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Efficacy of the amino-acetonitrile derivative, monepantel, against experimental and natural adult stage gastro-intestinal nematode infections in sheep

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ABSTRACT

Multiple drug resistance by nematodes, against anthelmintics has become an important economic problem in sheep farming worldwide. Here we describe the efficacy of monepantel, a developmental molecule from the recently discovered anthelmintic class, the amino-acetonitrile derivatives (AADs). Efficacy was tested against adult stage gastro-intestinal nematodes (GINs) in experimentally and naturally infected sheep at a dose of 2.5 mg/kg body weight when administered as an oral solution. Some of the isolates used in experimental infection studies were known to be resistant to the benzimidazoles or levamisole anthelmintics; strains resistant to the macrocyclic lactones were not available for these tests. Worm count-based efficacies of >98% were determined in these studies. As an exception, *Oesophagostomum venulosum* was only reduced by 88% in one study, albeit with a low worm burden in the untreated controls (geometric mean 15.4 worms). Similar efficacies for monepantel were also confirmed in naturally infected sheep. While the efficacy against most species was >99%, the least susceptible species was identified as *Nematodirus spathiger*, and although efficacy was 92.4% in one study it was generally >99%. Several animals were infected with *Trichuris ovis*, which was not eliminated after the treatment. Monepantel demonstrated high activity against a broad range of the important GINs of sheep, which makes this molecule an interesting candidate for use in this species, particularly in regions with problems of anthelmintic resistance. Monepantel was well tolerated by the treated sheep, with no treatment related adverse events documented.

1. Introduction

Infection with gastro-intestinal nematodes (GINs) is one of the most important economic problems in sheep husbandry worldwide. In contrast to cattle, sheep develop an insufficient immunoprotection against parasitic nematodes (Miller and Horohov, 2006). This may lead to substantial parasitic burdens in young and mature animals (e.g. breeding ewes during lambing time). In general, the control of GIN infections in sheep relies heavily on anthelmintic treatments (Getachew et al., 2007) and currently there are only three broad-spectrum anthelmintic groups commercially available for this purpose: (1) the benzimidazoles (BZ), (2) the imidazothiazoles (levamisole, LEV) and hydropyrimidines (pyrantel/morantel) and (3) the macrocyclic lactones (avermectins and milbemycins, ML); all have different mechanisms of action. Additionally, the salicylanilides and nitrophenols are used as narrow spectrum anthelmintics for the control of *Haemonchus contortus* in sheep,

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and in some countries, organophosphates are still marketed (Coles et al., 2006). However, the indiscriminate and long-term use of these anthelmintics has led to the widespread emergence of drug resistant populations. Work in South America (Waller et al., 1996), South Africa (van Wyk et al., 1999), Australia (Love and Coles, 2002), New Zealand (Wrigley et al., 2006) and Europe (Sargison et al., 2001; Traversa et al., 2007) stress that resistance is present to all three broad-spectrum anthelmintic groups. In Europe (Great Britain), sheep farm closure due to the failure of anthelmintics to adequately control multi-resistant nematode infection has now been reported (Sargison et al., 2005; Blake and Coles, 2007).

The widespread resistance in sheep GINs has necessitated the research and development of various control methods such as grazing management, biological agents and vaccines and the genetic selection of resistant and resilient animals, with or without the moderate use of anthelmintics. However, so far only limited success has been achieved.

Recently a novel class of anthelmintics, the amino-acetonitrile derivatives (AADs) was described by Kaminsky et al. (2008a). A drug development candidate from this class, monepantel (Kaminsky et al., 2008b), was successfully used for treatment of GIN in experimentally infected sheep (Hosking et al., 2008; Kaminsky et al., 2008c). This paper reports the further use of an oral monepantel formulation, administered at a dose of 2.5 mg/kg, against the adult stages of numerous GIN species in sheep after experimental infection and in naturally infected animals.

2. Materials and methods

2.1. Animals

Five studies with experimentally infected sheep were performed in Switzerland (studies CRA1–CRA3) and Australia (studies YAR1 and YAR2). Sheep used for the studies in Switzerland were of the Swiss White Alpine breed, partially inbred with La Romane (INRA 401, UPRA, Les Nauzes, France). Merino sheep were used in the Australian studies. The lambs were aged 4–5 months at study commencement. Treatment groups (monepantel-treated and untreated controls) in studies CRA1–CRA3 consisted of eight animals each. In study YAR1, there were nine animals in the untreated control group and 10 in the monepantel-treated group, while in YAR2 there were six animals in the control group and nine in the treated group. The sheep were housed indoors before and during their respective study to avoid natural GIN infection. Diets consisted of grass and maize silage and hay (studies CRA1–CRA3) and a hay/straw/oats chaff mix (studies YAR1 and YAR2). Fresh water was available *ad libitum*.

Healthy, naturally infected animals were selected for studies YAR3–YAR6 in Australia on the basis of fecal egg counts (FECs) and coproculture results. There were 10 animals in each treatment group (monepantel-treated and untreated controls). Second-cross Merino sheep were purchased from commercial producers at the age of 4 months (YAR3, YAR5 and YAR6) or 18 months (YAR4) and brought to the study site, where they were housed indoors for periods ranging from 3 to 5 weeks while their nematode burdens matured to the adult stage prior to treatment.

2.2. Experimental nematode infections

Sheep were infected with third stage larvae of recently isolated and characterized field strains by intra-ruminal injection (studies CRA1–CRA3) or *per os* (studies YAR1 and YAR2; Table 1). The number of infective larvae administered and the time of infection prior to treatment was based on international guidelines for evaluating the efficacy of anthelmintics in ruminants (Wood et al., 1995; Anon., 1999, 2000).

2.3. Treatment

Sheep in the treated group of each study were dosed orally with a 2.5% monepantel solution at 2.5 mg/kg body weight (0.1 mL/kg) between 34 and 46 days after experimental infection or 21–36 days after housing in the studies with naturally infected animals. Control animals did not receive any treatment.

2.4. Fecal egg counts and worm counts

Fecal egg counts at each study site were completed using modified McMaster methods (Kaufmann, 1996) and results were expressed as eggs per gram feces (epg). Strongylate and *Nematodirus* spp. eggs were identified and counted separately by morphological differentiation. Fecal egg counts of experimentally infected sheep were determined 4 days (CRA1–CRA3), 2 days (YAR2) and 1 day (YAR1) before treatment to confirm patency. Post-treatment FECs were completed on days 7 (CRA1–CRA3), day 13 (YAR1) and day 19 (YAR2).

Fecal egg counts in studies YAR3–YAR6 were between 400 and 2000 epg, 3–6 weeks before the planned anthelmintic treatment. Prior to enrolment into a study, feces from positive samples was used for coproculture in order to differentiate the nematode genera present (van Wyk et al., 2004). Further FECs were performed 1 day before and 13 days after treatment.

Worm counting techniques have been described by Hosking et al. (2008). The animals were euthanized 8–9 days (CRA1–CRA3), 14 days (YAR1 and YAR3–YAR6) or 21 days (YAR2) after treatment.

2.5. Evaluation of anthelmintic activity

For each study, the assessment of activity of monepantel against each nematode species was determined by a comparison of the treated and untreated groups worm counts, calculated using Abbott's formula as follows:

$$\%\,\text{efficacy} = \frac{100 \times (C - T)}{C},$$

where C was the geometric (arithmetic) mean worm count of each species for the untreated control group and T, the geometric (arithmetic) mean worm count of each species