Title: A VETERINARY INJECTABLE FORMULATION

Abstract: A veterinary single injectable formulation comprises buparvaquone and imidocarb or salts thereof. Both active ingredients are completely in solution and therefore bioavailable. The formulation is used in the treatment or prophylaxis of Theileria parasites and/or Anaplasma, Babesia and/or Ehrlichia parasites. The formulation may be administered in an amount of about 1ml per 20kg bodyweight in cattle and horses, or about 1 ml per 10kg bodyweight in calves, foals and dogs.
Introduction
This invention relates to a veterinary injectable formulation.

Buparvaquone has known activity against Theileria parasites.

Imidocarb dipropionate has known activity against Anaplasma, Babesia ("large" forms only) and Ehrlichia.

Statements of Invention
According to the invention there is provided a veterinary single intramuscular injectable formulation comprising buparvaquone and imidocarb or salts thereof. The composition may comprise buparvaquone and imidocarb dipropionate. Buparvaquone may be present in an amount of about 50mg/ml. Imidocarb dipropionate may be present in an amount of about 48mg/ml.

In one embodiment the composition comprises a solvent for buparvaquone. The solvent for buparvaquone may be N-methyl pyrollidone.

In one embodiment the composition comprises a solvent for imidocarb dipropionate. The imidocarb solvent may comprise water.

In one embodiment the composition comprises a co-solvent. In one case the co-solvent is a fractionated coconut oil.

The composition may comprise a surface active agent.

Buparvaquone is completely insoluble in water. Addition of water to organic solutions of buparvaquone causes the drug to be precipitated out of solution. Buparvaquone which precipitates remains at the injection site, so it has very little therapeutic effect.

The formulation of the invention is in one case a co-solution of both buparvaquone and imidocarb dipropionate. Both active ingredients are completely in solution and therefore bio-available. A formulation that is a solution of the active ingredients is greatly preferable to any
other injectable formulation type, such as a suspension or emulsion. Achievement of the formulation of the invention, therefore, is highly advantageous.

The invention also provides a method of treatment or prophylaxis of *Theileria* parasites and *Anaplasma, Babesia* and/or *Ehrlichia* parasites comprising administering an injectable formulation of the invention to a non-human animal.

In one case the formulation is administered in an amount of about 1ml per 20kg bodyweight in cattle and horses.

In another case the formulation is administered in an amount of about 1ml per 10kg bodyweight in calves, foals and dogs.

**Detailed Description**

The invention will be more clearly understood from the following description of an embodiment thereof, given by way of example only.

The injectable formulation of the invention is a unique combination of the actives Buparvaquone and Imidocarb Dipropionate. The two activities have a diverse solubility profile.

**Example**

**List of Actives and excipients**

1) Buparvaquone 50mg. per ml.
2) Imidocarb di propionate. 48mg. per ml.
3) N-Methyl Pyrrolidone. (NMP) Range 30% to 80% by weight.
4) Miglyol 812 (Fractionated coconut oil) and series of Miglyol e.g. 814, 840, 818. Cone. Range 3% to 36% by weight.
5) Water for Injection. 2% to 15% by weight.
6) Any other surface active agent e.g. Sorbitan Mono oleate (Span 80) cone. Range 1% to 5% by weight.
Preparation Method
Add Imidocarb to NMP and mix to dissolve. Add water for injection mix and add Buparvaquone mix. Add sorbitan and mix. Add Miglyol and mix until clear. Adjust volume with NMP, if necessary.

NMP is used as a solvent for Buparvaquone.

Miglyols are used as a co-solvent and to control the rate of depletion/absorption of Buparvaquone and also to reduce irritation due to Imidocarb dipropionate at the injection site.

Water for injection is used as a solvent for Imidocarb dipropionate.

Surface active agents such as Sorbitan, spans etc., are used as a co-solvent and as an emulsifier.

**Therapeutic advantages - in cattle.**

Cases of theileriosis in cattle are frequently complicated by concurrent infection with *Anaplasma* and/or *Babesia*. All three are transmitted by ticks. The clinical signs of theileriosis may mask the signs of the other infections, which, if untreated, are likely to kill the animal even if the theileriosis is cured. A single product that cures all three diseases has clear advantages.

Theileriosis causes severe immunodepression in its later stages. This can result in infections with *Anaplasma* and/or *Babesia*, that were not clinically apparent at the time of treatment for theileriosis, becoming clinically significant after the theileriosis is cured. The veterinarian may assume that these signs indicate that the theileriosis was not cured by the initial treatment, so he gives additional treatment with the "Theileria" drug, which has no effect on either *Anaplasma* or *Babesia*. The correct treatment would be with imidocarb dipropionate. The animal, already weakened by theileriosis, is therefore very likely to die of anaplasmosis or babesiosis. Use of the formulation of the invention, which cures all three diseases, avoids this mis-diagnosis problem.

A combination product containing both active ingredients is convenient and less costly than individual doses, in terms of drug and of syringes and needles, and it saves time and causes less stress to the treated animal.
Cattle can be immunised against all forms of theileriosis (East Coast fever, Corridor Disease and tropical theileriosis) by a simple procedure of natural exposure to infection by ticks and treatment (NEAT) with the antitheilerial drug buparvaquone during the early stages of the subsequent disease. However, because anaplasmosis and babesiosis are also transmitted by ticks, there is a risk that these diseases will also be contracted. The use of a single product to treat all three diseases removes the risk that the additional diseases will go undiagnosed and therefore untreated, and it may also result in immunisation against anaplasmosis and babesiosis, as well as theileriosis.

Therapeutic advantages - in horses.

Horses in countries from France to South Africa and Australia, and China to the Americas are affected by two tick-transmitted diseases of the blood, caused by *Babesia caballi* (a "large" babesia) and *Theileria equi* which, until recently, was regarded as a "small" Babesia - *Babesia equi*. Imidocarb dipropionate was the drug of choice. It was very effective, in a single dose, against *Babesia caballi* but less so against "*Babesia equi*", which required up to four injections, each of them at double the dose for *Babesia caballi*. The formulation of the invention will usually cure both diseases with a single injection - the imidocarb dipropionate treating *Babesia caballi* infections and buparvaquone treating the *Theileria equi* (with some addition effect from the imidocarb dipropionate). A single product that treats both diseases has obvious advantages over two separate ones, and the specific anti-theilerial effect of buparvaquone has inherent advantages over imidocarb dipropionate whose antitheilerial effects are relatively modest.

Mixed infections with both parasites are common in some areas, but *Theileria equi* infection is likely to be overlooked when blood smears are used as an aid to diagnosis because the *Babesia caballi* parasites are far more conspicuous, and the clinical signs of the two diseases (particularly anaemia) are similar. Despite its smaller size, *Theileria equi* infection is more frequently fatal than *Babesia caballi* so a single product that cures both without the need for differential diagnosis is clearly advantageous.

While treatment of horses with imidocarb dipropionate readily eliminates *Babesia caballi* infection totally, it does not consistently eliminate *Theileria equi*. Treated horses may therefore remain asymptomatic carriers of this infection. When severely stressed, such "carriers" may again develop clinical disease. Carrier and clinically sick horses can be a source of infection for ticks, so an otherwise disease-free area can become infected, posing a big risk to all other horses.
Treatment with a single injection containing buparvaquone and imidocarb dipropionate will greatly increase the chance, and perhaps ensure, that *Theileria equi* infections will be completely eliminated by the treatment, thus minimising or removing the risk from treated "carrier" horses.

Clinical theileriosis or babesiosis will adversely affect the performance of horses. There is also some evidence that even the asymptomatic carrier state of these diseases may reduce the performance of race horses. If so, then a product that could completely eliminate the infections could be valuable, while not infringing anti-doping regulations.

Because it is such a severe disease, and so difficult to cure, certain countries, most notably USA, prohibit the importation of horses from *Theileria equi-endemic* areas, including the Middle East and South America, unless it can be proven that they are not carriers of the infection. This involves quarantine for six months before importation, during which time the horses must be repeatedly "screened" to prove absence of the infection. This is clearly very expensive, particularly if, for example, an Arab stallion is to be imported. The formulations of the invention which reliably eliminates the infection, would be extremely valuable since proof that it had been used to treat the animal could be taken as proof that the horse was free of *Theileria equi* infection, obviating the need for quarantine.

**Therapeutic advantages - in dogs.**

Dogs in tropical and sub-tropical countries, and certain more temperate countries such as France, Italy and Spain and USA, are affected by at least three tick-transmitted blood diseases. *Babesia canis* (canine biliary fever) and most forms of *Ehrlichia (Anaplasma) canis* (including Nairobi bleeding disease) are readily treated by imidocarb dipropionate, albeit with relatively high treatment doses that may have to be repeated. However, several so-called "small babesias" are completely unaffected by this drug. These parasites, however, are highly susceptible to treatment with buparvaquone. While these parasites are not really babesias, they are not theilerias either, because theilerias are exclusively parasites of herbivores, not carnivores. Currently, no drugs are available to treat them.

Mixed infections of two or all three of these parasites are common, and the "small babesias" and ehrlichiosis can be difficult to detect, particularly in the presence of *Babesia canis*, so as with horse and cattle infections, a product that treats all three diseases, without requiring differential diagnosis, has clear advantages over imidocarb dipropionate alone.
Formulation considerations.

Buparvaquone at 50mg/ml, is regarded as the "ideal" formulation for the treatment of theileriosis in cattle. It reaches therapeutic concentrations within two hours of injection and its effect lasts for about three days. A single intramuscular injection cures most cases, although a second injection may be needed in more severe cases. The formulation of the invention is based on that of buparvaquone, so its therapeutic effect against theileriosis is predicted to be unchanged. Buparvaquone is completely insoluble in water. Addition of water to organic solutions of buparvaquone causes the drug to be precipitated out of solution. Buparvaquone which precipitates remains at the injection site, so it has very little therapeutic effect.

Imidocarb dipropionate, when used alone, is formulated as a 120mg/ml solution in water. This high concentration is used because lower concentrations are unstable over time. It reaches therapeutic concentrations in less than an hour after intramuscular injection and its effect against Anaplasma also lasts for about three days but it is effective against babesias for much longer.

Imidocarb, while highly soluble in water, is totally insoluble in organic solvents.

The formulation of the invention is in one case a co-solution of both buparvaquone and imidocarb dipropionate. Imidocarb is first dissolved in a minimum volume of water that is added to the buparvaquone formulation, but it does not cause precipitation of the buparvaquone. In an unexpected way, the imidocarb dipropionate appears to "occupy" the water so completely that there is no "spare" water to cause precipitation of the buparvaquone. Both active ingredients are completely in solution and therefore bio-available. A formulation that is a solution of the active ingredients is greatly preferable to any other injectable formulation type, such as a suspension or emulsion. Achievement of the formulation of the invention, therefore, is both novel and highly advantageous.

In the formulation of the invention both imidocarb dipropionate and buparvaquone are in solution in an overwhelmingly organic vehicle. This is surprising, particularly in view of the high concentrations of each of the active compounds in the formulation.

Pharmacokinetic and toxicological considerations.

Buparvaquone is almost uniquely non-toxic to treated animals and human operators. The absolute minimum toxic dose is at least 100-times the therapeutic dose. Imidocarb is significantly more toxic. Even at the normal therapeutic dose of 2.4 mg/kg bodyweight, it can
cause notable (but reversible) adverse side effects and severe pain and swelling at the injection site in some (indeed most) cases, particularly in dogs and horses. This is almost certainly because it reaches very high (unnecessarily high for therapeutic purposes) concentrations in the blood very soon after injection. However, its concentration then falls to more "acceptable" levels in a few hours. The minimum lethal dose of imidocarb dipropionate is only around 9.6 mg / Kg bodyweight (i.e. four times the therapeutic dose) when injected intramuscularly as an aqueous solution.

It is anticipated that in the formulation of the invention the imidocarb dipropionate will leave the injection site significantly more slowly, and over a much more protracted time-scale, than from a 120 mg / ml aqueous solution. This is expected to result in significantly less pain and swelling, and all other adverse side effects. This is a notable advantage over current imidocarb dipropionate products, particularly for horses and dogs, whose owners, understandably, dislike painful treatments.

While the time for the imidocarb dipropionate in the formulation of the invention to reach peak concentrations in the blood following injection will be delayed somewhat, its therapeutic effect and duration is unlikely to be reduced significantly. Thus, the therapeutic effect of the actives in the formulation against all its target diseases should be practically identical to that of the two active ingredients when injected as separate products.

The dose of the formulation of the invention will be 1ml / 20Kg bodyweight in cattle and horses - i.e. the same as for buparvaquone, and up to 1ml / 10 kg for some dog diseases. The dose volume for a 120 mg / ml aqueous formulation of imidocarb dipropionate, by contrast, is 1ml / 50 or 100 Kg, depending on the disease to be treated. This dose, while suitable for adult cattle and horses, is inconveniently small for calves, foals and dogs - particularly puppies, for which the dose may be less than 0.1 ml.

The invention is not limited to the embodiments hereinbefore described which may be varied in detail.
Claims

1. A veterinary single intramuscular injectable formulation comprising buparvaquone and imidocarb or salts thereof.

2. A composition as claimed in claim 1 comprising buparvaquone and imidocarb dipropionate.

3. A composition as claimed in claim 1 or 2 wherein buparvaquone is in an amount of about 50mg/ml.

4. A composition as claimed in claim 2 or 3 wherein imidocarb dipropionate is in an amount of about 48mg/ml.

5. A composition as claimed in any of claims 1 to 4 comprising a solvent for buparvaquone.

6. A composition as claimed in claim 5 wherein the solvent for buparvaquone comprises N-methyl pyrrolidone.

7. A composition as claimed in any of claims 1 to 6 comprising a solvent for imidocarb dipropionate.

8. A composition as claimed in claim 7 wherein the imidocarb solvent comprises water.

9. A composition as claimed in any of claims 1 to 8 comprising a co-solvent.

10. A composition as claimed in claim 9 wherein the co-solvent is a fractionated coconut oil.

11. A composition as claimed in any of claims 1 to 10 comprising a surface active agent.

12. A method of treatment or prophylaxis of Theileria parasites and Anaplasma, Babesia and/or Ehrlichia parasites comprising administering an injectable formulation as claimed in any of claims 1 to 11 to a non-human animal.

13. A method as claimed in claim 12 wherein the formulation is administered in an amount of about 1ml per 20kg bodyweight in cattle and horses.
14. A method as claimed in claim 12 wherein the formulation is administered in an amount of about 1ml per 10kg bodyweight in calves, foals and dogs.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K9/00 A61K31/122 A61K31/17
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Date of the actual completion of the international search
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