Title: COMPOSITIONS FOR CONTROLLING PARASITES COMPRISING A COMBINATION OF ABAMECTIN AND MILBEMYCIN

Abstract: A long acting composition for controlling parasites in and on animals, which comprises in a slow release vehicle a combination of ivermectin and abamectin, wherein the total percentage w/v of ivermectin and abamectin equals or exceeds 3% w/v and the concentration of ivermectin is higher than the concentration of abamectin.
COMPOSITIONS FOR CONTROLLING PARASITES COMPRISING A COMBINATION OF ABAECTIN AND MILBEMYCIN

The present invention relates to long acting compositions for controlling parasites, comprising a combination of ivermectin and abamectin in a slow release vehicle, the use of such compositions in the preparation of a medicament for controlling parasites.

Avermectins are well known anthelmintic compounds, belonging to the class of macrocyclic lactones. The avermectins are isolated from fermentation products of Streptomyces avermitilis and ivermectin is a compound which is a semisynthetic chemical compound formed by modification of avermectin. The basic structure of the avermectins is a 16-membered lactone ring to which are appended three main substituent groups: a hexahydrobenzofuran group, a disaccharide group (at C-13) and a spiroketal ring (C-17 to C-28). The avermectin series of compounds, including ivermectin, abamectin, doramectin, eprinomectin and selamectin, are potent anthelmintic and antiparasitic agents against internal and external parasites (endectocides). The natural product avermectins are disclosed in US-A-4,310, 519 to Albers Schonberg et al., and the 22,23-dihydro avermectin compounds are disclosed in Chabala et al., US-A- 4,199, 569.

Endoparasites cause widespread disease found in many animals. The most frequently encountered endoparasites are the group of worms referred to as nematodes. The nematodes are found in the intestinal tract, heart, lungs, blood vessels and other body tissues of animals and are a primary cause of anemia, weight loss and malnutrition in the infected animals. The nematodes most commonly found to be the infecting agents of ruminants include Haemonchus and Ostertagia generally found in abomasum; Cooperia, Trichostrongylus and Nematodirus generally found in the intestinal tract, and Dictyocaulus found in the lungs. Ectoparasites commonly infecting warm-blooded animals are arthropods such as ticks (e.g. Boophilus microplus), mites, lice, fleas, blowfly, and migrating dipterous larvae such as Hypoderma sp. in cattle. Treatment of animals to prevent infestation by any of the above-mentioned parasites, or to reduce or control the proliferation of these parasites in animals is thus important.

Meanwhile the problem has arisen that some parasites develop a resistance to drugs like ivermectin. The resistance occurs when a strain of a parasite is able to tolerate
doses of an active ingredient that is efficacious against other populations of parasites of the same species. This characteristic is inheritable.

After the use of macrocyclic lactones for almost two decades in cattle in Brazil the first reports on resistant parasites in cattle were published. In 2001 in Rio Grande do Sul State, Martins & Furlong observed low efficacy of doramectin, ivermectin and moxidectin against a strain of Boophilus microplus. In 2001 in Santa Catarina State, Souza et al. tested the efficacy of bovine anthelmintic on 7 farms (egg counting). Resistance to ivermectin was detected in 4 of them (3 to Cooperia and 1 to Haemonchus).

In vitro trials demonstrated resistance of H. placei and C. punctata to ivermectin in São Paulo State. The resistance to Cooperia and Haemonchus was also shown in other trials (CARDOSO et al., 2002). The endoparasites of the genus Cooperia and Haemonchus showed resistance to ivermectin in bovines in 11 farms of 12 evaluated in São Paulo State (SOUTELLO et al, 2003). These trials demonstrated that the problem of bovine parasites resistant to avermectins, particularly to ivermectin, might soon become as serious as it is in ovine species.

The discovery of novel anti-parasitics with equal or better qualities than macrocyclic lactones seems to be a distant reality in the veterinary pharmaceutical industry.

Therefore, the chemical groups available nowadays must be used in a rational way, with a view to achieving high percentages of efficacy against parasites and delaying the occurrence of resistant strains (GEARY & THOMPSON, 2003).

The avermectins have similar modes of action. Nevertheless, small differences in their structures affect their therapeutic efficiency. Where ivermectin resistant parasites will usually still be vulnerable to abamectin, abamectin has the disadvantage that it cannot be used at higher concentrations since it is toxic at higher concentrations. The use of low concentrations of abamectin however, has the disadvantage that the formulations based on abamectin only are short acting.

Many different concepts of prolonged release of pharmaceutical compositions in animals have been described, e.g. use of low water soluble forms or complexes of active ingredients, use of liposomes, microspheres and lipospheres formulations, polymer formulations, oil based formulations, gel formulations etc. Some of them
have been proposed for the use with endo and / or ectoparasitic compounds. Several oil formulations have been suggested in prior art for long acting formulations, e.g. ethyl oleate or sesam oil formulations.

5 A combination of ivermectin and abamectin for antiparasitic use in bovines has been mentioned in Brazilian Patent Application No. PI 0104761-2, that was filed on August 8, 2001 and published on August 12, 2003. This document does not disclose any specific amounts and ratio of the compounds of the combination that allows the composition to release ivermectin and avermectin in a way to show the desired effect even in parasites that are resistant to certain macrocyclic lactone compounds.

It would therefore be desirable to have a long acting composition available that allows the release of an effective amount of the specific combination of ivermectin and avermectin over a prolonged time period that are suitable to control parasites that show resistance to certain macrocyclic lactone compounds.

It has now been found that the combination of ivermectin and abamectin in defined concentration ranges in a long acting vehicle comprising castor oil result in such a broad-spectrum antiparasitic composition with long acting activity.

The present invention concerns a long acting composition for controlling parasites in and on animals, which comprises in a slow release vehicle a combination of ivermectin and abamectin, characterized in that the total percentage w/v of ivermectin and abamectin equals or exceeds 3% w/v and the concentration of ivermectin is higher than the concentration of abamectin.

In one embodiment the present invention provides a composition for controlling endo- and ecto-parasites in and on animals, which comprises as active ingredient, an effective amount of a combination of ivermectin and abamectin in the following concentrations, 1.75-5% w/v ivermectin, 0.5-2% w/v abamectin, the composition further comprising 30-60 % w/v castor oil, a suitable amount of a solvent for the active ingredients, optionally other pharmaceutically acceptable ingredients, and up to 100 % v/v of a diluent.

By "w/v" is meant weight/volume, i.e. "1% w/v" means 1 g in 100 ml of the composition. "v/v" means volume per volume, and 1% v/v means 1 ml, in a total of 100 ml.
Good results were obtained with a composition comprising 2.25% ivermectin and 1.25% abamectin, together amounting to a total concentration of 3.5% w/v of active ingredient. When benzyl alcohol is used as the solvent in a composition comprising 2.25% w/v ivermectin and 1.25% w/v abamectin,

Preferably the composition comprises a long acting vehicle that employs castor oil. Castor oil (ricinus oil, oleum ricini, ricinoleum, tangantangan) is a triglyceride of fatty acids. The fatty acid composition is approximately ricinoleic acid (87%); oleic acid (7%); linoleic acid (3%); palmitic acid (2%); stearic acid (1%) and trace amounts of dihydroxy stearic acid. Castor oil is the fixed oil obtained by cold-expression of the seeds of Ricinus communis Linnè (Fam. Euphorbiaceae). (Rowe R C et al: Handbook of Pharmaceutical Excipients, London, Pharmaceutical Press, GB, page 104-105).

The concentration of castor oil in the compositions according to the invention is between 30 and 60 %w/v. By raising or lowering the amount of the castor oil in the formulation the viscosity of the formulation is adjusted and the slow release effect is influenced. For example, by raising the concentration of castor oil, the viscosity is increased and the rate at which the active ingredients are released from the depot is lowered. Good results were obtained with concentrations of castor oil of 50.4% w/v (52.5 ml on a total of 100 ml) and 33.6 %w/v (35 ml on a total of 100 ml).

As shown in a pharmacokinetics study in example 4 and 5, although both sesame and castor oil are prolonged action vehicles, the castor oil based formulation provides a better long acting profile for a macrocyclic lactone based formulation. Each oil promoted a different ivermectin and abamectin release pattern that is possible to verify by Tmax and t1/2 values, which are 229.70h and 261.70h for formulation using castor oil and 63.43h and 197.87h for formulation using sesame oil. These results suggest that the castor oil formulation has longer persistency when compared to the sesame oil formulation what affects the residual efficacy of the product against parasites.

The novel compositions according to the invention have proven long action and good efficacy against internal and external parasites, especially in bovines. The novel compositions according to the invention result in a unique formulation which controls parasites resistant to ivermectin and doramectin, like, Cooperia and Haemonchus. It
is worth mentioning that these two nematode species account for approximately 90% of the parasites found in Brazilian cattle.

The diluent preferably is a vegetable oil. The vegetable oil used in the formulation may be any suitable vegetable oil of a pharmaceutical grade, such as soy bean oil, sesame oil, olive oil, sunflower oil and/or corn oil. Such vegetable oils may be used alone or in combinations. Preferably corn oil and/or olive oil is used, either alone or in combination.

The compositions according to the invention comprise a solvent for the active ingredient. Suitable solvents are, for example, benzyl alcohol, or caprylic/capric acid triglyceride. The amount of solvent used should be adjusted to match the amount of active ingredients present in the composition. Preferably the solvent is benzyl alcohol. The concentration of benzyl alcohol preferably is 7.3 % w/v.

An experiment conducted in cattle demonstrated that the association ivermectin + abamectin (3.5%) showed a higher anti-helminthic efficacy, when compared to a high concentration (3.15%) ivermectin commercial formulation, against four species of endoparasites: Haemonchus placei, Cooperia punctata, C. spatulata and Trichuris discolor. Both formulations showed a 100% efficacy against the others four species on the 14th DPT, 18 bovine hosts, divided into three groups of six animals each that were necropsied. Eight nematode species were identified.

Further pharmaceutically acceptable ingredients may be added such as an antioxidant. An antioxidant that is particularly useful for use in vegetable oils is tocopherol acetate, but other suitable antioxidants known in the art may also be used.

The compositions of the invention may be used for the preparation of a medicament for controlling parasites in and on host animals, particularly in bovines.

The compositions according to the invention can be used in injectable formulations. The composition of the invention may be delivered to the animal by injection, preferably subcutaneously, for example in the neck. The oily formulations of the invention will form a depot underneath the skin resulting in a slow release of the active ingredients.
The amount of the composition injected into a target animal depends on the body weight of the animal. Good results were obtained when 1 ml of a composition was administered per 50 kg bodyweight. When a composition is used comprising 2.25% w/v ivermectin and 1.25% w/v abamectin, this results in 450 microgram/kg bodyweight for ivermectin and 250 microgram/kg bodyweight for abamectin.

Alternatively the composition according to the invention can be used in pour-on formulations i.e. the parasiticidal active agents may be applied by a localised application to the outer surface of an animal, whereby the active ingredient migrates as to protect the whole external surface of the animal. By “localised” application it is meant that the active ingredient is only applied to a minor portion of the outer surface of the animal, generally as a line or spot on the animals back.

For such a pour on formulation a non-aqueous solvent as spreading agent may be added to the composition according to the invention in order to help dispersing the active ingredients so that they reach all parasites of the animal and control the level of skin penetration.

Suitable spreading agents are e.g. Crodamol® CAP and Crodamol PMP of Croda Chemicals Europe, East Yorkshire, United Kingdom which is an oil soluble emollient comprising fatty acid esters, produced by the direct esterification of natural fatty acids and alcohols.

Suitably pour on formulations normally include a colouring agent to enable the user to visually monitor the application of the composition to the animal. The nature of the coloring agent is unimportant and a wide variety of suitable dyes and pigments will be known to the skilled person.

A suitable pour-on formulation is disclosed in Example 3.
EXAMPLES:

EXAMPLE 1: Method to produce formulation 1

5 FORMULATION 1
IVERMECTIN 2.25g
ABAMECTIN 1.25g
BENZYL ALCOHOL 7.0mL
CASTOR OIL 52.5mL
10 TOCOPHEROL ACETATE 0.05g
CORN OIL up to 100mL

Method to manufacture formulation 1:
In a suitable recipient provided with stirrer and nitrogen bubbling system, add
abamectin, ivermectin and benzyl alcohol. Heat until 50 °C under agitation and
stirring until the solubilization of active ingredients occurs. Add castor oil and mix
during 30-60 minutes under heating (50 °C). Turn off the heat and diminish the
temperature until 30-40°C. Then, add acetate tocopherol and corn oil and mix until to
get homogeneous solution (around 60-90 minutes)
20 Filter the solution through suitable filter cartridge and sterilized in 0.22 µm cartridge
to obtain sterile solution.

Alternative method to manufacture formulation 1:
PART 1: In a suitable recipient provided with stirrer and nitrogen bubbling system,
add abamectin, ivermectin and benzyl alcohol. Heat until 50 °C under agitation and
stirring until the solubilization of active ingredients occurs.
PART 2: In another recipient add castor oil heat until 50 °C under stirring.
Transfer the part 1 to part 2 and mix during 30-60 minutes and then turn off the heat
and diminish the temperature until 30-40°C. Then, add acetate tocopherol and corn
oil and mix until to get homogeneous solution (around 60-90 minutes)
30 Filter the solution through suitable filter cartridge and sterilized in 0.22 µm cartridge
to obtain sterile solution.
EXAMPLE 2: Method to produce formulation 2

FORMULATION 2
IVERMECTIN 2.25g
5 ABAMECTIN 1.25g
BENZYL ALCOHOL 7.0mL
CASTOR OIL 35mL
TOCOPHEROL ACETATE 0.05g
CORN OIL 35mL
10 OLIVE OIL up to 100mL

PART 1: In a suitable recipient provided with stirrer and nitrogen bubbling system, add abamectin, ivermectin and benzyl alcohol. Heat until 50 oC under agitation and stirring until the solubilization of active ingredient occurs.

PART 2: In another recipient add castor oil and corn oil, and mix during 30 minute under 50 oC. Transfer the part 1 to part 2 and mix during 30-60 minutes and then turn off the heat and diminish the temperature until 30-40oC. Then, add acetate tocopherol and olive oil and mix until to get homogeneous solution (around one minute)

Filter the solution through suitable filter cartridge and sterilized in 0.22 μm cartridge to obtain sterile solution.

EXAMPLE 3: Pour-on formulation

IVERMECTIN
ABAMECTIN
Vehicle: BENZYL ALCOHOL, CAPRIC/ CAPRYLIC ACID TRIGLYCERIDE, ISOPROPYLALCOHOL, CASTOR OIL

30
EXAMPLE 4: Comparison of Ivermectin plasma concentrations after administration in different long acting vehicles by sc administration to cattle

Material and Methods: The pharmacokinetic profile of an 1% ivermectin formulation comprising 35% (v/v) castor oil was assessed in this study by a parallel comparison with an 1% ivermectin formulation in 72.2% (w/w) sesame oil. The formulations were administered at a single dose subcutaneous (sc) into the neck of cattle. Blood samples were collected by jugular venipuncture at times 0, 0.5, 1, 1.5, 2, 3, 5, 7, 10, 15, 20, 30, and 40 days after drug administration. The concentration of ivermectin in the plasma samples was determined by HPLC.

Results: Figure 1 shows bovine plasma ivermectin concentrations (individual animals in ng/mL) after single sc administration of the formulations. The results obtained show that the castor oil based long acting formulation maintained therapeutic blood levels for 40 days compared to 20 days for the sesame oil based formulation.

EXAMPLE 5: Comparison of Abamectin plasma concentrations after administration in different long acting vehicles by sc administration to cattle

Material and Methods: The pharmacokinetic profile of an 1% abamectin formulation comprising 35% (v/v) castor oil was assessed in this study by a parallel comparison with an 1% abamectin formulation in 72.2% (w/w) sesame oil. The formulations were administered at a single dose subcutaneous (sc) into the neck of cattle. Blood samples were collected by jugular venipuncture at times 0, 0.5, 1, 1.5, 2, 3, 5, 7, 10, 15, 20, 30, and 40 days after drug administration. The concentration of ivermectin in the plasma samples was determined by HPLC.

Results: Figure 2 shows bovine plasma abamectin concentrations (individual animals in ng/mL) after single sc administration of the formulations. The results obtained show that the castor oil based long acting formulation maintained therapeutic blood levels for 30 days compared to 15 days for the sesame oil based formulation.
CLAIMS:
1. A long acting composition for controlling parasites in and on animals, which comprises in a slow release vehicle a combination of ivermectin and abamectin, characterized in that the total percentage w/v of ivermectin and abamectin equals or exceeds 3% w/v and the concentration of ivermectin is higher than the concentration of abamectin.

2. The composition according to claim 1 characterised in that the slow release vehicle comprises 30 to 60% w/v of castor oil, a solvent and up to 100 % v/v of a diluent.

3. The composition according to any of claims 1 or 2, characterised in that it comprises 1.75-5% w/v ivermectin and 0.5-2% w/v abamectin.

4. The composition according to any of claims 1 to 3, characterized in that it comprises 2.25% ivermectin and 1.25% abamectin.

5. The composition according to any of claims 1 to 4, characterised in that the diluent is a vegetable oil.

6. The composition according to any of claims 1 to 5, characterised in that the vegetable oil is selected from corn oil or olive oil or a combination of corn oil and olive oil.

7. The composition according to any of claims 1 to 6, characterised in that the solvent is benzyl alcohol.

8. Use of a composition according to any of claims 1 to 7 for the preparation of a medicament for controlling parasites in and on host animals.

9. Use according to claim 8 characterized in that the composition is administered to the host animal by injection or as pour-on.

10. Use according to claim 8 or 9, characterized in that the host animal is bovine.
Figure 1: Plasma concentration of Ivermectin

![Ivermectin plasma concentrations graph]

Figure 2: Plasma concentration of Abamectin

![Abamectin plasma concentration graph]
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/7048 A61P33/00

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)
IPC 7 A61K

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 practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, CHEMABS Data, PAA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>BR 0 104 761 A (INSTITUTO DE PESQUISA EM SAUDE ANIMAL LTDA) 12 August 2003 (2003-08-12) cited in the application page 1, paragraph 1 page 2, paragraph 2</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>ROWE R C ET AL: &quot;Handbook of Pharmaceutical Excipients, PASSAGE&quot; 2003, HANDBOOK OF PHARMACEUTICAL EXCIPIENTS; LONDON: PHARMACEUTICAL PRESS, GB, PAGE(S) 104-105, X0002280157 page 104</td>
<td>1-10</td>
</tr>
<tr>
<td>A</td>
<td>US 5 183 814 A (DUKES MICHAEL) 2 February 1993 (1993-02-02) column 6, line 9 - column 22</td>
<td>1-10</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of box C

Patent family members are listed in annex.

* Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another of other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"L" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"X" document member of the same patent family

Date of the actual completion of the international search
31 March 2005

Date of mailing of the international search report
12/04/2005

Name and mailing address of the ISA
European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040, Fax (+31-70) 340-3016

Authorized officer
Leherte, C

Form PCT/ISA/210 (second sheet) (January 2004)
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR 0104761 A</td>
<td>12-08-2003</td>
<td>NONE</td>
<td></td>
</tr>
<tr>
<td>US 5183814 A</td>
<td>02-02-1993</td>
<td>AT 109351 T</td>
<td>15-08-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 622184 B2</td>
<td>02-04-1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 3592389 A</td>
<td>07-12-1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 1337591 C</td>
<td>21-11-1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 68917219 D1</td>
<td>08-09-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 68917219 T2</td>
<td>15-12-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DK 273489 A</td>
<td>07-12-1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0346014 A1</td>
<td>13-12-1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ES 2057124 T3</td>
<td>16-10-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FI 892762 A</td>
<td>07-12-1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IE 64368 B1</td>
<td>26-07-1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL 90410 A</td>
<td>31-07-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2042024 A</td>
<td>13-02-1990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO 892293 A</td>
<td>07-12-1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NZ 229392 A</td>
<td>29-01-1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT 90764 A ,B</td>
<td>29-12-1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 8903892 A</td>
<td>28-02-1990</td>
</tr>
</tbody>
</table>