Veterinary Parasitology xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

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

Characterisation of macrocyclic lactone resistance in two field-derived isolates of *Cooperia oncophora*

D.J. Bartley[a,*,1], C.L. McArthur[a,1], L.M. Devin[a], J.F. Sutra[b,c], A.A. Morrison[a], A. Lespine[b,c], J.B. Matthews[a]

[a] *Disease Control, Moredun Research Institute, Pentlands Science Park, Edinburgh EH26 0PZ, Scotland, United Kingdom*
[b] *INRA, UMR1331, Toxalim, Research Centre in Food Toxicology, F-31027 Toulouse, France*
[c] *Université de Toulouse, INP, UMR1331, Toxalim, F-31000 Toulouse, France*

ARTICLE INFO

Article history:
Received 9 May 2012
Received in revised form 11 July 2012
Accepted 17 July 2012

Keywords:
Anthelmintic resistance
Cattle
Cooperia
Ivermectin
Macrocyclic lactones
Moxidectin
Nematodes

ABSTRACT

The anthelmintic sensitivity of two field-derived isolates (designated FI001 and FI004) of cattle nematodes from beef farms in Scotland were investigated in a controlled efficacy test (CET). Efficacies of ivermectin pour-on (IVM-PO), IVM injectable (IVM-INJ) and moxidectin pour-on (MOX-PO) formulations were assessed. In each group, five helminth-naïve calves were infected experimentally with 50,000 third stage larvae from either isolate and administered with anthelmintic at the manufacturers' recommended dose rate 28 days later. For each isolate, nematode burdens were compared between treatment and control groups to determine efficacy. Nematode species composition, based on data derived from the untreated control groups' burden estimations, were 39 and 14% *Cooperia oncophora* and 61 and 86% *Ostertagia ostertagi* for isolates FI001 and FI004, respectively. Macrocyclic lactone resistance in *C. oncophora* was confirmed for both FI001 and FI004 isolates. Efficacies (as determined by nematode burden analysis) of 4, 21 and 31% for FI001, and 10, 1 and 74% for FI004, were obtained for IVM-INJ, IVM-PO and MOX-PO, respectively. Efficacy based on faecal egg count reduction at seven days post anthelmintic administration were 8, 99 and 100% for FI001, and 37, 20 and 100% for FI004 for IVM-INJ, IVM-PO and MOX-PO, respectively. In summary, this study details two macrocyclic lactone resistant isolates of *C. oncophora* obtained from cattle from two distinct geographical locales in the UK.

1. Introduction

Grazing cattle are susceptible to a number of gastrointestinal nematodes, with *Ostertagia ostertagi* and *Cooperia oncophora* being the most prevalent in temperate regions such as the UK (Anderson et al., 1965; Borgsteede, 1977; Claerebout et al., 1998; Rose, 1968). *O. ostertagi* is considered the more pathogenic of the two species (Bairden and Armour, 1981) and has been shown to cause profuse watery diarrhoea, inappetence and poor weight gain, with low grade infections leading to losses of around 30–60 kg in body weight gain in untreated beef cattle in their first 12 months compared to anthelmintic treated counterparts (Dimander et al., 2000, 2003). In addition to reduced weight gains, infection can reduce milk output significantly in dairy stock (Charlier et al., 2009). Although *C. oncophora* is generally considered to be a species of relatively low pathogenicity (Anderson et al., 1965; Coop et al., 1979) studies have indicated that, in co-infections with *O. ostertagi*, this parasitic nematode contributes to reduced productivity and inappetence (Hawkins, 1993; Stromberg et al., 2012; Sutherland and Leathwick, 2011).

The prophylactic use of anthelmintics is the control option most utilised by livestock producers and has been

* Corresponding author. Tel.: +44 131 4455111; fax: +44 131 4456111.
E-mail address: dave.bartley@moredun.ac.uk (D.J. Bartley).
[1] These authors contributed equally to the work described in this manuscript.

0304-4017/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2012.07.022

Table 1
Trial designs for the controlled efficacy tests, including dosage of anthelmintic, number of calves on trial per group, infective dose, days post-infection of treatment and necropsy. (P.M.)

Treatment (dosage)	Number of calves	Dose (L_3)	Day post infection	
			Treat	P.M.
Control – Untreated	5	50,000	–	35
Ivermectin injectable (0.2 mg/kg; IVM-INJ)	5	50,000	28	35
Ivermectin pour-on (0.5 mg/kg; IVM-PO)	5	50,000	28	35
Moxidectin pour-on (0.5 mg/kg; MOX-PO)	5	50,000	28	35

shown to increase productivity in both dairy (Gross et al., 1999) and beef (Dimander et al., 2000) cattle. Macrocyclic lactone (ML) anthelmintics hold the major market share for antiparasitics in cattle; partly because of their high efficacy and persistent effect against all developmental stages of parasitic nematodes of relevance, but also because of their additional efficacy against ectoparasites (Gonzalez et al., 2009). Currently, MLs are available for use in cattle as topical pour-on preparations or as a subcutaneous injection. The former method of application is most popular due to the associated reductions in animal handling time and the lower risk of injury to animals and handlers (Bogan and Armour, 1987).

Published reports of ML resistance/inefficacy in cattle nematodes have been documented in nine countries globally (Sutherland and Leathwick, 2011). In New Zealand and the US, for example, resistance to BZ and ML classes in the same nematode population have been identified (Gasbarre et al., 2009a, 2009b; Waghorn et al., 2006) and, in Argentina and Brazil, resistance of *Ostertagia*, *Cooperia*, *Haemonchus* and *Oesophagostumum* species to all three main classes of anthelmintic has been described (Anziani et al., 2004; Soutello et al., 2007). In the UK, the first reported case of ivermectin (IVM) resistant *Cooperia* nematodes in cattle was made in 1999 (Coles and Stafford, 1999; Stafford and Coles, 1999). Since then, there have been a few reports of reduced efficacy following administration of MLs in UK cattle (Orpin, 2010; Sargison et al., 2009, 2010; Stafford et al., 2010); however no extensive studies have been conducted. Previously, we reported reduced efficacy of injectable IVM against three field isolates of cattle nematodes (McArthur et al., 2011). Here, for two of these populations, we have investigated the anthelmintic sensitivity phenotype further by undertaking a controlled efficacy test (CET) in experimentally infected cattle.

2. Materials and methods

2.1. Parasite isolates

Faecal material was collected and cultured to generate infective larvae (L_3), from first season grazing calves from farms that had been identified through an ongoing anthelmintic sensitivity study (McArthur et al., 2011). In brief, L_3 were cultured from pre-treatment faecal material collected using the techniques described previously (Coop et al., 1995). Resultant L_3 were passaged through two helminth-naïve calves to produce sufficient material for use in the CET. The isolates used in this study were designated FI001 and FI004 and were derived from beef cattle farms in Dumfriesshire and Ayrshire, Scotland, respectively. On-farm IVM-faecal egg count reduction tests (FECRTs) using subcutaneous administration had demonstrated mean faecal egg count (FEC) reductions of 72% (95% confidence intervals: 41, 87) and 87% (95% confidence intervals: 77, 93) for FI001 and FI004, respectively (McArthur et al., 2011).

2.2. Controlled efficacy test

Forty helminth-free, male calves, 4–7 months-old, and housed since birth, were infected *per os* with 50,000 L_3 each (Day 0 post-infection; PI). A total of 20 calves were infected with each isolate. On day 27 PI, a FEC was performed and the calves weighed. On the basis of these parameters the calves were allocated into blocks, the blocks were then randomly assigned to a treatment group ($n = 5$ per group). Groups 1–4 and Groups 5–8 were infected with isolates FI001 and FI004, respectively. All groups were housed separately. Treatments to groups 1 and 5 were administered on Day 28 PI with IVM by subcutaneous injection (Ivomec Super®, 1% (w/v) IVM, 10% (w/v) clorsulon, Merial Animal Health, 0.2 mg/kg body weight; BW), Groups 2 and 6 were administered with pour-on IVM (Ivomec Pour-On®, 0.5% (w/v) IVM, Merial Animal Health; 0.5 mg/kg BW), Groups 3 and 7 were administered with MOX as a pour-on preparation (Cydectin Pour-On ®, 0.5% (w/v) MOX, Pfizer Animal Health Ltd; 0.5 mg/kg BW). The two remaining groups (4 and 8) received no anthelmintic and acted as infection controls for the experiment (Table 1). All anthelmintic treatment doses were calculated according to the respective manufacturer's instructions, with pour-on doses rounded up to the nearest 1 ml (dosage range 0.50–0.56 mg/kg BW) and injectable doses to the nearest 0.1 ml (0.2 mg/kg BW). Pour-on treatments were applied along the midline of the back from the withers to the tailhead using a syringe, animals were observed closely for 30 min after treatment for any licking behaviour. All experimental procedures described here were approved by the Moredun Research Institute Experiments and Ethics Committee and were conducted under the legislation of a UK Home Office License (reference PPL 60/03899) in accordance with the Animals (Scientific Procedures) Act of 1986.

2.3. Samples

Faecal samples were taken *per rectum* from each calf prior to infection to confirm that they were negative for helminth eggs, on Day 27 PI prior to treatment allocation, on the day of anthelmintic treatment (Day 28) and then

Please cite this article in press as: Bartley, D.J., et al., Characterisation of macrocyclic lactone resistance in two field-derived isolates of *Cooperia oncophora*. Vet. Parasitol. (2012), http://dx.doi.org/10.1016/j.vetpar.2012.07.022

daily until necropsy seven days later. Faecal egg counts (FECs) were conducted in duplicate using a modification of the technique described by Jackson (1974). Venous blood was collected via jugular venepuncture into 10 ml heparinised Vacutainer tubes (Becton Dickinson vacutainers systems) at 4, 8, 24, 48, 120, 144 and 168 h post administration of anthelmintic. All blood samples were stored in lidded cool boxes to prevent ML degradation. The samples were immediately centrifuged at 1272 × *g* for 15 min at 4 °C; plasma recovered and stored at −20 °C.

2.4. Necropsy and worm recovery

All animals were necropsied on Day 35 PI (Day 7 post treatment) using post mortem and nematode recovery methods described previously (Patterson et al., 1996). The full length of the small intestine was removed and processed to ensure that any worms that may have been temporarily paralysed and subsequently re-established to a more distal region of the gut were recovered (McKellar et al., 1988). Total nematode burdens were estimated from counts of a 2% sub-sample of the abomasal and intestinal washings and saline digests. Enumerated nematodes were classified to stage and species using criteria described in the Ministry of Agriculture, Fisheries and Food document (MAFF, 1986).

2.5. Ivermectin concentration and kinetic analysis

ML concentrations were determined in plasma by high performance liquid chromatography (HPLC) with fluorescence detection according to previously described and validated methods (Alvinerie et al., 1998). Data were analysed using a non-compartmental approach with version 4.2 of the Kinetica Tm computer program (InnaPhase, Philadelphia, USA). The partial area under the plasma concentration–time curve (AUC) from t_0 to t_{7d} was calculated by the linear trapezoidal rule. Data are expressed as mean ± standard error of the mean (SEM).

2.6. Statistical analyses

Nematode burdens and FECs were square-root transformed to successfully normalize for variance. Burdens were compared using one way ANOVA (Minitab version 13), followed by Fisher's pairwise comparisons when found to be significant ($p < 0.05$). The percentage efficacy (PE) of each treatment was calculated by means of the standard equation: $(1 - (T/C)) \times 100$ where C and T are the arithmetic mean total nematode burdens or FECs of the untreated control and treated groups, respectively (Coles et al., 1992). Anthelmintic resistance was deemed to be present when the PE in reducing nematode burden or FEC was <95%, with a lower 95% confidence limit of <90%. Bootstrap analysis was also conducted, with a re-sampling number of 2000 using the "BootStreat" program (Cabaret and Antoine, 2008) to calculate mean treatment efficacies and upper and lower 95% confidence limits. Statistical analysis for the comparison of mean AUC values was performed using one-way ANOVA followed by Fisher test (Statview software, Abacus concepts, Berkeley, CA, USA). Statistical significance was accepted as $p < 0.05$.

3. Results

3.1. Observations after treatment

One animal from the FI001 IVM-INJ group had no IVM detected in its plasma and was consequently removed from the trial and any subsequent analysis. No licking was observed in any of the animals administered with the pour-on applications over the entire observation period.

3.2. Nematode burden analysis

The average percentage establishment of nematodes in the control calves was 27% (13,630 nematodes) and 35% (17,560 nematodes) for FI001 and FI004, respectively. In terms of species composition identified at post mortem in these groups, FI001 comprised 61% *O. ostertagi* and 39% *C. oncophora* and FI004 comprised 86% *O. ostertagi* and 14% *C. oncophora*. In terms of efficacy, when compared to the untreated control group, for FI001, mean reductions in *C. oncophora* were 38%, 64% and 31% in the IVM-INJ, IVM-PO and MOX-PO treatment groups, respectively (Table 2). For FI004, the observed mean reductions in this nematode species were 10%, 0% and 74% for the IVM-INJ, IVM-PO and MOX-PO treatment groups, respectively. All three anthelmintic treatments produced a mean reduction in *O. ostertagi* of >99.5% in both isolates. For isolate FI001, no significant differences in nematode burdens were observed when the anthelmintic treated groups were compared with the non-treated control group, whereas with isolate FI004, significant reductions in nematode burdens were only observed in the group administered with MOX-PO ($p < 0.05$).

3.3. Faecal egg count analysis

Fig. 1A and B shows the mean FECs obtained daily for each group from 0 to 7 days after anthelmintic administration. FECs in the untreated control groups were consistently high throughout the sampling frame, with mean FECs across the 7 days of 1245 and 674 EPG for isolates FI001 and FI004, respectively. In animals that received isolate FI001, FECs declined steadily from 0 to 4 days after administration of IVM-PO and from 0 to 5 days after administration of IVM-INJ and MOX-PO. The pattern of faecal egg output after anthelmintic administration differed with isolate FI004, as only in those animals that received MOX-PO did the FECs reach 0 EPG (at four days after treatment). In the groups that received IVM, although the FECs declined after treatment, they remained >0 EPG throughout. For isolate FI001, at seven days after treatment, the mean FECR observed was 98%, 99% and 100% in the groups that received IVM-INJ, IVM-PO and MOX-PO, respectively. In contrast, for isolate FI004, mean FECRs of 37%, 20% and 100% were observed at seven days after treatment with IVM-INJ, IVM-PO and MOX-PO, respectively (Table 3). Significant differences ($p < 0.05$) in FEC compared to non-treated control animals were observed with all treatment groups with isolate FI001,

Please cite this article in press as: Bartley, D.J., et al., Characterisation of macrocyclic lactone resistance in two field-derived isolates of *Cooperia oncophora*. Vet. Parasitol. (2012), http://dx.doi.org/10.1016/j.vetpar.2012.07.022

Table 2
Arithmetic mean (± S.E.M.) small intestine worm counts, range of counts, differentiation of worm burdens into male, female and juvenile worms, and percentage efficacy (P.E.) of anthelmintic treated groups of calves relative to untreated control calves seven days post-treatment.

Treatment	Arithmetic mean worm burden (± S.E.M.) [range]				Percentage efficacy
	Male	Female	Juvenile	Total	
Isolate FI001					
Untreated	2480 (±635) [400–3800]	2810 (±633) [900–4400]	60 (±29) [0–150]	5350 (±1288) [1300–8350]	NA
Injectable ivermectin (IVM-INJ)	1425 (±429) [400–2600]	1900 (± 625) [200–3450]	0 (±0) [0–0]	3325 (±1051) [600–6050]	38
Pour-on ivermectin (IVM-PO)	920 (±543) [50–3000]	1010 (±557) [0–3050]	0 (±0) [0–0]	1930 (±1098) [50–6050]	64
Pour-on moxidectin (MOX-PO)	1590 (±837) [0–3700]	2090 (±983) [0–5000]	20 (±20) [0–100]	3700 (±292) [0–8550]	31
Isolate FI004					
Untreated	1170 (±326) [250–2050]	1270 (±339) [350–2050]	0 (±0) [0–0]	2440 (±679)[a] [850–3900]	NA
Injectable ivermectin (IVM-INJ)	1040 (±97) [850–1400]	1160 (±73) [1000–1350]	0 (±0) [0–0]	2200 (±104) [a] [1900–2400]	10
Pour-on ivermectin (IVM-PO)	1280 (±270) [750–2300]	1430 (±167) [1100–1950]	0 (±0) [0–0]	2710 (±428) [a] [1850–4250]	0
Pour-on moxidectin (MOX-PO)	350 (±183) [0–1000]	290 (±97) [100–650]	0 (±0) [0–0]	640 (±274) [b] [100–1650]	74

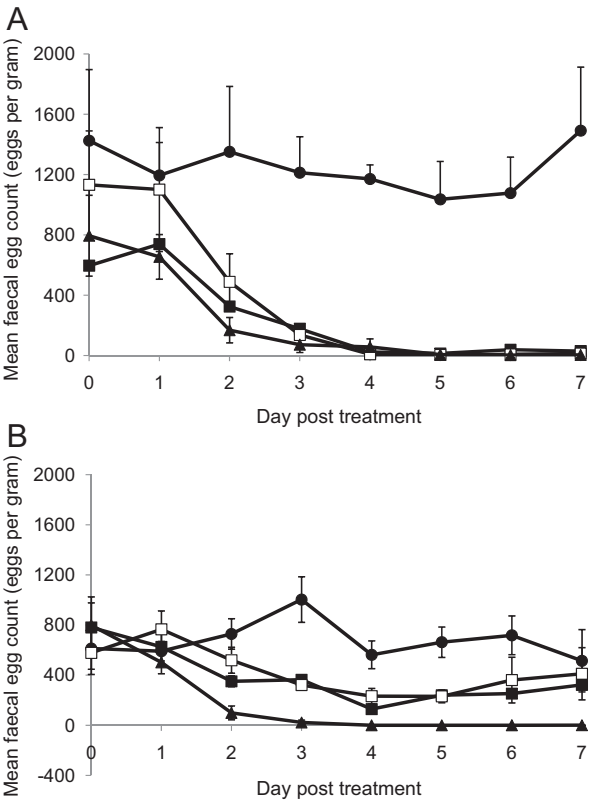

Fig. 1. Arithmetic mean faecal egg counts (±standard error of the mean, SEM) of the four groups of calves 28–35 days post infection with 50,000 infective larvae; FI001 (A) and FI004 (B). The groups are untreated control (●); injectable ivermectin (■); pour-on ivermectin (□) and pour-on moxidectin (▲).

whereas with isolate FI004, significant reductions in FEC were only observed with the group administered with MOX-PO.

3.4. Analysis of IVM and MOX concentration kinetics in plasma

Fig. 2A and B shows the mean plasma concentrations of IVM or MOX measured over time for each treatment group for both isolates and area under plasma concentration–time curves. In the IVM-INJ groups, the mean peak IVM concentration was between 20.4 and 38.6 ng/ml. In the IVM-PO groups, the mean peak IVM concentration was between 24.5 and 26 ng/ml, whereas the mean peak MOX concentration in MOX-PO treatment groups was 7.9 and 12.4 ng/ml.

4. Discussion

The current study demonstrated a lack of ML (IVM and MOX) efficacy against *C. oncophora* present in nematode isolates derived from two UK beef cattle enterprises. To date, reports of resistance/inefficacy in the UK have only described experiments in which pour-on IVM (Coles et al., 2001; Sargison et al., 2010) or doramectin (Sargison et al., 2009) were administered and these reports were

Table 3
Arithmetic mean (± S.E.M) [range] faecal egg counts and percentage efficacy (P.E.) of anthelmintic treated groups of calves relative to untreated control calves seven days post-treatment.

Treatment	Faecal egg count (eggs per gram)		Percentage efficacy (95% CI)	
	Day 0	Day 7	WAAVP	Bootstrap estimate
Isolate FI001				
Untreated	1425 (±472) [446–2624]	1491 (±421) [675–2741]	NA	NA
Injectable ivermectin (IVM-INJ)	596 (±91) [486–869]	30 (±9) [4–44]	98 (95, 99)	98 (95, 100)
Pour-on ivermectin (IVM-PO)	1132 (±357) [491–2453]	13 (±11) [2–57]	99 (95, 100)	99 (97, 100)
Pour-on moxidectin (MOX-PO)	795 (±268) [252–1715]	4 (±2) [0–12]	100 (99, 100)	100 (99, 100)
Isolate FI004				
Untreated	613 (±165) [189–1184]	515 (±249) [126–1458]	NA	NA
Injectable ivermectin (IVM-INJ)	781 (±243) [365–1562]	323 (±41) [221–428]	37 (0, 77)	22 (0, 69)
Pour-on ivermectin (IVM-PO)	578 (±172) [116–972]	411 (±208) [113–1215]	20 (0, 81)	0 (0, 77)
Pour-on moxidectin (MOX-PO)	798 (±178) [347–1305]	1 (±0) [3–0]	100 (99, 100)	100 (99, 100)

based on FEC reduction alone. Although there have been no reports of MOX resistance in cattle nematodes in the UK, there has been data describing this phenomenon in cattle from Argentina (Anziani et al., 2001), Belgium (El-Abdellati et al., 2010), Brazil (Condi et al., 2009), New Zealand (Vermunt et al., 1996) and the US (Gasbarre et al., 2009a, 2009b). As in the case of ovine nematodes (Pomroy and Whelan, 1993), reports of side resistance between members of the ML class in bovine nematodes are not unexpected (Conder et al., 1993; Vermunt et al., 1996). Here, for isolate FI004, the mean efficacy of MOX-PO was greater than IVM-PO and IVM-INJ, as assessed by nematode burden analysis, although this was not the case with isolate FI001. In previous reports of ML resistance in cattle, where both compounds were tested, MOX treatment generally resulted in higher percentage reductions in nematode burden and FEC than IVM (Anziani et al., 2001; El-Abdellati et al., 2010; Gasbarre et al., 2009b) although this was not always the case (Vermunt et al., 1996). The higher efficacy of MOX is believed to be due the compound's greater lipophilicity, as well as higher potency and persistency when compared to avermectins such as IVM (Kieran, 1994).

The drug concentration data indicates adequate uptake of the anthelmintics administered and confirms that the nematodes are ML resistant. Macrocyclic lactones have systemic action and their concentration and persistence in plasma and in tissues where parasites are located, contribute to their efficacy against the target parasite species. In this study, the ML concentration profiles in plasma were within the limits expected in cattle for each of the treatment groups (Gayrard et al., 1999; Lifschitz et al., 1999a, 1999b; Sallovitz et al., 2002). Generally, subcutaneous

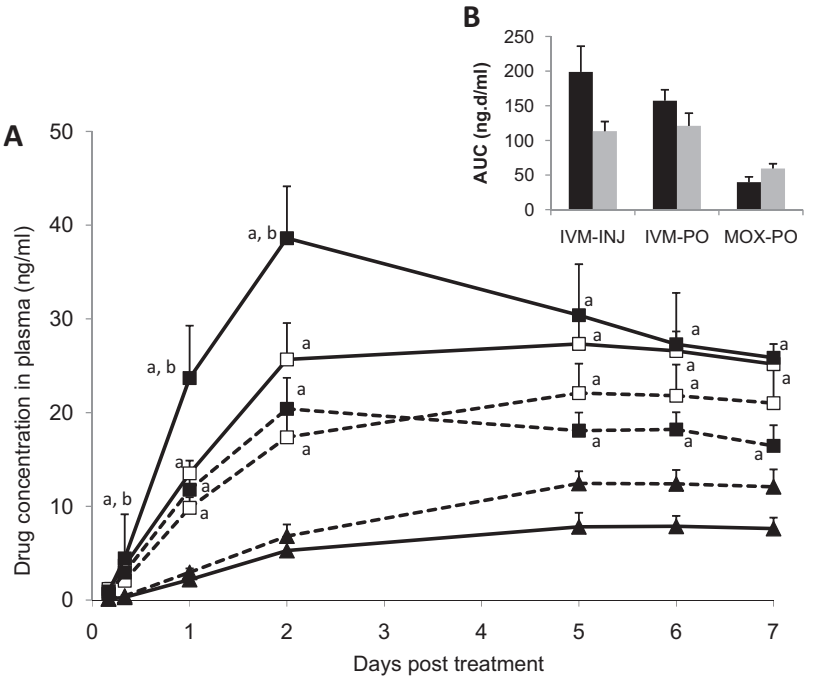

Fig. 2. (A) Plasma profile of macrocyclic lactone concentration in field derived nematode infected cattle: isolate FI001 (full line) and isolate FI004 (dotted lines) treated at time 0. IVM-INJ (■), IVM-PO (□) and MOX-PO (▲). (B) Area under plasma concentration–time curve (AUC): isolate FI001 (■) and isolate FI004 (▩). Values are arithmetic means ± S.E.M. Values are significantly different when compared with MOX-PO (a) or when compared with IVM-PO (b).

Please cite this article in press as: Bartley, D.J., et al., Characterisation of macrocyclic lactone resistance in two field-derived isolates of *Cooperia oncophora*. Vet. Parasitol. (2012), http://dx.doi.org/10.1016/j.vetpar.2012.07.022

injection is the most efficient route for ML administration in terms of bioavailability in cattle and other species, when compared to oral and topical administration (Gayrard et al., 1999; Laffont et al., 2001; Lespine et al., 2005). Ivermectin and MOX have different plasma and tissue kinetics which affect the duration of activity and generally, MOX has a longer persistence of activity than IVM does in hosts infected with ML sensitive nematodes.

The principal species surviving ML treatment in both isolates was *C. oncophora*. This is consistent with previous findings in the EU, where this species predominated after IVM treatment (Demeler et al., 2009; El-Abdellati et al., 2010; Familton et al., 2001). *C. oncophora* was also demonstrated to dominate larval cultures derived from samples obtained after IVM treatment failure in the field in Scotland (Sargison et al., 2009). *Cooperia*, including *C. oncophora*, are known to be one of the dose-limiting species for IVM (Benz and Ernst, 1979; Bisset et al., 1990; Egerton et al., 1979; McKenna, 1995), with similar findings reported for MOX (Ranjan et al., 1992; Vermunt et al., 1996; Whang et al., 1994).

Cooperia species have often been cited as relatively non-pathogenic (Anderson et al., 1965; Coop et al., 1979); however, ill thrift has been reported in cattle harbouring suspected or confirmed anthelmintic resistant nematodes of this species (Anziani et al., 2001; McKenna, 1995; Sargison et al., 2010). Indeed, recent studies in New Zealand have indicated that failure to control this nematode species in yearlings results in a 14 kg difference in live-weight gain over a grazing season compared to uninfected control animals (Sutherland and Leathwick, 2011). Previous studies in Scotland also indicated that this was the case (Armour et al., 1987). Some authors have suggested that anthelmintic resistant *C. oncophora* are more pathogenic than ML sensitive worms of the same species (Coles et al., 2001; Njue and Prichard, 2004). The trial here was of too short a duration to assess the clinical effects of the parasite isolates investigated and further studies need to be performed to explore this further.

Lower FECs were observed in calves infected with FI004 compared to those infected with FI001. This difference may be attributable to higher proportions of *O. ostertagi* in the FI004 isolate. *O. ostertagi* is known to be less fecund than *Cooperia* species (Kloosterman, 1971).

Examination of the FEC data following anthelmintic administration demonstrated that there was suppression of egg production from worms that survived treatment. It is believed that although nematodes are able to survive in therapeutic concentrations of the compound, IVM is still able to paralyse the uterine musculature (McKenna, 1997; Scott et al., 1991) resulting in a suppression in egg output in surviving worms. Egg output may then resume as the local anthelmintic concentrations fall over time, although one report detailed the effect of IVM on the reproductive potential of *Cooperia curticei*, with reduced numbers of eggs observed in worms from IVM treated animals compared to worms in untreated controls (McKellar et al., 1988). Although assessed at 7 days after anthelmintic administration, all treatments for isolate FI001 gave FECR efficacies >95%, whereas for isolate FI004, only MOX-PO treatment resulted in a FECR >95%. Whilst the faecal egg counts of the treated groups may have increased if left until 14 days after anthelmintic administration, as per the current WAAVP guidelines (Coles et al., 2006), it highlights the limitation of examining FECs in isolation when assessing anthelmintic efficacy.

The data generated here and in other trials reaffirms the need to ensure that appropriate guidelines are followed when assessing anthelmintic efficacy (Coles et al., 2006).

Competing interest

The authors declare that they have no competing interests.

Acknowledgments

The authors gratefully acknowledge the Scottish Government RESAS for funding this work, the Perry Foundation and Virbac for funding CLM's PhD studentship and our colleagues in Bioservices and Fiona Kenyon, Charlotte Burgess, Dave McBean, Hannah Lester, Samantha Ellis, Valerie Relf, Danielle Gordon, Heather McDougall, Frank Turnbull and Glen Lauder at Moredun for their technical assistance with the CET.

References

Alvinerie, M., Escudero, E., Sutra, J.F., Eeckhoutte, C., Galtier, P., 1998. The pharmacokinetics of moxidectin after oral and subcutaneous administration to sheep. Vet. Res. 29, 113–118.
Anderson, N., Armour, J., Jarrett, W.F., Jennings, F.W., Ritchie, J.S., Urquhart, G.M., 1965. A field study of parasitic gastritis in cattle. Vet. Rec. 77, 1196–1204.
Anziani, O.S., Zimmermann, G., Guglielmone, A.A., Vazquez, R., Suarez, V., 2001. Avermectin resistance in *Cooperia pectinata* in cattle in Argentina. Vet. Rec. 149, 58–59.
Anziani, O.S., Suarez, V., Guglielmone, A.A., Warnke, O., Grande, H., Coles, G.C., 2004. Resistance to benzimidazole and macrocyclic lactone anthelmintics in cattle nematodes in Argentina. Vet. Parasitol. 122, 303–306.
Armour, J., Bairden, K., Holmes, P.H., Parkins, J.J., Ploeger, H., Salman, S.K., McWilliam, P.N., 1987. Pathophysiological and parasitological studies on *Cooperia oncophora* infections in calves. Res. Vet. Sci. 42, 373–381.
Bairden, K., Armour, J., 1981. A survey of abomasal parasitism in dairy and beef cows in south-west Scotland. Vet. Rec. 109, 153–155.
Benz, G.W., Ernst, J.V., 1979. Anthelmintic activities of B1a fraction of avermectin against gastrointestinal nematodes in calves. Am. J. Vet. Res. 40, 1187–1188.
Bisset, S.A., Brunsdon, R.V., Forbes, S., 1990. Efficacy of a topical formulation of ivermectin against naturally acquired gastrointestinal nematodes in weaner cattle. N. Z. Vet. J. 38, 4–6.
Bogan, J., Armour, J., 1987. Anthelmintics for ruminants. Int. J. Parasitol. 17, 483–491.
Borgsteede, F.H.M., 1977. The epidemiology of gastrointestinal helminth infections in young cattle in The Netherlands. Utrecht University, The Netherlands.
Cabaret, J., Antoine, T., 2008. Available: http://wcentre.tours.inra.fr/sfpar/stat.htm.
Charlier, J., Hoglund, J., von Samson-Himmelstjerna, G., Dorny, P., Vercruysse, J., 2009. Gastrointestinal nematode infections in adult dairy cattle: impact on production, diagnosis and control. Vet. Parasitol. 164, 70–79.
Claerebout, E., Dorny, P., Vercruysse, J., Agneessens, J., Demeulenaere, D., 1998. Effects of preventive anthelmintic treatment on acquired resistance to gastrointestinal nematodes in naturally infected cattle. Vet. Parasitol. 76, 287–303.
Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44.

Coles, G.C., Stafford, K.A., 1999. Anthelmintic resistance in cattle nematodes in the UK. Cattle Practice 7, 173–175.
Coles, G.C., Watson, C.L., Anziani, O.S., 2001. Ivermectin-resistant *Cooperia* in cattle. Vet. Rec. 148, 283–284.
Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 136, 167–185.
Conder, G.A., Thompson, D.P., Johnson, S.S., 1993. Demonstration of co-resistance of *Haemonchus contortus* to ivermectin and moxidectin. Vet. Rec. 132, 651–652.
Condi, G.K., Soutello, R.G., Amarante, A.F., 2009. Moxidectin-resistant nematodes in cattle in Brazil. Vet. Parasitol. 161, 213–217.
Coop, R.L., Sykes, A.R., Angus, K.W., 1979. Pathogenicity of daily intakes of *Cooperia oncophora* larvae in growing calves. Vet. Parasitol. 5, 261–269.
Coop, R.L., Huntley, J.F., Smith, W.D., 1995. Effect of dietary protein supplementation on the development of immunity to *Ostertagia circumcincta* in growing lambs. Res. Vet. Sci. 59, 24–29.
Demeler, J., Van Zeveren, A.M., Kleinschmidt, N., Vercruysse, J., Hoglund, J., Koopmann, R., Cabaret, J., Claerebout, E., Areskog, M., von Samson-Himmelstjerna, G., 2009. Monitoring the efficacy of ivermectin and albendazole against gastrointestinal nematodes of cattle in Northern Europe. Vet. Parasitol. 160, 109–115.
Dimander, S.O., Hoglund, J., Sporndly, E., Waller, P.J., 2000. The impact of internal parasites on the productivity of young cattle organically reared on semi-natural pastures in Sweden. Vet. Parasitol. 90, 271–284.
Dimander, S.O., Hoglund, J., Uggla, A., Sporndly, E., Waller, P.J., 2003. Evaluation of gastro-intestinal nematode parasite control strategies for first-season grazing cattle in Sweden. Vet. Parasitol. 111, 193–209.
Egerton, J.R., Ostlind, D.A., Blair, L.S., Eary, C.H., Suhayda, D., Cifelli, S., Riek, R.F., Campbell, W.C., 1979. Avermectins, new family of potent anthelmintic agents: efficacy of the B1a component. Antimicrob. Agents Chemother. 15, 372–378.
El-Abdellati, A., Geldhof, P., Claerebout, E., Vercruysse, J., Charlier, J., 2010. Monitoring macrocyclic lactone resistance in *Cooperia oncophora* on a Belgian cattle farm during four consecutive years. Vet. Parasitol. 171, 167–171.
Familton, A.S., Mason, P., Coles, G.C., 2001. Anthelmintic-resistant *Cooperia* species in cattle. Vet. Rec. 149, 719–720.
Gasbarre, L.C., Smith, L.L., Hoberg, E., Pilitt, P.A., 2009a. Further characterization of a cattle nematode population with demonstrated resistance to current anthelmintics. Vet. Parasitol. 166, 275–280.
Gasbarre, L.C., Smith, L.L., Lichtenfels, J.R., Pilitt, P.A., 2009b. The identification of cattle nematode parasites resistant to multiple classes of anthelmintics in a commercial cattle population in the US. Vet. Parasitol. 166, 281–285.
Gayrard, V., Alvinerie, M., Toutain, P.L., 1999. Comparison of pharmacokinetic profiles of doramectin and ivermectin pour-on formulations in cattle. Vet. Parasitol. 81, 47–55.
Gonzalez, C.A., Sahagun Prieto, A.M., Jose Diez, L.M., Martinez, N.F., Vega, M.S., Vieitez, J.J., 2009. The pharmacokinetics and metabolism of ivermectin in domestic animal species. Vet. J. 179, 25–37.
Gross, S.J., Ryan, W.G., Ploeger, H.W., 1999. Anthelmintic treatment of dairy cows and its effect on milk production. Vet. Rec. 144, 581–587.
Hawkins, J.A., 1993. Economic benefits of parasite control in cattle. Vet. Parasitol. 46, 159–173.
Jackson, F., 1974. New technique for obtaining nematode ova from sheep faeces. Lab. Pract. 23, 65–66.
Kieran, P.J., 1994. Moxidectin against ivermectin-resistant nematodes—a global view. Aust. Vet. J. 71, 18–20.
Kloosterman, A., 1971. Observations on the Epidemiology of Trichostrongylosis of Calves. Agricultural University, Wageningen, The Netherlands.
Laffont, C.M., Alvinerie, M., Bousquet-Melou, A., Toutain, P.L., 2001. Licking behaviour and environmental contamination arising from pour-on ivermectin for cattle. Int. J. Parasitol. 31, 1687–1692.
Lespine, A., Alvinerie, M., Sutra, J.F., Pors, I., Chartier, C., 2005. Influence of the route of administration on efficacy and tissue distribution of ivermectin in goat. Vet. Parasitol. 128, 251–260.
Lifschitz, A., Virkel, G., Imperiale, F., Sutra, J.F., Galtier, P., Lanusse, C., Alvinerie, M., 1999a. Moxidectin in cattle: correlation between plasma and target tissues disposition. J. Vet. Pharmacol. Ther. 22, 266–273.
Lifschitz, A., Virkel, G., Pis, A., Imperiale, F., Sanchez, S., Alvarez, L., Kujanek, R., Lanusse, C., 1999b. Ivermectin disposition kinetics after subcutaneous and intramuscular administration of an oil-based formulation to cattle. Vet. Parasitol. 86, 203–215.
MAFF, 1986. Ministry of Agriculture, Fisheries and Food, Manual of veterinary parasitological laboratory techniques, Reference Book 418. Her Majesty's Stationery Office 3rd edition.
McArthur, C.L., Bartley, D.J., Shaw, D.J., Matthews, J.B., 2011. Assessment of ivermectin efficacy against gastrointestinal nematodes in cattle on four Scottish farms. Vet. Rec. 169, 658.
McKellar, Q.A., Bogan, J.A., Horspool, L., Reid, K., 1988. Effect of ivermectin on the reproductive potential of *Cooperia curticei*. Vet. Rec. 122, 444.
McKenna, P.B., 1995. Topically applied ivermectin and *Cooperia* infections in cattle. N. Z. Vet. J. 43, 44.
McKenna, P.B., 1997. Anthelmintic treatment and the suppression of egg production in gastro-intestinal nematodes of sheep and cattle: fact or fallacy? N. Z. Vet. J. 45, 173–177.
Njue, A.I., Prichard, R.K., 2004. Efficacy of ivermectin in calves against a resistant *Cooperia oncophora* field isolate. Parasitol. Res. 93, 419–422.
Orpin, P., 2010. Potential avermectin resistance in a cattle herd. Vet. Rec. 167, 69–70.
Patterson, D.M., Jackson, F., Huntley, J.F., Stevenson, L.M., Jones, D.G., Jackson, E., Russel, A.J., 1996. Studies on caprine responsiveness to nematodiasis: segregation of male goats into responders and non-responders. Int. J. Parasitol. 26, 187–194.
Pomroy, W.E., Whelan, N.C., 1993. Efficacy of moxidectin against an ivermectin-resistant strain of *Ostertagia circumcincta* in young sheep. Vet. Rec. 132, 416.
Ranjan, S., Trudeau, C., Prichard, R.K., Vonkutzleben, R., Carrier, D., 1992. Efficacy of moxidectin against naturally acquired nematode infections in cattle. Vet. Parasitol. 41, 227–231.
Rose, J.H., 1968. Species of gastro-intestinal nematodes of cattle in S.E. England. Vet. Rec. 82, 615–617.
Sallovitz, J., Lifschitz, A., Imperiale, F., Pis, A., Virkel, G., Lanusse, C., 2002. Breed differences on the plasma availability of moxidectin administered pour-on to calves. Vet. J. 164, 47–53.
Sargison, N., Wilson, D., Scott, P., 2009. Relative inefficacy of pour-on macrocyclic lactone anthelmintic treatments against *Cooperia* species in Highland calves. Vet. Rec. 164, 603–604.
Sargison, N.D., Wilson, D.J., Penny, C.D., Bartley, D.J., 2010. Unexpected production loss caused by helminth parasites in weaned beef calves. Vet. Rec. 167, 752–754.
Scott, E.W., Baxter, P., Armour, J., 1991. Fecundity of anthelmintic resistant adult *Haemonchus contortus* after exposure to ivermectin or benzimidazoles *in vivo*. Res. Vet. Sci. 50, 247–249.
Soutello, R.G., Seno, M.C., Amarante, A.F., 2007. Anthelmintic resistance in cattle nematodes in northwestern Sao Paulo State, Brazil. Vet. Parasitol. 148, 360–364.
Stafford, K., Coles, G.C., 1999. Nematode control practices and anthelmintic resistance in dairy calves in the south west of England. Vet. Rec. 144, 659–661.
Stafford, K., Morgan, E., Coles, G., 2010. Sustainable anthelmintic use in cattle. Vet. Rec. 167, 309.
Stromberg, B.E., Gasbarre, L.C., Waite, A., Bechtol, D.T., Brown, M.S., Robinson, N.A., Olson, E.J., Newcomb, H., 2012. *Cooperia punctata*: effect on cattle productivity? Vet. Parasitol. 183, 284–291.
Sutherland, I.A., Leathwick, D.M., 2011. Anthelmintic resistance in nematode parasites of cattle: a global issue? Trends Parasitol. 27, 176–181.
Vermunt, J.J., West, D.M., Pomroy, W.E., 1996. Inefficacy of moxidectin and doramectin against ivermectin-resistant *Cooperia* spp of cattle in New Zealand. N. Z. Vet. J. 44, 188–193.
Waghorn, T.S., Leathwick, D.M., Rhodes, A.P., Jackson, R., Pomroy, W.E., West, D.M., Moffat, J.R., 2006. Prevalence of anthelmintic resistance on 62 beef cattle farms in the North Island of New Zealand. N. Z. Vet. J. 54, 278–282.
Whang, E.M., Bauer, C., Kollmann, D., Burger, H.J., 1994. Efficacy of two formulations (injectable and pour-on) of moxidectin against gastrointestinal nematode infections in grazing cattle. Vet. Parasitol. 51, 271–281.

Please cite this article in press as: Bartley, D.J., et al., Characterisation of macrocyclic lactone resistance in two field-derived isolates of *Cooperia oncophora*. Vet. Parasitol. (2012), http://dx.doi.org/10.1016/j.vetpar.2012.07.022