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(54) **Title:** ANTI-PARASITIC COMPOSITION COMPRISING A MACROCYCLIC LACTONE AND LEVAMISOLE AND METHOD OF TREATMENT OF PARASITIC INFESTATION

(57) **Abstract:** This invention relates to a veterinary antiparasitic solubilised composition including a macrocyclic lactone or a pharmaceutically equivalent salt thereof, and levamisole or a pharmaceutically equivalent salt thereof, characterised in that the pH of the composition is in the range of 2.0 to 5.0 and wherein the composition includes at least one surfactant and at least one antioxidant.

**ANTI-PARASITIC COMPOSITION COMPRISING A MACROCYCLIC LACTONE AND  
LEVAMISOLE AND METHOD OF TREATMENT OF PARASITIC INFESTATION**

**TECHNICAL FIELD**

This invention relates to an anti-parasitic composition.

**BACKGROUND ART**

5 In preferred embodiments of the present invention, this invention relates to a composition for the use in the treatment of non-human animals, such as domesticated and farm animals.

There is a considerable loss of productivity worldwide due to the effect of parasites on farm animals. Such parasites can include gastrointestinal round worms, lung worms, eye worms, parasitic stage grubs, biting and sucking lice, ticks, mites, screw worm and horn flies.

10 As a consequence, a considerable amount of money, time and effort has been spent in developing compositions to treat animals for these parasites.

Typically, treatment compositions are based upon having a broad spectrum macrocyclic lactone as the primary active ingredient.

15 However, with the wide spread usage of the macrocyclic lactones, resistance has built up in the parasites and treatment compositions are becoming less effective.

Increasing the dosage level is not a ready solution. Firstly, an increased dosage could cause significant side effects and may affect the health of the animal.

20 The main concern, however, is that increased dosages lead to residual drug concentrations in the animal being higher. This causes a longer withhold time on the treated animals before they can be slaughtered for human consumption. The flow on effect of the animals not being timely slaughtered increases herd management costs and subsequently consumer cost.

It should be therefore appreciated that there could be provided a composition and method of treatment that addresses the issues of combating resistance amongst parasites as well as minimal side effects and withhold times.

It is an object of the present invention to address the foregoing problems or at least to provide the public with a useful choice.

All references, including any patents or patent applications cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents form part of the common general knowledge in the art, in New Zealand or in any other country.

Throughout this specification, the word "comprise", or variations thereof such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Further aspects and advantages of the present invention will become apparent from the ensuing description which is given by way of example only.

#### **DISCLOSURE OF THE INVENTION**

According to one aspect of the present invention there is provided a veterinary anti-parasitic solubilised composition including

a macrocyclic lactone or a pharmaceutically equivalent salt thereof, and  
levamisole or a pharmaceutically equivalent salt thereof,  
characterised in that the pH of the composition is the range of 2.0 to 5.0 and wherein the composition includes at least one surfactant and at least one antioxidant.

According to another aspect of the present invention there is provided a method of treating non-human animals for parasitic infestation, the composition including a macrocyclic lactone or a pharmaceutically equivalent salt thereof, and levamisole or a pharmaceutically equivalent salt

thereof, wherein the pH of the composition is in the range of 2.0 to 5.0 and wherein the composition includes at least one surfactant and at least one antioxidant

characterised by the step of administering a veterinary anti-parasitic solubilised composition to a non-human animal in need thereof.

- 5 Preferred macrocyclic lactones are selected from the avermectin and milbemycin derivatives, including but not limited to abamectin, doramectin, eprinomectin, ivermectin, moxidectin, and milbemectin.

A particularly preferred macrocyclic lactone is doramectin, which is well recognised as being useful as an antiparasitic agent. A popular product which incorporates doramectin is  
10 Dectomax™, which is widely used on New Zealand cattle.

However, like many other macrocyclic lactones, doramectin is only fully effective against a proportion of parasites as a result of increasing resistance being built up. Despite this, there is still less resistance with doramectin than other products (such as ivermectin), and it is comparatively cost effective (compared to, for example Moxidectin).

- 15 Levamisole was chosen as the other active to be used in the composition because the inventors recognised that it would provide a synergistic effect by being effective against those parasites resistant to doramectin.

The combination of doramectin and levamisole is not one that has to the inventors' knowledge been contemplated because these two actives are thought to be incompatible with each other  
20 due to quite different pH ranges being preferred.

In preferred embodiments, the present invention is provided in the form of an injectable composition. However this can be administered via other methods such as orally or as a pour on.

A discussion of the trial results follows, but in summary significant and unexpected advantages  
25 were found by using the present invention in cattle, when compared against the use of the incumbent Dectomax™ formulation and untreated animals.

The applicants experience in the veterinary industry leads it to believe that the present invention will also be effective when applied to other farm species such as sheep. For example, they expect lower injection site residues as per cattle and better efficacy as resistance to doramectin in sheep becomes more common.

5 These results included a significant improvement in efficacy when doramectin resistance was found to be present. In particular, the present invention was highly effective against such resistant *Cooperia* and *Trichostrongylus* when compared to Dectomax™.

Another significant advantage found by the use of the present invention is that residues, specifically at the injection site, were found not to persist for as long and at the high levels found  
10 for a standard doramectin product.

In preferred embodiments of the present invention, the effective amount of macrocyclic lactone within the composition is in the order of 0.2 to 1.2% w/v. This compares with the effective amount of macrocyclic lactone (doramectin) in Dectomax™ which is in the order of 1% w/v.

In preferred embodiments of the present invention, the effective amount of levamisole in  
15 composition is the range of 6 to 27% w/v. This has been confirmed by the applicants to be an effective amount in terms of killing off parasites but also maintaining animal health and co-existing with doramectin in a stable solution.

It is envisaged that levamisole may be provided in the form of levamisole hydrochloride or levamisole phosphate. Levamisole phosphate is preferred as there are less irritation issues at  
20 site of injection than levamisole hydrochloride. In the composition it is preferred to be in the range of 10 to 40% w/v.

One of the difficulties of formulating with levamisole is that it prefers a pH of around about 3, whereas other actives tend to be stable in solution with a more neutral pH.

In preferred embodiment of the present invention, the pH range of the composition is in the  
25 order of 2.0 to 5.0.

More preferably the pH range of the composition is in the order of 3.0 to 4.5.

As will be seen in subsequent trial data, the careful choice of preferred excipients and concentrations was required to help maintain both the macrocyclic lactone and levamisole in solution and with an appropriate pH.

In preferred embodiments of the present invention, one of the excipients is a chelating agent to  
5 chelate any heavy metals which would facilitate the oxidation of actives. A preferred chelating agent is EDTA disodium, although other agents can be used. Other salts of EDTA may be used as well including sodium, calcium and potassium. Further chelating agents that may be used include citric acid monohydrate, fumaric and malic acid.

In preferred embodiments, the composition also includes an antioxidant. This is a preferred  
10 inclusion in the composition because it stabilise the actives in the product from oxidation. In particular, the inventors found the incorporation of an antioxidant to be beneficial in preventing degradation of the macrocyclic lactone, which may otherwise be unstable at the preferred low pH of the composition.

Suitable antioxidants include BHA, vitamin E, propyl gallate, TBHQ, sodium metabisulphate.

15 A preferred antioxidant is butylated hydroxytoluene (BHT) because BHT is the usual stabilizer for mectins.

Preferably, the antioxidant is present in the composition at or above 0.15% w/v.

More preferably, the antioxidant is present in the composition at or above 0.20% w/v.

Most preferably, the antioxidant is present in the composition at approximately 0.25% w/v.

20 Surprisingly the inventors found that the conventional amount of antioxidant (0.1 to 0.15 % w/v) used in injectable compositions may not be sufficient to stabilise the actives, and in particular the macrocyclic lactone. However, when the level of antioxidant was increased beyond conventional amounts, the stability may be significantly improved, which was difficult to achieve at this lower pH. It is thought that in combination with other excipients, such as stabilisers  
25 and/or surfactant, this stability may be synergistically improved.

Preferably, the composition also includes preservatives. Suitable preservatives include parabenz, thiomersals, cresols and chlorbutanol.

The preferred preservative is benzyl alcohol because it acts as a solubiliser and preservative at the same time.

- 5 Preferably, the composition also includes a stabiliser. Suitable stabilisers include polyols with preferred stabiliser being stabilised glycerine formal because it is already in a stabilised form.

In preferred embodiments, the composition also includes a surfactant which in the present invention assists in stabilising the macrocyclic lactone (e.g. doramectin) from the low pH of the product. It should be noted that the lower pH is required to stabilize the levamisole.

- 10 Preferably, the amount of surfactant in the composition is above 15% w/v.

More preferably, the amount of surfactant in the composition is approximately 20% w/v.

- This is different to many past compositions, particularly those which strived, albeit unsuccessfully, to achieve a stable solubilised composition at a low pH below 4.0 (for example NZ 508969). The inventors found that increasing the level of surfactant helped to stabilise the  
15 macrocyclic lactone, in particular, from crystallisation at a pH between 2.0 to 5.0.

Again, the inventors saw that the stability may be synergistically improved when the surfactant is included together with the stabiliser and/or antioxidant.

Suitable surfactants include polysorbate 20, cremophor and peg-12 oleate. The preferred surfactant is Polysorbate 80, it is soluble in water and widely used in injectables.

- 20 Aspects of the present invention will now be described by way of example only with reference to various experiments and trials conducted by the applicant.

### **BEST MODES FOR CARRYING OUT THE INVENTION**

- As alluded previously in the patent specification, it can be very difficult to formulate a stable composition containing an effective amount of macrocyclic lactone (such as doramectin) and  
25 levamisole.

With regard to the present invention, it should be appreciated that the inventors are highly experienced formulation chemists particularly in the field of veterinary medicine. It is therefore significant that it took over forty attempts at formulating the present invention before a formulation was found that was storage stable and did not have crystal formation at key temperatures.

Table 1 below is a summary of some of the trialled formulations.

**TABLE 1: Trialled Formulations**

| no | Ingredients                 | PF-1 | PF-2  | PF-3 | PF-4  | PF-5 | PF-6  | PF-7  | PF-8  | PF-9  | PF-10 | PF-11 | PF-12 | PF-13 |
|----|-----------------------------|------|-------|------|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|
|    |                             | %w/v | %w/v  | %w/v | %w/v  | %w/v | %w/v  | %w/v  | %w/v  | %w/v  | %w/v  | %w/v  | %w/v  | %w/v  |
| 1  | Doramectin**                | 0.42 | 0.525 | 0.42 | 0.525 | 0.42 | 0.525 | 0.525 | 0.525 | 0.525 | 0.63  | 0.84  | 0.525 | 0.525 |
| 2  | Levamisole Hydrochloride ** | 15.8 | 21    | 15.8 | 21    | 15.8 | 21    | 21    | 21    | 21    | 31.5  | 42    | 0     | 0     |
| 3  | Levamisole Phosphate**      | 0    | 0     | 0    | 0     | 0    | 0     | 0     | 0     | 0     | 0     | 0     | 26.25 | 26.25 |
| 4  | Disodium edetate            | 0    | 0     | 0.1  | 0.1   | 0.1  | 0.1   | 0.1   | 0.1   | 0.1   | 0.1   | 0.1   | 0.1   | 0.1   |
| 5  | Butylated hydroxyl toluene  | 0.25 | 0.05  | 0.25 | 0.25  | 0.25 | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  |
| 6  | Benzyl alcohol              | 0    | 0     | 2.5  | 2.5   | 2    | 2.5   | 3     | 3     | 3     | 4     | 4     | 3     | 3     |
| 7  | Glycerine formal stabilised | 40   | 40    | 0    | 0     | 0    | 0     | 0     | 0     | 20    | 0     | 0     | 0     | 20    |
| 8  | Propylene glycol            | qs   | qs    | 0    | 0     | 0    | 0     | 0     | 20    | 0     | 20    | 20    | 20    | 0     |
| 9  | Polysorbate 80              | 0    | 0     | 8    | 8     | 10   | 12    | 20    | 20    | 20    | 20    | 20    | 20    | 20    |
| 10 | Citric acid                 | 0    | 0     | 2    | 2     | 2    | 2     | 2.2   | 2.2   | 2.2   | 2.2   | 2.2   | 2.2   | 2.2   |
| 11 | Sodium hydroxide            | 0    | 0     | 0.6  | 0.6   | 0.6  | 0.6   | 0.6   | 0.5   | 0.5   | 0.4   | 0.4   | 0.3   | 0.3   |
| 12 | Water for injection         | 0    | 0     | qs   | qs    | qs   | qs    | qs    | qs    | qs    | qs    | qs    | qs    | qs    |

\*\* 5% overages added

PF-01 - solvent based - stability issue -not stable

PF-02- solvent based - stability issue -not stable

PF-03- water based- stability issue-not stable

PF-04- water based- stability issue-not stable

PF-05- water based- stability issue-not stable

PF-06- water based- stability issue-not stable

PF-07,08 & 09- water based- crystal formation at 4°C

PF-11- water based- changed to levamisole phosphate- due to the irritation of levamisole hydrochloride

PF-12- water based- crystal formation at 4 & 40°C

Different excipients, concentrations of actives and the buffer system were varied to determine how the stability of the macrocyclic lactone and levamisole actives were affected.

Surprisingly it was found that it is possible to prevent or significantly decrease degradation of a macrocyclic lactone an aqueous composition at low pH. This permits the use of a pH suitable

for stabilising the levamisole component dissolved in the aqueous composition. This approach is preferred over a compromise that would require a pH in-between the low value suitable for levamisole and a neutral pH suitable for a macrocyclic lactone.

Even more surprisingly, it was found that the combination of macrocyclic lactone and levamisole could be stabilised in a composition containing the relatively high concentrations of actives that would generally be required for an injectable. The development of a composition suitable for injection can be complicated by the desire to increase the concentration of actives as compared to other types of compositions, to limit or reduce the volume of the composition that needs to be injected.

The macrocyclic lactone is solubilised using solvent that is fully soluble within the composition. Accordingly a true solution or micellar solution is formed rather than an emulsion. A preferred solvent is benzyl alcohol, which is an effective solvent for macrocyclic lactones, and also functions as a preservative. The solubility of the solvent within the composition can be increased with the use of co-solvents, such as the water miscible solvents glycerine formal and propylene glycol, which can also function as stabilisers. A skilled person would readily recognise other solvents systems that would also be suitable.

Using surfactant to enhance the solubility and/or stability of the actives is particularly preferred. The formation of a micellar solution is a preferred method of solubilising the macrocyclic lactone within the aqueous composition.

Table 2 below represents a preferred formulation in accordance with the present invention. This formulation has doramectin and levamisole in quantities that were shown to be highly effective against parasites. The formulation is stable without crystal formation and has minimal, if any, side effects on the animals.

**TABLE 2: Preferred Formulation**

| DL Injection    |            |                |          |
|-----------------|------------|----------------|----------|
| Ingredient name | CAS number | Quantity (g/L) | Function |
|                 |            |                |          |

|                                |                        |      |                   |
|--------------------------------|------------------------|------|-------------------|
| Doramectin                     | 117704-25-3            | 4.2* | Active ingredient |
| Levamisole phosphate           | 32093-35-9             | 210* | Active ingredient |
| EDTA disodium                  | 139-33-3               | 1    | Chelating agent   |
| Butylated hydroxytoluene (BHT) | 128-37-0               | 2.5  | Antioxidant       |
| Benzyl alcohol                 | 100-51-6               | 35   | Preservative      |
| Glycerine formal stabilised    | 4740-78-7<br>5464-28-8 | 200  | Stabiliser        |
| Polysorbate 80                 | 9005-65-6              | 200  | Surfactant        |
| Citric acid                    | 77-92-9                | 22   | pH adjuster       |
| Sodium hydroxide               | 1310-73-2              | 3    | pH adjuster       |
| Water for injection            | 7732-18-5              | q.s. | Solvent/vehicle   |

\* 5% overage added

The pH of the composition was adjusted to around 3.9 using the citrate buffer system.

In particular, it was noted that to achieve stability and to keep the formulation in solution, a high percentage of surfactant was required. Without the presence of a surfactant in sufficient quantity, crystallisation and colour changes in the solution were observed.

## 10 Manufacturing method

A preferred method of manufacturing the preferred embodiment comprises the steps of:

Step 1:

- a. In a clean and dry manufacturing vessel load 30% of water for injection
- b. Add and dissolve EDTA disodium with stirring
- c. Add and dissolve citric acid with stirring

15

- d. Add and dissolve sodium hydroxide with stirring
- e. Add and mix glycerine formal stabilized
- f. Add and dissolve levamisole phosphate with stirring
- g. Check the clarity of the solution

5 Step 2:

- h. In a separate clean and dry manufacturing vessel load benzyl alcohol and heat to 50 – 55 °C
- i. Add and dissolve BHT with stirring
- j. Add and dissolve doramectin with stirring

- 10 k. Add warm Polysorbate 80 and mix well
- l. Check the clarity of the solution

Step 3:

- m. Add 'Step 2' to 'Step 1' and mix well
- n. Rinse the vessel from Step 2 with some water for injection and add the rinsate to the  
15 main bulk
- o. Make up the final volume with water for injection and stir well
- p. Check the pH – it should be 3.6 – 4.2

A considerable number of trials were conducted using a formulation in accordance with the present invention and comparing its effects against Dectomax™ and a control untreated group.

- 20 Table 3 below shows the mean faecal egg counts found in non-slaughtered cattle following treatment with the present invention (DL injection) and Dectomax™. This is from a sample size of 36 cattle.

As can be clearly seen, the egg counts 35 days after treatment for the present invention are still zero, whereas the Dectomax™ treated and the untreated cattle have significant faecal egg counts. This suggested resistance may be present in this trial.

5 **TABLE 3: Mean Faecal Egg Counts in the non-slaughtered cattle following treatment with DL Injection and Dectomax**

| Product applied | Days after treatment |                |                |                |                |                 |                 |                |
|-----------------|----------------------|----------------|----------------|----------------|----------------|-----------------|-----------------|----------------|
|                 | -3                   | 7              | 14             | 21             | 28             | 35              | 42              | 49             |
| DL Injection    | 121.4                | 0<br>(100)     | 0<br>(100)     | 0<br>(100)     | 0<br>(100)     | 0<br>(100)      | 50.0<br>(73.1)  | 50.0<br>(70.8) |
| Dectomax        | 128.6                | 57.1<br>(71.5) | 21.4<br>(85.7) | 28.6<br>(80.0) | 57.1<br>(63.7) | 107.1<br>(40.0) | 135.7<br>(26.9) | 128.6<br>(25)  |
| Untreated       | 128.6                | 200.0          | 150.0          | 142.9          | 157.1          | 178.6           | 185.7           | 171.4          |

Table 4 below shows the mean faecal egg counts in slaughtered cattle of the same trial. Again, it indicates that the present invention may have significantly improved results when doramectin

10 resistance is present compared to the industry's standard of Dectomax™.

15 **TABLE 4: Mean Faecal Egg Counts in the slaughtered cattle following treatment with DL Injection, Dectomax or untreated**

| Product applied | Days after treatment |                |                |
|-----------------|----------------------|----------------|----------------|
|                 | -3                   | 7              | 13/14          |
| DL Injection    | 288                  | 0<br>(100)     | 0<br>(100)     |
| Dectomax        | 250                  | 22.2<br>(90.5) | 77.8<br>(67.0) |

|           |       |       |       |
|-----------|-------|-------|-------|
| Untreated | 271.4 | 233.3 | 235.7 |
|-----------|-------|-------|-------|

Likewise, Table 5 shows significant differences between the effect of the present invention on larval cultures compared with Dectomax™.

**TABLE 5: Quantitative larval culture data for 50g samples collected at varying times after treatment with DL Injection, Dectomax or untreated in the non-slaughtered cattle**

| Product applied | Days after Treatment |                |               |            |                |                |                |
|-----------------|----------------------|----------------|---------------|------------|----------------|----------------|----------------|
|                 | -3                   | 7              | 15            | 21         | 28             | 35             | 42             |
| DL Injection    | 22000                | 0<br>(100)     | 15<br>(99.7)  | 0<br>(100) | 8<br>(99.8)    | 0<br>(100)     | 6500<br>(19.8) |
| Dectomax        | 22000                | 7600<br>(19.1) | 490<br>(90.9) | 410 (94.6) | 2300<br>(53.1) | 1100<br>(64.5) | 3100<br>(61.7) |
| Untreated       | 22000                | 9400           | 5400          | 7600       | 4900           | 3100           | 8100           |

5 Number in brackets refers to the % control compared with the untreated group.

The interpretation of these results along with more detailed data (not provided in the specification) is given below.

The quantitative measuring of larval numbers can provide a more sensitive measure of the efficacy of the test products than egg counts, and the results in the non-slaughtered cattle indicated that DL injection appeared to effectively suppress the excretion of viable eggs for a period of at least 35 days.

Significantly, the level of control indicated by this single ingredient doramectin product was relatively low throughout the study, most likely because of resistant *Trichostrongylus* and *Cooperia*.

15 The quantitative larval culture results, also provided information about the species make up of the eggs being excreted. In both the slaughter and non-slaughter parts of the study, *Cooperia* were the dominant species of larvae found in the group treated with Dectomax 7, 13/14, 15, 21 and 28 days after treatment. In addition, significant numbers of *Trichostrongylus* larvae were

found in the cattle treated with Dectomax at each of the post treatment samplings. Interestingly, this trend is consistent with the pattern found in the total worm count results.

As mentioned previously, the present invention appeared to be particularly effective in targeting doramectin resistant species and this was confirmed by the total worm count as can be seen in

5 Table 6.

**TABLE 6: Efficacy (%) <sup>1</sup> of the test and reference products against, *Trichostrongylus* spp. and *Cooperia* spp. based on total worm counts**

| Product      | Parasite species and life stage |   |                      |
|--------------|---------------------------------|---|----------------------|
|              | <i>Trichostrongylus axei</i>    | Small intestinal <i>Trichostrongylus</i> spp. | <i>Cooperia</i> spp. |
| DL Injection | >99.9                           | >99.9   | >99.9                |
| Dectomax     | 79.3                            | 83.8  | 97.6                 |

<sup>1</sup>based on geometric means

The presence of doramectin resistant species in this trial is unequivocally confirmed for  
 10 abomasal and small intestinal *Trichostrongylus* spp. when the individual total worm counts collected at slaughter for Dectomax is compared with the present invention (DL injection), see Table 7 below. The results, from the individual worm counts and % reduction in arithmetic means, against *Cooperia* are also very suggestive of emerging resistance to doramectin by this species. It can also be noted *Cooperia* spp. resistance to macrocyclic lactones has been  
 15 documented in cattle in New Zealand.

**Table 7:**

| DL Injection |                              |   |                      |
|--------------|------------------------------|---|----------------------|
|              | <i>Trichostrongylus axei</i> | Small intestinal <i>Trichostrongylus</i> spp. | <i>Cooperia</i> spp. |
| Tag No       | 5 <sup>th</sup> Stage        | 5 <sup>th</sup>                               | 5 <sup>th</sup>      |
| 48           | -                            | -   | -                    |
| 57           | -                            | -   | -                    |
| 239          | -                            | -   | -                    |
| 242          | 50                           | -   | -                    |

|                 |                 |                 |                 |
|-----------------|-----------------|-----------------|-----------------|
| 309             | -               | -               | -               |
| 438             | -               | -               | -               |
| PVS080          | -               | -               | -               |
| R74             | -               | -               | -               |
| <b>AM</b>       | <b>6.3</b>      | <b>0.0</b>      | <b>0.0</b>      |
| <b>GM</b>       | <b>0.6</b>      | <b>0.0</b>      | <b>0.0</b>      |
| <b>% Red AM</b> | <b>&gt;99.9</b> | <b>&gt;99.9</b> | <b>&gt;99.9</b> |
| <b>% Red GM</b> | <b>&gt;99.9</b> | <b>&gt;99.9</b> | <b>&gt;99.9</b> |

| <b>Dectomax</b>                           |             |              |              |
|---|-------------|--------------|--------------|
| 59  | 450         | 200          | 450          |
| 236                                       | 600         | -            | 400          |
| 237                                       | 500         | 250          | 8600         |
| 560                                       | 11000       | 100          | -            |
| 573                                       | 15500       | 450          | -            |
| 1039                                      | 850         | 350          | 2750         |
| 2066                                      | 6250        | 200          | -            |
| 65  | 300         | 100          | 5150         |
| 233                                       | 3750        | -            | 250          |
| <b>AM</b>                                 | <b>4356</b> | <b>183.3</b> | <b>1956</b>  |
| <b>GM</b>                                 | <b>1728</b> | <b>63.1</b>  | <b>120.8</b> |
| <b>% Red AM</b>                           | <b>71.9</b> | <b>76.5</b>  | <b>83.8</b>  |
| <b>% Red GM</b>                           | <b>79.3</b> | <b>83.8</b>  | <b>97.6</b>  |
| AM = arithmetic mean, GM = geometric mean |             |              |              |

In particular, the results can be summarised as below:

- 5 1. DL Injection provided >99.9% control based on geometric means of *Ostertagia* spp, *Trichostrongylus axei*, small intestinal *Trichostrongylus* spp, *Cooperia* spp, *Oesophagostomum* spp, *Chabertia* sp and *Trichuris* spp.
2. Dectomax™ and DL Injection provided comparable levels of control of *Ostertagia* spp, *Oesophagostomum* spp, *Chabertia* sp and *Trichuris* spp.

3. Dectomax™ provided low levels efficacy of *T. axei* and small intestinal *Trichostrongylus* spp that were significantly different to those provided by DL Injection

It can clearly be seen that the present invention is highly effective in its own right, but also in comparison with the current product where resistance to Doramectin is present.

5 Use of the present invention also has the added advantage of having much lower doramectin residues than a standard doramectin product particularly at the injection site. The JECFA (1996) Doramectin Monograph, in 'Residues of Some Veterinary Drugs in Animals and Foods, FAO Food and Nutrition Paper', No 41/8, pp 85-98 noted high concentrations of doramectin at the injection site during the 35 day period after parental administration of the unlabelled dose of  
 10 a doramectin injection at the recommended label rate. The carrier used in this injection composition comprised 75% sesame oil and 25% ethyl oleate.

Within that report, table 8, shown below for day 21, 28 and 35, had mean doramectin residues at the injection site of 1900, 380 and 930 ug/kg after 21, 28 and 35 days respectively after treatment of unlabelled doramectin at 0.2mg/kg bodyweight.

15 **Table 8: Residues of Doramectin (ug/kg) in tissues of cattle after treatment with unlabelled doramectin at 0.2mg/kg bodyweight.**

| Tissue    | Day 21 | Day 28 | Day 35 |
|-----------|--------|--------|--------|
| Muscle    | <7     | <4     | <3     |
| Liver     | 107    | 66     | 29     |
| Kidney    | 11     | 8.8    | <4.5   |
| Fat       | 182    | 94     | 57     |
| Injection | 1900   | 380    | 930    |

In comparison to that treatment of cattle with our DL Injection had average doramectin residues in the injection site of 6.1, 13.7 and 2.8 ug/kg. Our tissue levels were also lower as shown in

20 Table 9 below.

**Table 9: Average doramectin residues (ug/kg) in various tissues of cattle treated with DL injection at 0.25 mg/kg b.w. doramectin**

| Tissue site | Day 21 | Day 28 | Day 35 |
|-------------|--------|--------|--------|
| Muscle      | 2.5    | 2.5    | 2.5    |

|                  |      |      |     |
|------------------|------|------|-----|
| Liver            | 15   | 2.5  | 2.5 |
| Kidney           | 4.8  | 2.5  | 2.5 |
| Peri-renal fat   | 19.1 | 2.5  | 2.5 |
| Subcutaneous fat | 28.0 | 2.5  | 2.5 |
| Injection site   | 6.1  | 13.7 | 2.8 |

A preferred dosage regime that provided all of the previous results is provided in Table 10 below.

**TABLE 10: Doramectin + Levamisole Injection Label Dosage Instructions**

| Weight (kg) | Vol (mL) |
|-------------|----------|
| 61-70       | 3.5      |
| 71-80       | 4.0      |
| 81-100      | 5.0      |
| 101-120     | 6.0      |
| 121-140     | 7.0      |
| 141-160     | 8.0      |
| 161-180     | 9.0      |
| 181-220     | 11.0     |
| 221-260     | 13.0     |
| 261-300     | 15.0     |

The dose rate for DL injection for cattle greater than 300kg will be 1mL/20kg (0.2mg/kg doramectin and 10mg/kg levamisole phosphate).

Table 11 is the Dectomax™ label dosage instructions.

**TABLE 11: Dectomax label dosage Instructions**

| Liveweight | Dose        |
|------------|-------------|
|            | Volume (mL) |
| 40-50      | 1           |
| 51-75      | 1.5         |
| 75-100     | 2.0         |

|         |     |
|---------|-----|
| 101-150 | 3.0 |
| 151-200 | 4.0 |
| 201-250 | 5.0 |
| 251-300 | 6.0 |
| 301-350 | 7.0 |
| 351-400 | 8.0 |

Cattle heavier than 400kg are to be dosed at the rate of 1 mL/50kg.

**Site reactions to DL injections**

A safety study was performed to evaluate the safety of the preferred formulation of the invention. The study was performed on four groups of cattle when dosed subcutaneously at 1x  
 5 with a single dose and with a split dose, and 2x the maximum label dose rate, and intramuscularly at 1x the maximum label dose rate.

Injection site swellings were seen in most of the groups for the first four days following treatment. The resolution of the swelling at the injection sites was almost complete at seven days for the animals in the intramuscular injection group, but continued to resolve over the next  
 10 14 days following treatment for animals in the other groups.

It was noted that a large proportion of the swellings were only detected because the site of injection was carefully examined as part of the requirements of the study. However, under normal farm conditions where the site of injection is not viewed closely it was observed by the Investigator and the farmer that the majority of the site reactions that occurred in the current  
 15 study would not have been noticed. It was concluded that administration of the DL injection, as recommended, was not associated with undue pain, inflammation, or distress.

Aspects of the present invention have been described by way of example only and it should be appreciated that modifications and additions may be made thereto without departing from the scope thereof as defined in the appended claims.

**WHAT WE CLAIM IS:**

1. A veterinary antiparasitic solubilised composition including  
  
a macrocyclic lactone or a pharmaceutically equivalent salt thereof, and  
  
levamisole or a pharmaceutically equivalent salt thereof,  
  
characterised in that the pH of the composition is in the range of 2.0 to 5.0 and wherein the composition includes at least one surfactant and at least one antioxidant.
2. A composition as claimed in claim 1 wherein the effective amount of levamisole in the composition is in the range of 6.0% to 27.0% w/v.
3. A composition as claimed in either claim 1 or claim 2 wherein the levamisole is provided in the form of levamisole hydrochloride.
4. A composition as claimed in any one of claims 1 to 3 wherein the levamisole is provided in the form of levamisole phosphate.
5. A composition as claimed in any one of claims 1 to 4 wherein the macrocyclic lactone is in the range of 0.2 to 1.2 % w/v.
6. A composition as claimed in any one of claims 1 to 5 which includes a chelating agent.

7. A composition as claimed in claim 6 wherein the chelating agent is EDTA disodium.
8. A composition as claimed in any one of the above claims wherein the antioxidant is butylated hydroxytoluene.
9. A composition as claimed in any one of the above claims wherein the concentration of antioxidant in the composition is above 0.15% w/v.
10. A composition as claimed in any one of claims 1 to 9 which includes a preservative.
11. A composition as claimed in claim 10 wherein the preservative is benzyl alcohol.
12. A composition as claimed in any one of claims 1 to 11 wherein the composition includes a stabiliser.
13. A composition as claimed in claim 12 wherein the stabiliser is glycerine formal.
14. A composition as claimed in any one of the above claims wherein the concentration of surfactant in the composition is above 15% w/v.
15. A composition as claimed in any one of the above claims wherein the concentration of surfactant in the composition is approximately 20% w/v.
16. A composition as claimed in any one of the above claims wherein the surfactant is Polysorbate 80.
17. A method of treating non human animals for parasitic infestation  
  
characterised by the step of

administering the veterinary composition as claimed in any one of claims 1 to 16.

18. A method of treating non human animals as claimed in claim 17 wherein the composition is administered by injection.
19. A composition substantially as herein described with reference to and as illustrated in the Best Modes section of the specification.
20. A method of treating non-human animals substantially as herein described with reference to and as illustrated in the Best Modes section of the specification.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ2012/000104

## A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/366 (2006.01) A61K 31/429 (2006.01) A61P 33/00 (2006.01)

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

EPOQUE (EPODOC, WPI, MEDLINE, NPL); STN (CAPLUS, AGRICOLA):

Levamisole, ergamisol, R12564, 14769-73-4, tetramisole, 32093-35-9, macrocyclic lactone, avermectin, ivermectin, abamectin, doramectin, eprinomectin, selamectin, milbemycin, moxidectin, nematictin, dectomax, parasite, ectoparasite, worm, eyeworm, lungworm, kidneyworm, roundworm, heartworm, hookworm, whipworm, grub, louse, lice, tick, mite, fly, flies, anthelmintic, antihelminth, pesticide, insecticide, acaricide, nematode, cooperia, trichostrongylus, surfactant, polysorbate, cremophor, wetting agent, emulsifier, antioxidant, butylated hydroxytoluene, BHA, BHT, vitamin E, tocopherol, propyl gallate, sodium metabisulfate, sodium metabisulphite, tert butylhydroquinone, stabiliser, stabilizer, pH

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category*   | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|--|-----------------------|
| Documents are listed in the continuation of Box C |  |                       |



Further documents are listed in the continuation of Box C



See patent family annex

|   |  |
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| * Special categories of cited documents:  | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  |
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| "P" document published prior to the international filing date but later than the priority date claimed  |  |
| Date of the actual completion of the international search<br>17 August 2012   | Date of mailing of the international search report<br>22 August 2012   |
| Name and mailing address of the ISA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>Email address: pct@ipaustalia.gov.au<br>Facsimile No.: +61 2 6283 7999 | Authorized officer<br>Michael Grieve<br>AUSTRALIAN PATENT OFFICE<br>(ISO 9001 Quality Certified Service)<br>Telephone No. +61 2 6283 2267  |

## INTERNATIONAL SEARCH REPORT

International application No.

C (Continuation).

DOCUMENTS CONSIDERED TO BE RELEVANT

**PCT/NZ2012/000104**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.  |
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| X<br>Y    | WO 2010/021555 A1 (SCHERING-PLOUGH ANIMAL HEALTH LIMITED) 25 February 2010<br>Claims 1, 4, 6, 22; page 5<br>Claims 1, 4, 6, 22; page 5   | 1 to 3, 5, 9 to 12, 14 to 15<br>and 17 to 20<br>4, 6 to 8, 13 and 16 |
| Y         | WO 2008/072985 A2 (BOMAC RESEARCH LIMITED) 19 June 2008<br>Claims 19 to 21; page 8 lines 16 to 18, page 9 line 8, page 15 line 1         | 4, 8, 13 and 16  |
| Y         | WO 2004/009080 A1 (ASHMONT HOLDINGS LIMITED) 29 January 2004<br>Study 7 and 8, pages 11 to 13  | 6 and 7  |
| P,X       | WO 2011/143479 A1 (MERIAL LIMITED) 17 November 2011<br>Claims 1, 2, 4, 5, 6, 7; page 12 lines 6, 10 to 11 and 13 to 21, page 29 Table 10 | 1 to 5, 8 to 12 and 16 to 20   |
| P,X       | NZ 570591 A (SCHERING-PLOUGH ANIMAL HEALTH LIMITED) 12 January 2012<br>Claim 1, 6, 8, 22; page 6 line 15                                 | 1 to 3, 5, 9 to 12, 14 to 15<br>and 17 to 20                         |

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

**PCT/NZ2012/000104**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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**End of Annex**

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

Form PCT/ISA/210 (Family Annex)(July 2009)