Veterinary Parasitology 227 (2016) 85–92

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

The efficacy and plasma profiles of abamectin plus levamisole combination anthelmintics administered as oral and pour-on formulations to cattle

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ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form 25 July 2016
Accepted 28 July 2016

Keywords:
Cattle
Macrocyclic lactone
Levamisole
Formulation
Pharmacokinetics

ABSTRACT

In phase I, faecal egg count reduction tests (FECRT) were conducted on six commercial cattle farms to compare the performance of two pour-on and one oral combination anthelmintic. Groups of 12–15 calves were sampled for faecal nematode egg count (FEC) before treatment with either abamectin oral, levamisole oral, an abamectin + levamisole oral combination or one of two abamectin + levamisole combination pour-ons. Samples were collected again 14 days after treatment to calculate the percentage reduction in FEC. The proportions of infective stage larvae (L3) in faecal cultures were used to apportion egg counts to, and calculate efficacy against, the main parasite genera.

Abamectin oral was effective against *Ostertagia* except on one farm where resistance was indicated, but had reduced efficacy against *Cooperia* on four farms. Levamisole oral was effective against *Cooperia* on all farms, but had variable efficacy against *Ostertagia*. The abamectin + levamisole oral was effective against both species on all farms. The abamectin + levamisole pour-ons were effective on some farms but not on others. In particular, pour-on 2 failed to achieve 95% efficacy in 45% of evaluations, 4/6 against *Cooperia* and 1/5 against *Ostertagia*. On some farms the combination pour-ons were less effective than their constituent actives administered alone as orals.

In phase II, 8 groups of 6 calves, grazing parasite-free pasture, were infected with putatively ML-resistant isolates of *Cooperia oncophora* and *Ostertagia ostertagi*. Once infections were patent groups were treated with oral or pour-on formulations of abamectin alone, levamisole alone, abamectin + levamisole (two pour-ons) or remained untreated. Blood samples were collected for analysis and after 8 days all calves were euthanized and abomasa and intestines recovered for worm counts.

All treatments were effective against *O. ostertagi* and all treatments containing levamisole were effective against *C. oncophora*. Animals treated with the oral combination had higher Cmax and AUC values for abamectin in plasma than animals treated orally with abamectin alone. In contrast, animals treated with the combination pour-ons tended to have lower plasma levels for abamectin than those treated with abamectin alone as a pour-on, with differences in the Cmax and AUC values approaching statistical significance (p-values $\leq$0.07). There were no differences detected in plasma concentrations of levamisole.

The inconsistent and sometimes poor efficacy of the combination pour-ons on-farm is likely due to reduced levels of abamectin in the plasma and hence less active reaching the target worms in the gut.

1. Introduction

Anthelmintic resistance in nematode parasites of cattle has been confirmed in many parts of the world and is seen as a potentially serious threat in the future (Sutherland and Leathwick, 2011). The identification of practices and products which are likely to select strongly for anthelmintic resistance is an important aspect of managing resistance problems before they become too severe (Leathwick and Besier, 2014). One practice, which has raised concerns with respect to selecting resistance in cattle parasites, is the delivery of anthelmintics as topical or pour-on formulations (Bliss et al., 2008; Sutherland and Leathwick, 2011; Gasbarre, 2014). Not only is the amount of active delivered to the plasma of the treated animal lower than with other routes of administration (Leathwick

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http://dx.doi.org/10.1016/j.vetpar.2016.07.031
0304-4017/

Table 1
Brief description of the trials conducted in Phase I and II of this study.

Phase I – Faecal egg count reduction tests	
Number of trials	6
Measurements	Faecal nematode egg count
	Larval differentiation to genus
Animals per group	12–15
Treatments	Untreated control
	Abamectin (0.2 mg/kg) oral
	Levamisole (7.5.0 mg/kg) oral
	Abamectin (0.2 mg/kg) + levamisole (8.0 mg/kg) oral
	Abamectin (0.5 mg/kg) + levamisole (10.0 mg/kg) pour-on
	Abamectin (0.5 mg/kg) + levamisole (10.0 mg/kg) pour-on
Outputs	Efficacy (reduction in faecal egg count) against *Cooperia* and *Ostertagia*
Phase II – Pharmacokinetics and efficacy study	
Number of trials	1
Measurements	Plasma concentrations
	Worm burden
Animals per group	6
Treatments	Untreated control
	Abamectin (0.2 mg/kg) oral
	Levamisole (8.0 mg/kg) oral
	Abamectin (0.2 mg/kg) + levamisole (8.0 mg/kg) oral
	Abamectin (0.5 mg/kg) pour-on
	Levamisole (10 mg/kg) pour-on
	Abamectin (0.5 mg/kg) + levamisole (10.0 mg/kg) pour-on
	Abamectin (0.5 mg/kg) + levamisole (10.0 mg/kg) pour-on
Outputs	Efficacy (reduction in worm count) against *Cooperia* and *Ostertagia*
	Cmax, AUC and elimination half-life for abamectin in plasma
	Plasma concentrations for levamisole

and Miller, 2013), but concerns have been raised regarding the influence of licking (Laffont et al., 2001; Bousquet-Mélou et al., 2004), breed of cattle (Sallovitz et al., 2002) and weather (Sargent et al., 2009) on the penetration of active through the hide.

A recent study involving the macrocyclic lactone (ML) moxidectin, found that oral delivery resulted in higher overall efficacy, and lower variation in efficacy, than either the injectable or pour-on formulations (Leathwick and Miller, 2013). These results were interpreted to indicate that the oral route resulted in higher concentrations of active reaching the target worms in the intestine than did either the injectable or pour-on routes (Leathwick and Miller, 2013), a conclusion supported by the results of studies in other host species (Bogan and McKellar, 1988; Gokbulut et al., 2010; Lloberas et al., 2012). It was hypothesised that this higher concentration of active resulted in the higher efficacy achieved by oral administration.

The studies reported here were initiated in response to several reports from farmers of continuing ill thrift, and in one case positive FEC, in calves treated with an abamectin + levamisole combination pour-on product. While ML resistance is common in *C. oncophora* in New Zealand (Waghorn et al., 2006; Leathwick and Miller, 2013) there is not as yet any confirmed resistance to levamisole in this species, and at that time ML-resistance in *O. ostertagi* had not been confirmed. It seemed unlikely, therefore, that these reports represented inefficacy due to resistance in either of these species and so it was unclear why the treatments should have failed.

The aims of the study were to determine whether there was any indication of resistance to abamectin and/or levamisole on the study farms, and then to compare the performance (efficacy) of two pour-on and one oral combination products, each containing both abamectin and levamisole. We hypothesised that if the combination pour-on products were showing variable performance in the field, then the combination oral would achieve higher and/or more consistent efficacy by delivering higher doses of active to the target worms in the gut. Following the results of this first phase of the study, a second phase was undertaken to investigate aspects of the pharmacology of these combination products.

2. Materials and methods

In the first phase of the study FECRT were conducted on six commercial sheep and beef farms in the North Island of New Zealand. In the second phase an artificial challenge experiment was undertaken to compare both the efficacy and plasma profiles of a range of oral and pour-on anthelmintics (Table 1). All animal manipulations were approved by the AgResearch Grasslands animal ethics committee under approval number 12177.

2.1. Faecal egg count reduction tests

The study farms were located in different regions of the North Island of New Zealand, two farms in the northern Hawkes Bay, two in the Manawatu and two in the Ruapehu district. The first two farms were those which had previously reported inefficacy of a combination pour-on product. The remaining four farms were selected by local veterinary practitioners on the basis of number of rising 1-year old cattle available, access to suitable yards and weighing facilities and the willingness of the farmer to be involved. For these farms, no attempt was made to select farms on the basis of pre-existing resistance or worm control problems. All on-farm sampling and administration of treatments was carried out by the veterinary practitioners.

All tests were carried out in beef cattle breeds (Angus or Hereford) in the autumn-early winter period (April–June in New Zealand) and all calves had been recently weaned. Once a mob of 60–75 calves had been identified as being suitable for the trial, routine monitor samples consisting of 10 faecal samples collected rectally from randomly selected animals were submitted to the laboratory for FEC. Once the mean FEC of these samples exceeded 250 eggs per g of fresh faeces (epg) and there were no zero values, the trial commenced.

On day zero calves were drafted into five groups of between 12 and 15 animals per group. Each group was then sampled for FEC, weighed and treated, based on their individual liveweight, with one of the five anthelmintic treatments. Treatments were; 1) abamectin administered orally at 0.2 mg/kg liveweight (Genesis Hi-Mineral,