unresponsive ("non-responders") to treatment with a macrocyclic lactone for parasitic infection, as well as parasitic-infected mammals who suffer a relapse following treatment with a macrocyclic lactone for parasitic infection ("responder-relapsers").

The term "pharmaceutically acceptable" as used herein means it is, within the scope of sound judgement in veterinary medicine, suitable for use in contact with the cells of a mammal without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio.

In a specific embodiment, the term "about" or "approximately" means within 20%, preferably within 10%, and more preferably within 5% of a given value or range.

In cases where compounds of the invention are sufficiently basic or acidic to form stable non-toxic acid or base salts, the compounds may be in the form of a pharmaceutically acceptable salt. Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic or organic bases and acids. Suitable salts include those derived from alkali metals such as potassium and sodium, alkaline earth metals such as calcium and magnesium, among numerous other acids well
known in the art. In particular, examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids, which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, a-ketoglutarate, and a-glycerophosphate. Suitable inorganic salts may also be formed, including, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

In one embodiment, the compositions of the invention comprises an effective amount of:

a) at least one cyclic depsipeptide;
b) at least one macrocyclic lactone; and
c) a pharmaceutically acceptable carrier;

for the treatment or prophylaxis of parasitic infection in a mammal wherein the parasite shows resistance to at least one macrocyclic lactone.

In another embodiment, the compositions of the invention further comprises praziquantel.

In another embodiment of the invention, the parasite is *Dirofilaria immitis*, more particularly a resistant *Dirofilaria immitis* strain containing single-nucleotide polymorphisms encoding a P-glycoprotein transporter, comprised of homozygous guanosine residues at sites 11 and 618 (GG-GG) of a gene encoding for P-glycoprotein ("GG-GG" genotype). In another embodiment of the invention, the parasite is a JYD 34 *Dirofilaria immitis* strain, MP3 *Dirofilaria immitis* strain or a combination thereof.

In another embodiment of the invention, the parasite is third-stage larvae (L3) or fourth-stage larvae (L4) of *Dirofilaria immitis* or a combination thereof.

In another embodiment of the invention, the cyclic depsipeptide is 24-membered cyclooctadepsipeptide.

In another embodiment of the invention, the cyclic depsipeptide is emodepside, PF1022A, a PF1022A derivative, or a combination thereof, more particularly, emodepside.
In another embodiment of the invention, the macrocyclic lactone of the composition is an avermectin, a milbemycin or a combination thereof, more particularly, a macrocyclic lactone selected from the group consisting of abamectin, dimedectin, doramectin, emamectin, eprinomectin, ivermectin, latidectin, lepimectin, selamectin, milbemycin oxime and moxidectin or a combination thereof, more particularly, ivermectin, eprinomectin, or moxidectin.

In another embodiment of the invention, the effective amount of macrocyclic lactone and cyclic depsipeptide is a synergistic effective amount.

In another embodiment of the invention the weight ratio of macrocyclic lactone to cyclic depsipeptide is about 1:500 to about 1:1000, about 1:833, 1:750 to about 1:1000, 1:500 to about 1:750, about 1:250 to about 1:500, about 1:417, about 1:100 to about 1:250, about 1:167, 1:150 to about 1:200, or about 1:50 to about 1:100. More preferably, the weight ratio of macrocyclic lactone to cyclic depsipeptide is about 1:100 to about 1:1000, or about 1:500 to about 1:1000.

In another embodiment of the invention, the weight ratio of macrocyclic lactone to praziquantel is about 1:50 to 1:5000, more preferably about 1:500 to about 1:5000, or about 1:3500 to about 1:5000.
In another embodiment of the invention, the veterinary composition is an oral formulation, injectable formulation, topical formulation, pour-on formulation, dermal formulation or sub-dermal formulations, preferably an oral formulation, a soft chewable composition or chewable tablet composition.

In another embodiment of the invention, the parasites controlled by the compositions and methods of the invention show resistance to at least one macrocyclic lactone selected from the group consisting of abamectin, dimadectin, doramectin, emamectin, eprinomectin, ivermectin, latidectin, lepimectin, selamectin, milbemycin-oxime, and moxidectin or a combination thereof, more particularly ivermectin.

In another embodiment of the invention, the mammal is selected from the group consisting of humans, dogs and catsmore particularly dogs or cats.

Another embodiment of the invention is a veterinary composition comprising a synergistically effective amount of:

a) emodepside; and
b) ivermectin; and
a pharmaceutically acceptable carrier;

for the treatment or prevention of a parasitic infection, wherein the parasite is a resistant *Dirofilaria immitis* strain, more particularly the parasite is third-stage larvae (L3) or fourth-stage larvae (L4) of a resistant *Dirofilaria immitis* strain or a combination thereof.

In another embodiment of the invention, the veterinary composition further comprises praziquantel.

Another aspect of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal comprising administering to said mammal an effective amount of:

a) at least one cyclic depsipeptide; and
b) at least one macrocyclic lactone;
and a pharmaceutically acceptable carrier;
wherein the parasitic infection comprises a parasite that is resistant to at least one macrocyclic lactone.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection wherein the parasitic infection comprises a parasite that is resistant to treatment or prophylaxis of the macrocyclic lactone when used alone.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal further comprising praziquantel or episiprantel or a combination thereof.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal, wherein the effective amount is a synergistic effective amount.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal, wherein the parasite is *Dirofilaria immitis*. 

9
Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the parasite is a resistant *Dirofilaria immitis* strain.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the parasite is third-stage larvae (L3) or fourth-stage larvae (L4) of a resistant *Dirofilaria immitis* strain or a combination thereof.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the cyclic depsipeptide is 24-membered cyclooctadepsipeptide, more particularly, emodepside, PF1022A, a PF1022A derivative or a combination thereof. Examples of a PF1022A derivative include those cyclic depsipeptide compounds described in Table 2 of Ohyama, M., et al., Biosci. Biotechnol. Biochem., 75 (7), 1354-1363, 2011, which are incorporated herein in its entirety. More particularly, a PF1022A derivative comprises a cyclic depsipeptide compound selected from the group consisting of:
Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the cyclic depsipeptide is emodepside.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the macrocyclic lactone administered is an avermectin, a milbemycin or a combination thereof.
Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the macrocyclic lactone administered is selected from the group consisting of abamectin, dimadectin, doramectin, emamectin, eprinomectin, ivermectin, latidectin, lepimectin, selamectin, milbemycin oxime and moxidectin or a combination thereof.

In another embodiment of the invention, a method for the treatment or prophylaxis of a parasite infection in a mammal is provided comprising administering to the mammal an effective amount of a combination comprising a macrocyclic lactone selected from the group consisting of abamectin, dimadectin, doramectin, emamectin, eprinomectin, ivermectin, latidectin, lepimectin, selamectin, milbemycin oxime and moxidectin or a combination thereof; and a cyclic depsipeptide selected from emodepside, PF1022A, a PF1022A derivative or a combination thereof.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the macrocyclic lactone of the composition is ivermectin.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal which includes first treating the mammal with an adulticide such as thiacetarsamide sodium or melarsomine dihydrochloride, followed 3 to 6 weeks later by treatment with a) at least one cyclic depsipeptide; and b) at least one macrocyclic lactone; and a pharmaceutically acceptable carrier; wherein the parasitic infection comprises a parasite that is resistant to at least one macrocyclic lactone. Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal which further includes treatment with afoxolaner.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal, wherein the veterinary composition is an oral formulation, injectable formulation, topical formulation, pour-on formulation, dermal formulation or sub-dermal formulation, or more preferably an oral formulation with a soft chewable composition or a chewable tablet composition.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the parasites shows resistance to at least one macrocyclic lactone selected from the group consisting of abamectin, dimadectin, doramectin, emamectin, eprinomectin, ivermectin, latidectin, lepimectin, selamectin, milbemycin-oxime, and moxidectin or a combination thereof, more particularly ivermectin.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the mammal is selected from the group consisting of humans, dogs, and cats, more particularly dogs or cats.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal comprising administering to the mammal a synergistically effective amount of:

a) emodepside; and
b) ivermectin;
and a pharmaceutically acceptable carrier;
wherein the parasite is a resistant *Dirofilaria immitis* strain.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal which further comprises praziquantel.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the parasitic infection causes heartworm associated respiratory disease in the mammal.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the administration of the cyclic depsipeptide and macrocyclic lactone is concomitant.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the administration of the cyclic depsipeptide and macrocyclic lactone is sequential.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal which further comprises detecting the presence of the resistant parasitic strain in the mammal prior to administering said composition to the mammal.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the resistant parasitic strain is a resistant *Dirofilaria immitis* strain.

Another embodiment of the invention is a method of treatment or prophylaxis of a parasitic infection in a mammal wherein the administration is selected from the group consisting of enteral, oral, parenteral, topical, or transdermal.

A further embodiment of the invention is a kit, wherein the kit comprises:

i) at least one container;

ii) a synergistic effective amount of at least one cyclic depsipeptide and at least one macrocyclic lactone; and a pharmaceutically acceptable carrier; and

iii) instructions for use of the cyclic depsipeptide and macrocyclic lactone for treating or preventing a parasitic infection by a resistant *Dirofilaria immitis* strain.

A further embodiment of the invention is a kit which further comprises praziquantel.

A further embodiment of the invention is a kit wherein the cyclic depsipeptide and macrocyclic lactone are in the same container or separate containers.

A further embodiment of the invention is a kit wherein the container is selected from the group consisting of a blister pack, bottle, sachet, ampoule, syringe, pill popper device, drench gun, spray gun, pour-on device, pipette, dropper, spot-on device, or by any other container suitable for holding pesticides, or a combination thereof.

A further embodiment of the invention is a kit which further comprises an administration device for administering the cyclic depsipeptide and macrocyclic to a mammal.
A further embodiment of the invention is a kit wherein the administration device is selected from the group consisting of a syringe, pill popper device, drench bottle, drench gun, spray gun, transdermal patch, pour-on device, pipette dropper, spot-on device, ear-tag, collar, or by any other device suitable for administering drugs to mammals, or a combination thereof.

A further embodiment of the invention is a kit which further comprises a diagnostic tool for detecting the presence or absence of heartworm e.g. the *Dirofilaria immitis* strain.

A further embodiment of the invention is a kit wherein the diagnostotic tool is a detection assay which detects the presence or absence of nucleic acid primer pairs, combinations of nucleic acid primer pairs, nucleic acid arrays (e.g., diagnostic cards) containing nucleic acid primer pairs or combinations of nucleic acid primer pairs of heartworm e.g. the *Dirofilaria immitis* strain.

A further embodiment of the invention is a method of preventing parasitic infection in a mammal comprising administering a composition of the invention to said mammal, wherein the parasitic infection comprises at least one parasite resistant to at least one macrocyclic lactone.

A further embodiment of the invention is a method of preventing parasitic infection in a mammal by administering a composition of the invention to the mammal so as to kill the third-stage larva (L3), as well as the young and maturing fourth-stage larva (L4) of *Dirofilaria immitis* so that they do not mature into adult worms.

A further embodiment of the invention is a dosage, formulation, route of administration or dosing regimen as described for Treatment Group 4 or 5 in the Examples. Also provided are uses and methods comprising the compositions of the invention for the prevention or treatment of a parasitic infestation in birds or mammals or for in the manufacture of a medicament for the prevention or treatment of a parasitic infestation in birds or mammals.

Macrocyclic lactone anthelmintic compounds may be used for treating endo- and ectoparasite infections in mammals and birds. Compounds that belong to this class of macrocyclic lactones include, but are not limited to, the avermectin and milbemycin series of compounds. These compounds are potent antiparasitic agents against a wide range of internal and external parasites. Avermectins and milbemycins share the same common 16-membered macrocyclic lactone ring; however, milbemycins do not possess the disaccharide substituent on the 13-position of the lactone ring. In addition to treating parasitic insects, avermectins and milbemycins are used to treat endoparasites, e.g., round worm infections, in mammals.

The avermectins may be isolated from the fermentation broth of an avermectin producing strain of *Streptomyces avermitilis* and derivatives thereof. The production, isolation and structural determination of the avermectins are documented in Albers-Schonberg, et al, *J. Am. Chem. Soc.* 1981, 103, 4216-4221 and references cited therein. The description of the morphological characteristics of the culture is described in U.S. Patent No. 4,310,519. Naturally occurring milbemycins are described in Aoki *et al.*, U.S. Patent 3,950,360, which is hereby incorported by

The avermectin and milbemycin series of compounds either are natural products or are semi-synthetic derivatives. The natural product avermectins are disclosed in U.S. Patent No. 4,310,519, and the 22,23-dihydro avermectin compounds are disclosed in U.S. Patent No. 4,199,569, both of which are hereby incorporated by reference in their entirety. The synthesis of avermectins has been documented (J. Am. Chem. Soc. 1989, 111, 2967; J. Am. Chem. Soc. 1986, 108, 2776) and research on deconjugation and epimerization of avermectin derivatives is also described in Hanessian, et al (J. Am. Chem. Soc. 1987, 109, 7063) and Fraser-Reid, et al (J. Am. Chem. Soc. 1987, 109, 933). For a general discussion of avermectins, which includes a discussion of their uses in humans and animals, see “Ivermectin and Abamectin,” W.C. Campbell, ed., Springer-Verlag, New York (1989). Examples of avermectins include, but are not limited to, abamectin, dimadectin, doramectin, emamectin, eprinomectin, ivermectin, latidectin, lepimectin, and selamectin.

The milbemycins are the aglycone derivatives of the avermectins, such as those described, for example in U.S. Patent Nos. 4,144,352; 4,791,134; and 6,653,342, all of which are hereby incorporated by reference in their entirety. Particularly important anthelmintics of this family include moxidectin, as described, for example in U.S. Patent Nos. 7,348,417; and 4,916,154 (and references cited therein), which are hereby incorporated by reference in their entirety. Examples of milbemycins also include milbemectin, milbemycin D and nemadectin. Also included are the 5-oxo and 5-oxime derivatives of said avermectins and milbemycins, respectively.

The macrocyclic lactone compounds are known in the art and can easily be obtained commercially or through synthesis techniques known in the art. Reference is made to the widely available technical and commercial literature. For avermectins, such as ivermectin and abamectin, reference may be made, for example, to the work "Ivermectin and Abamectin", 1989, by M.H. Fischer and H. Mrozik, William C. Campbell, published by Springer Verlag., or Albers-Schonberg et al. (1981), "Avermectins Structure Determination", J. Am. Chem. Soc, 103, 4216-4221. For doramectin, "Veterinary Parasitology", vol. 49, No. 1, July 1993, 5-15 may be consulted. For milbemycins, reference may be made, inter alia, to Davies H.G. et al., 1986, "Avermectins and Milbemycins", Nat. Prod. Rep., 3, 87-121, Mrozik H. et al., 1983, Synthesis of Milbemycins from Avermectins, Tetrahedron Lett., 24, 5333-5336, U.S. Patent No. 4,134,973 and EP 0 677 054.

The avermectins and milbemycins demonstrate potent antiparasitic activity while being relatively non-toxic to most mammalian species. As a result, the avermectin/milbemycin family has been the focus of extensive chemical modification studies, which are outlined, for example, in U.S. Patents 4,199,569; 4,285,963; 4,310,519; 4,423,209; 4,427,663; 4,457,920, 4,806,527; 4,831,016; 4,855,317; 4,859,657; 4,871,719; 4,873,224; 4,874,749; 4,895,837; 4,906,619, 4,920,148; 4,963,582; 4,973,711; 4,978,677; 5,015,630, 5,023,241, 5,030,622; 5,055,454; 5,055,596; 5,057,499; 5,077,308; 5,162,363; 5,169,839; 5,208,222; 5,244,879; 5,262,400; 5,637,703; 5,830,875; 7,250,402; and EP 0
212 867; 0 237 339; 0 241 146; 0 214 731; 0 194 125; and 0 170 006, all of which are hereby incorporated by reference in their entirety. Further modifications of members of the avermectin family are outlined, for example, in U.S. patent application nos 10/488,225; 10/498,858; 10/513,247; 10/539,274; 10/543,637; 10/543,638; 10/543,643, 10/544,274; 10/544,281; 10/560,390; 10/568,715; 10/599,671; 11/317,932; 11/319,686; and 11/319,687, all of which are hereby incorporated by reference in their entirety. Chemical modifications have also been induced via spiking the fermentation broth with acids, which are subsequently incorporated at the C-25 position of the avermectins (EP 0 214 731, and Arch. Biochem. Biophys 1989, 269, 544-547). All of these documents and references cited therein, as well as the references cited herein, are expressly incorporated by reference.

Notwithstanding the excellent progress in antiparasitic research, concerns remain with respect to increasingly common reports of resistance among veterinary parasites (Parasitology 2005, 131, S179-190). Thus, there remains an ongoing need for novel compositions and treatments in veterinary medicine. It is an object of this invention to provide novel formulations comprising cyclic depsipeptides and macrocyclic lactones, as well as methods of treatment using such compounds. That the invention performs as herein described is surprising, unexpected and nonobvious.

While the macrocyclic lactones are well known antiparasitic compounds, there remains an ongoing need to combat the constantly evolving resistance of parasites. To this end, we have found that a combination of macrocyclic lactones and cyclic depsipeptides are effective in treating certain resistant parasites, in particular Dirofilaria immitis, more particularly JYD-34 Dirofilaria immitis strain.


The compositions of the invention may also include paraherquamide compounds and derivatives of these compounds, including derquantel (see Ostlind et al., Research in Veterinary Science, 1990, 48, 260-61; and Ostlind et al., Medical and Veterinary Entomology, 1997, 11, 407-408). The paraherquamide family of compounds is a known class of compounds that include a spirodioxepino indole core with activity against certain parasites (see Tet. Lett. 1981, 22, 135; J
In addition, the structurally related marcfortine family of compounds, such as marcfortines A-C, are also known and may be combined with the formulations of the invention (see *J. Chem. Soc. - Chem. Comm.* 1980, 601 and *Tet. Lett.* 1981, 22, 1977). Further references to the para-herquamide derivatives can be found, for example, in WO 91/09961, WO 92/22555, WO 97/03988, WO 01/076370, WO 09/004432 and US 2010/0197624, U.S. Patent 5,703,078 and U.S. Patent 5,750,695, all of which are hereby incorporated by reference in their entirety. The compositions of the invention may also include at least one additional systemically-acting active agents described herein including, but not limited to, one or more isoxazoline active agents, or anthelmintics of other classes including one or more amino acetonitrile active agents, one or more aryloazol-2-yl cyanoethylamino active agents, or a combination thereof.

The compositions of the invention may also include a spinosyn active agent produced by the soil actinomycete *Saccharopolyspora spinosa* (see, for example Salgado V.L. and Sparks T.C., "The Spinosyns: Chemistry, Biochemistry, Mode of Action, and Resistance," in Comprehensive Molecular Insect Science, vol. 6, pp. 137-173, 2005) or a semisynthetic spinosoid active agent. The spinosins are typically referred to as factors or components A, B, C, D, E, F, G, H, J, K, L, M, N, 0, P, Q, R, S, T, U, V, W, or Y, and any of these components, or a combination thereof, may be used in the compositions of the invention. The spinosyn compound may be a 5,6,5-tricyclic ring system, fused to a 12-membered macrocyclic lactone, a neutral sugar (rhamnose), and an amino sugar (forosamine). These and other natural spinosyn compounds, including 21-butenyl spinosyn produced by *Saccharopolyspora pagona*, which may be used in the compositions of the invention, may be produced via fermentation by conventional techniques known in the art. Other spinosyn compounds that may be used in the compositions of the invention are disclosed in U.S. Patent Nos. 5,496,931; 5,670,364; 5,591,606; 5,571,901; 5,202,242; 5,767,253; 5,840,861; 5,670,486; 5,631,155 and 6,001,981, all incorporated by reference herein in their entirety. The spinosyn compounds may include, but are not limited to, spinosyn A, spinosyn D, spinosad, spinetoram, or combinations thereof. Spinosad is a combination of spinosyn A and spinosyn D, and spinetoram is a combination of 3'-ethoxy-5,6-dihydro spinosyn J and 3'-ethoxy spinosyn L.

In some embodiments, the compositions may contain a combination of two or more spinosyn and/or spinosoid active agents. For example, in one embodiment, the compositions may include spinosad, which is a combination of spinosyn A and spinosyn D. Other combinations are also contemplated. In another embodiment, the compositions may include a spinosyn and/or a spinosoid active agent, or a combination thereof, in combination with one or more additional systemically-acting active agents described herein including, but not limited to, one or more isoxazoline active agents, one or more macrocyclic lactone active agents, one or more benzimidazole agents including thiabendazole, oxibendazole, mebendazole, fenbendazole, oxfendazole, albendazole, triclabendazole and febantel, or anthelmintics of other classes including levamisole, pyrantel, morantel, praziquantel, closantel, clorsulon, one or more amino acetonitrile active agents, one or more insect growth
regulators, one or more neonicotinoid active agents or an aryloazol-2-yl cyanoethylamino active agent, or a combination thereof.

The phenylpyrazoles as a class are known in the art and are described, for example in U.S. Patent No. 5,885,607; U.S. Patent No. 6,010,710; U.S. Patent No. 6,083,519; U.S. Patent No. 6,096,329; U.S. Patent No. 6,395,765, U.S. Patent No. 6,867,229, EP-A-295,217, EP-A-352,944 as well as in U.S. Patent No. 5,576,429; U.S. Patent No. 5,122,530, U.S. Patent application No. 11/825,050, and EP 295 177, the disclosures of which, as well as the references cited herein, are incorporated by reference. This class of insecticides is known to possess excellent activity against insects such as ticks and fleas, and one of these compounds, 1-[2,6-Cl₂-4-CF₃ phenyl]-3-CN-4-[SO-CF₃]-5-NH₂pyrazole, or fipronil, may be included in the compositions and methods of the invention in certain embodiments.

The combinations according to the invention, comprising an effective amount of at least one cyclic depsipeptide and at least one macrocyclic lactone exhibit an unexpected synergistic effect in treating parasites showing resistance to at least one anthelmintic macrocyclic lactone.

The combinations according to the invention, comprising an effective amount of at least one cyclic depsipeptide and at least one macrocyclic lactone exhibit an unexpected synergistic effect in treating parasites showing resistance to Ivermectin.

The combinations according to the invention, comprising an effective amount of at least one cyclic depsipeptide, for example emodepside, and at least one macrocyclic lactone, for example ivermectin, exhibit an unexpected synergistic effect in preventing third-stage larvae (L₃) as well as fourth-stage larvae (L₄) of a resistant Dirofilaria immitis strain, from maturing into adult worms.

Synergism has been described as "the cooperative action of two components (e.g., component (a) and component (b)) in a mixture, such that the total effect is greater or more prolonged than the sum of the effects of the two (or more) taken independently" (see P. M. L. Tames, Neth. J Plant Pathology 1964, 70, 73-80). Mixtures containing an effective amount of at least one cyclic depsipeptide and at least one macrocyclic lactone are found to exhibit synergistic effects against certain important pests. Successful combinations of an effective amount of at least one cyclic depsipeptide and at least one macrocyclic lactone provides improved and even synergistic effect over mono-therapy, i.e. pharmaceutical treatment limited to one drug e.g. macrocyclic lactones or cyclic depsipeptides, particularly against resistant strains of parasites such as a resistant strain of Dirofilaria immitis.

If the macrocyclic lactone and cyclic depsipeptide in the combinations according to the invention are present in certain weight ratios, the synergistic effect is particularly pronounced. However, the weight ratios of the macrocyclic lactone and cyclic depsipeptide in the combinations can be varied within a relatively wide range. In general, the combinations according to the invention comprise macrocyclic lactone and cyclic depsipeptide in the preferred ratios given.
An additive or synergistic effect may be attained when the macrocyclic lactone and cyclic depsipeptide are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, an additive or synergistic effect may be attained when the macrocyclic lactone and cyclic depsipeptide are administered or delivered sequentially, e.g., by separate oral administrations in different unit dosage forms. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e., serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

The composition of the invention may also be in a variety of forms which include, but are not limited to, oral formulations, injectable formulations, and topical, pour-on, dermal or subdermal formulations. The formulations are intended to be administered to a mammal. Examples of mammals include but are not limited to humans, dogs, cats and other livestock or domestic mammals. The composition of the invention may be in a form suitable for oral use, for example, as baits (see, e.g., U.S. Patent No. 4,564,631, which is hereby incorporated by reference in its entirety), dietary supplements, troches, lozenges, chewables, tablets, hard or soft capsules, bolus, emulsions, aqueous or oily suspensions, aqueous or oily solutions, oral drench formulations, dispersible powders or granules, premixes, syrups or elixirs, enteric formulations or pastes. Compositions intended for oral use may be prepared according to any method known in the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, bittering agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations.

Tablets may contain the active ingredient in admixture with non-toxic, pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc, the tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glycercyl distearate may be employed. They may also be coated by the technique described in U.S. Patent Nos. 4,256,108; 4,166,452; and 4,265,874 (all incorporated herein by reference in their entirety) to form osmotic therapeutic tablets for controlled release.

Formulations for oral use may be hard gelatin capsules, wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. Capsules may also be soft gelatin capsules, wherein the active ingredient is mixed with water or miscible solvents such as propylene glycol, PEGs and ethanol, or an oil medium, for example peanut oil, liquid paraffin, or olive oil.
The compositions of the invention may also be in the form of oil-in-water or water-in-oil emulsions. The oily phase may be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example, liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phosphatides, for example, soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example, sorbitan monoleate, and condensation products of the said partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monoooleate. The emulsions may also contain sweetening agents, bittering agents, flavoring agents, and/or preservatives.

In one embodiment of the formulation, the composition of the invention may be in the form of a microemulsion. Microemulsions are well suited as the liquid carrier vehicle. Microemulsions are quaternary systems comprising an aqueous phase, an oily phase, a surfactant and a cosurfactant. They are translucent and isotropic liquids.

Microemulsions are composed of stable dispersions of microdroplets of the aqueous phase in the oily phase or conversely of microdroplets of the oily phase in the aqueous phase. The size of these microdroplets is less than 200 nm (1000 to 100,000 nm for emulsions). The interfacial film is composed of an alternation of surface-active (SA) and co-surface-active (Co-SA) molecules which, by lowering the interfacial tension, allows the microemulsion to be formed spontaneously.

In one embodiment of the oily phase, the oily phase may be formed from mineral or vegetable oils, from unsaturated polyglycosylated glycerides or from triglycerides, or alternatively from mixtures of such compounds. In one embodiment of the oily phase, the oily phase may be comprised of triglycerides; in another embodiment of the oily phase, the triglycerides are medium-chain triglycerides, for example C₈-C₁₀ caprylic/capric triglyceride. In another embodiment of the oily phase may represent a % v/v range selected from the group consisting of about 2 to about 15%; about 7 to about 10%; and about 8 to about 9% v/v of the microemulsion.

The aqueous phase may include, for example water or glycol derivatives, such as propylene glycol, glycol ethers, polyethylene glycols or glycerol. In one embodiment of the glycol derivatives, the glycol may be selected from the group consisting of propylene glycol, diethylene glycol monoethyl ether, dipropylene glycol monoethyl ether and mixtures thereof. Generally, the aqueous phase will represent a proportion from about 1 to about 4% v/v in the microemulsion.

Surfactants for the microemulsion may include diethylene glycol monoethyl ether, dipropylene glycol monomethyl ether, polyglycolized C₈-C₁₀ glycerides or polyglyceryl-6 dioleate. In addition to these surfactants, the cosurfactants may include short-chain alcohols, such as ethanol and propanol.

In one embodiment for the amount of surfactant/cosurfactant, the cosurfactant to surfactant ratio will be from about 1/7 to about 1/2. In another embodiment for the amount of cosurfactant, there will be from about 25 to about 75% v/v of surfactant and from about 10 to about 55% v/v of cosurfactant in the microemulsion.
Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example, atachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example, beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as sucrose, saccharin or aspartame, bittering agents, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid, or other known preservatives.

Aqueous suspensions may contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example, sodium carboxymethylcellulose, methylcellulose, hydroxy-propylmethylcellulose, sodium alginate, polvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example, heptadecaethylenoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide, with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or w-propyl, p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents and/or bittering agents, such as those set forth above.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water may provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, sweetening, bittering, flavoring and coloring agents, may also be present.

Syrups and elixirs may be formulated with sweetening agents, for example, glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, flavoring agent(s) and/or coloring agent(s).

In another embodiment of the invention, the composition may be in paste form. Examples of embodiments in a paste form include but are not limited to those described in U.S. Patent Nos. 6,787,342 and 7,001,889 (each of which are incorporated herein by reference). In addition to the compounds of the invention, the paste may further contain fumed silica; a viscosity modifier; a carrier; optionally, an absorbent; and optionally, a colorant, stabilizer, surfactant, or preservative.

In one embodiment of the formulation, the formulation may be a paste containing the compounds of the invention, fumed silica, a viscosity modifier, an absorbent, a colorant; and a hydrophilic carrier which is triacetin, a monoglyceride, a diglyceride, or a triglyceride.

The paste may also include, but is not limited to, a viscosity modifier selected from the group consisting of PEG 200, PEG 300, PEG 400, PEG 600, monoethanolamine, triethanolamine, glycerol,
propylene glycol, polyoxyethylene (20) sorbitan mono-oleate (polysorbate 80 or Tween 80), and polyoxamers (e.g., Pluronic L 81); an absorbent selected from the group consisting of magnesium carbonate, calcium carbonate, starch, and cellulose and its derivatives; and a colorant selected from the group consisting of titanium dioxide iron oxide, and FD&C Blue #1 Aluminum Lake.

The compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. Cosolvents such as ethanol, propylene glycol glycerol formal or polyethylene glycols may also be used. Preservatives, such as phenol or benzyl alcohol, may be used.

In addition, sterile, fixed oils may be conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Topical, dermal and subdermal formulations may include, by way of non-limiting example, emulsions, creams, ointments, gels, pastes, powders, shampoos, pour-on formulations, ready-to-use formulations, spot-on solutions and suspensions, dips and sprays. Topical application of an inventive composition including a spot-on, spray-on or pour-on composition, may allow for the inventive composition to be absorbed through the skin to achieve systemic levels, distributed through the sebaceous glands or on the surface of the skin achieving levels throughout the haircoat. When the compound is distributed through the sebaceous glands, they may act as a reservoir, whereby there may be a long-lasting effect (up to several months) effect. Spot-on formulations are typically applied in a localized region which refers to an area other than the entire mammal. In one embodiment of a localized region, the location may be between the shoulders. In another embodiment of a localized region it may be a stripe, e.g. a stripe from head to tail of the mammal.

Pour-on formulations are described in U.S. Patent No. 6,010,710, also incorporated herein by reference. The pour-on formulations may be advantageously oily, and generally comprise a diluent or vehicle and also a solvent (e.g. an organic solvent) for the active ingredient if the latter is not soluble in the diluent.

Organic solvents that may be used in the invention include but are not limited to: acetyltributyl citrate, fatty acid esters such as the dimethyl ester, diisobutyl adipate, acetone, acetonitrile, benzyl alcohol, butyl diglycol, dimethylacetamide, dimethylformamide, dipropylene glycol ra-butyl ether, ethanol, isopropanol, methanol, ethylene glycol monoethyl ether, ethylene glycol monomethyl ether, monomethylacetamide, dipropylene glycol monomethyl ether, liquid polyoxyethylene glycols, propylene glycol, 2-pyrrolidone (e.g. N-methylpyrrolidone), diethylene
glycol monoethyl ether, ethylene glycol and diethyl phthalate, or a mixture of at least two of these solvents.

As vehicle or diluent, mention may be made of plant oils such as, but not limited to soybean oil, groundnut oil, castor oil, corn oil, cotton oil, olive oil, grape seed oil, sunflower oil, coconut oils etc.; mineral oils such as, but not limited to, petrolatum, paraffin, silicone, etc.; aliphatic or cyclic hydrocarbons or alternatively, for example, medium-chain (such as C8 to C12) triglycerides.

In another embodiment of the invention, an emollient and/or spreading and/or film-forming agent may be added. In one embodiment, the emollient and/or spreading and/or film-forming agent may be:

(a) polyvinylpyrrolidone, polyvinyl alcohols, copolymers of vinyl acetate and vinylpyrrolidone, polyethylene glycols, benzyl alcohol, mannitol, glycerol, sorbitol, polyoxyethylenated sorbitan esters; lecithin, sodium carboxymethylcellulose, silicone oils, polydiorganosiloxane oils (such as polydimethylsiloxane (PDMS) oils), for example those containing silanol functionalities, or a 45V2 oil,

(b) anionic surfactants such as alkaline stearates, sodium, potassium or ammonium stearates; calcium stearate, triethanolamine stearate; sodium abietate; alkyl sulphates (e.g. sodium lauryl sulphate and sodium cetyl sulphate); sodium dodecylbenzenesulphonate, sodium dioctylsulphosuccinate; fatty acids (e.g. those derived from coconut oil),

(c) cationic surfactants such as water-soluble quaternary ammonium salts of formula \( \text{N}^+ \text{R}' \text{R}'' \text{R}''' \), \( \text{Y}^- \) in which the radicals \( \text{R} \) are optionally hydroxylated hydrocarbon radicals and \( \text{Y}^- \) is an anion of a strong acid such as the halide, sulphate and sulphonate anions; cetyltrimethylammonium bromide is among the cationic surfactants which can be used,

(d) amine salts of formula \( \text{N}^+ \text{HR}' \text{R}'' \) in which the radicals \( \text{R} \) are optionally hydroxylated hydrocarbon radicals; octadecylamine hydrochloride is among the cationic surfactants which can be used,

(e) nonionic surfactants such as sorbitan esters, which are optionally polyoxyethylenated (e.g. polysorbate 80), polyoxyethylenated alkyl ethers; polyoxypropylated fatty alcohols such as polyoxypropylene-styrol ether; polyethylene glycol stearate, polyoxyethylenated derivatives of castor oil, polyglycerol esters, polyoxyethylenated fatty alcohols, polyoxyethylenated fatty acids, copolymers of ethylene oxide and propylene oxide,

(f) amphoteric surfactants such as the substituted lauryl compounds of betaine; or

(g) a mixture of at least two of these agents.

The solvent will be used in proportion with the concentration of the compounds of the invention and their solubilities in this solvent. It will be sought to have the lowest possible volume. The vehicle makes up the difference to 100%.

In one embodiment of the amount of emollient, the emollient used may be in a proportion of from about 0.1 to 50% or 0.25 to 5%, by volume. In another embodiment, the emollient used may be
in a proportion of from about 0.1% to about 30%, about 1% to about 30%, about 1% to about 20%, or about 5% to about 20% by volume.

In another embodiment of the invention, the composition may be in ready-to-use solution form as is described in U.S. Patent No. 6,395,765, incorporated herein by reference. In addition to the compounds of the invention, the ready-to-use solution may contain a crystallization inhibitor, an organic solvent and an organic co-solvent.

In one embodiment of the amount of crystallization inhibitor, the crystallization inhibitor may be present in a proportion of about 1 to about 30% (w/v) or about 5 to about 15%. In other embodiments, the amount of crystallization inhibitor in the inventive formulations may be about 1% to about 20%, about 1% to about 15%, or about 1% to about 10% (w/w).

In some embodiments, the organic solvent may have a dielectric constant of between 10 and 35 or between about 20 and 30, the content of this organic solvent in the overall composition representing the complement to 100% of the composition.

In some embodiments, the organic co-solvent may have a boiling point of below about 100°C, or below about 80°C. In other embodiments, the organic co-solvent may have a boiling point of below about 250°C, below about 230°C, below about 210°C or below about 200°C. In other embodiments, the organic co-solvent may have a dielectric constant of between about 10 and 40 or between about 20 and 30. In some embodiments, the co-solvent may be present in the composition in a organic co-solvent/organic solvent weight/weight (WAV) ratio of between about 1/15 and 1/2. The solvent may act as to improve solubility or as a drying promoter, and is miscible with water and/or with the organic solvent.

The formulation may also comprise an antioxidizing agent intended to inhibit oxidation in air, this agent being present in a proportion selected from a range consisting of about 0.005 to about 1% (w/v) and about 0.01 to about 0.05%.

The type of crystallization inhibitor used in the inventive formulations is not limited as long as it functions to inhibit crystallization or precipitation of the active or inactive agents from the formulation. Crystallization inhibitors which are useful for the invention may include but are not limited to:

(a) polyvinylpyrrolidone, polyvinyl alcohols, copolymers of vinyl acetate and of vinylpyrrolidone, polyethylene glycols, benzyl alcohol, N-methylpyrrolidone, mannitol, glycerol, sorbitol or polyoxyethyleneated esters of sorbitan; lecithin or sodium carboxymethylcellulose; or acrylic derivatives, such as methacrylates and others;

(b) anionic surfactants, such as alkaline stearates (e.g. sodium, potassium or ammonium stearate); calcium stearate or triethanolamine stearate; sodium abietate; alkyl sulphates, which include but are not limited to sodium lauryl sulphate and sodium cetyl sulphate; sodium dodecylbenzenesulphonate or sodium dioctyl sulphosuccinate; or fatty acids (e.g. coconut oil);
(c) cationic surfactants, such as water-soluble quaternary ammonium salts of formula
N^+R'R''R'''R''''Y^-, in which the R radicals are identical or different optionally hydroxylated
hydrocarbon radicals and Y^- is an anion of a strong acid, such as halide, sulphate and sulphonate
anions; cetyltrimethylammonium bromide is one of the cationic surfactants which can be used;
(d) amine salts of formula N^+HR'R''R''' in which the R radicals are identical or different
optionally hydroxylated hydrocarbon radicals; octadecylamine hydrochloride is one of the cationic
surfactants which can be used;
(e) non-ionic surfactants, such as optionally polyoxyethylenated esters of sorbitan, e.g.
Polysorbate 80, or polyoxyethylenated alkyl ethers; polyethylene glycol stearate, polyoxyethylenated
derivatives of castor oil, polyglycerol esters, polyoxyethylenated fatty alcohols, polyoxyethylenated
fatty acids or copolymers of ethylene oxide and of propylene oxide;
(f) amphoteric surfactants, such as substituted lauryl compounds of betaine; or
(g) a mixture of at least two of the compounds listed in (a)-(f) above.

In one embodiment of the crystallization inhibitor, a crystallization inhibitor pair will be used.
Such pairs include, for example, the combination of a film-forming agent of polymeric type and of a
surface-active agent. These agents will be selected from the compounds mentioned above as
crystallization inhibitor.

In one embodiment of the film-forming agent, the agents are of the polymeric type which
include but are not limited to the various grades of polyvinylpyrrolidone, polyvinyl alcohols, and
copolymer of vinyl acetate and of vinylpyrrolidone.

In one embodiment of the surface-active agents, the agents include but are not limited to those
made of non-ionic surfactants; in another embodiment of the surface active agents, the agent is a
polyoxyethylenated esters of sorbitan and in yet another embodiment of the surface-active agent, the
agents include the various grades of polysorbate, for example Polysorbate 80.

In another embodiment of the invention, the film-forming agent and the surface-active agent
may be incorporated in similar or identical amounts within the limit of the total amounts of
crystallization inhibitor mentioned elsewhere.

The pair thus constituted secures, in a noteworthy way, the objectives of absence of
crystallization on the coat and of maintenance of the cosmetic appearance of the skin or fur; that is to
say without a tendency towards sticking or towards a sticky appearance, despite the high
concentration of active material.

In one embodiment of the antioxidizing agents, the agents are those conventional in the art
and include but are not limited to butylated hydroxyanisole, butylated hydroxytoluene, ascorbic acid,
sodium metabisulphite, propyl gallate, sodium thiosulphate or a mixture of not more than two of
them.

The formulation adjuvants discussed above are well known to the practitioner in this art and
may be obtained commercially or through known techniques. These concentrated compositions are
generally prepared by simple mixing of the constituents as defined above; advantageously, the starting point is to mix the active material in the main solvent and then the other ingredients or adjuvants are added.

The volume applied may be of the order of about 0.3 to about 1 ml. In one embodiment for the volume, the volume may be on the order of about 0.5 ml, for cats and on the order of about 0.3 to about 3 ml for dogs, depending on the weight of the mammal.

In another embodiment of the invention, application of a spot-on formulation according to the present invention may also provide long-lasting and broad-spectrum efficacy when the solution is applied to the mammal or bird. The spot-on formulations provide for topical administration of a concentrated solution, suspension, microemulsion or emulsion for intermittent application to a spot on the mammal, generally between the two shoulders (solution of spot-on type).

For spot-on formulations, the carrier may be a liquid carrier vehicle as described in U.S. Patent No. 6,426,333 (incorporated herein by reference), which in one embodiment of the spot-on formulation may comprise a solvent and a cosolvent wherein the solvent is selected from the group consisting of acetone, acetonitrile, benzyl alcohol, butyl diglycol, dimethylacetamide, dimethylformamide, dipropylene glycol n-butyl ether, ethanol, isopropanol, methanol, ethylene glycol monoethyl ether, ethylene glycol monomethyl ether, monomethylacetamide, dipropylene glycol monomethyl ether, liquid polyoxyethylene glycols, propylene glycol, 2-pyrrolidone (e.g. N-methylpyrrolidone), diethylene glycol monoethyl ether, ethylene glycol, diethyl phthalate fatty acid esters, such as the diethyl ester or diisobutyl adipate, and a mixture of at least two of these solvents and the cosolvent is selected from the group consisting of absolute ethanol, isopropanol or methanol.

The liquid carrier vehicle may optionally contain a crystallization inhibitor selected from the group consisting of an anionic surfactant, a cationic surfactant, a non-ionic surfactant, an amine salt, an amphoteric surfactant or polyvinylpyrrolidone, polyvinyl alcohols, copolymers of vinyl acetate and vinylpyrrolidone, polyethylene glycols, benzyl alcohol, mannitol, glycerol, sorbitol, polyoxyethylenated sorbitan esters; lecithin, sodium carboxymethylcellulose, and acrylic derivatives, or a mixture of these crystallization inhibitors.

Spot-on formulations may be prepared by dissolving the active ingredients into the pharmaceutically or veterinary acceptable vehicle. Alternatively, the spot-on formulation may be prepared by encapsulation of the active ingredient to leave a residue of the therapeutic agent on the surface of the mammal. These formulations will vary with regard to the weight of the therapeutic agent in the combination depending on the species of host mammal to be treated, the severity and type of infection and the body weight of the host.

Dosage forms may contain from about 0.5 mg to about 5 g of each active agent. In one embodiment of the dosage form, the dosage is from about 1 mg to about 500 mg of an active agent, typically about 25 mg, about 50 mg, about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 800 mg, or about 1000 mg.
In one embodiment of the invention, each active agent may be present in the formulation at a concentration of about 0.05 to 10% weight/volume. In another embodiment of the invention, the active agent may be present in the formulation as a concentration from about 0.1 to 2% weight/volume. In yet another embodiment of the invention, the active agent may be present in the formulation as a concentration from about 0.25 to about 1.5% weight/volume. In still another embodiment of the invention, the active agent may be present in the formulation as a concentration about 1% weight/volume.

In one embodiment of the invention, administration of the active agents may be performed at any of various intervals (e.g., daily, weekly, or monthly) and the dosage, frequency, and mode of administration of each agent can be determined individually. For example, administration of cyclic depsipeptide and macrocyclic lactone; may be hourly, daily, weekly, monthly, yearly, or a single event. In a preferred embodiment the administration of cyclic depsipeptide and macrocyclic lactone is monthly. In addition, administration can have a duration of from six months to one year or more.

It will be appreciated in another embodiment of the invention, the active agents of the combination may be administered concomitantly, either in the same or different pharmaceutical formulation or sequentially. In another embodiment of the invention, the active composition may be administered via a drench, and may be administered either topically or orally. Drench formulations are those in which the liquid containing the compositions of the invention is administered to the mouth or throat of the mammal, or poured onto the skin or coat of the mammal.

The invention is also directed toward a method of treating a mammal against ectoparasitic infection by administering an ectoparasiticidally effective amount of the composition of the invention. Mammals which can be treated include but are not limited to humans, cats, and dogs.

In one embodiment for treatment against ectoparasites, the ectoparasite is one or more insect or arachnid including those of the genera Ctenocephalides, Rhipicephalus, Dermacentor, Ixodes, Boophilus, Amblyomma, Haemaphysalis, Hyalomma, Sarcoptes, Psoroptes, Otodectes, Chorioptes, Hypoderma, Damalinia, Linognathus, Haematoptinus, Solenoptes, Trichodectes, and Felicola.

In another embodiment for the treatment against ectoparasites, the ectoparasite is from the genera Ctenocephalides, Rhipicephalus, Dermacentor, Ixodes and/or Boophilus. The ectoparasites treated include but are not limited to fleas, ticks, mites, mosquitoes, flies, lice, blowfly and combinations thereof. Specific examples include but are not limited to cat and dog fleas (Ctenocephalides felis, Ctenocephalides sp. and the like), ticks (Rhipicephalus sp., Ixodes sp., Dermacentor sp., Amblyomma sp. and the like), and mites (Demodex sp., Sarcoptes sp., Otodectes sp. and the like), lice (Trichodectes sp., Cheyletiella sp., Lignonathus sp., and the like), mosquitoes (Aedes sp., Culex sp., Anopheles sp., and the like) and flies (Hematobia sp., Musca sp., Stomoxys sp., Dematobia sp., Cochliomyia sp., and the like). In yet another embodiment for the treatment against ectoparasites, the ectoparasite is a flea and/or tick.
Additional examples of ectoparasites include but are not limited to the tick genus *Boophilus*, especially those of the species *microplus* (cattle tick), *decoloratus* and *annulatus*; myiases such as *Dermatobia hominis* (known as Berne in Brazil) and *Coelhiomyia hominivorax* (greenbottle); sheep myiases such as *Lucilia sericata*, *Lucilia cuprina* (known as blowfly strike in Australia, New Zealand and South Africa). Flies proper, namely those whose adult constitutes the parasite, such as *Haematobia irritans* (horn fly); lice such as *Linognathus vitulorum*, etc.; and mites such as *Sarcoptes scabiei* and *Psoroptes ovis*. The above list is not exhaustive and other ectoparasites are well known in the art to be harmful to animals and humans. These include, for example migrating dipterous larvae.

The compositions of the invention can also be used to treat against endoparasites such as those helminths selected from the group consisting of *Anaplocephala*, *Ancylostoma*, *Anecator*, *Ascaris*, *Capillaria*, *Cooperia*, *Dipylidium*, *Dirofilaria*, *Echinococcus*, *Enterobius*, *Fasciola*, *Haemonchus*, *Oesophagostomum*, *Ostertagia*, *Toxocara*, *Strongyloides*, *Toxascaris*, *Trichinella*, *Trichuris*, and *Trichostrongylus*.

In another embodiment of the invention, the compounds and compositions of the invention are suitable for controlling pests such as insects selected from the group consisting of *Blatella germanica*, *Heliothis virescens*, *Leptinotarsa decemlineata*, *Tetramorium caespitum* and combinations thereof.


In addition, with or without the other pesticidal agents added to the composition, the invention can also be used to treat other pests which include but are not limited to pests:

1. from the order of Isopoda, for example *Oniscus asellus*, *Armadillidium vulgare* and *Porcellio scaber*;
2. from the order of Diplopoeda, for example *Blanius gattulatus*;
3. from the order of Chilopoda, for example *Geophilus carpophagus* and *Scutigera* spp.;
4. from the order of Symphyla, for example *Scutigerella immaculata*;
5. from the order of Thysanura, for example *Lepisma saccharina*;
6. from the order of Collembola, for example *Onychiurus armatus*;
7. from the order of Blattaria, for example *Blatta orientalis*, *Periplaneta americana*, *Leucophaea maderae* and *Blatella germanica*;
8. from the order of Hymenoptera, for example *Diprion* spp., *Hoplocampa* spp., *Lasius* spp., *Monomorium pharaonis* and *Vespa* spp.;
9. from the order of Siphonaptera, for example *Xenopsylla cheopis* and *Ceratophyllum* spp.;
(10) from the order of Anoplura (Phthiraptera), for example, Damalinia spp., Haematopinus spp., Linognathus spp., Pediculus spp., Trichodectes spp.;


(12) from the order of Bivalva, for example, Dreissena spp.;


(15) from the class of Gastropoda, for example, Arion spp., Biomphalaria spp., Bulinus spp., Deroceras spp., Galba spp., Lymnaea spp., Oncomelania spp., Succinea spp.;

(16) from the class of helminths, for example, Ancylostoma duodenale, Ancylostoma ceylanicum, Acylostoma braziliensis, Ancylostoma spp., Ascaris lubricoides, Ascaris spp., Brugia malayi, Brugia timori, Bunostomum spp., Chabertia spp., Clonorchis spp., Cooperia spp., Dicrocoelium spp., Dictyocaulus filaria, Diphyllobothrium latum, Dracunculus medinensis,


(19) from the order of Isoptera, for example, Reticulitermes spp., Odontotermes spp.;

(21) from the order of Orthoptera, for example, Acheta domesticus, Blatta orientalis, Blattella germanica, Gryllotalpa spp., Leucophaea maderae, Locusta spp., Melanoplus spp., Periplaneta americana, Schistocerca gregaria;

(22) from the order of Thysanoptera, for example, Batiothrips biformis, Eriothrips flavens, Frankliniella spp., Heliothrips spp., Hercinothrips femoralis, Kakothrips spp., Rhipiphorothrips cruentatus, Scirtothrips spp., Taeniothrips cardamoni, Thrips spp.;

(23) from the class of Protozoa, for example, Eimeria spp..

In each aspect of the invention, the compounds and compositions of the invention can be applied against a single pest or combinations thereof.

Additional pharmaceutical, pesticidal or veterinarily active ingredients, which include, but are not limited to, parasiticidals including acaricides, anthelmintics, endectocides and insecticides, may also be added to the compositions of the invention. Anti-parasitic agents may include both ectoparasiticidal and endoparasiticidal agents. Veterinary pharmaceutical agents are well-known in the art (see e.g. Plumb’ Veterinary Drug Handbook, 5th Edition, ed. Donald C. Plumb, Blackwell Publishing, (2005) or The Merck Veterinary Manual, 9th Edition, (January 2005)) and include but are not limited to acarbose, acepromazine maleate, acetaminophen, acetazolamide, acetazolamide sodium, acetic acid, acetyldroxyamic acid, acetylcysteine, acitretin, acyclovir, afoxolaner, albendazole, albuterol sulfate, alfentanil HCl, allopurinol, alprazolam, altrenogest, amantadine HCl, amikacin sulfate, aminacaproic acid, aminopentamide hydrogen sulfate, aminophylline/theophylline, amiodarone HCl, aminz, amitriptyline HCl, amloidipine besylate, ammonium chloride, ammonium molybdenate, amoxicillin, amoxicillin, clavulanate potassium, amphotericin B desoxycholate, amphotericin B lipid-based, ampicillin, amprolium HCl, antacids (oral), antivenin, apomorphine HCl, apramycin sulfate, ascorbic acid, aspirin, aspirin, atipamezole HCl, atracurium besylate, atropine sulfate, aurofino, aurothioglucone, azaperone, azathioprine, azithromycin, baclofen, barbituates, benazepril HCl, betamethasone, betanecochol chloride, bisacodyl, bismuth subsalicylate,
bleomycin sulfate, boldenone undecylenate, bromides, bromocriptine mesylate, budenoside,
buprenorphine HCl, buspirone HCl, busulfan, butorphanol tartrate, cabergoline, calcitonin salmon,
calcitrol, calcium salts, captopril, carbencillin indanyl sodium, carbimazole, carboplatin, carnitine,
carprofen, carvedilol, cefadroxil, cefazolin sodium, cefixime, cefoperazone sodium, cefotaxime
sodium, cefotetan disodium, cefoxitin sodium, cepodoxime proxetil, ceftazidime, ceftiofur sodium,
ceftiofur HCl, cefixime sodium, cephalixin, cephalosporins, cepahpin, charcoal (activated),
chlorambucil, chloramphenicol, chloridiazepoxide, chlordiazepoxide +/- clidinium bromide,
chlorothiazide, chlorpheniramine maleate, chlorpromazine HCl, chlorpropamide, chlortetracycline,
chorionic gonadotropin (HCG), chromium, cimetidine, ciprofloxacin, cisapride, cisplatin, citrate salts,
clarithromycin, clemastine fumarate, clenbuterol HCl, clindamycin, clofazimine, clomipramine HCl,
claonazepam, clonidine, cloprostenol sodium, clorazepate dipotassium, clorsulon, cloxacinil, codeine
phosphate, colchicine, corticotropin (ACTH), cosyntropin, cyclophosphamide, cyclosporine,
cyproheptadine HCl, cytarabine, dacarbazine, dactinomycin/actinomycin D, dalteparin sodium,
danazol, dantrolene sodium, dapsone, decoquinate, deferoxamine mesylate, deracoxib, desloretin
acetate, desmopressin acetate, desoxycorticosterone pivalate, detomidine HCl, dexamethasone,
dexpanthenol, dexraazoxane, dextan, diazepam, diazoxide (oral), dichlorphenamide, dichlorvos,
diclofenac sodium, dicloxacillin, diethylcarbamazine citrate, diethylstilbestrol (DES), difloxacin HCl,
digoxin, dihydratroxy sterol (DHT), diltiazem HCl, dimenhydrinate, dimercaprol/BAL, dimethyl
sulfoxide, dinoprost tromethamine, diphenylhydramine HCl, disopyramide phosphate, dobutamine
HCl, docusate/DSS, dolasetron mesylate, domperidone, dopamine HCl, doramectin, doxapram HCl,
doxepin HCl, doxorubicin HCl, doxycycline, edetate calcium disodium, calcium EDTA, edrofonium
chloride, enalapril/enalaprilat, enoxaparin sodium, enrofloxacin, ephedrine sulfite, epinephrine,
epoetin/erythropoietin, epirinomectin, epsiprantel, erythromycin, esmolol HCl, estradiol cypionate,
etacrynac acid/ethacrymate sodium, ethanol (alcohol), etidronate sodium, etodolac, etomidate,
euthanasia agents w/pentobarbital, famotidine, fatty acids (essential/omega), felbamate, fenbendazole,
fentanyl, ferrous sulfate, filgrastim, finasteride, fipronil, florfenicol, fluconazole, flucytosine,
fludrocortisone acetate, flumazenil, flumethasone, flunixin meglumine, fluoroouracil (5-FU),
fluoxetine, fluticasone propionate, fluvoxamine maleate, fomepizole (4-MP), furazolidone,
furosemide, gabapentin, gemcitabine HCl, gentamicin sulfate, glimepiride, glipizide, glucagon,
glucocorticoid agents, glucosamine/chondroitin sulfate, glutamine, glyburide, glycerine (oral),
glycopyrrolate, gonadorelin, griseofulvin, guaifenesin, halothane, hemoglobin glutamer-200
(oxyglobin®), heparin, hetastarch, hyaluronate sodium, hydrazaline HCl, hydrochlorothiazide,
hydrocortone bitartrate, hydrocortisone, hydromorphone, hydroxyurea, hydroxyzine, ifosfamide,
imidacloprid, imidocarb dipropionate, impenem-cilastatin sodium, imipramine, inamrinone lactate,
insulin, interferon alfa-2a (human recombinant), iodide (sodium/potassium), ipecac (syrup), ipodate
sodium, iron dextran, isoflurane, isoproterenol HCl, isotretinoin, isoxsuprine HCl, itraconazole,
ivermectin, kaolin/pectin, ketamine HCl, ketoconazole, ketoprofen, ketorolac tromethamine, lactulose,
leuprolide, levamisole, levetiracetam, levothyroxine sodium, lidocaine HCl, lincomycin HCl, liothyronine sodium, lisinopril, lonustine (CCNU), lufenuron, lysine, magnesium, mannitol, marbofloxacin, mecloretamine HCl, meclizine HCl, meclofenamic acid, medetomidine HCl, medium chain triglycerides, medroxyprogesterone acetate, megestrol acetate, melarsomine, melatonin, meloxicam, melphalan, meperidine HCl, mercaptopurine, meropenem, metformin HCl, methadone HCl, methazolamide, methenamine mandelate/hippurate, methimazole, methionine, methocarbamol, methohexital sodium, methotrexate, methoxyflurane, methylene blue, methylphenidate, methylprednisolone, metoclopramide HCl, metoprolol, metronidazole, mefoxetine HCl, mibolerlone, midazolam HCl milbemycin oxime, mineral oil, minocycline HCl, misoprostol, mitotane, mitoxantrone HCl, morantel tartrate, morphine sulfate, moxidectin, naloxone HCl, mandrolone decanoate, naproxen, narcotic (opiate) agonist analogics, neomycin sulfate, neostigmine, niacinamide, nitazoxanide, nitenpyram, nitrofurantoin, nitroglycerin, nitroprusside sodium, nizatidine, novobiocin sodium, nystatin, octreotide acetate, olsalazine sodium, omeprazole, ondansetron, opiate antidiarrheals, orfivoxacin, oxacillin sodium, oxazepam, oxendazole, oxibutynin chloride, oxymorphone HCl, oxytetracycline, oxytocin, pamidronate disodium, pancrelipase, panceuronium bromide, paromomycin sulfate, paroxetine HCl, pencillamine, general information penicillins, penicillin G, penicillin V potassium, pentazocine, pentobarbital sodium, pentosan polysulfate sodium, pentoxifylline, pergolide mesylate, phenobarbital, phenoxybenzamine HCl, phyllobutazone, phenylephrine HCL, phenypropanolamine HCl, phenytoin sodium, phenomones, parenteral phosphate, phytonadione/vitamin K-1, pimobendan, piperazone, pirlimycin HCL, piroxicam, polysulfated glycosaminoglycan, ponazuril, potassium chloride, pralidoxime chloride, praziquantel, prazosin HCl, prednisolone/prednisone, primidone, procainamide HCl, procarbazine HCl, prochlorperazine, propantheline bromide, propionibacterium acnes injection, propofol, propranolol HCl, protamine sulfate, pseudoephedrine HCl, psyllium hydrophilic mucilloid, pyrantel pamoate, pyridostigmine bromide, pyrilidine maleate, pyrimethamine, quinacrine HCl, quinidine, ranitidine HCl, rifampin, s-adenosyl-methionine (SAMe), saline/hyposmotic laxative, selamectin, selegiline HCL/l-depenyl, sertraline HCl, sevelamer HCl, sevoflurane, silymarin/milk thistle, sodium bicarbonate, sodium polystyrene sulfonate, sodium stibogluconate, sodium sulfate, sodium thiosulfate, somatotropin, sotalol HCl, spectinomycin HCl, spironolactone, stanozolol, streptokinase, streptococin, succimer, succinylcholine chloride, sucrafate, sufentanil citrate, sulfachlorpyridazine sodium, sulfadiazine/trimethoprim, sulfamethoxazole/trimethoprim, sulfadimethoxine, sulfadimethoxine/ormetoprin, sulfasalazine, taurine, tepoxaline, terbinafine HCl, terbutaline sulfate, testosterone, tetracycline HCl, thiamphenicol, thiabendazole, thiacetarsamide sodium, thiamine HCl, thioguanine, thiopental sodium, thiopental sodium, thiotepa, thyrotropin, tiamulin, ticarcillin disodium, tiletamine HCl/zolazepam HCl, tilmicosin, tiopronin, tobramycin sulfate, tocainide HCl, tolazoline HCl, telfenamic acid, topiramate, trimadol HCl, trimcinolone acetonide, trientine HCl, trilostane, trimeprazine tartrate w/prednisolone, tripeleennamine HCl, tylosin, ursosiol, valproic acid, vanadium, vancomycin HCl, vasopressin,
vecuronium bromide, verapamil HC1, vinblastine sulfate, vincristine sulfate, vitamin E/selenium, warfarin sodium, xylazine HC1, yohimbine HC1, zafirlukast, zidovudine (AZT), zinc acetate/zinc sulfate, zonisamide and mixtures thereof.

In one embodiment of the invention, arylpyrazole compounds may be added to the compositions of the invention. Arylpyrazoles may include but are not limited to those described in U.S. Patent Nos. 6,001,384; 6,010,710; 6,083,19; 6,096,329; 6,174,540; 6,685,954 and 6,998, 131, all of which are hereby incorporated by reference in their entirety, - each assigned to Merial, Ltd., Duluth, GA).

In another embodiment of the invention, nodulisporic acid and its derivatives (a class of known acaricidal, anthelminitic, anti-parasitic and insecticidal agents) may be added to the compositions of the invention. These compounds are used to treat or prevent infections in humans and animals and are described, for example, in U.S. Patent No. 5,399,582, 5,962,499, 6,221,894 and 6,399,786, all of which are hereby incorporated by reference in their entirety. The compositions may include one or more of the known nodulisporic acid derivatives in the art, including all stereoisomers, such as those described in the literature cited above.

In another embodiment of the invention, the class of acaricides or insecticides known as insect growth regulators (IGRs) may also be added to the compositions of the invention. Compounds belonging to this group are well known to the practitioner and represent a wide range of different chemical classes. These compounds all act by interfering with the development or growth of the insect pests. Insect growth regulators are described, for example, in U.S. Patent No. 3,748,356; U.S. Patent No. 3,818,047; U.S. Patent No. 4,225,598; U.S. Patent No. 4,798,837; U.S. Patent No. 4,751,225, EP 0 179 022 or U.K. 2 140 010 as well as U.S. Patent Nos. 6,096,329 and 6,685,954, all of which are hereby incorporated by reference in their entirety, (both assigned to Merial Ltd., Duluth, GA). Examples of IGRs suitable for use may include but are not limited to methoprene, pyriproxyfen, hydroprene, cyromazine, fluaizuron, lufenuron, novaluron, pyrethroids, formamidines and 1-(2,6-difluorobenzoyl)-3-(2-fluoro-4-(trifluoromethyl)phenylurea.

An anthelmintic agent that may be combined with the compositions of the invention may be a benzenedisulfonamide compound, which includes but is not limited to clorsulon; or a cestodal agent, which includes but is not limited to praziquantel, pyrantel or morantel.

A parasiticidal agent that may be combined with the compositions of the invention may be a biologically active peptide or protein including, but not limited to, depsipeptides, which act at the neuromuscular junction by stimulating presynaptic receptors belonging to the secretin receptor family resulting in the paralysis and death of parasites. In one embodiment the depsipeptide may be emodepside.

An insecticidal agent that may be combined with the compositions of the invention may be a spinosyn (e.g. spinosad) or a substituted pyridymethyl derivative compound such as imidacloprid. Agents of this class are described above, and for example, in U.S. Patent No. 4,742,060 or in EP 0
892 060, both of which are hereby incorporated by reference in their entirety. It would be well within the skill level of the practitioner to decide which individual compound may be used in the inventive formulation to treat a particular infection of an insect. For endoparasites, parasiticides which may be combined include but are not limited to pyrantel, morantel, the benzimidazoles (including albendazole, cambendazole, thiabendazole, fenbendazole, febantel, oxfendazole, oxibendazole, triclabendazole mebendazole and netobimin), levamisole, closantel, rafoxanide, nitroxynil, disophenol and paraenderam. For ectoparasites, insecticides which may be combined also include but are not limited to pyrethroids, organophosphates and neonicotinoids such as imidaclorpid, as well as compounds such as metaflumizone, amitraz and ryanodine receptor antagonists.

Where appropriate, the anthelmintic, parasiticidal and insecticidal agent may also be selected from the group of compounds described above as suitable for agrochemical use.

In general, the additional pesticidal agent may be included in a dose of between about 0.1 µg and about 10 mg. In one embodiment of the invention, the additional pesticidal agent may be included in a dose of between about 1 µg and about 10 mg. In another embodiment of the invention, the additional pesticidal agent may be included in a dose of about 5 to about 200 µg/kg of weight of mammal. In yet another embodiment of the invention, the additional pesticidal agent may be included in a dose between about 0.1 to about 10 mg/kg of weight of mammal. In still another embodiment of the invention, the additional pesticidal agent may be included in a dose between about 0.5 to 50 mg/kg.

A further embodiment of the invention includes a diagnostic tool for testing the presence or absence of a parasitic strain. For example, US20070042354, US20110223599, 20030129680, 20110223599 discloses systems, methods, and compositions for identifying a subject infected with a parasite.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims. This invention includes all modifications and equivalents of the subject matter recited in the aspects or claims presented herein to the maximum extent permitted by applicable law.

Certain aspects of the invention are further described by the following Examples:

**Examples**

The following examples are provided to illustrate certain embodiments of the invention and are not to be construed in any way as limiting the scope of the invention.
Study of the Efficacy of Ivermectin and Emodepside. Separately and in Combination.

Against *Dirofilaria immitis*:

Ten male and ten female healthy Beagle dogs, 5.2 to 6.2 months of age, weighing 7.3 to 10.3 kg were studied.

Dogs were tested for microfilaria and heartworm antigen and received a full physical examination prior to inclusion in the study. Each dog was inoculated with 50 infective third-stage *D. immitis* larvae on Day -7 (JYD-34 isolate). Antigen testing performed on blood collected on Day 111 confirmed that animals had not been exposed to *D. immitis* prior to the induced infection.

Four blocks of five dogs each were formed based on descending Day -2 body weights within sex. Within blocks, dogs were randomly allocated to one of five treatment groups by lottery and treated orally five times at monthly intervals with an oral solution of ivermectin, Profender tablets or a combination of an ivermectin solution and Profender tablets at monthly dosing intervals for according to the following table 1:

**Table 1:**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Investigational Material</th>
<th>Dose</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated Control</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>ivermectin oral solution</td>
<td>6 mcg/kg (0.15 mL/kg)</td>
<td>Less than 50%</td>
</tr>
<tr>
<td>3</td>
<td>Profender Tablet(s): emodepside + praziquantel</td>
<td>1 mg/kg 5 mg/kg</td>
<td>Less than 50%</td>
</tr>
<tr>
<td>4</td>
<td>ivermectin oral solution and Profender Tablet(s): emodepside + praziquantel</td>
<td>6 mcg/kg (0.15 mL/kg) and 1 mg/kg 5 mg/kg</td>
<td>81%</td>
</tr>
<tr>
<td>5</td>
<td>ivermectin oral solution and Profender Tablet(s): emodepside + praziquantel</td>
<td>6 mcg/kg (0.15 mL/kg) and 5 mg/kg 25 mg/kg</td>
<td>100%</td>
</tr>
</tbody>
</table>
All animals were humanely euthanized on Day 160 and a necropsy was performed for parasite recovery and live *D. immitis* counts for individual dogs. The percent efficacies by treatment group are listed in Table 1.

In this study, ivermectin solution (6 mcg/kg), administered orally in combination with Profender tablets (5 mg/kg emodepside plus 25 mg/kg praziquantel) for five months, provided 100% efficacy against induced infections of the JYD-34 isolate of *Dirofilaria immitis*.

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.
WHAT IS CLAIMED IS:

1. A method of treatment or prophylaxis of a parasitic infection in a mammal comprising administering to said mammal an effective amount of:
   a) at least one cyclic depsipeptide; and
   b) at least one macrocyclic lactone;
and a pharmaceutically acceptable carrier;
wherein the parasitic infection comprises a parasite that is resistant to treatment or prophylaxis with the macrocyclic lactone alone.

2. A method according to claim 1, further comprising praziquantel or epsiprantel or a combination thereof.

3. A method according to claim 1, wherein the effective amount is an additive or synergistic effective amount.

4. A method according to claim 1, wherein the parasite is Dirofilaria immitis.

5. A method according to claim 1, wherein the parasite is a resistant Dirofilaria immitis strain.

6. A method according to claim 1, wherein the parasite is third-stage larvae (L3) or fourth-stage larvae (L4) of a resistant Dirofilaria immitis strain or a combination thereof.

7. A method according to claim 1, wherein the cyclic depsipeptide is 24-membered cyclooctadepsipeptide.

8. A method according to claim 1, wherein the cyclic depsipeptide is emodepside, PF1022A, a PF1022A derivative or a combination thereof.

9. A method according to claim 1, wherein the cyclic depsipeptide is emodepside.

10. A method according to claim 1, wherein the macrocyclic lactone administered is an avermectin, a milbemycin or a combination thereof.

11. A method according to claim 1, wherein the macrocyclic lactone administered is selected from the group consisting of abamectin, dimadectin, doramectin, emamectin, eprinomectin,
ivermectin, latidectin, lepimectin, selamectin, milbemycin oxime and moxidectin or a combination thereof; and
the cyclic depsipeptide is selected from the group consisting of emodepside, PF1022A, and a PF1022A derivative or a combination thereof.

12. A method according to claim 1, wherein the macrocyclic lactone administered is selected from the group consisting of abamectin, dimadectin, doramectin, emamectin, eprinomectin, ivermectin, latidectin, lepimectin, selamectin, milbemycin oxime and moxidectin or a combination thereof.

13. A method according to claim 1, wherein the macrocyclic lactone of the composition is ivermectin.

14. A method according to claim 1, wherein the weight ratio of macrocyclic lactone to cyclic depsipeptide is 1:100 to about 1:1000, or about 1:500 to about 1:1000.

15. A method according to claim 1, wherein the veterinary composition is an oral formulation, injectable formulation, topical formulation, pour-on formulation, dermal formulation or sub-dermal formulations.

16. A method according to claim 1, wherein the veterinary composition is an oral formulation.

17. A method according to claim 1, wherein the parasitic infection is an endoparasitic infection.

18. A method according to claim 1, wherein the parasites shows resistance to at least one macrocyclic lactone selected from the group consisting of abamectin, dimadectin, doramectin, emamectin, eprinomectin, ivermectin, latidectin, lepimectin, selamectin, milbemycin-oxime, and moxidectin or a combination thereof.

19. A method according to claim 1, wherein the parasites shows resistance to ivermectin.

20. A method according to claim 1, wherein the mammal is selected from the group consisting of humans, dogs, and cats.

21. A method according to claim 1, wherein the mammal is dogs or cats.
22. A method according to claim 1, wherein treatment or prophylaxis of the parasitic infection in the mammal kills the third-stage larva (L3) or fourth-stage larva (L4) of *Dirofilaria immitis* so that they do not mature into adult worms.

23. A method according to claim 1, wherein the parasitic infection causes heartworm associated respiratory disease in the mammal.

24. A method according to claim 1, wherein the administration of the cyclic depsipeptide and macrocyclic lactone is concomitant.

25. A method according to claim 1, wherein the administration of the cyclic depsipeptide and macrocyclic lactone is sequential.

26. A method according to claim 1, wherein the route of administration of the cyclic depsipeptide and macrocyclic lactone is the same.

27. A method according to claim 1, wherein the administration of the cyclic depsipeptide and macrocyclic lactone is five times at monthly intervals.

28. A method according to claim 1, comprising administering to said mammal an effective amount of:
   a) cyclic depsipeptide which is emodepside or PF1022A; and
   b) a macrocyclic lactone selected from the group consisting of abamectin, dimadectin, doramectin, emamectin, eprinomectin, ivermectin, latidectin, lepimectin, selamectin, milbemycin oxime and moxidectin;
   and a pharmaceutically acceptable carrier;
   wherein the parasitic infection comprises a parasite that is resistant to treatment or prophylaxis with the macrocyclic lactone alone.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K38/15 A61K31/7Q48 A61P33/00 A61P33/14

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US 6 159 932 A (MENCKE NORBERT [DE] ET AL) 12 December 2000 (2000-12-12) cited in the application on col umn 1, line 47 - col umn 2, line 56; claims 1-9; example C col umn 11, line 42 - col umn 12, line 15</td>
<td>1-28</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"Z" document member of the same patent family

Date of the actual completion of the international search

20 June 2016

Date of mailing of the international search report

05/07/2016

Name and mailing address of the ISA/Authorized officer

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel: (+31-70) 340-2040, Fax: (+31-70) 340-3016

Escolar Blasco, P

Form PCT/ISA/210 (second sheet) (April 2000)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>
| Y        | KRÜDEWAGEN: "concomitant simul taneous and consecuti ve treatment of imidaclorpid/moxic  
emodepside/praziqantel tablet in dogs", INTERN J APPL RES VET MED,  
vol. 9, no. 3, 1 January 2011 (2011-01-01)  
, XP055280966, abstract  
Introducti on;  
paragrap h [0001] ------ 1-28 |
| Y        | SAMSON-HIMMELSTJERNA G VON ET AL:  
"Effi cacy of two cyclooctadepsi peptides, PF1022A and emodepside, against an  
thelminti c-resi stant nematodes in sheep and catlle",  
PARASITOLOGY,  
vol. 130, March 2005 (2005-03) , pages  
343-347,  
XP9190568, ISSN: 0031-1820  
abstract  
page 346, left-hand col umn, paragraph 3 -  
page 347, left-hand col umn, paragraph 1 ------ 1-28 |
| Y        | HARDER A ET AL:  
"Cyclooctadepsi peptides-an an  
thelminti cal ly acti ve class of compounds exhi bi ting a novel mode of action",  
INTERNATIONAL JOURNAL OF ANTIMICROBIAL AGENTS, ELSEVIER SCIENCE, AMSTERDAM, NL,  
vol. 22, no. 3,  
1 September 2003 (2003-09-01) , pages  
318-331,  
XP002995541, ISSN: 0924-8579, DOI:  
10.1016/S0924-8579(03)00219-X  
abstract  
page 321, right-hand col umn, last  
paragraph - page 322, right-hand col umn,  
paragraph 4 ------ 1-28 |
| Y        | NwOSU UZOMA ET AL:  
"Effi cacy of the cyclooctadepsi peptidePF1022A agai nst Hel igmosomoides bakeri i n vi tro and i n vi vo",  
PARASITOLOGY, CAMBRIDGE UNIVERSITY PRESS, LONDON, GB,  
vol. 138, no. 9,  
1 August 2011 (2011-08-01) , pages  
1193-1201,  
XP009190563, ISSN: 0031-1820, DOI:  
10.1017/S003118201100076X  
I n vi vo studi es;  
page 1195, right-hand col umn, paragraph 4  
page 1198, right-hand col umn, paragraph 2 ------ 1-28 |
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

abstract
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 6159932</td>
<td>12-12-2000</td>
<td>AR 005640 AI</td>
<td>14-07-1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT 213645 T</td>
<td>15-03-2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 703048 B2</td>
<td>11-03-1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 5900496 A</td>
<td>18-12-1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BR 9608961 A</td>
<td>29-06-1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2222680 AI</td>
<td>05-12-1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 1191489 A</td>
<td>26-08-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CZ 9703825 A3</td>
<td>18-03-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 19520750 A</td>
<td>05-12-1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DK Q828506 T3</td>
<td>10-06-2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0828506 A2</td>
<td>18-03-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ES 2173284 T3</td>
<td>16-10-2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HU 9900346 A2</td>
<td>28-06-1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL 118518 A</td>
<td>27-12-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 4104653 B2</td>
<td>18-06-2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP H11506438 A</td>
<td>08-06-1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 100434388 B1</td>
<td>25-08-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO 975516 A</td>
<td>06-01-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NZ 309073 A</td>
<td>23-12-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PL 323595 AI</td>
<td>14-04-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT 828506 E</td>
<td>30-08-2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SK 159979 A3</td>
<td>08-07-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TR 9701484 T1</td>
<td>21-03-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TW 469133 B</td>
<td>21-12-2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 6159932 A</td>
<td>12-12-2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9638165 A2</td>
<td>05-12-1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 9604473 A</td>
<td>29-01-1997</td>
</tr>
<tr>
<td>US 2011046072</td>
<td>24-02-2011</td>
<td>AR 071620 AI</td>
<td>30-06-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 2009243759 AI</td>
<td>12-11-2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BR P10912428 A2</td>
<td>16-02-2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2723553 AI</td>
<td>12-11-2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 102215824 A</td>
<td>12-10-2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO 6311067 A2</td>
<td>22-08-2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 10200822520 AI</td>
<td>12-11-2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DK 2285359 T3</td>
<td>26-11-2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DO P2010000331 A</td>
<td>15-12-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EC SP10010588 A</td>
<td>30-12-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2285359 A2</td>
<td>23-02-2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ES 2393169 T3</td>
<td>19-12-2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HK 1162936 AI</td>
<td>06-05-2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 5539966 B2</td>
<td>02-07-2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2011519878 A</td>
<td>14-07-2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 20110015592 A</td>
<td>16-02-2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NZ 589017 A</td>
<td>30-11-2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE 19492009 AI</td>
<td>25-12-2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT 2285359 E</td>
<td>26-11-2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RU 2010149561 A</td>
<td>20-06-2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TW 201010714 A</td>
<td>16-03-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UA 103025 C2</td>
<td>10-09-2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2011046072 AI</td>
<td>24-02-2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2015150985 AI</td>
<td>04-06-2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UY 31788 A</td>
<td>14-12-2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2009135593 A2</td>
<td>12-11-2009</td>
</tr>
</tbody>
</table>