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(54) **INJECTABLE PARASITICIDAL FORMULATIONS OF LEVAMISOLE AND MACROCYCLIC LACTONES**

INJIZIERBARE PARASITIZIDE FORMULIERUNGEN AUS LEVAMISOL UND MAKROCYCLISCHEN LACTONEN

FORMULATIONS PARASITICIDES INJECTABLES DE LÉVAMISOLE ET DE LACTONES MACROCYCLIQUES

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- **SARI PEYAMI ET AL: "HPLC assay of levamisole and abamectin in sheep plasma for application to pharmacokinetic studies", JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES, vol. 29, no. 15, 2006, pages 2277-2290, XP009151151, ISSN: 1082-6076**

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Description**INCORPORATION BY REFERENCE**

5 [0001] This application claims priority to U.S. provisional patent application No. 61/333,882, filed May 12, 2010. All documents cited or referenced in the applicant 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
10 practice of the invention.

FIELD OF THE INVENTION

15 [0002] The present invention is directed to new injectable parasitocidal formulations comprising levamisole and macrocyclic lactones. The present invention also provides methods for eradicating, controlling, and preventing parasite infestation and/or infection in birds, fish and mammals. The formulations of the invention may be administered to animals, particularly mammals, fish and birds, to prevent or treat parasitic infestation and/or infection.

BACKGROUND OF THE INVENTION

20 [0003] Animals, such as mammals, fish and birds are often susceptible to parasite infestation and/or infection. These parasites may be ectoparasites, such as insects and acarine species, and endoparasites such as filariae and other worms. Endoparasites pose a significant problem to those who raise production animals such as bovines, ovines, and porcines. Thus, there is an ongoing need to develop active pesticidal and parasitocidal compounds to protect animals
25 against attack or infestation/infection by pests.

[0004] Many endoparasiticides or anthelmintics exist in the art for treating internal parasites and other animal pests. These endoparasiticides vary in cost as well as their effectiveness to a particular parasite. However, the results of treatment with these pesticides are not always satisfactory because of continued development of resistance by the parasite to the available therapeutic agents. Thus, there is a need in the art for more effective parasitocidal agents for
30 treatment and protection of animals, e.g. mammals, fish and birds from infestation by animal parasites.

[0005] The avermectin and milbemycin series of compounds are potent anthelmintic and antiparasitic agents against a wide range of internal and external parasites. The compounds which belong to this series are either natural products or are semi-synthetic derivatives thereof. The structure of these two series of compounds are closely related and they both share a complex 16-membered macrocyclic lactone ring; however, the milbemycins do not contain the disaccharide
35 substituent in the 13-position of the lactone ring. The natural product avermectins are disclosed in U.S. Patent No. 4,310,519 to Albers-Schonberg, et al., and the 22, 23-dihydro avermectin compounds are disclosed in Chabala, et al., U.S. Patent No. 4,199,569. For a general discussion of avermectins, which include a discussion of their uses in humans and animals, see "Ivermectin and Abamectin," W. C. Campbell, ed., Springer-Verlag, N.Y. (1989). Naturally occurring milbemycins are described in Aoki et al., U.S. Patent No. 3,950,360 as well as in the various references cited in "The Merck Index" 12th ed., S. Budavari, Ed., Merck & Co., Inc. Whitehouse Station, N.J. (1996). Semi-synthetic derivatives of these classes of compounds are well known in the art and are described, for example, in U.S. Patent No. 5,077,308, U.S. Patent No. 4,859,657, U.S. Patent No. 4,963,582, U.S. Patent No. 4,855,317, U.S. Patent No. 4,871,719, U.S. Patent No. 4,874,749, U.S. Patent No. 4,427,663, U.S. Patent No. 4,310,519, U.S. Patent No. 4,199,569, U.S. Patent No. 5,055,596, U.S. Patent No. 4,973,711, U.S. Patent No. 4,978,677, and U.S. Patent No. 4,920,148, all incorporated
45 herein by reference in their entirety. A particularly useful macrocyclic lactone is eprinomectin, which is described in US6,174,540, US6,733,767, US5,602,107 to Merck (all incorporated by reference). This compound provides for a very short milk withdrawal period due to its tendency to partition away from the milk of a lactating mammal.

[0006] To overcome the problem of resistance, various combinations of anthelmintics have been extensively researched. Unfortunately, combinations of macrocyclic lactone anthelmintics with anthelmintics of other families have proven difficult to formulate at high concentration and with an acceptable viscosity for injection. For example, Closamectin (manufactured by Norbrook) contains ivermectin at 0.5% and closantel at 12.5% and is so viscous that the product is supplied in a special pack that has a warming device. The warming device is activated 15 minutes before administration and the pack requires periodic shaking throughout this time to ensure temperature reduces the viscosity sufficiently for acceptable administration.

55 [0007] Another example, which has been the subject of considerable research, is combining a macrocyclic lactone (ML) with levamisole. The difficulties of combining these two actives lies in the stability of each being incompatible in the environment in which the other is stable. Levamisole is stable in acid conditions usually a pH of about 4 or below, while macrocyclic lactones are unstable in conditions where the pH is acidic. Both levamisole and macrocyclic lactones

are well known in the art. In particular, levamisole has been used as an anthelmintic to treat nematodes and respiratory worm infestations in both humans and animals. Levamisole may be combined with other actives, although often with significant difficulty, as is described in various patents and applications. For example, US 2009/0075918 A1 to Neto et al. appears to contemplate adding various organic salts of levamisole to macrocyclic lactones, but provides no working examples. Other applications/documents that mention levamisole in combination with macrocyclic lactones include WO 00/74489, Sari et al., Journal of liquid chromatography and related technologies. Vol. 29, no. 15, 2006, pages 2277-2290, WO 2007/068073; WO 2004/069242; WO 00/061068, which describes non-aqueous pour-on formulations that further contain triclabendazole, and WO 04/009080, which describes stable pyrrolidone-based formulations of avermectins or milbemycins combined with levamisole. Even more recently, WO2010021555 (to Intervet) has described oral drenches containing levamisole and a macrocyclic lactone, where the macrocyclic lactone is apparently stabilized via the inclusion of a protective agent, namely hydroxypropyl starch phosphate. Since this type of "protective agent" also serves as a thickener and emulsion stabilizer, this particular approach to stabilizing the macrocyclic lactone component may be ill-suited to developing injectable formulations. Hydroxypropyl starch phosphate (HP starch) is typically used for creams & lotions, foundations, mascara, color cosmetics, liquid make-up, liquid talc, conditioners, shampoos, sunscreen products, and antiperspirants. Finally, AU200310102 (to Nufarm and Novartis) describes formulations having levamisole, avermectins, and benzimidazole, said formulations appearing to make use of a biphasic solvent system, in an attempt to overcome the problematic instability of formulations which comprise levamisole and macrocyclic lactones. Several patents describe long-acting injectable combination formulations comprising macrocyclic lactones and other actives, for example US patent numbers US 6,174,540 and US 6,733,767 (to Merck), which use castor oil and liquid polymers, respectively, to achieve the sustained release of the macrocyclic lactones and the other actives. US 4,381,397 describes injectable formulations of ivermectin. After reviewing the current literature, applicants are unaware of any previously described, effective combinations of levamisole and eprinomectin, injectable or otherwise.

[0008] It is expressly noted that 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. 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.

SUMMARY OF THE INVENTION

[0009] The invention provides novel parasitocidal formulations comprising levamisole and macrocyclic lactones, particularly avermectins such as ivermectin and eprinomectin, and milbemycins, such as moxidectin, milbemectin, and milbemycin oxime. The formulations are storage-stable and provided in a single solvent system that is miscible in water. Therefore, the formulations according to the present invention may be classified as micellar solutions as opposed to emulsions or biphasic emulsions. The invention also provides methods for the treatment and prevention of parasitic infestation and/or infection of animals.

[0010] The inventive formulations comprising the levamisole and macrocyclic lactones are highly effective for the treatment or prophylaxis of parasites in or on mammals, fish and birds, and in particular, cats, dogs, horses, chickens, pigs, sheep and cattle with the aim of ridding these hosts of all the parasites commonly encountered by mammals, fish and birds. The invention also provides for effective and long-lasting defense against endoparasites, such as helminths, nematodes, filariae, hookworms, whipworms and roundworms of the digestive tract of animals and humans.

[0011] Accordingly, in a first aspect the present invention provides a pharmaceutically or veterinarily acceptable formulation adapted to be injected into an animal, which formulation comprises;

- a. an effective amount of a levamisole;
- b. an effective amount of at least one macrocyclic lactone;
- c. a pharmaceutically acceptable system of solvents and surfactants wherein the system provides for acceptable storage stability for both the levamisole and the macrocyclic lactone and wherein the system provides for a viscosity which is suitable for injection;

wherein the system comprises at least one surfactant, wherein the surfactant is CREMAPHOR EL, and a water miscible organic solvent which is dimethylacetamide (DMA).

[0012] In a second aspect, the present invention provides a pharmaceutically or veterinarily acceptable formulation adapted to be injected into an animal, which formulation comprises;

- a. an effective amount of a levamisole;

- b. an effective amount of at least one macrocyclic lactone;
- c. a pharmaceutically acceptable system of solvents and surfactants wherein the system provides for acceptable storage stability for both the levamisole and the macrocyclic lactone and wherein the system provides for a viscosity which is suitable for injection;

wherein the system comprises at least one surfactant, wherein the surfactant is CREMAPHOR EL, and a water miscible organic solvent which is dimethylacetamide (DMA), wherein the macrocyclic lactone is eprinomectin and its concentration is about 0.5% to about 0.9% (w/v); the levamisole is levamisole phosphate and its concentration is about 18% to about 25% (w/v); the surfactant is POLYSORBATE 80, CREMAPHOR EL, or combinations thereof, and its concentration is about 3% to about 7% (w/v); the antioxidant is BHT, the solvent is DMA and its concentration is about 1% to about 10% (w/v); and the preservative is methylparaben.

[0013] In a third aspect, the present invention provides a formulation according to the first and second aspects or use in preventing or treating internal parasite infestations in animals.

[0014] In a fourth aspect, the present invention provides a method for producing the formulation according to claim 1 or 2 comprising the steps of:

- a. adding water for injection to a vessel,
- b. dissolving the Levamisole in the water,
- c. in a separate vessel dissolving Eprinomectin and BHT in DMA,
- d. adding the surfactant to the DMA/Eprinomectin blend,
- e. transferring the Eprinomectin/DMA blend to the bulk aqueous phase with mixing, and
- f. optionally, bringing the batch to volume with added WFI and mix.

[0015] It is an object of the invention to not encompass within the invention any previously known product, process of making the product, or method of using the product such that the Applicants reserve the right to this invention and hereby disclose a disclaimer of any previously known product, process, or method.

[0016] It is 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; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law; e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0017] These and other embodiments are disclosed or are obvious from, and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF DRAWINGS

[0018] The following Detailed Description, given by way of example, and not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying Figures, incorporated herein by reference, in which:

FIG. 1 is a flow diagram outlining the preparation of formulations according to the instant invention

FIG. 2 depicts levamisole plasma concentration at time points after administration of formulations according to the instant invention

FIG. 3 depicts eprinomectin plasma concentration at time points after administration of formulations according to the instant invention

DETAILED DESCRIPTION

[0019] The present invention provides novel injectable levamisole / macrocyclic lactone formulations with parasitocidal activity, for the treatment or prevention of parasitic infestations and/or infection in an animal.

[0020] An important aspect of formulations containing eprinomectin as the macrocyclic lactone component is that said formulations may have shortened withdrawal times before it is appropriate to obtain milk from lactating mammals. This is because of the macrocyclic lactones, eprinomectin has relatively the lowest milk to plasma partition ratio (-0.2). For a point of reference and definition, a milk to plasma partition ratio of 1 would indicate the active tends to distribute evenly between milk and plasma.

[0021] Formulations described herein are particularly effective for controlling endoparasites. Endoparasites include, but are not limited to, nematodes (such as roundworms, hookworms, whipworms and heartworms) and cestodes (tapeworms) and trematodes (flukes). Therefore, the inventive formulations have substantial utility in preventing damage to

crops, plants, plant propagation material and wood-containing property, and in controlling and preventing the infestation and/or infection of animals by parasites.

[0022] Also contemplated within the scope of the invention are acid or base salts of the compounds in the compositions of the invention, where applicable.

[0023] The term "acid" contemplates all pharmaceutically acceptable inorganic or organic acids. Inorganic acids include mineral acids such as hydrohalic acids such as hydrobromic acid and hydrochloric acid, sulfuric acid, phosphoric acids and nitric acid. Organic acids include all pharmaceutically acceptable aliphatic, alicyclic and aromatic carboxylic acids, dicarboxylic acids, tricarboxylic acids and fatty acids. In one embodiment of the acids, the acids are straight chain or branched, saturated or unsaturated C₁-C₂₀ aliphatic carboxylic acids, which are optionally substituted by halogen or by hydroxyl groups, or C₆-C₁₂ aromatic carboxylic acids. Examples of such acids are carbonic acid, formic acid, acetic acid, propionic acid, isopropionic acid, valeric acid, α -hydroxy acids such as glycolic acid and lactic acid, chloroacetic acid, benzoic acid, methane sulfonic acid, and salicylic acid. Examples of dicarboxylic acids include oxalic acid, malic acid, succinic acid, tartaric acid, fumaric acid, and maleic acid. An example of a tricarboxylic acid is citric acid. Fatty acids include all pharmaceutically acceptable saturated or unsaturated aliphatic or aromatic carboxylic acids having 4 to 24 carbon atoms. Examples include butyric acid, isobutyric acid, sec-butyric acid, lauric acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and phenylstearic acid. Other acids include gluconic acid, glycoheptonic acid and lactobionic acid.

[0024] The term "base" contemplates all pharmaceutically acceptable inorganic or organic bases, including hydroxides, carbonates or bicarbonates of alkali metal or alkaline earth metals. Salts formed with such bases include, for example, the alkali metal and alkaline earth metal salts, including, but not limited to, as the lithium, sodium, potassium, magnesium or calcium salts. Salts formed with organic bases include the common hydrocarbon and heterocyclic amine salts, which include, for example, ammonium salts (NH₄⁺), alkyl- and dialkylammonium salts, and salts of cyclic amines such as the morpholine and piperidine salts.

[0025] In addition, the compounds within the compositions of the invention may exist as hydrates or solvates, in which a certain stoichiometric amount of water or a solvent is associated with the molecule in the crystalline form. The compositions of the invention may include hydrates and solvates of the active agents.

Definitions:

[0026] Terms used herein will have their customary meanings in the art unless specified. As used herein the term "anthelmintic" and variations thereof encompasses one or more nematocidal, trematocidal and cestocidal active compounds. The term "pesticidal" and variations thereof includes any said anthelmintic, any endectoparasitocidal, and/or any ectoparasitocidal compound. Where the context allows the term "ectoparasitocidal" includes compounds effective against any one or more ectoparasites including ticks, lice, fleas, mites and the like. Further, as used herein the "endectoparasitocidal" and variants thereof includes compounds and/or formulations that are active against internal (endo) and external (ecto) parasites.

[0027] As used herein the term "solubility" refers to the ability of a compound to be dissolved in a specific phase; and "lipophilic" means a greater tendency to an organic, oil or the like phase as opposed to another phase (for example, the aqueous phase).

[0028] As referred to herein the term "injectable" in the context of fluids or liquids covers viscosities ranging from a free flowing liquid to a thin gel consistency that is capable of being expelled by syringe and suitable for being administered to an animal via injection. As used herein, a suitable or appropriate viscosity for injection may be between 1-50 cp or from 1-10 cp. Routes of injection may be parenteral, for example intramuscular (IM), intraperitoneal (IP), or subcutaneous (SQ).

[0029] As referred to herein the term "acceptable storage stability" means stable for greater than 3 months at room temperature.

Formulations of the Invention:

[0030] The formulations of the invention comprise at least levamisole and at least one macrocyclic lactone active compound, and are provided as micellar solutions of actives, which are miscible in water for injection, and are particularly effective at controlling / combating parasites, particularly endoparasites. In certain embodiments, the formulations of the invention are useful in veterinary applications, including for controlling parasites in or on an animal.

[0031] In one embodiment, the at least one macrocyclic lactone active agent in the formulations of the invention may be at least one avermectin or milbemycin compound. In various embodiments, the at least one macrocyclic lactone compound may be abamectin, avermectin, dimadectin, doramectin, emamectin, eprinomectin, ivermectin, latidectin, lepimectin, selamectin, milbemectin, milbemycin D, milbemycin oxime, moxidectin or nemadectin.

[0032] In another embodiment, the at least one macrocyclic lactone active agent may be any one of the avermectins

or avermectin monosaccharides modified in the 4' or 4" position described in US 7,704,961, US 7,521,429, US 2006-0105970 A1, US 7,678,740, US 7,632,820, US 7,678,773, US 7,605,134, and US 6,933,260 (each to Merial). It will be appreciated by a skilled person that any of the above-recited avermectins exhibiting a relatively low milk to plasma partition ratio (for example less than about 1, less than about 0.5, and even more favorably, less than about 0.2) will be a particularly desirable component of formulations according to the instant invention.

[0033] In an embodiment, the levamisole is levamisole phosphate and is present in an amount sufficient to deliver a dose of at least 4 mg/kg animal bodyweight. In another embodiment, the macrocyclic lactone is either ivermectin or eprinomectin and is present in an amount sufficient to deliver a dose of at least 150 µg/kg animal bodyweight.

[0034] In a particular embodiment, the present invention is a stable injectable formulation comprising eprinomectin and levamisole and is suitable for use in cattle. In an embodiment, the formulation is an injectable combination anthelmintic containing about 7 g/L eprinomectin and about 223 g/L levamisole phosphate.

[0035] In an embodiment, the formulation requires not more than a 35 day withholding period for either milk or meat.

[0036] In an embodiment, the formulation is administered at a dose rate of 1 mL per 35 kg (200 µg eprinomectin and 6.4 mg levamisole phosphate per kg, equivalent to 5 mg levamisole HCl per kg). In another embodiment, the formulation is effective against a broad range of internal parasites including *Ostertagia spp.*, *Trichostrongylus axei*, *Cooperia spp.*, sucking lice, and certain strains resistant to certain macrocyclic lactones and/or benzimidazoles.

[0037] In an embodiment, the formulations have a shelf life and storage condition of at least 2 years at 2-6 °C. In another embodiment, the formulations are prepared as pack sizes of 100 to 1000 mL. In a particular embodiment, the pack sizes are 500 mL flexible packages, or flexipacks.

[0038] In another preferred embodiment, the formulations of the invention will comprise a pharmaceutically acceptable amide including, but not limited to, dimethylformamide, dimethylacetamide, 2-pyrrolidone, N-methylpyrrolidone and the like.

[0039] Surfactants are well known in the art and may include non-ionic surfactants, cationic surfactants and anionic surfactants. Anionic surfactants include, but are not limited to, alkaline stearates (e.g. sodium, potassium or ammonium stearate); calcium stearate or triethanolamine stearate; sodium abietate; alkyl sulfates, which include but are not limited to sodium lauryl sulfate and sodium cetyl sulfate; sodium dodecylbenzenesulphonate or sodium dioctyl sulphosuccinate; or fatty acids (e.g. coconut oil);

[0040] Cationic surfactants include, but are not limited to, any known water-soluble quaternary ammonium salts such as cetyltrimethylammonium bromide and the like; and known amine salts such as octadecylamine hydrochloride and the like.

[0041] Non-ionic surfactants include, but are not limited to, 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. Other surfactants that are suitable for the formulations of the present invention include amphoteric surfactants, such as substituted lauryl compounds of betaine. The surfactant of the inventive formulations may also be a mixture of at least two different surfactants.

[0042] In another embodiment, the levamisole is levamisole phosphate and the macrocyclic lactone is either eprinomectin or ivermectin. Thus in an embodiment, the formulation of the present invention may comprise:

- (a) Levamisole phosphate;
- (b) Eprinomectin, ivermectin, abamectin, doramectin or moxidectin;
- (c) DMA
- (d) CREMOPHOR EL; and
- (e) WFI

[0043] The inventive formulations may contain other inert ingredients such as antioxidants, preservatives, or pH stabilizers. These compounds are well known in the formulation art. Antioxidant such as an alpha tocopherol, ascorbic acid, ascorbyl palmitate, fumaric acid, malic acid, sodium ascorbate, sodium metabisulfate, n-propyl gallate, BHA (butylated hydroxy anisole), BHT (butylated hydroxy toluene) monothioglycerol and the like, may be added to the present formulation. In certain embodiments, the antioxidants are generally added to the formulation in amounts of from about 0.01 to about 2.0%, based upon total weight of the formulation, with about 0.05 to about 1.0% being especially preferred.

[0044] In some embodiments, preservatives, such as the parabens (methylparaben and/or propylparaben), are suitably used in the formulation in amounts ranging from about 0.01 to about 2.0%, with about 0.05 to about 1.0% being especially preferred. Other preservatives include benzalkonium chloride, benzethonium chloride, benzoic acid, benzyl alcohol, bronopol, butylparaben, cetrimide, chlorhexidine, chlorobutanol, chlorocresol, cresol, ethylparaben, imidurea, methylparaben, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric acetate, phenylmercuric borate, phenylmercuric nitrate, potassium sorbate, sodium benzoate, sodium propionate, sorbic acid, thimerosal, and the like. Preferred ranges for these compounds include from about 0.01 to about 5%.

[0045] Compounds which stabilize the pH of the formulation are also contemplated and may be used in certain embodiments of the inventive formulations. Again, such compounds are well known to a practitioner in the art as well as how to use these compounds. Buffering systems include, for example, systems selected from the group consisting of acetic acid/acetate, malic acid/malate, citric acid/citrate, tataric acid/tartrate, lactic acid/lactate, phosphoric acid/phosphate, glycine/glycinate, tris, glutamic acid/glutamates and sodium carbonate.

Parasites Controlled:

[0046] The injectable levamisole / macrocyclic lactone formulations are particularly effective for efficiently controlling endoparasites, such as flukes, hookworms, and helminths such as cestodes, nematodes, and trematodes. Endoparasites further include helminths such as *Anaplocephala*,

[0047] *Ancylostoma*, *Anecator*, *Ascaris*, *Capillaria*, *Cooperia*, *Dipylidium*, *Dirofilaria*, *Echinococcus*, *Enterobius*, *Fasciola*, *Haemonchus*, *Oesophagostomum*, *Ostertagia*, *Toxocara*, *Strongyloides*, *Toxascaris*, *Trichinella*, *Trichuris*, and *Trichostrongylus*. Or others from the class of helminths, such as from the class of helminths, for example, *Arccylostoma duodenale*, *Ancylostoma ceylanicum*, *Ancylostoma braziliensis*, *Ancylostoma* spp., *Ascaris lubricoides*, *Ascaris* spp., *Brugia malayi*, *Brugia timori*, *Bunostomum* spp., *Chabertia* spp., *Clonorchis* spp., *Cooperia* spp., *Dicrocoelium* spp., *Dictyocaulus filaria*, *Diphyllbothrium latum*, *Dracunculus medinensis*, *Echinococcus granulosus*, *Echinococcus multilocularis*, *Enterobius vermicularis*, *Fasciola* spp., *Haemonchus* spp., *Heterakis* spp., *Hymenolepis nana*, *Hyostrogylus* spp., *Loa Loa*, *Nematodirus* spp., *Oesophagostomum* spp., *Opisthorchis* spp., *Onchocerca volvulus*, *Ostertagia* spp., *Paragonimus* spp., *Schistosoma* spp., *Strongyloides fuelleborni*, *Strongyloides stercoralis*, *Strongyloides* spp., *Taenia saginata*, *Taenia solium*, *Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi*, *Trichinella nelsoni*, *Trichinella pseudospiralis*, *Trichostrongylus* spp., *Trichuris trichuria*, *Wuchereria bancrofti*.

[0048] In one embodiment for use in the 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*, *Haematopinus*, *Solenopotes*, *Trichodectes*, and *Felicola*.

[0049] In another embodiment for use in 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., *Linognathus* 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 use in 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*; myiasis such as *Dermatobia hominis* (known as Berne in Brazil) and *Cochliomyia hominivorax* (greenbottle); sheep myiasis 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.

When an anthelmintic agent is added to the composition of the invention, the composition can also be for use 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.

[0050] The phytoparasitic nematodes include, for example, *Anguina* spp., *Aphelenchoides* spp., *Belonoaimus* spp., *Bursaphelenchus* spp., *Ditylenchus dipsaci*, *Globodera* spp., *Helicotylenchus* spp., *Heterodera* spp., *Longidorus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Radopholus similis*, *Rotylenchus* spp., *Trichodorus* spp., *Tylenchorhynchus* spp., *Tylenchulus* spp., *Tylenchulus semipenetrans*, *Xiphinema* spp.

[0051] In addition, with or without the other pesticidal agents added to the composition, the invention can also be for use 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 Diplopoda, for example *Blaniulus guttulatus*;
- (3) from the order of Chilopoda, for example *Geophilus carpophagus* and *Scutigera* spp.;

- (4) from the order of Symphyla, for example *Scutigera 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 *Blattella 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 *Ceratophyllus* spp.;
- (10) from the order of Anoplura (Phthiraptera), for example, *Damalinia* spp., *Haematopinus* spp., *Linognathus* spp., *Pediculus* spp., *Trichodectes* spp.;
- (11) from the class of Arachnida, for example, *Acarus siro*, *Aceria sheldoni*, *Aculops* spp., *Aculus* spp., *Amblyomma* spp., *Argas* spp., *Boophilus* spp., *Brevipalpus* spp., *Bryobia praetiosa*, *Chorioptes* spp., *Dermanyssus gallinae*, *Eotetranychus* spp., *Epitrimerus pyri*, *Eutetranychus* spp., *Eriophyes* spp., *Hemitarsonemus* spp., *Hyalomma* spp., *Ixodes* spp., *Latrodectus mactans*, *Metatetranychus* spp., *Oligonychus* spp., *Ornithodoros* spp., *Panonychus* spp., *Phyllocoptura oleivora*, *Polyphagotarsonemus latus*, *Psoroptes* spp., *Rhipicephalus* spp., *Rhizoglyphus* spp., *Sarcoptes* spp., *Scorpio maurus*, *Stenotarsonemus* spp., *Tarsonemus* spp., *Tetranychus* spp., *Vasates lycopersici*;
- (12) from the class of Bivalva, for example, *Dreissena* spp.;
- (13) from the order of Coleoptera, for example, *Acanthoscelides obtectus*, *Adoretus* spp., *Agelastica alni*, *Agriotes* spp., *Amphimallon solstitialis*, *Anobium punctatum*, *Anoplophora* spp., *Anthonomus* spp., *Anthrenus* spp., *Apogonia* spp., *Atomaria* spp., *Attagenus* spp., *Bruchidius obtectus*, *Bruchus* spp., *Ceuthorrhynchus* spp., *Cleonus mendicus*, *Conoderus* spp., *Cosmopolites* spp., *Costelytra zealandica*, *Curculio* spp., *Cryptorhynchus lapathi*, *Dermestes* spp., *Diabrotica* spp., *Epilachna* spp., *Faustinus cubae*, *Gibbium psyllioides*, *Heteronychus arator*, *Hylamorphia elegans*, *Hylotrupes bajulus*, *Hypera postica*, *Hypothenemus* spp., *Lachnosterna consanguinea*, *Leptinotarsa decemlineata*, *Lissorhoptrus oryzophilus*, *Lixus* spp., *Lyctus* spp., *Meligethes aeneus*, *Melolontha melolontha*, *Migdolus* spp., *Monochamus* spp., *Naupactus xanthographus*, *Niptus hololeucus*, *Oryctes rhinoceros*, *Oryzaephilus surinamensis*, *Otiorrhynchus sulcatus*, *Oxycetonia jucunda*, *Phaedon cochleariae*, *Phyllophaga* spp., *Popillia japonica*, *Premnotypes* spp., *Psylliodes chrysocephala*, *Ptinus* spp., *Rhizobius ventralis*, *Rhizopertha dominica*, *Sitophilus* spp., *Sphenophorus* spp., *Sternechus* spp., *Symphyletes* spp., *Tenebrio molitor*, *Tribolium* spp., *Trogoderma* spp., *Tychius* spp., *Xylotrechus* spp., *Zabrus* spp.;
- (14) from the order of Diptera, for example, *Aedes* spp., *Anopheles* spp., *Bibio hortulanus*, *Calliphora erythrocephala*, *Ceratitis capitata*, *Chrysomya* spp., *Cochliomyia* spp., *Cordylobia anthropophaga*, *Culex* spp., *Cuterebra* spp., *Dacus oleae*, *Dermatobia hominis*, *Drosophila* spp., *Fannia* spp., *Gastrophilus* spp., *Hylemyia* spp., *Hyppobosca* spp., *Hypoderma* spp., *Liriomyza* spp., *Lucilia* spp., *Musca* spp., *Nezara* spp., *Oestrus* spp., *Oscinella frit*, *Pegomyia hyoscyami*, *Phorbia* spp., *Stomoxys* spp., *Tabanus* spp., *Tannia* spp., *Tipula paludosa*, *Wohlfahrtia* 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*, *Ancylostoma braziliensis*, *Ancylostoma* spp., *Ascaris lubricoides*, *Ascaris* spp., *Brugia malayi*, *Brugia timori*, *Bunostomum* spp., *Chabertia* spp., *Clonorchis* spp., *Cooperia* spp., *Dicrocoelium* spp., *Dictyocaulus filaria*, *Diphyllbothrium latum*, *Dracunculus medinensis*, *Echinococcus granulosus*, *Echinococcus multilocularis*, *Enterobius vermicularis*, *Faciola* spp., *Haemonchus* spp., *Heterakis* spp., *Hymenolepis nana*, *Hyostrogylus* spp., *Loa Loa*, *Nematodirus* spp., *Oesophagostomum* spp., *Opisthorchis* spp., *Onchocerca volvulus*, *Ostertagia* spp., *Paragonimus* spp., *Schistosomen* spp., *Strongyloides fuelleborni*, *Strongyloides stercoralis*, *Strongyloides* spp., *Taenia saginata*, *Taenia solium*, *Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi*, *Trichinella nelsoni*, *Trichinella pseudopsiralis*, *Trichostrongylus* spp., *Trichuris trichuria*, *Wuchereria bancrofti*;
- (17) from the order of Heteroptera, for example, *Anasa tristis*, *Antestiopsis* spp., *Blissus* spp., *Calocoris* spp., *Campylomma livida*, *Cavelerius* spp., *Cimex* spp., *Creontiades dilutus*, *Dasynus piperis*, *Dichelops furcatus*, *Diconocoris hewetti*, *Dysdercus* spp., *Euschistus* spp., *Eurygaster* spp., *Heliopeltis* spp., *Horcias nobilellus*, *Leptocoris* spp., *Leptoglossus phyllopus*, *Lygus* spp., *Macropes excavatus*, *Miridae*, *Nezara* spp., *Oebalus* spp., *Pentomidae*, *Piesma quadrata*, *Piezodorus* spp., *Psallus seriatus*, *Pseudacysta perseae*, *Rhodnius* spp., *Sahlbergella singularis*, *Scotinophora* spp., *Stephanitis nashi*, *Tibraca* spp., *Triatoma* spp.;
- (18) from the order of Homoptera, for example, *Acyrtosiphon* spp., *Aeneolamia* spp., *Agonosceana* spp., *Aleurodes* spp., *Aleurolobus barodensis*, *Aleurothrix* spp., *Amrasca* spp., *Anuraphis cardui*, *Aonidiella* spp., *Aphanostigma piri*, *Aphis* spp., *Arboridia apicalis*, *Aspidiella* spp., *Aspidiotus* spp., *Atanus* spp., *Aulacorthum solani*, *Bemisia* spp., *Brachycaudus helichrysi*, *Brachycolus* spp., *Brevicoryne brassicae*, *Calligypona marginata*, *Carneiocephala fulgida*, *Ceratovacuna lanigera*, *Cercopidae*, *Ceroplastes* spp., *Chaetosiphon fragaefolii*, *Chionaspis tegalensis*, *Chlorita onukii*, *Chromaphis juglandicola*, *Chrysomphalus ficus*, *Cicadulina mbila*, *Coccomytilus halli*, *Coccus* spp., *Cryptomyzus ribis*, *Daibulus* spp., *Dialeurodes* spp., *Diaphorina* spp., *Diaspis* spp., *Doralis* spp., *Drosicha* spp., *Dysaphis*

spp., *Dysmicoccus* spp., *Empoasca* spp., *Eriosoma* spp., *Erythroneura* spp., *Euscelis bilobatus*, *Geococcus coffeae*, *Homalodisca coagulata*, *Hyalopterus arundinis*, *Icerya* spp., *Idiocerus* spp., *Idioscopus* spp., *Laodelphax striatellus*, *Lecanium* spp., *Lepidosaphes* spp., *Lipaphis erysimi*, *Macrosiphum* spp., *Mahanarva fimbriolata*, *Melanaphis sacchari*, *Metcalfiella* spp., *Metopolophium dirhodum*, *Monellia costalis*, *Monelliopsis pecanis*, *Myzus* spp., *Nasonovia ribisnigri*, *Nephotettix* spp., *Nilaparvata lugens*, *Oncometopia* spp., *Orthezia praelonga*, *Parabemisia myricae*, *Paratrioza* spp., *Parlatoria* spp., *Pemphigus* spp., *Peregrinus maidis*, *Phenacoccus* spp., *Phloeomyzus passerinii*, *Phorodon humuli*, *Phylloxera* spp., *Pinnaspis aspidistrae*, *Planococcus* spp., *Protopulvinaria pyriformis*, *Pseudaulacaspis pentagona*, *Pseudococcus* spp., *Psylla* spp., *Pteromalus* spp., *Pyrilla* spp., *Quadraspidotus* spp., *Quesada gigas*, *Rastrococcus* spp., *Rhopalosiphum* spp., *Saissetia* spp., *Scaphoides titanus*, *Schizaphis graminum*, *Selenaspidus articulatus*, *Sogata* spp., *Sogatella furcifera*, *Sogatodes* spp., *Stictocephala festina*, *Tenalaphara malayensis*, *Tinocallis caryaefoliae*, *Tomaspsis* spp., *Toxoptera* spp., *Trialeurodes vaporariorum*, *Trioza* spp., *Typhlocyba* spp., *Unaspis* spp., *Viteus vitifolii*;

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

(20) from the order of Lepidoptera, for example, *Acronicta major*, *Aedia leucomelas*, *Agrotis* spp., *Alabama argillacea*, *Anticarsia* spp., *Barathra brassicae*, *Bucculatrix thurberiella*, *Bupalus piniarius*, *Cacoecia podana*, *Capua reticulana*, *Carpocapsa pomonella*, *Cheimatobia brumata*, *Chilo* spp., *Choristoneura fumiferana*, *Clysia ambiguella*, *Cnaphalocerus* spp., *Earias insulana*, *Ephestia kuehniella*, *Euproctis chrysorrhoea*, *Euxoa* spp., *Feltia* spp., *Galleria mellonella*, *Helicoverpa* spp., *Heliothis* spp., *Hofmannophila pseudospretella*, *Homona magnanima*, *Hyponomeuta padella*, *Laphygma* spp., *Lithocolletis blancardella*, *Lithophane antennata*, *Loxagrotis albicosta*, *Lymantria* spp., *Malacosoma neustria*, *Mamestra brassicae*, *Mocis repanda*, *Mythimna separate*, *Oria* spp., *Oulema oryzae*, *Panolis flammea*, *Pectinophora gossypiella*, *Phyllocnistis citrella*, *Pieris* spp., *Plutella xylostella*, *Prodenia* spp., *Pseudaletia* spp., *Pseudoplusia includens*, *Pyrausta nubilalis*, *Spodoptera* spp., *Thermesia gemmatilis*, *Tinea pellionella*, *Tineola bisselliella*, *Tortrix viridana*, *Trichoplusia* 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, *Baliothrips biformis*, *Enneothrips 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.

EXAMPLES

[0052] 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.

[0053] Example 1 is particularly, though not exclusively, representative of injectable eprinomectin / levamisole formulations according to the present invention. Example 2 provides evidence that ivermectin-containing formulations according to the present invention are stable and safe in animals. Finally, example 3 provides extensive, though not sole evidence of the significant amount of time and effort that was invested in developing the inventive formulations.

Example 1 - Development of stable injectable levamisole / eprinomectin formulations

[0054] Levamisole is water soluble and eprinomectin is nearly water insoluble. Eprinomectin is soluble, for example, in methanol, ethanol, ethyl acetate, and dimethyl acetate (DMA). Applicants are aware of no existing combination injectable formulation comprising eprinomectin and levamisole. The development work fully disclosed and described herein out was carried out to provide a formulation in which the anthelmintics eprinomectin and levamisole (as the phosphate) could be combined in a formulation having physical and chemical properties suitable for injection.

[0055] Stress studies have identified at least two formulations that are stable and suitable in terms of irritancy.

[0056] Constituents of these formulations are provided in Table 1.

Table 1: Formulation of Eprinomectin levamisole Injection

Ingredient	Formulation 1	Formulation 2	Function
	% w/v Composition	% w/v Composition	
Eprinomectin	0.70*	0.70*	Active
Levamisole Phosphate	22.3*	22.3*	Active
BHT	0.1	0.1	Antioxidant

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(continued)

Ingredient	Formulation 1	Formulation 2	Function
	% w/v Composition	% w/v Composition	
Dimethylacetamide	5.0	5.0	Solvent
Methyl Paraben	0.15	0.15	Preservative
Polysorbate 80	5.0	-	Surfactant
Cremophor EL	-	5.0	Surfactant
Water For Injection	q.s. to 100%		Vehicle
*Corrected for potency			

[0057] Stability of both formulations has been demonstrated in real-time storage conditions.

Table 2: Stability of Eprinomectin Levamisole Injection Formulation 1

Batch No.		2-8°C	25°C / 60% RH			
		Control	1 month	2 month	3 month	
Formulation 1.	Description	VLYS	VLYS	VLYS	VLYS	
	Assay of Eprinomectin	100.9	99.6	98.0	98.6	
	Assay of Levamisole Phosphate	98.0	97.9	98.3	99.2	
VLYS: Very Light Yellow Solution LYS: Light Yellow Solution YS: Yellow Solution						

Table 3: Stability of Eprinomectin Levamisole Injection Formulation 2

Batch No.		2-8°C	25°C / 60% RH			
		Control	1 month	2 month	3 month	
Formulation 2.	Description	VLYS	VLYS	VLYS	VLYS	
	Assay of Eprinomectin	101.1	100.0	98.0	99.6	
	Assay of Levamisole Phosphate	98.9	99.3	98.8	100.3	
VLYS: Very Light Yellow Solution LYS: Light Yellow Solution YS: Yellow Solution						

Table 4: Administration of Treatments

[0058] Both formulations were also tested in cattle studies to determine the absorption characteristics and injection site acceptability. Twenty-one Hereford Cross 15 month old female cattle were selected for the study from a larger group held at the study site. On Day -1 the study animals were individually identified with uniquely numbered ear tags.

[0059] The study animals were weighed and ranked according to body weight. The animal with the median body weight (305 kg) was selected as the control animal. The remaining animals were assigned to two groups of ten by restrictive randomization based on body weight. Treatments were administered as per Table 4.

[0060] Animals in Group 1 were treated with Formulation 1 at a dose rate of 1ml/35kg body

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Animal ID	Group 1D	Product/ BN	Weight (kg)	Dose (ml)
2	1	Formulation 1 BN:08	324.0	9.3
3			294.0	8.4
5			334.0	9.5
6			315.0	9.0
9			343.0	9.8
11			297.0	8.5
12			285.0	8.1
13			289.0	8.3
14			302.0	8.6
20			313.0	8.9
1	2	Formulation 2 BN:09	270.0	7.7
4			303.0	8.7
7			288.0	8.2
8			317.0	9.1
10			326.0	9.3
16			331.0	9.5
17			291.0	8.3
18			309.0	8.8
19			296.0	8.5
21			340.0	9.7
15	3	Control	305.0	-----
weight. Animals in Group 2 were treated with Formulation 2 at a dose rate of 1ml/35kg bodyweight. All calculated doses were rounded up to the nearest 0.2ml. The control animal received no treatment.				

[0061] Treatments were administered by subcutaneous injection to the anterior half of the neck, using a new sterile 18 gauge 25mm needle for each injection. The area for injection was clipped and swabbed with 70% isopropyl alcohol prior to injection. The injection sites were inspected and palpated at 24, 48 and 96 hours post treatment and on Days 10, 21 and 35. All observations were recorded.

[0062] The degree of injection site swelling was recorded according to the following scale:

- 0 = no edema
- 1 = very slight edema (barely noticed)
- 2 = slight edema (well defined by definite raising)
- 3 = moderate edema (raised approximately 1mm)
- 4 = (raised more than 1 mm and extending beyond the area of injection)

[0063] The maximum length, width and height of any visible or palpable site reaction was determined using calipers, and all findings were recorded.

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Table 5: Injection Site Reactions Formulation 1

Formulation 1 Batch no:08								
Animal ID	Weight (kg)	Dose (mL)	24 hours	48 hours	96 hours	Day 10	Day 21	Day 35
2	324.0	9.3	4	4	4	4	1	0
3	294.0	8.4	4	2	1	0	0	0
5	334.0	9.5	4	1	1	0	0	0
6	315.0	9.0	4	4	4	4	3	1
9	343.0	9.8	0	0	1	0	0	0
11	297.0	8.5	4	3	1	1	0	0
12	285.0	8.1	4	2	1	1	0	0
13	289.0	8.3	4	4	3	2	0	0
14	302.0	8.6	4	4	1	1	0	0
20	313.0	8.9	4	4	1	0	0	0

Table 6: Injection Site Reactions Formulation 2

Formulation 2 Batch no:09								
Animal ID	Weight (kg)	Dose (mL)	24 hours	48 hours	96 hours	Day 10	Day 21	Day 35
1	270.0	7.7	4	4	3	1	0	0
4	303.0	8.7	4	4	1	0	0	0
7	288.0	8.2	4	4	4	0	0	0
8	317.0	9.1	4	4	4	3	0	0
10	326.0	9.3	4	4	0	0	0	0
16	331.0	9.5	4	1	0	0	0	0
17	291.0	8.3	4	4	4	1	0	0
18	309.0	8.8	4	3	1	0	0	0
19	296.0	8.5	1	1	1	0	0	0
21	340.0	9.7	4	4	1	1	0	0

Blood plasma profile

[0064] On Day -1, (3 Jan 2010) five animals were randomly selected from each of the two treated groups for blood sampling as illustrated in Table 7:

Table 7: Eprinomectin Levamisole Blood Sampling Schedule

Blood Plasma Group 1	Blood Plasma Group 2
3	1
11	10
12	16
14	18
20	21

[0065] These ten animals plus the untreated control were blood sampled via jugular venipuncture on Day -1 prior to

treatment. These same animals were blood sampled at 1, 2, 6, 12, 24, 48 and 96 hours post treatment, and then at Day 10, Day 21 and Day 35 after treatment. Blood samples were collected by caudal venipuncture into green top heparinised Vacutainer's, and spun in a centrifuge for ten minutes at 3000 RPM. Plasma for each animal was decanted into individually labeled plasma vials and frozen within two hours of collection.

[0066] At the conclusion of the animal phase of the study (Day 35) the frozen plasma samples were submitted to an analytical laboratory for Eprinomectin and Levamisole analysis. Samples from 0, 1, 2, 6, 12, 24 and 48 hours were analyzed for Levamisole concentration. Samples from 0, 48, 96 hours and 10, 20 and 35 days were analyzed for Eprinomectin concentration.

[0067] The mean eprinomectin and levamisole pharmacokinetic profiles in cattle plasma after treatment with the two formulations are presented in the figures below. Results demonstrate that both formulations resulted in almost identical plasma profiles for both levamisole and eprinomectin. FIGs. 2 and 3 summarize the data.

Table 8: Excipients used in the Eprinomectin / Levamisole injectable formulations

Ingredient	CAS No.	Formulation 1	Formulation 2	Function
		% w/v Composition	% w/v Composition	
BHT	128-37-0	0.1	0.1	Antioxidant
Dimethylacetamide	127-19-5	5.0	5.0	Solvent
Methyl Paraben	99-76-3	0.15	0.15	Preservative
Polysorbate 80	9005-65-6	5.0	-	Surfactant
Cremophor EL	61796-12-6	-	5.0	Surfactant
Water For Injection	7789-20-0	q.s. to 100%	q.s. to 100%	Vehicle

Formulation Development

[0068] A number of solvents were evaluated including Glycerol Formal, 2-Pyrol, Propylene Glycol, Miglyol 840 and DMA from 2 - 20% (see Example 2). The preferred batches contained a surfactant such as Cremophor EL, Polysorbate 80 and modified Lecithin in the range 2 - 5%. Vehicles trialed included Water, Glycerol Formal and modified vegetable oil. The combination of 5% DMA and 5% CREMAPHOR EL or POLYSORBATE 80 was demonstrated to be quite useful for the levamisole / ivermectin formulations according to the present invention.

[0069] Formulations comprising eprinomectin instead of ivermectin were evaluated using CREMAPHOR EL and several other surfactants. The stability data provided above indicated that eprinomectin performed at least as well as ivermectin. A series of batches were prepared comparing the addition of BHT as an antioxidant to batches without antioxidant. Stress studies showed a significant improvement in stability of the actives when BHT was added to the formulation. Finally, Methyl Paraben was selected for a preservative as the marketed product may be provided as a multi-dose pack.

Physicochemical Properties

[0070] Assessment of developmental formulation batches was generally performed on the basis of appearance, including color, of the solution and the assay content of both of the active ingredients. The description of appearance for the proposed formulation is that of a clear light yellow to yellow solution. The physicochemical properties investigated during the stress study monitored the assay of the two actives. Density and pH values were also monitored. Density was in the range 1.05 - 1.10 and pH in the range 3.0 - 5.0. Visual assessment shows the product to be flowable with a water-like appearance.

[0071] The bulk of the WFI was added to the main manufacturing vessel and methyl paraben, the water soluble preservative, was added first to maintain microbial resistance. Levamisole Phosphate was added next as it water soluble and at a relatively high concentration. In a separate vessel, eprinomectin and BHT were dissolved in DMA and the surfactant added next. This combination was then added to the bulk aqueous phase with mixing. Eprinomectin is not water soluble, so this carried the eprinomectin into and maintained it in solution. Finally the batch was made to volume with WFI and mixed. All mixing was accomplished using simple mechanical agitation. Heat was only required to dissolve the Methyl Paraben, and once all soluble components were dissolved, the WFI was cooled before further processing occurs.

Example 2

[0072] Preliminary work focused on stability of ivermectin / levamisole-containing formulations, though subsequent studies (summarized in Example 1) shifted to the related macrocyclic lactone eprinomectin. As mentioned, eprinomectin offers a significant advantage in terms of short withdrawal time due its relatively low milk to plasma partition ratio. Table 9 summarizes batches 2-13.

Table 9. Formulation summary: IVM is ivermectin and LEV is levamisole phosphate

Batch No	Batch size	Formulation		Source and batch number of APIs		Purpose, method of manufacture and comments																											
			% w/v	IVM	LEV																												
02 (REF)	100m L	Ivermectin Levamisole PO ₄ Glycerol Formal Benzyl Alcohol Polysorbate 80 WFI	0.70 22.3 20.0 1.0 5.0 to 100	Hisun 080340	Guilin 020903	Purpose: Preliminary screening trial to determine a suitable base for Ivermectin and Levamisole Phosphate <table><tr><td></td><td colspan="2">55°C 5 days</td></tr><tr><td></td><td colspan="2">Assay compared to initial</td></tr><tr><td></td><td>IVM</td><td>LEV</td></tr><tr><td>02</td><td>78.0%</td><td>94.4%</td></tr><tr><td>03</td><td>101.2%</td><td>94.1%</td></tr><tr><td>04</td><td>96.5%</td><td>86.3%</td></tr><tr><td>06</td><td>99.6%</td><td>99.7%</td></tr><tr><td>07</td><td>98.0%</td><td>94.5%</td></tr><tr><td>08</td><td>125.3%</td><td>96.1%</td></tr></table>		55°C 5 days			Assay compared to initial			IVM	LEV	02	78.0%	94.4%	03	101.2%	94.1%	04	96.5%	86.3%	06	99.6%	99.7%	07	98.0%	94.5%	08	125.3%	96.1%
	55°C 5 days																																
	Assay compared to initial																																
	IVM	LEV																															
02	78.0%	94.4%																															
03	101.2%	94.1%																															
04	96.5%	86.3%																															
06	99.6%	99.7%																															
07	98.0%	94.5%																															
08	125.3%	96.1%																															
03	100m L	Ivermectin Levamisole PO ₄ DMA Cremophor EL WFI	0.70 22.3 2.0 2.0 to 100	Hisun 080340	Guilin 020903																												
04 (REF)	100m L	Ivermectin Levamisole PO ₄ Propylene Glycol Benzyl Alcohol WFI	0.70 22.3 20.0 1.5 to 100	Hisun 080340	Guilin 020903																												
06 (REF)	100m L	Ivermectin Levamisole PO ₄ Miglyol 840 Phospholipon Glycerol Formal WFI	0.70 22.3 20.0 1.8 2.2 to 100	Hisun 080340	Guilin 090601																												
07 (Repe at 03)	100m L	Ivermectin Levamisole PO ₄ DMA Cremophor EL WFI	0.70 22.3 2.0 2.0 to 100	Hisun 070403	Guilin 090601																												
						Result: Batch 03 (07) batch 06 most suitable formulations. Batch 03, a solution preferred over batch 06 an emulsion.																											

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(continued)

Batch No	Batch size	Formulation		Source and batch number of APIs		Purpose, method of manufacture and comments
			% w/v	IVM	LEV	
08 (REF)	100m L	Ivermectin Levamisole PO ₄ WFI Montanide	0.70 22.3 30.0 to 100	Hisun 070403	Guilin 090601	
09	100m L	Ivermectin Levamisole PO ₄ DMA Cremophor EL WFI	0.70 22.3 2.0 2.0 to 100	Hisun 080340	Guilin 090601	55°C 5 days Percent initial IVM LEV 09 83.6% 97.9% 10 58.4% 97.6% Result: Crystallization observed.
10	100m L	Ivermectin Levamisole PO ₄ DMA Cremophor EL WFI	0.70 22.3 2.0 2.0 to 100	Hisun 070403	Guilin 090601	
11	100m L	Ivermectin Levamisole PO ₄ DMA Cremophor EL WFI	0.70 22.3 5.0 5.0 to 100	Hisun 080340	Guilin 090601	</

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(continued)

Batch No	Batch size	Formulation		Source and batch number of APIs		Purpose, method of manufacture and comments													
			% w/v	IVM	LEV														
13	300mL	Ivermectin	0.70	Hisun	Guilin	<u>Purpose:</u> Repeat batch 12 with stress testing at 40°C and 55°C. <table><tr><td rowspan="3"></td><td colspan="2">5 days</td></tr><tr><td colspan="2">Percent initial</td></tr><tr><td>IVM</td><td>LEV</td></tr><tr><td>40° C</td><td>98.3%</td><td>99.6%</td></tr><tr><td>55° C</td><td>85.2%</td><td>97.3%</td></tr></table> <u>Result:</u> Stability improved.		5 days		Percent initial		IVM	LEV	40° C	98.3%	99.6%	55° C	85.2%	97.3%
	5 days																		
	Percent initial																		
	IVM	LEV																	
40° C	98.3%	99.6%																	
55° C	85.2%	97.3%																	
		Levamisole	22.3	070403	090601														
		PO ₄																	
		DMA	5.0																
		Cremophor EL	5.0																
		WFI	to 100																

Example 3 (Reference) -Alternative Formulations:

[0073] The following example illustrates the significant time and effort that was invested in developing the stable, injectable formulations according to the present invention. A person of ordinary skill will immediately appreciate the rigorous experimentation that was, and is very often required in order to arrive at the proper blend of actives and excipients to produce an effective, pharmaceutically acceptable formulation. Injectable formulations tend to be particularly challenging to develop, as factors such as excessive viscosity and injection site irritation can preclude the use of otherwise effective excipients (e.g. excipients which provide good stability for the combination of active pharmaceutical ingredients).

[0074] Pre-formulation development efforts commenced using the hydrochloride salt of Levamisole. Preliminary solubility data strongly contra-indicated the use of NMP and Glycerin as a single solvent system to solubilize Levamisole and the use of vegetable oil to solubilize the Ivermectin. A large number of surfactants and stabilizers including CAPMUL MCM, TWEEN 80, SPAN 20, CREMAPHOR RH 40, PVP K-30 and SODIUM CMC were trialed. Also trialed were several buffers, including phosphate and acetate salts before citrate was finally selected based upon the desirability / stability of the resulting formulations.

[0075] Early indications were that CAPMUL MCM (medium chain mono and diglycerides) aqueous based formulations might provide a clinically acceptable base for injection. Batches INJ0003-01 to INJ0003-11 were therefore formulated with Levamisole using either the hydrochloride or the phosphate salts thereof (formulation compositions are summarized in Table 10). CAPMUL MCM and TWEEN 80 with a citrate buffer was the basis of this aqueous formulation. Although considered stable at the planned storage of 2 - 8°C, unacceptable site reactions occurred during clinical investigation.

Table 10: Formulation summary - batches INJ003-01 through INJ003-11

Batch No	Batch size	Formulation		Purpose, method of manufacture and comments					
			% w/v						
INJ003-01	400mL	Ivermectin	0.48	<u>Purpose:</u> Preliminary screening trial to determine a suitable base for Ivermectin and Levamisole HCl <table><tr><td rowspan="2"></td><td>25°C</td></tr><tr><td>Assay compared to initial</td></tr></table>				25°C	Assay compared to initial
			25°C						
			Assay compared to initial						
		Levamisole HCl	20.0						
		Capmul MCM	25.0						
		Tween 80	15.0						
		Tri Sodium Citrate	1.5						
Conc. HCl	0.25 mL								

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(continued)

Batch No	Batch size	Formulation		Purpose, method of manufacture and comments				
			% w/v					
		WFI	to 100			Ivermectin	Levamisole HCl	
					1 M	99%	98%	
					2 M	98%	99%	
					4 M	97%	97%	
					6 M	95%	98%	
					9 M	93%	97%	
					12 M	92%	97%	
						40°C		
						Assay compared to initial		
						Ivermectin	Levamisole HCl	
					1 M	95%	95%	
					2 M	91%	96%	
					4 M	86%	92%	
					6 M	78%	90%	
						55°C		
						Assay compared to initial		
						Ivermectin	Levamisole HCl	
						2 wk	84%	
4 wk	70%	93%						

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(continued)

Batch No	Batch size	Formulation		Purpose, method of manufacture and comments
			% w/v	
INJ003-02	400mL	Ivermectin	0.48	25°C Assay compared to initial Ivermectin Levamisole HCl 1 M 97% 97% 2 M 95% 96% 4 M 93% 97% 6 M 91% 95% 9 M 90% 96% 12 M 87% 96%
		Levamisole HCl	20.0	
		Capmul MCM	10.0	
		Tween 80	10.0	
		Tri Sodium Citrate	2.0	
		Cone. HCl	0.25 mL	
		WFI	to 100	
INJ003-03	1000mL	Ivermectin	0.48	40°C Assay compared to initial Ivermectin Levamisole HCl 1 M 90% 94% 2 M 83% 91% 4 M 79% 91% 6 M 75% 88%
		Levamisole HCl	16.11	
		Capmul MCM	25.0	
		Tween 80	15.0	
		Tri Sodium Citrate	1.5	
		Conc. HCl	0.25 mL	

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(continued)

Batch No	Batch size	Formulation		Purpose, method of manufacture and comments					
			% w/v						
		WFI	to 100		1 M	98%	99%		
					2 M	94%	96%		
					4 M	94%	99%		
INJ003-04	500 mL	Ivermectin	0.48	<u>Purpose:</u> Same formula as batch INJ003-01		25°C			
		Levamisole HCl	20.0			Assay compared to initial			
		Capmul MCM	25.0						
		Tween 80	15.0			Ivermectin	Levamisole HCl		
		Tri Sodium Citrate	1.5						
		Conc. HCl	0.25 mL			1 M	97%		100%
		WFI	to 100			2 M	97%		99%
						4 M	96%		98%
INJ003-05	500 mL	Ivermectin	0.48	<u>Purpose:</u> Same formula as batch INJ003-01		25°C			
		Levamisole HCl	20.0			Assay compared to initial			
		Capmul MCM	25.0						
		Tween 80	15.0			Ivermectin	Levamisole HCl		
		Tri Sodium Citrate	1.5						
		Conc. HCl	0.25 mL			1 M	89%		79%
		WFI	to 100			2 M	92%		81%
						4 M	87%		77%
INJ003-06	500 mL	Ivermectin	0.48	<u>Purpose:</u> Same formula as batch INJ003-01 - with parabens		25°C			
		Levamisole HCl	20.0			Assay compared to initial			
		Capmul MCM	25.0						
		Tween 80	15.0			Ivermectin	Levamisole HCl		
		Tri Sodium Citrate	1.5						
		Methylparaben	0.18			1 M	99%		99%
		Propylparaben	0.20						
		Conc. HCl	0.25 mL						
WFI	to 100								
					2 M	100%	98%		
					4 M	96%	99%		

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(continued)

Batch No	Batch size	Formulation		Purpose, method of manufacture and comments																		
			% w/v																			
INJ003-07	1500 mL	Ivermectin Levamisole PO ₄ Capmul MCM Tween 80 BHT Methylparaben Propylparaben Tri Sodium Citrate Propyl gallate Conc. HCl WFI	0.57 20.0 25.0 15.0 0.02 0.18 0.20 1.5 0.02 0.25 mL to 100	<u>Purpose:</u> Batch with Levamisole PO ₄ & BHT Results: Sample kept at 55°C were separated into two layers in three weeks																		
INJ003-08	1500 mL	Ivermectin Levamisole PO ₄ Capmul MCM Tween 80 Methylparaben Propylparaben Tri Sodium Citrate Propyl gallate Conc. HCl WFI	0.57 20.0 25.0 15.0 0.18 0.20 1.5 0.02 0.25 mL to 100	<u>Purpose:</u> Batch with Levamisole PO ₄ & nil BHT Results: Sample kept at 55°C were separated into two layers in three weeks																		
INJ003-09	1500 mL	Ivermectin Levamisole PO ₄ Capmul MCM Tween 80 BHT Methylparaben Propylparaben Tri Sodium Citrate Propyl gallate Conc. HCl WFI	0.57 20.0 25.0 15.0 0.03 0.18 0.20 1.5 0.02 0.25 mL to 100	<u>Purpose:</u> Batch with Levamisole PO ₄ & BHT with pH 4.5 <table><tr><td></td><td colspan="2">2-8°C</td></tr><tr><td></td><td colspan="2">Assay compared to initial</td></tr><tr><td></td><td>Ivermectin</td><td>Levamisole PO₄</td></tr><tr><td>1 M</td><td>103</td><td>104</td></tr><tr><td>2 M</td><td>105</td><td>100</td></tr><tr><td>3 M</td><td>100</td><td>99</td></tr></table>		2-8°C			Assay compared to initial			Ivermectin	Levamisole PO ₄	1 M	103	104	2 M	105	100	3 M	100	99
	2-8°C																					
	Assay compared to initial																					
	Ivermectin	Levamisole PO ₄																				
1 M	103	104																				
2 M	105	100																				
3 M	100	99																				
		WFI	to 100	<table><tr><td>6 M</td><td>103</td><td>101</td></tr><tr><td>9 M</td><td>101</td><td>99</td></tr></table>	6 M	103	101	9 M	101	99												
6 M	103	101																				
9 M	101	99																				

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(continued)

Batch No	Batch size	Formulation		Purpose, method of manufacture and comments										
			% w/v											
						25°C								
						Assay compared to initial								
						Ivermectin	Levamisole PO ₄							
					1 M	103	103							
					2 M	103	99							
					3 M	100	95							
					6 M	101	94							
					9 M	93	90							
INJ003 -10	1500 mL	Ivermectin	0.57	<u>Purpose:</u> Batch with Levamisole PO ₄ & BHT with pH 4.0										
		Levamisole PO ₄	20.0											
		Capmul MCM	25.0											
		Tween 80	15.0											
		BHT	0.03											
		Methylparaben	0.18											
		Propylparaben	0.20											
		Tri Sodium Citrate	1.5											
		Propyl gallate	0.02											
		Conc. HCl	0.25 mL											
		WFI	to 100											

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(continued)

Batch No	Batch size	Formulation		Purpose, method of manufacture and comments																																												
			% w/v																																													
INJ003-11	1500 mL	Ivermectin	0.57	<u>Purpose:</u> Batch with Levamisole PO₄ & BHT with pH 5.0 <table><tr><td rowspan="3"></td><td colspan="2">2-8°C</td></tr><tr><td colspan="2">Assay compared to initial</td></tr><tr><td>Ivermectin</td><td>Levamisole PO₄</td></tr><tr><td>1 M</td><td>101</td><td>104</td></tr><tr><td>2 M</td><td>102</td><td>102</td></tr><tr><td>3 M</td><td>101</td><td>10</td></tr><tr><td>6 M</td><td>102</td><td>100</td></tr><tr><td>9 M</td><td>98</td><td>99</td></tr></table> <table><tr><td rowspan="3"></td><td colspan="2">25°C</td></tr><tr><td colspan="2">Assay compared to initial</td></tr><tr><td>Ivermectin</td><td>Levamisole PO₄</td></tr><tr><td>1 M</td><td>102</td><td>102</td></tr><tr><td>2 M</td><td>102</td><td>101</td></tr><tr><td>3 M</td><td>95</td><td>96</td></tr><tr><td>6 M</td><td>98</td><td>94</td></tr><tr><td>9 M</td><td>97</td><td>86</td></tr></table>		2-8°C		Assay compared to initial		Ivermectin	Levamisole PO ₄	1 M	101	104	2 M	102	102	3 M	101	10	6 M	102	100	9 M	98	99		25°C		Assay compared to initial		Ivermectin	Levamisole PO ₄	1 M	102	102	2 M	102	101	3 M	95	96	6 M	98	94	9 M	97	86
			2-8°C																																													
			Assay compared to initial																																													
			Ivermectin		Levamisole PO ₄																																											
		1 M	101		104																																											
		2 M	102		102																																											
		3 M	101		10																																											
		6 M	102		100																																											
		9 M	98		99																																											
			25°C																																													
			Assay compared to initial																																													
			Ivermectin		Levamisole PO ₄																																											
		1 M	102		102																																											
		2 M	102		101																																											
3 M	95	96																																														
6 M	98	94																																														
9 M	97	86																																														
Levamisole PO ₄	20.0																																															
Capmul MCM	25.0																																															
Tween 80	15.0																																															
BHT	0.03																																															
Methylparaben	0.18																																															
Propylparaben	0.20																																															
Tri Sodium Citrate	1.5																																															
Propyl gallate	0.02																																															
Conc. HCl	0.25 mL																																															
WFI	to 100																																															

INJ0003/11 Method of Preparation

[0076]

1. Ivermectin was dissolved in CAPMUL MCM under stirring.
2. To this Tween 80 was added under stirring.
3. BHT was added to step 1 under stirring.
4. Methyl paraben and Propyl paraben dissolved in 600ml of water under heating.
5. Levamisole phosphate was added to step 3 under stirring at room temperature.
6. Propyl gallate was added to step 4 under stirring.
7. Trisodium citrate and 50% HCl solution were added to step 5 under stirring.
8. Step 2 was added to step 6 under stirring
9. Adjusted the pH to around 5.00 using 20% HCl solution, if necessary.
10. The resulting solution was made up to volume with water.

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Table 11: Site reaction test for ivermectin / levamisole injection for cattle - INJ 0003/11

Species:	Bovine
Number:	2 animals
Type:	mixed
Age:	~6 months
Sex:	either
Weight:	Greater than 125kg
Source:	Commercial farm
Health status:	Normal animals in good body condition.
Special requirements:	No anthelmintic treatment in the past 4 weeks
Medication history:	Herd-based treatments as part of normal animal husbandry practice.
Housing and management:	The study animals were maintained as a single mob and rotationally grazed on pasture with continuous access to water throughout the experimental period.

[0077] *Results:* Two calves were treated. No sting in either animal was noted upon injection. A site reaction occurred in both animals within 1 hour of injection, lasting for the entire 28 days of the trial. The reaction was a slightly raised area at the point of injection getting as large as 150 x 170mm and 110 x 140mm. Such reactions would be unacceptable in a commercial farming situation.

Example 4 - Formulations including other macrocyclic lactones

[0078] Tests were conducted to determine whether the invention would be able to be used as a vehicle for delivering a wider range of macrocyclic lactone active ingredients. The test formulations were prepared using POLYSORBATE 80 as the surfactant of choice according to Table 12.

Table 12. Formulation ingredients

Ingredient	% w/v Composition	Function
Macrocyclic lactone active	0.70*	Active
Levamisole Phosphate	22.3*	Active
BHT	0.1	Antioxidant
Dimethylacetamide	5.0	Solvent
Methyl Paraben	0.15	Preservative
Polysorbate 80	5.0	Surfactant
Water For Injection	q.s. to 100%	Vehicle
*Corrected for potency		

[0079] The results demonstrated that abamectin, moxidectin and doramectin are suitable macrocyclic lactones for formulations according to the instant invention. When compared to the eprinomectin/levamisole formulation, the general physical parameters are very similar.

Table 13a: Formulations of various macrocyclic lactones levamisole

	Eprinomectin/Levamisole Inj.			Abamectin/Levamisole Inj. (REF)		
B.No.	SOW 21-08			SOW 21-23		
Condition	2-8°C	25°C	40°C	2-8°C	25°C	40°C
Storage Period	5 Days	5 Days	5 Days	5 Days	5 Days	5 Days
Description	*	*	*	*	*	*

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(continued)

	Eprinomectin/Levamisole Inj.			Abamectin/Levamisole Inj. (REF)		
B.No.	SOW 21-08			SOW 21-23		
Wt/mL	1.0843	1.0843	1.0843	1.0831	1.0831	1.0831
Viscosity	3.5 cP	3.5 cP	3.5 cP	3.3 cP	3.3 cP	3.3 cP
pH	4.22	4.21	4.06	4.17	4.16	4.05

Table 13b (REF): Formulations of various macrocyclic lactones levamisole

	Moxidectin/Levamisole Inj.			Doramectin/Levamisole Inj.		
	Inj.			Inj.		
B.No.	SOW 21-24			SOW 21-25		
Condition	2-8°C	25°C	40°C	2-8°C	25°C	40°C
Storage Period	5 Days	5 Days	5 Days	5 Days	5 Days	5 Days
Description	*	*	*	*	*	*
Wt/mL	1.0825	1.0825	1.0825	1.0837	1.0837	1.0837
Viscosity	4.2 cP	4.2 cP	4.2 cP	3.3 cP	3.3 cP	3.3 cP
pH	4.15	4.14	4.02	4.13	4.12	4.01
* Clear very light yellow solution						

Example 5 - Other Formulations

[0080] Formulations including novel avermectins would be prepared in accordance with Table 12, and could include the compounds and amounts recited in Table 14. The formulations could also include any one of the avermectins or avermectin monosaccharides, modified in the 4' or 4" position, described in US 7,704,961, US 7,521,429, US 2006-0105970 A1, US 7,678,740, US 7,632,820, US 7,678,773, US 7,605,134, and US 6,933,260 (each to Merial).

Table 14. Amounts and identities of novel avermectin compounds

Avermectin	% w/v Composition
4"-diallylamino-sulfonyloxy-avermectin B ₁	0.5 - 0.9
4"-(2-Hydroxyethylamino-sulfonyloxy)-avermectin B ₁	0.5 - 0.9
4"-dipropargylamino-sulfonyloxy-avermectin B ₁	0.5 - 0.9
4"-bis(cyanomethyl)amino-sulfonyloxy-avermectin B ₁	0.5 - 0.9
4"-Dicyanomethylamino-sulfonyloxy-avermectin B ₁	0.5 - 0.9
4"-pyrrolidinamino-sulfonyloxy-avermectin B ₁	0.5 - 0.9
4"-benzoylamino-sulfonyloxy-avermectin B ₁	0.5 - 0.9
4"-epi-sulfamoyloxy-avermectin B ₁	0.5 - 0.9
4"-methylamino-sulfonyloxy-avermectin B ₁	0.5 - 0.9
4"-ethylamino-sulfonyloxy-avermectin B ₁	0.5 - 0.9
4"-sulfamoyloxy-avermectin B ₁	0.5 - 0.9
4"-allylamino-sulfonyloxy-avermectin B ₁	0.5 - 0.9
Any dimeric avermectin Any hetero-dimeric avermectin/milbemycin	0.5 - 0.9

(continued)

Avermectin	% w/v Composition
Any 4" substituted avermectin	0.5 - 0.9
Any 4'-4" dually substituted avermectin	0.5 - 0.9
Any avermectin monosaccharide	0.5 - 0.9

Example 6 - Determination of the chemical residues and depletion profiles for eprinomectin and levamisole following subcutaneous injection of cattle.

[0081] These studies were conducted to determine the residue levels and depletion profile of eprinomectin and levamisole in the tissue of cattle, following the subcutaneous injection of an injectable formulation according to the instant invention, containing 7 g/L eprinomectin and 223 g/L levamisole phosphate. Thirty Short Horn cross cattle were sourced from a commercial farm. On the day of treatment (Day 0) all cattle were weighed, and then, based on live weight, 24 animals were allocated to treatment and tissue collection groups. The treatment groups and sampling times are presented in Table 15.

Table 15. Treatment groups, active ingredients and dose rates

Treatment group	Active ingredient concentration	Dose rate	Number of cattle treated (tissue collection times)
1. Untreated	-	-	2 cattle (2 on Day 21)
2. Test Item	7 g/L eprinomectin + 223 g/L levamisole phosphate	1.25 mL/35 kg	20 cattle (5 on each of Days 21, 28, 35 and 42)
3. Test Item contingency	7 g/L eprinomectin + 223 g/L levamisole phosphate	1.25 mL/35 kg	2 cattle

[0082] *Residues.* The untreated cattle were removed from the site of treatment before application of the test item. A single injection was made on Day 0 using pre-calibrated plastic graduated disposable syringes and 18 G x 1" hypodermic needles. The calculated dose was administered subcutaneously into the neck (20 cm above and on an angle of approximately 45 degrees from the shoulder). The following biological samples were collected on Days 21, 28, 35 and 42, and were submitted to the analytical laboratory for residue analysis: injection site, muscle (*latissimus dorsi*), kidney, liver and peri-renal fat. Both primary and reserve samples were stored frozen until shipment of the "primary samples" to the lab. "Reserve" samples remained in frozen storage. No detectable concentrations of eprinomectin nor levamisole were found in any tissues from untreated cattle indicating no contamination at the field or analytical phases. Statistical analyses and withholding periods were calculated. No adverse effects were noted in any of the treated cattle for the duration of the study.

Table 16. Study design

Parameter	Description
Number	24
Species	Bovine
Breed	Short Horn cross (Hereford x Friesian dams, Short Horn sires)
Sex	Females and castrated males
Age	Approximately 8 Months
Body weight Range	200-246 kg
Condition	Excellent condition, healthy

[0083] The field study was conducted according to the details outlined in Table 16. In this GLP tissue residue study, twenty (20) treated cattle and two (2) untreated control cattle were sacrificed for tissue collection following the subcutaneous injection of a developmental parasiticide. Two (2) further "contingency" animals were treated and managed with

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the treated mob. All animals were chosen from a mob of thirty (30) cattle. Upon arrival at the research facility, all cattle were already individually identified with an ear tag with a unique number in their left ear so it was unnecessary to remove that tag and/or replace it with another. On the day of treatment (Day 0) all cattle were weighed and ranked from highest to lowest based on live weight. In order to keep the live weight range as narrow as possible, the heaviest four and lightest two animals from the 30 potential study animals were removed from the study. Labelled discs (20 x treated, 2 x contingency and 2 x untreated) were drawn randomly from a box and assigned to each animal as per ranking, in order of drawing. This meant that the cattle were randomly allocated to treatment groups (treated or untreated). Slaughter days were randomly allocated to treated cattle in blocks of four. Average live weights for the treatment group by collection day are shown in Table 17.

Table 17. Average live weight of each tissue collection day for treated and untreated cattle

Treatment Group		Collection day			
	0	21	28	35	42
Untreated		223			
Treated		231	228	227	227
Contingency	215				

[0084] Cattle that were treated received a white uniquely numbered tag in their right ear while the untreated control cattle received a red uniquely numbered tag in their right ear. Contingency animals received a blue numbered tag in their right ear. Treatment details are presented in Table 18.

Table 18. Active ingredient, dose rates and cattle numbers in treatment groups in accordance with ACVAM guidelines for maximum allowable residues in animal meat, edible offal and fat tissue

Treatment group	Active ingredient concentration	Dose rate		Number of cattle
1. Untreated	-		-	2
2. Test Item	7 g/L eprinomectin and 223 g/L levamisole phosphate	1.25mL/35 kg		20
3. Contingency	7 g/L eprinomectin and 223 g/L levamisole phosphate	1.25mL/35 kg		2

Table 19. Presents the maximum residue limits for eprinomectin (mg/kg), and levamisole (mg/kg) limit (PQL) for eprinomectin (mg/kg) and levamisole (mg/kg) in animal meat, edible offal and fat tissue

Eprinomectin (mg/kg)		Levamisole (mg/kg)
Fat	0.25	0.01
Liver	1.5	0.1
Kidney	0.3	0.01
Muscle	0.05	0.01

Table 20. Limit of quantitation (LOQ), limit of detection (LOD) and practical quantitation limit (PQL) for eprinomectin (mg/kg) and levamisole (mg/kg) in animal meat, edible offal and fat tissue

Eprinomectin (mg/kg)				Levamisole (mg/kg)		
LOD LOQ PQL				LOD LOQ PQL		
Fat tissue	0.001	0.005	0.01	0.001	0.005	0.01
Edible offal & meat	0.001	0.005	0.01	0.002	0.006	0.01

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Table 21. Eprinomectin B1a and levamisole concentrations in **liver** tissue

5	Ear Tag	Interval	Product	Eprinomectin B1a (mg/kg)		Levamisole (mg/kg)	
				(Corrected)	(Uncorrected)	(Corrected)	(Uncorrected)
	1	21	UTC	< LOD	< LOD	< LOD	< LOD
	2	21	UTC	< LOD	< LOD	< LOD	< LOD
	3	21	Eprinomectin/Levamisole	0.21	0.22	0.011	0.01
10	4	21	Eprinomectin/Levamisole	0.15	0.16	< LOQ	< LOQ
	5	21	Eprinomectin/Levamisole	0.024	0.024	< LOQ	< LOQ
	6	21	Eprinomectin/Levamisole	0.023	0.023	< LOQ	< LOQ
	7	21	Eprinomectin/Levamisole	0.022	0.023	< LOQ	< LOQ
15	8	28	Eprinomectin/Levamisole	0.013, 0.013	0.013, 0.014	< LOQ, < LOQ	< LOQ, < LOQ
	9	28	Eprinomectin/Levamisole	0.04	0.04	< LOQ	< LOQ
	10	28	Eprinomectin/Levamisole	0.048	0.049	< LOQ	< LOQ
	11	28	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
20	12	28	Eprinomectin/Levamisole	0.099	0.009	< LOD	< LOQ
	13	35	Eprinomectin/Levamisole	< LOQ	< LOQ	< LOQ	< LOQ
	14	35	Eprinomectin/Levamisole	0.019	0.019	< LOD	< LOD
	15	35	Eprinomectin/Levamisole	0.012	0.013	< LOD	< LOD
	16	35	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
	17	35	Eprinomectin/Levamisole	0.005	0.005	< LOD	< LOD
25	18	42	Eprinomectin/Levamisole	< LOD, < LOD	< LOD, < LOD	< LOQ, < LOQ	< LOQ, < LOQ
	19	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOQ	< LOQ
	20	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOQ	< LOQ
30	21	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOQ	< LOQ
	22	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD

Table 22. Eprinomectin B1a and levamisole concentrations in **kidney** tissue

35	Ear Tag	Interval	Product	Eprinomectin B1a (mg/kg)		Levamisole (mg/kg)	
				(Corrected)	(Uncorrected)	(Corrected)	(Uncorrected)
	1	21	UTC	< LOD	< LOD	< LOD	< LOD
	2	21	UTC	< LOD, < LOD	< LOD, < LOD	< LOD, < LOD	< LOD, LOD
	3	21	Eprinomectin/Levamisole	0.044	0.045	< LOD	< LOD
40	4	21	Eprinomectin/Levamisole	0.02	0.02	< LOQ	< LOD
	5	21	Eprinomectin/Levamisole	0.006	0.006	< LOD	< LOD
	6	21	Eprinomectin/Levamisole	< LOQ	< LOQ	< LOD	< LOD
	7	21	Eprinomectin/Levamisole	< LOQ, < LOQ	< LOQ, LOQ	< LOD, < LOD	< LOD, < LOD
45	8	28	Eprinomectin/Levamisole	< LOQ	< LOQ	< LOD	< LOD
	9	28	Eprinomectin/Levamisole	0.005	0.006	< LOD	< LOD
	10	28	Eprinomectin/Levamisole	0.006	0.007	< LOD	< LOD
	11	28	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
50	12	28	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
	13	35	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
	14	35	Eprinomectin/Levamisole	< LOQ	< LOQ	< LOD	< LOD
	15	35	Eprinomectin/Levamisole	< LOQ	< LOQ	< LOD	< LOD
	16	35	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
55	17	35	Eprinomectin/Levamisole	< LOD, < LOD	< LOS, < LOD	< LOD, < LOD	< LOD, < LOD
	18	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
	19	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD

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(continued)

Ear Tag	Interval	Product	Eprinomectin B1a (mg/kg)		Levamisole (mg/kg)	
			(Corrected)	(Uncorrected)	(Corrected)	(Uncorrected)
20	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
21	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
22	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD

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Table 23. Eprinomectin B1a and levamisole concentrations in injection site tissue

Ear Tag	Interval	Product	Eprinomectin B1a (mg/kg)		Levamisole (mg/kg)	
			(Corrected)	(Uncorrected)	(Corrected)	(Uncorrected)
1	21	UTC	< LOD	< LOD	< LOD	< LOD
2	21	UTC	< LOD	< LOD	< LOD	< LOD
3	21	Eprinomectin/Levamisole	0.12	0.12	< LOQ	< LOQ
4	21	Eprinomectin/Levamisole	0.015	0.015	< LOD	< LOD
5	21	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
6	21	Eprinomectin/Levamisole	< LOQ	< LCQ	< LOD	< LOD
7	21	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
8	28	Eprinomectin/Levamisole	< LOD, < LOQ	< LOD, < LOQ	< LOD, < LOD	< LOD, < LOD
9	28	Eprinomectin/Levamisole	0.11	0.11	< LOD	< LOD
10	28	Eprinomectin/Levamisole	< LOQ	< LOQ	< LOD	< LOD
11	28	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
12	28	Eprinomectin/Levamisole	< LOQ	< LOQ	< LOD	< LOD
13	35	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
14	35	Eprinomectin/Levamisole	0.005	0.006	< LOD	< LOD
15	35	Eprinomectin/Levamisole	< LOQ	< LOQ	< LOD	< LOD
16	35	Eprinomectin/Levamisole	< LOQ	< LOQ	< LOD	< LOD
17	35	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
18	42	Eprinomectin/Levamisole	< LOD, LOD	< LOD, < LOD	< LOD, < LOD	< LOD, < LOD
19	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
20	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
21	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
22	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD

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Table 24. Eprinomectin B1a and levamisole concentrations in peri-renal tissue of treated and untreated cattle

Ear Tag	Interval	Product	Eprinomectin B1a (mg/kg)		Levamisole (mg/kg)	
			(Corrected)	(Uncorrected)	(Corrected)	(Uncorrected)
1	21	UTC	< LOD	< LOD	< LOD	< LOD
2	21	UTC	< LOD	< LOD	< LOD	< LOD
3	21	Eprinomectin/Levamisole	< LOQ	< LOQ	< LOD	< LOD
4	21	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
5	21	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
6	21	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
7	21	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
8	28	Eprinomectin/Levamisole	< LOD, < LOD	< LOD, < LOD	< LOD, < LOD	< LOD, < LOD
9	28	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
10	28	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD
11	28	Eprinomectin/Levamisole	< LOD	< LOD	< LOD	< LOD

(continued)

Ear Tag	Interval	Product	Eprinomectin B1a (mg/kg)		Levamisole (mg/kg)	
			(Corrected)	(Uncorrected)	(Corrected)	(Uncorrected)
5	12	28	Eprinomectin/Levamisole	< LOD	< LOD	< LOD
	13	35	Eprinomectin/Levamisole	< LOD	< LOD	< LOD
	14	35	Eprinomectin/Levamisole	< LOD	< LOD	< LOD
	15	35	Eprinomectin/Levamisole	< LOD	< LOD	< LOD
10	16	35	Eprinomectin/Levamisole	< LOD	< LOD	< LOD
	17	35	Eprinomectin/Levamisole	< LOD	< LOD	< LOD
	18	42	Eprinomectin/Levamisole	< LOD, < LOD	< LOD, < LOD	< LOD, < LOD
	19	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD
15	20	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD
	21	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD
	22	42	Eprinomectin/Levamisole	< LOD	< LOD	< LOD

Example 7 - Efficacy of a combination eprinomectin and levamisole injection against sucking lice (*Linognathus vituli*) in cattle.

[0085] Four species of louse have been recorded on cattle in New Zealand. Of these, the biting louse *Bovicola bovis* and one of the three species of sucking louse, *Linognathus vituli* are common and dual infections with *L. vituli* and *B. bovis* occur. The remaining two species of sucking louse, *Haematopinus eurysternus* and *Solenopotes capillatus* are uncommon (Chalmers and Charleston, 1980). The 56 day post-treatment assessment period covered at least two complete lifecycles of the louse.

[0086] This study was a randomized efficacy study in naturally infected animals with a negative control group. Prior to the start of the study, a mob of suitably infected animals were identified on a commercial grazing property located in the Waikato region of New Zealand. On Day -13 sixteen animals from the larger mob were purchased and relocated to the study site and identified using uniquely numbered ear tags. Samples of biting and sucking lice were collected from seven study animals and identified by the Study Investigator. On Day 0 all sixteen study animals were lice counted and allocated to two groups (n=8) based on individual sucking lice counts. All study animals were weighed and treatments were administered subcutaneously. Group 1 was treated with the instant eprinomectin (7g/L) / levamisole phosphate (223g/L) formulation at 1mL/35kg, and Group 2 remained untreated as the negative control group for the study. Individual lice counts were performed on each study animal on Days 7, 13 and 48. Group 1 was counted prior to Group 2, to ensure no transmission of lice between untreated animals and animals in the treated group. Injection sites were inspected on Days 7, 13 and 48 post-treatment for lesions or abnormalities.

Table 25. Treatment regime

Group	No. Animals	Treatment	Dose	Route
1	10	Eprinomectin/levamisole injection	1mL/35kg	Subcutaneous
2	10	Dectamax® injectable endectocide	1mL/50kg	Subcutaneous
3	10	Negative control	NA	NA

[0087] *Statistical methods.* The efficacy of the Investigational Veterinary Product (IVP, i.e. the eprinomectin / levamisole injectable formulation recited above) against both sucking and biting lice at each time point was calculated as the percent reduction in arithmetic and geometric mean lice count on the treated group compared to the untreated control group using Equation 1 and Equation 2, as recommended in WAAVP guidelines.

Equation 1: Abbott's formula

$$\text{Efficacy \%} = \left(1 - \frac{n \text{ in T after treatment}}{n \text{ in Co after treatment}} \right) * 100$$

Equation 2: Henderson-Tilton's formula

$$\text{Efficacy \%} = \left(1 - \frac{n \text{ in Co before treatment} * n \text{ in T after treatment}}{n \text{ in Co after treatment} * n \text{ in T before treatment}} \right) * 100$$

[0088] Where Efficacy % = efficacy, n = mean number of lice of the relevant species, T = Treatment group and Co = Control group. All statistical analyses were performed using Minitab® for Windows® Release 16.1.0 (Minitab, Inc.). Where data were considered to be normally distributed, as determined by Levene's test of equal variance, means were compared using a Two Sample T -Test. Data that were not normally distributed were compared using an equivalent non-parametric method. Results were considered significant at P<0.05.

[0089] Tables 26 and 27 show arithmetic and geometric mean sucking and biting lice counts recorded for Groups 1 and 2 throughout the study. Both biting and sucking lice were present on all animals prior to treatment and at the termination of the study on Day 48. Both tables incorporate the results of the statistical evaluation.

Table 26. Arithmetic and geometric mean sucking lice counts

Arithmetic mean sucking lice				
Group	Day 0	Day 7	Day 13	Day 48
1	36.3 ^a	5.1 ^a	13.6 ^a	12.3 ^a
2	26.8 ^a	32.3 ^b	32.1 ^a	13.3 ^a
Geometric mean sucking lice				
Group	Day 0	Day 7	Day 13	Day 48
1	27.6 ^a	3.8 ^a	5.8 ^a	10.8 ^a
2	24.6 ^a	30.6 ^b	31.0 ^b	16.2 ^a
^{ab} means within the same column with the same superscript are not significantly different at p<0.05				

Table 27. Arithmetic and geometric mean biting lice counts

Arithmetic mean biting Lice				
Group	Day 0	Day 7	Day 13	Day 48
1	673 ^a	12.4 ^a	21.8 ^a	17.1 ^a
2	64.9 ^a	46.6 ^b	43.9 ^b	26.0 ^b
Geometric mean biting Lice				
Group	Day 0	Day 7	Day 13	Day 48
1	60.5 ^a	10.5 ^a	20.3 ^a	15.7 ^a
2	58.2 ^a	45.9 ^b	41.2 ^b	25.0 ^b
^{ab} means within the same column with the same superscript are not significantly different at p<0.05				

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[0090] The efficacy of the IVP against sucking and biting lice are show in Tables 28 and 29. Each table presents calculations based on Equation 1 and Equation 2. The efficacy of the IVP against sucking lice peaked at 84% and 88%, based on arithmetic mean counts on Day 7 using Equation 1 and Equation 2, respectively. Efficacy had declined over the subsequent two counts to 36% and 53% by Day 48, based on Equation 1 and Equation 2, respectively. The efficacy of the IVP against sucking lice based on geometric mean counts peaked at 88% and 89% on Day 7, but declined to 33% and 41 % by Day 48, using the same two equations.

Table 28. Efficacy against sucking lice based on arithmetic and geometric mean lice counts calculated using Equation 1 and Equation 2.

Arithmetic Efficacy Abbott's Formula sucking lice			
Group	Day 7	Day 13	Day 48
2	0.84	0.58	0.36
Arithmetic Efficacy Henderson-Tilton's Formula sucking lice			
Group	Day 7	Day 13	Day 48
2	0.88	0.69	0.53
Geometric Efficacy Abbott's Formula sucking lice			
Group	Day 7	Day 13	Day 48
2	0.88	0.81	0.33
Geometric Efficacy Henderson-Tilton's Formula sucking lice			
Group	Day 7	Day 13	Day 48
2	0.89	0.83	0.41

[0091] Efficacy based on arithmetic mean lice counts against biting lice peaked at a lower level with the IVP achieving a maximum reduction of 73% and 74% in lice numbers, using Equation 1 and Equation 2, respectively and declined to 34% (Equation 1) and 36% (Equation 2) by Day 48. When efficacy was calculated using the geometric mean lice counts, maximum reductions of 77% and 78% were achieved on Day 7 and had declined to 37% and 40%, based on Equation 1 and Equation 2.

Table 29. Efficacy against biting lice based on arithmetic and geometric mean lice counts calculated using Equation 1 and 2

Arithmetic Efficacy Abbott's Formula biting lice			
Group	Day 7	Day 13	Day 48
2	0.73	0.50	0.34
Arithmetic Efficacy Henderson-Tilton's Formula biting lice			
Group	Day 7	Day 13	Day 48
2	0.74	0.52	0.36
Geometric Efficacy Abbott's Formula biting lice			
Group	Day 7	Day 13	Day 48
2	0.77	0.49	0.37
Geometric Efficacy Henderson-Tilton's Formula biting lice			
Group	Day 7	Day 13	Day 48
2	0.78	0.51	0.40

[0092] As expected, the IVP was more effective against sucking lice than biting lice, given that its route of administration was by subcutaneous injection. The highest level of efficacy against sucking lice (89%) was recorded on Day 7 compared to a 78% reduction in biting lice numbers at the same time point. At 13 days post treatment, the highest level of efficacy

against sucking lice was 83% compared to 52% against biting lice.

Example 8 - Safety of eprinomectin/levamisole formulations

[0093] Thirty male Hereford x Friesian cattle, each weighing between 105-123kgs, were treated according to preceding examples. The animals were healthy and had not been treated with a macrocyclic lactone or levamisole within 3 months prior to Study Day 0. One group received the 1x concentration of eprinomectin and levamisole phosphate (see Table 18) administered by subcutaneous injection, while the other group received 3x this concentration. Animals were monitored for pain and swelling, and the results indicated the eprinomectin / levamisole injection is safe to administer the recommended dose, or at least up to 3x the recommended dose (for example, in the event of an accidental overdose).

Example 9. Fecal egg count reduction efficacy of a subcutaneous combination eprinomectin and levamisole injection against a natural infection of gastrointestinal nematodes in cattle.

[0094] This study was a randomized fecal egg count reduction field efficacy study in animals carrying a natural infection, and included positive and negative control groups for comparison. Seven days prior to commencement of the study, ten individual unidentified fecal samples were collected from the paddock and individual fecal egg counts were conducted to confirm infection. Four days prior to commencement of the study, individual fecal samples were collected per rectum from 60 animals otherwise meeting the inclusion criteria. Fecal egg counts (FECs) and a pooled larval culture were performed. Forty-five animals with positive FECs were selected for the study and randomly allocated to three groups each of 15 animals based on FEC. They remained at pasture in a single mob on the source farm for the duration of the study.

[0095] On Day 0, the animals were weighed and a fecal sample was collected from each animal per rectum for individual FECs and a pooled larval culture. Group 1 was treated with the IVP, Group 2 was treated with Control Product (CP) and Group 3 remained untreated as the negative control. Treated animals were observed at 30 minutes and 2 hours after treatment. On Days 14, 16 and 28 post-treatment, fecal samples were collected per rectum for individual FECs and pooled group larval cultures. No sample was collected from Animal #60 (Group 3) on Day 14. Injection sites of animals in Group 1 were examined on Days 14 and 28. Further samples were due to be collected on days 42 and 56 but the study was concluded after day 28 due to evidence of nematode resistance to macrocyclic lactone anthelmintics. After the conclusion of the study, the study animals were treated with an oral abamectin, oxfendazole and levamisole anthelmintic (MATRIX C HI-MIN; Merial Ancare).

[0096] *Randomization Procedure.* FECs were determined for 60 animals on Day -4. Fourteen animals were excluded for negative FECs and one animal with a FECs of 1050 eggs per gram was excluded. The remaining 45 animals with positive FECs (range 50-550 eggs per gram) were selected for the study. They were ranked in ascending order of FECs and blocked into blocks of 3 animals. A random number was allocated to each animal using Microsoft Excel random number generation. The animals were ranked in ascending order of random number within each block. The animal with the lowest random number in each block was allocated to Group 1, the next to Group 2 and the highest to Group 3. Each of the three groups comprised 15 animals and had a similar average FECs.

[0097] *Blinding/masking procedure.* Laboratory staff performing FECs and group coprocultures were advised which group each animal was in after Day 0, but were not aware of the treatment allocated to each group. Forty-five Friesian/Jersey cross cattle, aged 10 months, female, and weighing on average 158.6 kg (range 106.5-208.5 kg) on Day 0, were used for the study. All animals were FEC positive, which was determined by the presence in the animals of a mean egg per gram fecal (epg) of 206, with the range being 50-550 epg on Day -4. No anthelmintic had been administered to the cattle within 30 days prior to Study Day 0. Study animals were transferred from the home dairy farm to the study site (a grazing block) approximately 6 weeks prior to Day 0. On Day 0 they were harboring an existing gastrointestinal nematode infection derived from the study site farm and/or the home dairy farm. The IVP (Eprinomectin 7g/L and levamisole phosphate 223 g/L) was administered at 1 mL/35 kg via subcutaneous route. The Control Product (CP) was DECTOMAX® (Pfizer Animal Health), which consists of 10mg/mL doramectin was administered to the animals via subcutaneous route at a dose of 1mL/50kg. Meat and milk withholding were each 35 days.

[0098] *Method of administration/application.* The IVP and CP were administered 1 time at Day 0 by subcutaneous injection in the anterior part of the neck. Individual doses were drawn up into a graduated 10mL syringe immediately prior to treatment and were checked by a second person before administration. Doses of IVP and CP were calculated on the basis of the heaviest bodyweight in the relevant group as determined on Day 0. Calculated doses were rounded up to the nearest minor increment of the syringe. The IVP was administered at 1mL / 35kg. The heaviest animal in Group 1 weighed 208.5 kg and the dose of IVP administered to each animal in Group 1 was 6.0 mL. The CP was administered at 1mL/50kg. The heaviest animal in Group 2 weighed 199.0 kg and the dose of CP administered to each animal in Group 2 was 4.0 mL.

[0099] All animals were returned to the source herd at the conclusion of the study. All animals were treated with MATRIX C Hi-Min (Merial Ancare) after the conclusion of the study and relevant withholding periods applied. Efficacy

of the IVP and CP was determined by fecal egg count reduction for the treated groups compared to the negative control group at each time point.

[0100] Efficacy (%E) of the IVP and CP was calculated at each time point, using the formula: $%E = [C - T]/C \times 100$. Where: C = mean FEC of the control group; T = mean FEC of the treated group. Arithmetic and geometric mean FECs were then calculated. The significance of differences between group FECs was statistically evaluated. Where data were considered to be normally distributed as determined by Levene's test of equal variance, means were compared using One-way Analysis of Variance (ANOVA). Data that were not normally distributed were compared using an equivalent non-parametric method. Where differences were considered significant, ($p < 0.05$), pair-wise comparisons were used to evaluate differences between groups.

[0101] Results. No injection site reactions were observed in Group 1 animals at any time point. FEC and larval culture results are shown in Tables 30-34. Table 30 shows arithmetic mean Day -4 FEC and Day 0 bodyweight. Means in the same column that share a letter (A) are not significantly different ($p < 0.05$). Table 31 shows arithmetic and geometric mean FEC at Days 0 to 42. Based on arithmetic and geometric means, the FECs for Group 1 (treated with the IVP) were significantly less ($p < 0.05$) than both the control group (Group 3) at every timepoint post-treatment and significantly less than Group 2 (treated with DECTOMAX) at Day 14 and 28. Based on arithmetic and geometric means, the FECs for Group 2 (treated with DECTOMAX) were not significantly different from the control group at any timepoint.

Table 30. Group details.

Group	n	Mean (range) FEC Day -4 (epg)	Mean (range) bodyweight Day 0 (kg)
1	15	20 ^A (50-500)	162 (112.5-208.5)
2	15	207 ^A (50-550)	156 (106.5-199)
3	15	207 ^A (50-550)	158 (128.5-201)

Table 31. Group FECs.

Group	Treatment	Arithmetic mean FEC (epg)			
		D0	D 14	D 16	D 28
1	IVP	337 ^A	37 ^A	23 ^A	17 ^A
2	Dectomax	320 ^A	150 ^B	80 ^{AB}	183 ^a
3	Control	237 ^A	261 ^B	183 ^B	180 ^B
		Geometric mean FEC (epg)			
		D 0	D 14	D 16	D 28
1	IVP	219.3 ^A	4.8 ^B	4.0 ^A	2.0 ^B
2	Dectomax	262.6 ^A	93.5 ^A	27.2 ^{AB}	44.7 ^A
3	Control	121.8 ^A	136.9 ^A	87.7 ^A	45.5 ^A

Table 32. Percentage FEC reduction efficacy.

Group	Treatment	% Efficacy (arithmetic means)			% Efficacy (geometric means)		
		D 14	D 16	D 28	D14	D16	D28
1	IVP	859	87.3	90.7	96.0	95.4	95.6
2	Dectomax	42.5	56.4	-1.9	32.0	69.0	1.8

[0102] Efficacy of the IVP and CP at each timepoint (based on FEC reduction compared to the untreated control group) is shown in Table 32. The percentage of each nematode genus identified in 100 larvae from group coprocultures at each timepoint is shown in Table 33.

Table 33. Larval culture results (%)

Day	Group	Treatment	Ostertagia	Trichostrongylus	Cooperia	Number of larvae speciated
-4	Pooled	NA	50	7	43	100
0	Pooled	NA	46	3	51	100
14	1	IVP	100*	0	0	1
	2	Dectomax	0	4	96	100
	3	NA	27	2	71	100
16	1	IVP	100**	0	0	6
	2	Dectomax	18	4	78	100
	3	NA	30	1	69	100
28	1	IVP	46***	0	54***	35
	2	Dectomax	1	5	94	100
	3	NA	35	1	64	100
*1 larva recovered ** 6 larvae recovered ***% of 35 larvae recovered						

[0103] The larval differential in a coproculture is usually determined from the percentage of each genus identified in the first 100 larvae counted. Small numbers of larvae were obtained from coprocultures from group 1 at days 14, 16 and 28, despite positive FECs. This suggests that egg viability or larval development may have been suppressed in this group. Individual FEC results at each timepoint are shown in Table 34.

Table 34. Individual FEC results

Group	ID	STRONGYLE FEC(epg)				
		D-4	D0	D14	D16	D28
1	11	100	100	150	50	0
	15	150	100	0	0	0
	20	100	150	0	0	0
	21	200	400	100	0	0
	22	300	250	0	0	0
	35	400	1050	50	0	0
	36	50	200	0	0	100
	38	50	100	0	0	0
	42	500	400	50	50	0
	46	250	300	150	100	0
	47	150	100	0	50	0
	57	350	1500	0	50	50
	70	250	100	0	50	50
	72	50	150	50	0	50
	86	150	150	0	0	0

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Group	ID	STRONGYLE FEC(epg)				
		D-4	D0	D14	D16	D28
2	3	200	550	100	200	150
	7	550	600	200	100	450
	18	100	100	200	0	50
	23	300	600	250	0	300
	26	100	200	50	50	50
	27	50	100	200	0	0
	29	200	300	100	200	150
	30	150	200	0	0	0
	55	50	200	150	50	350
	56	350	600	200	50	550
	64	50	150	50	50	0
	81	300	250	250	100	50
	91	350	600	50	100	400
	96	150	200	400	200	0
	97	200	150	50	100	250
3	13	50	50	50	0	0
	16	250	50	100	150	200
	19	100	100	100	150	200
	28	300	350	750	250	400
	33	550	1000	600	600	150
	40	200	300	250	250	150
	45	150	350	150	100	0
	49	50	0	100	50	100
	51	200	400	250	200	150
	53	100	100	150	0	0
	60	450	200	No sample	300	650
	85	300	150	100	100	50
	89	150	50	400	200	100
	94	50	100	0	100	0
	102	200	350	650	300	550

[0104] Conclusions. Based on arithmetic mean FECs, the levamisole/eprinomectin injection was over 85% effective and this effect was maintained up to 28 days after treatment. DECTOMAX (doramectin) was insufficiently effective. Based on geometric mean FECs, the levamisole/eprinomectin injection was over 95% effective and this effect was maintained up to 28 days after treatment. DECTOMAX was insufficiently effective. Efficacies were evaluated in animals carrying a mixed infection of *Ostertagia*, *Trichostrongylus* and *Cooperia* spp. There was strong evidence of anthelmintic resistance, and in particular, resistance by *Cooperia* spp. to doramectin.

Example 10 - Efficacy of a combination injectable anthelmintic containing eprinomectin and levamisole against common gastrointestinal nematodes in cattle.

[0105] *Experimental design.* This study was a randomized total worm count efficacy and dose confirmation study, with a negative control group. The study determined efficacy against a natural infection of common gastrointestinal nematodes in cattle. Prior to the study, a group of 21 animals meeting the inclusion criteria and suspected of harboring a natural infection of gastrointestinal nematodes were individually identified with uniquely numbered ear tags. The animals were fecal sampled and individual fecal egg counts (FECs) were performed. A bulk fecal sample was cultured and larval differentiation performed to identify the nematode species present. The fourteen study animals with the highest FEC results were selected for the study and introduced to the when study diet while in the paddock on day -5. On Day 0, the study animals were removed from pasture to the indoor facility. The animals were fecal sampled for individual FEC and randomly allocated into two groups of seven. One animal in each group was identified as a spare. Animals were weighed and the NP treatment was administered. Animals in Group 1 were treated with the IVP. Animals in Group 2 were left untreated. Treated animals were observed for adverse reactions at approximately 30 minutes and 1, 2 and 4 hours after treatment.

[0106] On Day 11, fecal samples were collected for individual fecal egg counts. On Day 13, animals were held off food overnight but continued to have *ad libitum* access to water. On Day 14, all animals (excluding spares) were humanely euthanized. The gastrointestinal tract of each animal was collected and forwarded directly to the laboratory for processing and subsequent worm counts. The animal phase of the study concluded after the Day 14 procedures had been performed. Bodyweights were recorded immediately prior to treatment Fecal egg count and total worm count data was entered into an excel spreadsheet for statistical analysis. Individual fecal samples were collected from study animals by rectal grab prior to the study and on Day 0, and Day 11. Samples were delivered to the Laboratory on the day of collection or the day after collection. Individual fecal egg counts were performed on samples collected prior to the study and on Day 0 and 11. Bulk larval culture and larval differentiation was performed on samples collected prior to the study. Gastrointestinal tracts (GITs) were collected immediately after euthanasia. The GIT was ligated at each end of the small intestine, large intestine and abomasum. The GIT was allowed to cool at ambient temperature for approximately 2 hours after collection. Each individual GIT was sealed (together with the animal's ear tag) in an individual bag identified by the animal's ear tag number. GITs were delivered to the Analytical Laboratory as soon as possible on the day of collection, accompanied by a sample submission form. Randomization / blinding was carried out as described.

[0107] All study animals had positive FECs prior to the study with a mean egg count of 121 epg. Larvae cultured from feces from all animals sampled prior to the study showed the following parasite species, 86% *Cooperia* spp., and 14% *Ostertagia* (*Teladorsagia*) spp. The mean body weight of the treatment group (excluding the reserve animal) on day zero of the study was 98.0kg. The mean body weight of the control group (excluding the reserve animal) on day zero of the study was 96.5kg. IVP was prepared and administered as in Example 9, and comprised eprinomectin (at 7 g/L) and levamisole phosphate (at 223 g/L). The injection site was cleaned and dried and was clipped then swabbed with 70% alcohol before treatment. A new sterile 20G 1.5 inch needle was used for each injection. Administration was performed once during the study, on Day 0, based upon bodyweight at 1 mL/35kg bodyweight. After the conclusion of the study, the spare animals were returned to the source herd. The treated spare was subjected to observation of a 91 day withholding period.

[0108] Worm counts were performed according to the *VICH Guideline on the efficacy of anthelmintics: general requirements* (1998). Counts were made on 2% aliquots of abomasa and small intestine washings that had been sieved through a 38µm sieve. Counts were also made on 10% aliquots of large intestine washings that had been sieved through a 150µm sieve. Total Worm Counts & Fecal Egg Counts: Calculation of IVP percentage efficacy (%E) was performed for each of the worm species (WS) present using the following formula: %E (Mean of (WS) in controls - mean of (WS) in treatment group) x 100 Mean of (WS) in controls. The same %E calculations were used to analyze the fecal egg count data. Statistics were calculated as described in the preceding Examples.

[0109] *Results.* Fecal egg counts: Mean fecal egg count (FEC) results are shown in Table 35. Based on FEC results the IVP was > 99.9% effective at reducing FECs at eleven days post treatment.

Table 35. Arithmetic mean FECs and % reduction in FECs of treated animals relative to controls in calves treated with a combination eprinomectin and levamisole injection

Study Day	Control Grp FEC (epg)	Treatment Grp FEC (epg)	Efficacy
Day 0	108.3	91.7	/
Day 11	250	0*	>99.9%
* = Statistically significant difference from the control group			

Table 36. Arithmetic mean worm counts and % reduction in worm numbers of treated animals relative to controls in calves treated with a combination eprinomectin and levamisole injection.

Parasite	Control	Treatment	Efficacy
<i>Ostertagia</i> (adult)	1867	0	>99.9%
<i>Ostertagia</i> (immature)	50	0	>99.9%
<i>Trichostrongylus axei</i> (adult)	367	0	>99.9%
<i>Haemonchus contortus</i> (adult)	42	0	>99.9%
<i>Haemonchus contortus</i> (immature)	17	0	>99.9%
<i>Cooperia</i> (adult)	18550	0	>99.9%
<i>Cooperia</i> (immature)	192	0	>99.9%
<i>Trichostrongylus</i> (adult)	33	0	>99.9%
<i>Nematodirus</i> (adult)	17	0	>99.9%
<i>Oesophagostomum</i> (adult)	12	0	>99.9%

[0110] *Total worm counts.* Arithmetic and Geometric mean total worm counts and percentage efficacy of the treatment group relative to the control group are presented in Table 36 and 37. Results demonstrate that the IVP was highly effective in the control of a mixed infection of gastrointestinal parasites in cattle. The IVP was >99.9% effective against the following parasite spp; *Ostertagia* (adult & immature), *Trichostrongylus axei* (adult), *Haemonchus contortus* (adult & immature), *Cooperia* (adult & immature), *Trichostrongylus* (adult), *Nematodirus* (adult), *Oesophagostomum* (adult).

Table 37. Geometric mean worm counts and % reduction in worm numbers of treated animals relative to controls in calves treated with a combination eprinomectin and levamisole injection.

Parasite	Control	Treatment	Efficacy
<i>Ostertagia</i> (adult)	1338	0*	>99.9%
<i>Ostertagia</i> (immature)	8	0	>99.9%
<i>Trichostrongylus axei</i> (adult)	237	0*	>99.9%
<i>Haemonchus contortus</i> (adult)	8	0	>99.9%
<i>Haemonchus contortus</i> (immature)	3	0	>99.9%
<i>Cooperia</i> (adult)	14015	0*	>99.9%
<i>Cooperia</i> (immature)	147	0*	>99.9%
<i>Trichostrongylus</i> (adult)	7	0	>99.9%
<i>Nematodirus</i> (adult)	3	0	>99.9%
<i>Oesophagostomum</i> (adult)	8	0*	>99.9%
* = Statistically significant difference from the control group			

[0111] *Conclusions.* This study was designed to determine the efficacy of an eprinomectin / levamisole injection against a mixed infection of common gastrointestinal parasites in cattle. Results demonstrate that the IVP was highly efficacious. Efficacies of >99.9% were recorded against the following parasite spp; *Ostertagia* (adult and immature), *Trichostrongylus axei* (adult), *Haemonchus contortus* (adult and immature), *Cooperia* (adult and immature), *Trichostrongylus* (adult), *Nematodirus* (adult), *Oesophagostomum* (adult).

Example 11. Efficacy of investigational eprinomectin / levamisole formulation versus commercial product

[0112] This study was performed using the methods described in the preceding Examples, except that an additional DECTOMAX treatment group was added.

[0113] *Results.* No injection site reactions were observed in Group 1 animals at either the Day 14 or the Day 28 observations. Table 38 below shows group arithmetic and geometric mean FEC at Days -8 to 50. Means in the same column that do not share a letter (A/B/C) are significantly different ($p < 0.05$). Based on both arithmetic and geometric means, the FECs for Group 1 (treated with the IVP) and Group 2 (treated with DECTOMAX) were significantly less ($p < 0.05$) than the control group (Group 3) at each of the post-treatment timepoints up to and including Day 43. The FECs for Group 1 (treated with the IVP) were also significantly less ($p < 0.05$) than the control group (Group 3) at day 50.

Table 38. Group mean FECs (epg)

Group	Treatment	Arithmetic mean FEC					
		D-8	D 0	D 14	D 28	D 43	D50
1	IVP	146.7 ^A	96.4 ^A	6.7 ^A	13.3 ^A	13.3 ^A	23.3 ^A
2	Dectomax	140.0 ^A	83.3 ^A	43.3 ^A	46.7 ^A	46.7 ^A	43.3 ^{AB}
3	Control	133.3 ^A	121.4 ^A	166.7 ^B	133.3 ^B	156.7 ^B	100.0 ^B
		Geometric mean FEC					
		D-8	D 0	D 14	D 28	D 43	D50
1	IVP	134.1 ^A	52.2 ^A	0.4 ^B	0.8 ^C	1.3 ^B	4.0 ^B
2	Dectomax	129.1 ^A	30.1 ^A	4.0 ^B	12.0 ^A	7.5 ^B	9.0 ^{AB}
3	Control	131.4 ^A	65.0 ^A	59.5 ^A	87.8 ^A	109.9 ^A	52.4 ^A

[0114] Efficacy of the IVP and CP at each timepoint (based on FEC reduction compared to the untreated control group) is shown in Table 39.

Table 39. Percentage FEC reduction efficacy

Group	Treatment	% Efficacy (arithmetic means)				% Efficacy (geometric means)			
		D14	D 28	D 43	D 50	D14	D28	D 43	D 50
1	IVP	96.0	90.0	91.5	76.7	99.0	99.1	98.8	92.3
2	Dectomax	74.0	65.0	70.2	56.7	93.0	86.3	93.2	82.8

[0115] The percentage of each nematode genus identified in 100 larvae from group coprocultures at each timepoint is shown in Table 40. Only 18 larvae were recovered and identified from the coproculture for Group 1 on Day 14. This suggests that egg viability or larval development may have been suppressed in this Group.

Table 40. Larval culture results (%).

Day	Group	Treatment	Ostertagia	Trichostrongylus	Cooperia	Haemonchus
0	Pooled	NA	14	1	85	0
14	1	IVP	100*	0	0	0
	2	Dectomax	0	0	100	0
	3	NA	28	13	59	0
28	1	IVP	86	0	14	0
	2	Dectomax	0	0	100	0
	3	NA	19	0	81	0

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Day	Group	Treatment	Ostertagia	Trichostrongylus	Cooperia	Haemonchus
43	1	IVP	41	0	59	0
	2	Dectomax	6	2	92	0
	3	NA	19	0	81	0
50	1	IVP	14	2	84	0
	2	Dectomax	22	0	78	0
	3	NA	34	6	60	0
*18 larvae recovered.						

[0116] Table 41 shows group body weights at Day 0 and Day 50. Means in the same column that do not share a letter (A/B) are significantly different ($p < 0.05$). The mean live weight gain for Group 1 (treated with the IVP) over the 50 day period of the study was significantly greater than for the untreated control group (Group 3).

Table 41. Bodyweights

Group	Treatment	Mean (range) bodyweight Day 0 (kg)	Mean (range) bodyweight Day 50 (kg)	Mean (range) weight gain (kg)
1	IVP	177.2 (138-221)	233.5 (201-266)	56.3 ^A (43-80)
2	Dectomax	184.1 (128-229)	234.5 (153-285)	50.4 ^{AB} (25-71)
3	Control	189.6 (163-234)	235.4 (218-291)	45.8 ^B (33-58)

Table 42. Individual FEC results

Group	ID	STRONGYLE FEC (epg)					
		D-8	D0	D14	D28	D43	D50
1	101	100	100	0	0	0	50
	103	150	200	0	50	50	0
	112	100	150	0	0	100	50
	113	200	50	0	0	0	100
	116	150	50	100	150	0	50
	118	100	100	0	0	0	50
	126	400	0	0	0	0	0
	127	100	100	0	0	0	0
	128	100	150	0	0	0	0
	138	150	50	0	0	0	0
	143	100	100	0	0	0	50
	149	150	150	0	0	0	0
	153	200	NS	0	0	50	0
	158	100	0	0	0	0	0
	163	100	150	0	0	0	0

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Group	ID	STRONGYLE FEC (epg)					D50
		D-8	D0	D14	D28	D43	
2	102	150	100	0	50	50	0
	111	100	100	0	100	100	0
	114	100	50	100	50	0	0
	119	300	0	0	0	100	150
	121	100	100	100	100	0	100
	123	200	100	0	0	100	50
	124	150	0	100	50	0	50
	129	50	150	0	50	0	50
	136	100	0	0	0	0	0
	137	200	150	200	150	150	0
	139	150	100	0	0	0	50
	146	100	100	0	50	0	100
	150	100	0	150	100	0	100
	160	150	100	0	0	100	0
	164	150	200	0	0	100	0
3	106	100	100	0	50	100	0
	108	100	NS	100	50	150	0
	110	150	50	150	100	150	50
	115	100	100	0	0	0	50
	117	250	0	0	50	200	200
	120	100	150	50	100	100	250
	122	350	100	300	150	250	200
	130	100	100	450	200	150	100
	131	150	200	300	350	200	50
	132	50	150	150	150	50	50
	144	200	200	150	100	150	50
	145	100	200	200	200	150	150
	147	100	200	200	150	250	100
	159	150	0	300	250	150	100
	162	200	150	150	100	300	150
NS=No sample							

[0117] *Conclusions.* Based on arithmetic mean FECs, the levamisole/eprinomectin injection had an efficacy of 90% or greater and this effect was maintained up to 43 days after treatment. DECTOMAX (doramectin) was insufficiently effective at all time points. Based on geometric mean FECs, the levamisole/eprinomectin injection was over 98% effective and this effect was maintained up to 43 days after treatment. DECTOMAX was over 86% effective during the same period. Efficacies were evaluated in animals carrying a mixed infection of *Ostertagia*, *Trichostrongylus* and *Cooperia* spp.

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Example 12 - Efficacy of investigational eprinomectin / levamisole compared with doramectin-containing product

[0118] Methods. This study was performed in accordance with Example 11. The doses of IVP and CP were calculated according to individual body weights. Group 1 was treated with the IVP at the dose rate of 1mL/ 35 kg live weight; Group 2 was treated with the Control Product at the recommended dose rate of 1mL/50kg; and Group 3 was left untreated as the negative control.

[0119] Results. No injection site reactions were observed in Group 1 animals on either Day 17 or Day 28. Table 43 shows group arithmetic mean FEC at Day -6 and mean bodyweights at Day 0 and Day 56. Means in the same column that share a letter (A) are not significantly different ($p < 0.05$).

Table 43. Group details

Group	n	Mean (range) FEC Day-6 (epg)	Mean (range) bodyweight Day 0 (kg)	Mean (range) bodyweight Day 56 (kg)
1	15	170 ^A (100-300)	159 (129-189)	184 (155-205)
2	15	180 ^A (100-350)	158 (136-209)	183 (152.5-240)
3	15	187 ^A (100-450)	156 (127-197)	178 (149-220)

[0120] Table 44 shows group arithmetic and geometric mean FEC at Days 0-42. Means in the same column that do not share a letter (A/B) are significantly different ($p < 0.05$). Based on geometric means, the FECs for Group 1 (treated with the IVP) were significantly less ($p < 0.05$) than both the control group (Group 3) and the group treated with DECTOMAX (Group 2) on both of Days 16 and 28.

Table 44. Group FECs

Group	Treatment	Arithmetic mean (range) FEC (epg)			
		D 0	D 16	D 28	D 42
1	IVP	503 ^A (0-1800)	3 ^B (0-50)	3 ^B (0-50)	60 ^A (0-200)
2	Dectomax	393 ^A (0-1050)	160 ^A (0-1050)	103 ^A (0-700)	86 ^A (0-200)
3	Control	254 ^A (0-550)	137 ^{AB} (0-600)	125 ^A (0-250)	83 ^A (0-150)
		Geometric mean FEC (epg)			
		D 0	D 16	D 28	D 42
1	IVP	249.2 ^A	0.3 ^A	0.3 ^A	17.6 ^A
2	Dectomax	161.7 ^A	28.1 ^B	16.8 ^B	35.8 ^A
3	Control	137.8 ^A	52.7 ^B	68.0 ^B	30.4 ^A

[0121] The efficacy of the IVP and CP at each timepoint (based on FEC reduction compared to the untreated control group) is shown in Table 45. The percentage of each nematode genus identified in 100 larvae from group coprocultures at each timepoint is shown in Table 46.

Table 45. Percentage FEC reduction efficacy compared to untreated control

Group	Treatment	% Efficacy (arithmetic means)			% Efficacy (geometric means)		
		D 16	D 28	D 42	D16	D28	D42
1	IVP	97.6	97.3	28	99.0	99.6	42.2
2	Dectomax	-17.1	17.3	-2.9	47.0	75.3	-18.0

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Table 46. Larval culture results (%)

Day	Group	Treatment	Ostertagia	Trichostrongylus	Cooperia	Haemonchus
0	Pooled	NA	12	8	80	0
16	1	IVP	97	0	3	0
	2	Dectomax	2	0	98	0
	3	NA	5	0	95	0
28	1	IVP	56	0	44	0
	2	Dectomax	11	0	89	0
	3	NA	23	9	68	0
42	1	IVP	2	0	98	0
	2	Dectomax	3	0	97	0
	3	NA	8	0	92	0

Table 47. Individual FEC results

Group	ID	STRONGYLE FEC (epg)				
		D-6	D0	D16	D28	D42
1	77	150	50	50	50	0
	88	150	0	0	0	150
	91	200	1800	0	0	200
	94	300	850	0	0	50
	97	100	650	0	0	0
	102	200	150	0	0	100
	105	100	250	0	0	100
	112	150	200	0	0	0
	116	200	300	0	0	0
	119	150	400	0	0	50
	120	300	700	0	0	0
	123	200	1050	0	0	50
	124	100	100	0	0	50
	133	100	600	0	0	100
	136	150	450	0	0	50

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(continued)

Group	ID	STRONGYLE FEC (epg)				
		D-6	D0	D16	D28	D42
2	76	350	850	0	50	200
	82	100	650	100	0	200
	83	200	250	100	100	0
	85	150	350	100	100	100
	92	100	0	0	0	50
	98	150	200	100	50	0
	100	200	150	0	0	150
	103	200	250	200	150	100
	111	150	1050	1050	700	100
	121	100	850	400	200	50
	122	200	100	50	100	100
	127	150	0	0	0	ns
	129	150	250	100	0	100
	132	300	700	200	100	0
	137	200	250	0	0	50
3	78	150	250	100	150	0
	81	100	50	150	100	150
	84	150	200	100	250	100
	87	100	50	150	150	0
	90	150	400	100	100	100
	95	200	150	100	150	100
	99	450	150	100	100	100
	101	200	550	0	0	0
	104	100	ns	200	100	0
	106	150	0	100	100	50
	110	150	550	150	200	100
	117	400	350	200	200	150
	118	250	300	0	ns	100
	125	100	500	600	150	150
	128	150	50	0	0	150
ns = no sample						

[0122] *Conclusions.* Based on arithmetic mean FECs, the levamisole/eprinomectin injection was over 97.6% effective and this effect was maintained up to 28 days after treatment. DECTOMAX was insufficiently effective at all time points. Based on geometric mean FECs, the levamisole / eprinomectin injection was over 99% effective and this effect was maintained up to 28 days after treatment. DECTOMAX was insufficiently effective at all time points. Efficacies were evaluated in animals carrying a mixed infection of *Ostertagia*, *Trichostrongylus* and *Cooperia* spp. There was strong evidence of resistance by *Cooperia* spp. to doramectin.

Claims

1. A pharmaceutically or veterinarily acceptable formulation adapted to be injected into an animal, which formulation comprises;
5
a. an effective amount of a levamisole;
b. an effective amount of at least one macrocyclic lactone;
c. a pharmaceutically acceptable system of solvents and surfactants wherein the system provides for acceptable storage stability for both the levamisole and the macrocyclic lactone and wherein the system provides for a
10 viscosity which is suitable for injection;
wherein the system comprises at least one surfactant, wherein the surfactant is CREMOPHOR EL, and a water miscible organic solvent which is dimethylacetamide (DMA).
2. The formulation of claim 1 wherein the levamisole is levamisole phosphate and wherein the levamisole is present
15 in an amount sufficient to deliver a dose of at least 4 mg/kg animal bodyweight.
3. The formulation of claim 1, wherein the macrocyclic lactone is either ivermectin or eprinomectin and is present in an amount sufficient to deliver a dose of at least 150 µg/kg animal bodyweight.
- 20 4. The formulation of any one of claims 1 or 3 which further comprises an antioxidant, a preservative, and water for injection wherein the water for injection is provided in a quantity sufficient to bring the formulation to a suitable injection volume.
- 25 5. The formulation of any one of claims 1 to 4 wherein the macrocyclic lactone concentration is from about 0.1% to about 2% (w/v); the levamisole concentration is from about 15% to about 30% (w/v); and the surfactant concentration is from about 1% to about 10% (w/v).
- 30 6. The formulation of claim 5 wherein the macrocyclic lactone is eprinomectin and its concentration is about 0.5% to about 0.9% (w/v); the levamisole is levamisole phosphate and its concentration is about 18% to about 25% (w/v); the surfactant is CREMOPHOR EL, or a combination of CREMOPHOR EL and POLYSORBATE 80, and its concentration is about 3% to about 7% (w/v); the antioxidant is BHT; the solvent is DMA and its concentration is about 1% to about 10% (w/v); and the preservative is methyl paraben.
- 35 7. The formulation of any one of claims 1-6 wherein the macrocyclic lactone is eprinomectin and wherein the eprinomectin has a specification such that its B1a composition is greater than or equal to 85.0% and its B1a + B1b composition is greater than or equal to 90.0%; and wherein the density of the formulation is between 0.95 and 1.20 and pH is between 3.0 - 5.0.
- 40 8. A pharmaceutically or veterinarily acceptable formulation adapted to be injected into an animal, which formulation comprises;
a. an effective amount of a levamisole;
b. an effective amount of at least one macrocyclic lactone;
45 c. a pharmaceutically acceptable system of solvents and surfactants wherein the system provides for acceptable storage stability for both the levamisole and the macrocyclic lactone and wherein the system provides for a viscosity which is suitable for injection;
wherein the system comprises at least one surfactant, wherein the surfactant is CREMOPHOR EL, and a water miscible organic solvent which is dimethylacetamide (DMA), wherein the macrocyclic lactone is eprinomectin and its concentration is about 0.5% to about 0.9% (w/v); the levamisole is levamisole phosphate and its concentration is about 18% to about 25% (w/v); the surfactant is POLYSORBATE 80, CREMOPHOR EL, or combinations thereof, and its concentration is about 3% to about 7% (w/v); the antioxidant is BHT, the solvent is DMA and its concentration is about 1% to about 10% (w/v); and the preservative is methyl paraben.
50
9. A formulation according to any one of claims 1 to 8 for use in preventing or treating internal parasite infestations in
55 animals.
10. A formulation for use according to claim 9 wherein the formulation is injected.

11. A formulation for use according to claim 9 or 10 wherein the macrocyclic lactone concentration is from 0.5% to 0.9% (w/v) and the levamisole concentration is from 15% to 25% (w/v).
12. A formulation for use according to claim 9 or 10 wherein the parasites are helminths.
13. A formulation for use according to claim 12 wherein the helminths are nematodes, cestodes, trematodes.
14. A method for producing the formulation according to claim 1 or 2 comprising the steps of:
 - a. adding water for injection to a vessel,
 - b. dissolving the Levamisole in the water,
 - c. in a separate vessel dissolving Eprinomectin and BHT in DMA,
 - d. adding the surfactant to the DMA/Eprinomectin blend,
 - e. transferring the Eprinomectin/DMA blend to the bulk aqueous phase with mixing, and
 - f. optionally, bringing the batch to volume with added WFI and mix.

Patentansprüche

1. Pharmazeutisch oder veterinärmedizinisch verträgliche Formulierung, die angepasst ist, um in ein Tier injiziert zu werden, wobei die Formulierung umfasst:
 - a. eine wirksame Menge an einem Levamisol;
 - b. eine wirksame Menge an mindestens einem makrocyclischen Lacton ;
 - c. ein pharmazeutisch verträgliches System an Lösungsmitteln und Tensiden, wobei das System eine verträgliche Lagerungsstabilität sowohl für das Levamisol und das makrocyclische Lacton bereitstellt und wobei das System eine Viscosität bereitstellt, die zur Injektion geeignet ist; und wobei das System mindestens ein Tensid umfasst, wobei das Tensid CREMOPHOR EL ist, und ein wassermischbares organisches Lösungsmittel, das Dimethylacetamid (DMA) ist.
2. Formulierung nach Anspruch 1, wobei das Levamisol Levamisol-Phosphat ist und wobei das Levamisol in einer Menge vorliegt, die ausreicht, um eine Dosis von mindestens 4 mg/kg Tierkörpergewicht zu verabreichen.
3. Formulierung nach Anspruch 1, wobei das makrocyclische Lacton entweder Ivermectin oder Eprinomectin ist und in einer Menge vorliegt, die ausreicht, um eine Dosis von mindestens 150 µg/kg Tierkörpergewicht zu verabreichen.
4. Formulierung nach einem der Ansprüche 1 oder 3, die des Weiteren ein Antioxidans, ein Konservierungsmittel und Wasser zur Injektion umfasst, wobei das Wasser zur Injektion in einer Menge vorhanden ist, die ausreicht, um die Formulierung auf ein geeignetes Injektionsvolumen zu bringen.
5. Formulierung nach einem der Ansprüche 1 bis 4, wobei die makrocyclische Lactonkonzentration von etwa 0,1% bis etwa 2% (w/v) ist, die Levamisolkonzentration von etwa 15% bis etwa 30% (w/v) ist; und die Tensidkonzentration von etwa 1% bis etwa 10% (w/v) ist.
6. Formulierung nach Anspruch 5, wobei das makrocyclische Lacton Eprinomectin ist und seine Konzentration etwa 0,5% bis etwa 0,9% (w/v) ist; das Levamisol Levamisol-Phosphat ist und seine Konzentration etwa 18% bis 25% (w/v) ist; das Tensid CREMOPHOR EL, oder eine Kombination von CREMOPHOR EL und POLYSORBAT 80 ist und seine Konzentration etwa 3% bis etwa 7% (w/v) ist; das Antioxidans BHT ist; das Lösungsmittel DMA ist und seine Konzentration etwa 1% bis etwa 10% (w/v) ist; und das Konservierungsmittel Methylparaben ist.
7. Formulierung nach einem der Ansprüche 1 bis 6, wobei das makrocyclische Lacton Eprinomectin ist und wobei Eprinomectin die Spezifikation hat, dass sein Gehalt an B1 a größer oder gleich 85,0 % und sein Gehalt an B1a + B1b größer oder gleich 90,0% ist; und wobei die Dichte der Formulierung zwischen 0,95 und 1,20 ist und der pH-Wert zwischen 3,0 bis 5,0 ist.
8. Pharmazeutisch oder veterinärmedizinisch verträgliche Formulierung, die angepasst ist, um in ein Tier injiziert zu werden, wobei die Formulierung umfasst:

a. eine wirksame Menge an einem Levamisol;
 b. eine wirksame Menge an mindestens einem makrocyclischen Lacton;
 c. ein pharmazeutisch verträgliches System an Lösungsmitteln und Tensiden, wobei das System eine verträgliche Lagerungsstabilität sowohl für das Levamisol und das makrocyclische Lacton bereitstellt und wobei das System eine Viskosität bereitstellt, die zur Injektion geeignet ist;
 und wobei das System mindestens ein Tensid umfasst, wobei das Tensid CREMOPHOR EL ist, und ein wassermischbares organisches Lösungsmittel, das Dimethylacetamid (DMA) ist, wobei das makrocyclische Lacton Eprinomectin ist und seine Konzentration etwa 0,5% bis etwa 0,9% (w/v) ist; das Levamisol Levamisol-Phosphat ist und seine Konzentration etwa 18% bis etwa 25% (w/v) ist; das Tensid POLYSORBAT 80, CREMOPHOR EL oder eine Kombination davon ist und seine Konzentration etwa 3% bis etwa 7% ist; das Antioxidans BHT ist, das Lösungsmittel DMA ist und seine Konzentration etwa 1% bis etwa 10% (w/v) ist; und das Konservierungsmittel Methylparaben ist.

9. Formulierung nach einem der Ansprüche 1 bis 8, zur Verwendung bei der Vorbeugung oder Behandlung von innerlichem Parasitenbefall bei Tieren.

10. Formulierung zur Verwendung nach Anspruch 9, wobei die Formulierung injiziert wird.

11. Formulierung zur Verwendung nach Anspruch 9 oder 10, wobei die makrocyclische Lactonkonzentration von 0,5% bis 0,9% (w/v) ist und die Levamisolkonzentration von 15% bis 25% (w/v) ist.

12. Formulierung zur Verwendung nach Anspruch 9 oder 10, wobei die Parasiten Helminthen sind.

13. Formulierung zur Verwendung nach Anspruch 12, wobei die Helminthen Nematoden, Cestoden, Trematoden sind.

14. Verfahren zur Herstellung der Formulierung nach Anspruch 1 oder 2, umfassend die Schritte:

a. Hinzufügen von Wasser zur Injektion in ein Gefäß,
 b. Lösen des Levamisols in dem Wasser,
 c. Lösen von Eprinomectin und BHT in DMA in einem separaten Gefäß,
 d. Hinzufügen des Tensids zu der DMA/Eprinomectin-Mischung,
 e. Transferieren der DMA/Eprinomectin-Mischung in die wässrige Bulk-Phase durch Mischen, und
 f. gegebenenfalls die Charge durch das Wasser zur Injektion (WFI) bis zum Injektionsvolumen auffüllen und Mischen.

Revendications

1. Formulation de qualité pharmaceutique ou vétérinaire conçue pour être injectée à un animal, ladite formulation comprenant :

a. une quantité efficace de lévamisole ;
 b. une quantité efficace d'au moins une lactone macrocyclique ;
 c. un système de solvants et de tensioactifs de qualité pharmaceutique, dans laquelle le système présente une stabilité acceptable au stockage à la fois pour le lévamisole et pour la lactone macrocyclique, et dans laquelle le système a une viscosité qui est appropriée pour une injection ;
 dans laquelle le système comprend au moins un tensioactif, dans laquelle le tensioactif est le CREMOPHOR EL, et un solvant organique miscible avec l'eau qui est le diméthylacétamide (DMA).

2. Formulation selon la revendication 1, dans laquelle le lévamisole est le phosphate de lévamisole et dans laquelle le lévamisole est présent en une quantité suffisante pour administrer une dose d'au moins 4 mg/kg de poids corporel de l'animal.

3. Formulation selon la revendication 1, dans laquelle la lactone macrocyclique est soit l'ivermectine soit l'éprinomectine et est présente en une quantité suffisante pour administrer une dose d'au moins 150 µg/kg de poids corporel de l'animal.

4. Formulation selon l'une quelconque des revendications 1 ou 3, qui comprend en outre un antioxydant, un conser-

vateur, et de l'eau pour l'injection, dans laquelle l'eau pour l'injection est prévue en une quantité suffisante pour amener la formulation à un volume d'injection approprié.

5 5. Formulation selon l'une quelconque des revendications 1 à 4, dans laquelle la lactone macrocyclique est située dans la plage allant d'environ 0,1 % à environ 2 % (p/v) ; la concentration en lévamisole est située dans la plage allant d'environ 15 % à environ 30 % (p/v) ; et la concentration en tensioactif est située dans la plage allant d'environ 1 % à environ 10 % (p/v).

10 6. Formulation selon la revendication 5, dans laquelle la lactone macrocyclique est l'éprinomectine et sa concentration est située dans la plage allant d'environ 0,5 % à environ 0,9 % (p/v) ; le lévamisole est le phosphate de lévamisole et sa concentration est située dans la plage allant d'environ 18 % à environ 25 % (p/v) ; le tensioactif est le CREMOPHOR EL, ou une combinaison de CREMOPHOR EL et de POLYSORBATE 80, et sa concentration est située dans la plage allant d'environ 3 % à environ 7 % (p/v) ; l'antioxydant est le BHT ; le solvant est le DMA et sa concentration est située dans la plage allant d'environ 1 % à environ 10 % (p/v) ; et le conservateur est le p-hydroxybenzoate de méthyle.

15 7. Formulation selon l'une quelconque des revendications 1 à 6, dans laquelle la lactone macrocyclique est l'éprinomectine et dans laquelle l'éprinomectine a une spécification telle que sa composition en constituant B1a est supérieure ou égale à 85,0 % et que sa composition en constituants B1a + B1b est supérieure ou égale à 90,0 % ; et dans laquelle la densité de la formulation est comprise entre 0,95 et 1,20 et le pH est compris entre 3,0 et 5,0.

20 8. Formulation de qualité pharmaceutique ou vétérinaire conçue pour être injectée à un animal, ladite formulation comprenant :

- 25 a. une quantité efficace de lévamisole ;
 b. une quantité efficace d'au moins une lactone macrocyclique ;
 c. un système de solvants et de tensioactifs de qualité pharmaceutique, dans laquelle le système présente une stabilité acceptable au stockage à la fois pour le lévamisole et pour la lactone macrocyclique, et dans laquelle le système a une viscosité qui est appropriée pour une injection ;
 30 dans laquelle le système comprend au moins un tensioactif, dans laquelle le tensioactif est le CREMOPHOR EL, et un solvant organique miscible avec l'eau qui est le diméthylacétamide (DMA), dans laquelle la lactone macrocyclique est l'éprinomectine et sa concentration est située dans la plage allant d'environ 0,5 % à environ 0,9 % (p/v) ; le lévamisole est le phosphate de lévamisole et sa concentration est située dans la plage allant d'environ 18 % à environ 25 % (p/v) ; le tensioactif est le POLYSORBATE 80, le CREMOPHOR EL, ou des combinaisons de ceux-ci, et sa concentration est située dans la plage allant d'environ 3 % à environ 7 % (p/v) ; l'antioxydant est le BHT, le solvant est le DMA et sa concentration est située dans la plage allant d'environ 1 % à environ 10 % (p/v) ; et le conservateur est le p-hydroxybenzoate de méthyle.

35 9. Formulation selon l'une quelconque des revendications 1 à 8, destinée à être utilisée dans la prévention ou le traitement d'infestations d'animaux par des endoparasites.

40 10. Formulation destinée à être utilisée selon la revendication 9, dans laquelle la formulation est injectée.

45 11. Formulation destinée à être utilisée selon la revendication 9 ou la revendication 10, dans laquelle la concentration en lactone macrocyclique est située dans la plage allant de 0,5 % à 0,9 % (p/v) et la concentration en lévamisole est située dans la plage allant de 15 % à 25 % (p/v).

50 12. Formulation destinée à être utilisée selon la revendication 9 ou la revendication 10, dans laquelle les parasites sont des helminthes.

55 13. Formulation destinée à être utilisée selon la revendication 12, dans laquelle les helminthes sont des nématodes, des cestodes, des trématodes.

14. Procédé de production de la formulation selon la revendication 1 ou la revendication 2, comprenant les étapes suivante :

- a. ajouter de l'eau pour l'injection dans un récipient,
 b. dissoudre le lévamisole dans l'eau,

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- c. dans un récipient séparé, dissoudre l'éprinomectine et le BHT dans le DMA,
- d. ajouter le tensioactif au mélange DMA/éprinomectine,
- e. transférer le mélange éprinomectine/DMA à la phase aqueuse mère tout en mélangeant, et
- f. éventuellement, amener le lot au volume souhaité avec un ajout d'eau pour l'injection et sous mélange.

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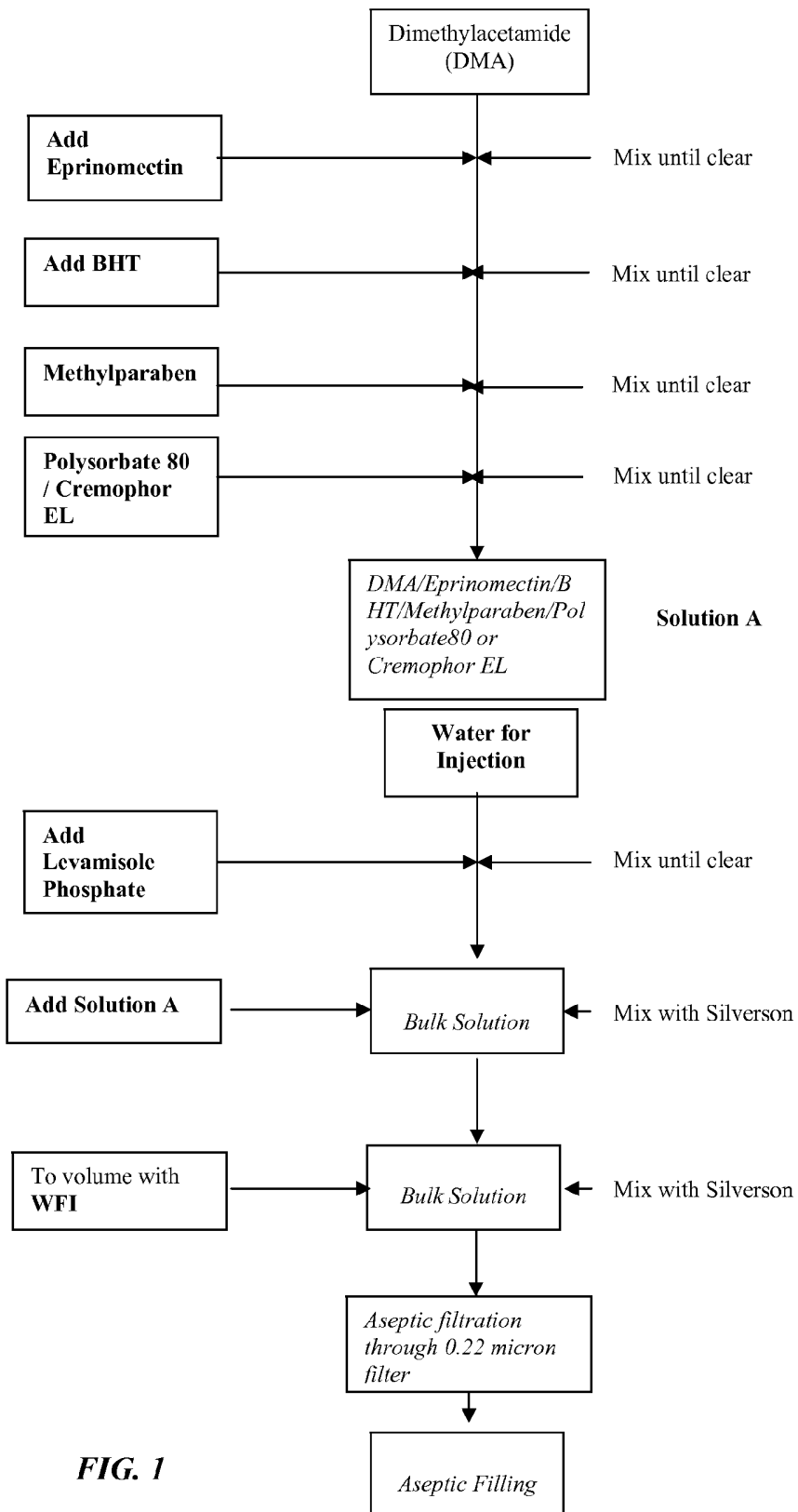
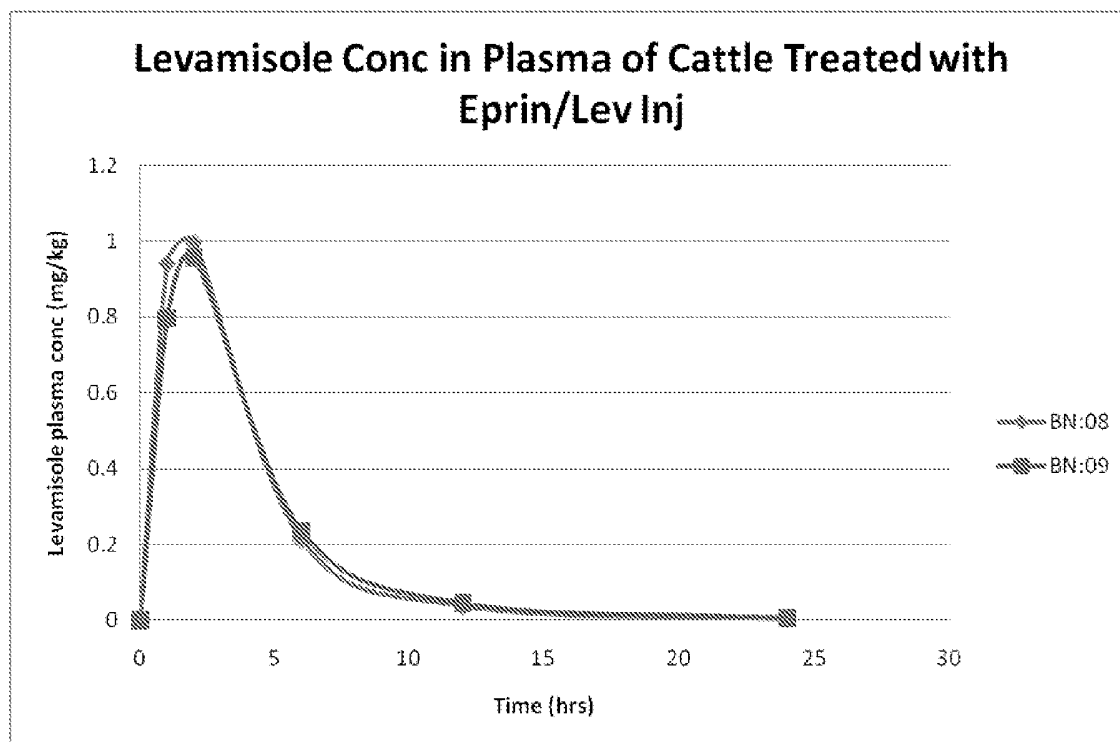
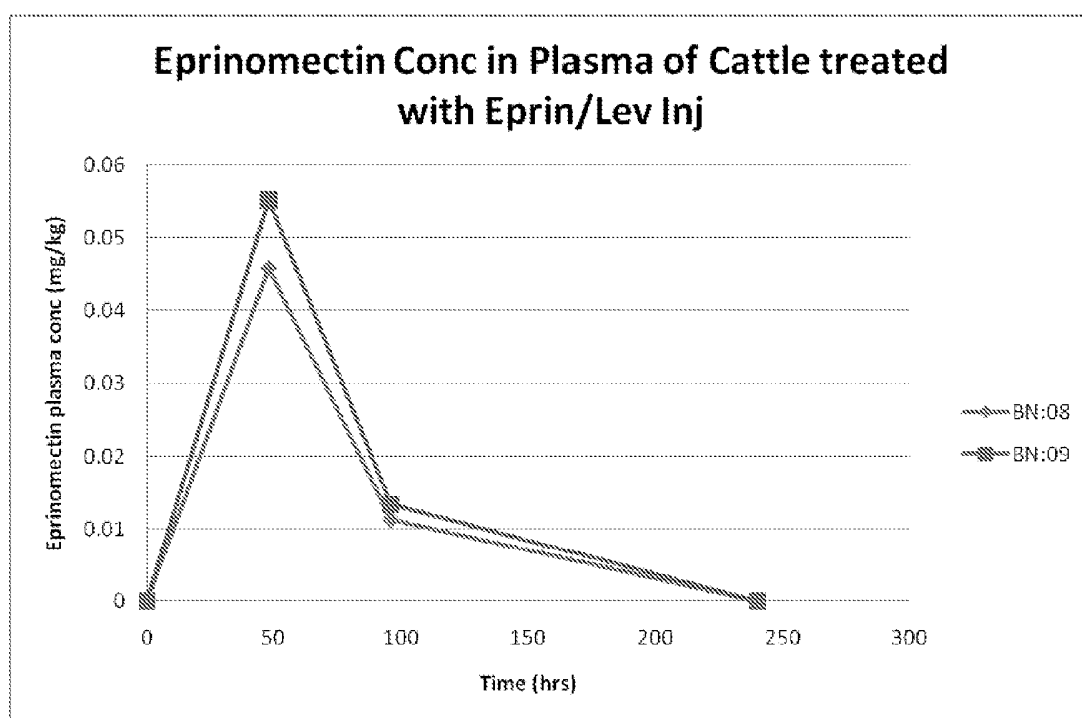


FIG. 1

**FIG. 2**

**FIG. 3**

REFERENCES CITED IN THE DESCRIPTION

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Szabadalmi igénypontok

1. Gyógyászatiilag vagy állatgyógyászatiilag elfogadható, állatba vagy emberbe történő injektálásra adaptált készítmény, amely készítmény tartalmazza:

- a. levamizol hatékony mennyiségét,
- b. legalább egy makrociklusos lakton hatékony mennyiségét,
- c. oldószerek és felületaktív anyagok gyógyászatiilag elfogadható rendszerét, ahol a rendszer mind a levamizol, mind a makrociklusos lakton elfogadható tárolási stabilitását biztosítja, és ahol a rendszer injektáláshoz megfelelő viszkozitást biztosít,

ahol a rendszer tartalmaz legalább egy felületaktív anyagot, ahol a felületaktív anyag CREMOPHOR EL,

és vízzel elegyedő szerves oldószert, ami dimetil-acetamid (DMA).

2. Az 1. igénypont szerinti készítmény, ahol a levamizol levamizol-foszfát és ahol a levamizol az állat vagy ember testtömegére számított legalább 4 mg/kg dózis bevitelére elegendő mennyiségben van jelen.

3. Az 1. igénypont szerinti készítmény, ahol a makrociklusos lakton ivermektin vagy eprinomektin, és az állat vagy ember testtömegére számított legalább 150 µg/kg dózis bevitelére elegendő mennyiségben van jelen.

4. Az 1. vagy 3. igénypont szerinti készítmény, amely tartalmaz továbbá antioxidánst, tartósítószert és injektálásra alkalmas vizet, ahol az injektálásra alkalmas víz olyan mennyiségben van biztosítva, amely elegendő ahhoz, hogy a készítményt megfelelő injekciós térfogatra egészítse ki.

5. Az 1-4. igénypontok bármelyike szerinti készítmény, ahol a makrociklusos lakton koncentrációja mintegy 0,1-2 vegyes%, a levamizol koncentrációja mintegy 15-30 vegyes% és a felületaktív anyag koncentrációja mintegy 1-10 vegyes%.

6. Az 5. igénypont szerinti készítmény, ahol a makrociklusos lakton eprinomektin és koncentrációja mintegy 0,5-0,9 vegyes%, a levamizol levamizol-foszfát és koncentrációja mintegy 18-25 vegyes%, a felületaktív anyag CREMOPHOR EL vagy CREMOPHOR EL és polisorbát 80 kombinációja, és koncentrációja mintegy 3-7 vegyes%, az antioxidáns BHT, az oldószer DMA és koncentrációja mintegy 1-10 vegyes% és a tartósítószer metil-parabén.

7. Az 1-6. igénypontok bármelyike szerinti készítmény, ahol a makrociklusos lakton eprinomektin és ahol az eprinomektin specifikációja olyan, hogy B1a összetétele 85% vagy annál nagyobb és B1a+B1b összetétele 90% vagy annál nagyobb; és ahol a készítmény sűrűsége 0,95-1,20 és pH-ja 3,0-5,0.

8. Gyógyászatiilag vagy állatgyógyászatiilag elfogadható, állatba vagy emberbe történő injektálásra adaptált készítmény, amely készítmény tartalmazza:

- a. levamizol hatékony mennyiségét,
- b. legalább egy makrociklusos lakton hatékony mennyiségét,
- c. oldószerek és felületaktív anyagok gyógyászatiilag elfogadható rendszerét, ahol a rendszer mind a levamizol, mind a makrociklusos lakton elfogadható tárolási stabilitását biztosítja, és ahol a rendszer injektáláshoz megfelelő viszkozitást biztosít,

ahol a rendszer tartalmaz legalább egy felületaktív anyagot, ahol a felületaktív anyag CREMOPHOR EL.

és vízzel elegyedő szerves oldószert, amely dimetil-acetamid (DMA), ahol a makrociklusos lakton eprinomektin és koncentrációja mintegy 0,5-0,9 vegyes%, a levamizol levamizol-foszfát és koncentrációja mintegy 18-25 vegyes%, a felületaktív szer polisorbát 80, CREMOPHOR EL vagy kombinációjuk, és koncentrációja mintegy 3-7 vegyes%, az antioxidáns BHT, az oldószer DMA és koncentrációja mintegy 1-10 vegyes%, és a tartósítószer metil-parabén.

9. Az 1-8. igénypontok bármelyike szerinti készítmény állatban vagy emberben belső élősködővel történő fertőződés megelőzésében vagy kezelésében történő alkalmazásra.

10. A 9. igénypont szerinti készítmény az ott meghatározott alkalmazásra, ahol a készítmény injektálva van.

11. A 9. vagy 10. igénypont szerinti készítmény az ott meghatározott alkalmazásra, ahol a makrociklusos lakton koncentrációja 0,5-0,9 vegyes% és a levamizol koncentrációja 15-25 vegyes%.

12. A 9. vagy 10. igénypont szerinti készítmény az ott meghatározott alkalmazásra, ahol az élősködő féreg.

13. A 12. igénypont szerinti készítmény az ott meghatározott alkalmazásra, ahol az élősködő féreg fonálféreg, galandféreg vagy mételevél.

14. Eljárás az 1. vagy 2. igénypont szerinti készítmény előállítására, amely tartalmazza a következő lépéseket:

- a. injektálásra alkalmas víz hozzáadása egy edényhez,
- b. a levamizol oldása a vízben,
- c. eprinomektin és BHT oldása DMA-ban egy másik edényben,
- d. a felületaktív anyag hozzáadása a DMA/eprinomektin elegyhez,
- e. az eprinomektin/DMA elegy átvitele a fő vízes fázisba keveréssel, és
- f. adott esetben az egység megfelelő térfogatra történő beállítása hozzáadott injektálásra alkalmas vízzel és keverés.

A meghatalmazott:

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