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# Ivermectin and moxidectin resistance characterization by larval migration inhibition test in field isolates of *Cooperia* spp. in beef cattle, Mato Grosso do Sul, Brazil

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ABSTRACT

Ivermectin (IVM) resistance of *Cooperia* spp. in cattle has become an increasing and global problem. The early detection of anthelmintic resistance (AR) is important to propose strategies to slow down the development of resistance and requires sensitive, reliable, economic high-throughput and practical tests. The purpose of the present study was to apply a larval migration inhibition test (LMIT) for evaluating IVM and MOX efficacy against well-characterized field isolates of *Cooperia* spp. infecting cattle in Brazil. Eight isolates were used for IVM and seven for MOX. The following EC50 values of IVM were observed for the isolates: susceptible, 1.16 ηmol; Nova Alvorada do Sul I, 4.09 ηmol (RF = 3.52); Campo Grande BNA, 3.57 ηmol (RF = 3.07); Campo Grande TBR, 4.09 ηmol (RF = 3,52); Nova Alvorada do Sul II, 2.50 ηmol (RF = 2.15); Bandeirantes, 11.35 ηmol (RF = 9.78); Campo Grande II, 6.03 ηmol (RF = 5.20); and Porto Mortinho, 8.63 ηmol (RF = 7.44). For MOX, the following EC50 values were observed: susceptible, 0.75 ηmol; Campo Grande BNA, 0.93 ηmol (RF = 1.24); Campo Grande TBR, 0.36 ηmol (RF = 0.48); Nova Alvorada do Sul II, 2.57 ηmol (RF = 3.42); Bandeirantes, 1.43 ηmol (RF = 1.90); Campo Grande II, 1.08 ηmol (RF = 1.44); and Porto Mortinho, 0.49 ηmol (RF = 0.65). The LMIT used in the present study can be a useful tool for *in vitro* evaluation of IVM, but not of MOX. However, such methodology cannot be used in large-scale studies yet. The isolates of *Cooperia* spp. showed various degrees of resistance to IVM, though remaining susceptible to MOX.



## 1. Introduction

Helminth infections in ruminants are usually subclinical but can determine significant economic losses due to both mortality and reduced productivity of animals. The helminthes of major importance in cattle in Brazil are *Cooperia* spp. and *Haemonchus placei*, which are the most prevalent, with greater infection intensity (Santos et al., 2010) and more reports of resistance to anthelmintics (Borges et al., 2005; Soutello et al., 2007; Souza et al., 2008). *Cooperia punctata* infection can reduce feed intake and live weight gain and influence the phosphorus kinetics, reducing P intake, absorption and retention (Louvandini et al., 2009). Recently, Stromberg et al. (2012) evaluated the effect of *C. punctata* in beef cattle and observed deleterious effect on dry feed uptake (0.68 kg/day) and weight gain (0.11 kg/day) in a period of 60 days.

There are a growing number of reports of ivermectin (IVM) resistance in beef cattle, mostly of the genus *Cooperia*, in many countries, such as New Zealand (Jackson et al., 1987), United Kingdom (Coles et al., 1998), Argentina

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(Anziani et al., 2000; Fiel et al., 2000), Brazil (Paiva et al., 2001; Soutello et al., 2007; Souza et al., 2008), Venezuela (Sandoval et al., 2001), Chile (Sievers and Fuentealba, 2003), Nigeria (Fashanu and Fagbemi, 2003), Mexico (Mena et al., 2008), Sweden, Belgium and Germany (Demeler et al., 2009), the United States (Gasbarre et al., 2009; Stromberg et al., 2012) and Australia (Lyndal-Murphy et al., 2010; Rendell, 2010). Most of these reports are related to resistance to ivermectin (IVM). However, there are few reports for resistance to moxidectin (MOX) (Vermunt et al., 1996; Anziani et al., 2000; Gasbarre et al., 2009; Condi et al., 2009).

The early detection of anthelmintic resistance (AR) requires sensitive, reliable, economic high-throughput and practical tests. The fecal egg count reduction test (FECRT) is considered to be the least sensitive and reliable test for the detection of AR and the limitations of this methodology are more evident in cattle than in sheep and goats because of the lower fecal egg output (Coles et al., 2006; El-Abdellati et al., 2010; Sutherland and Leathwick, 2011).

Up to now, only benzimidazole (BZD) resistance can be detected by using one or more of the three well-known SNPs in the beta-tubulin isotype-1 gene at codons 200 (Phe200Tyr) (Kwa et al., 1994), 167 (Phe167Tyr) (Silvestre and Cabaret, 2002) and 198 (Glu198Ala) (Ghisi et al., 2007). The limited knowledge of the molecular basis of resistance to other anthelmintic (AH) classes in parasitic nematodes and the polygenic resistance to levamisole (Sangster et al., 1991) and ivermectin (McCavera et al., 2007) make it more complex to develop resistance markers.

The limitations of FECRT in cattle and the lack of molecular markers to detect macrocyclic lactone (ML) resistance stimulated the development of assays based on parasite motility, specifically the larvae (L3) stage. The phenotypic AR characterization can be also performed by several *in vitro* tests that are more sensitive and in some cases more rapid, economic and practical than FECRT. Larval migration inhibition test (LMIT) showed sensitivity of 10% for detecting ML resistance in *Haemonchus contortus* (Kotze et al., 2006).

Previous *in vitro* migration assay for detection of IVM resistance in ruminants was described for *H. contortus* (Gill et al., 1991), *Trichostrongylus colubriformis* and *T. circumcincta* (Gill and Lacey, 1998), but the assessment of motility was quite subjective, so the use of agar and sieves for the separation of migrating (survival) and non-migrating (dead) L3 was assessed and validated for reliable quantification of migration (D'assonville et al., 1996; Kotze et al., 2006). Recently, a ring test was carried out in six different laboratories to evaluate a larval migration inhibition test (LMIT) for detection of IVM resistance in *O. ostertagi*, *Cooperia oncophora*, and *H. contortus* (Demeler et al., 2010a). Special attention was given to the preparation of IVM solutions and the authors observed high reproducibility when using this standardized protocol. Unfortunately, there are no validated *in vitro* tests available for IVM diagnosis in *C. punctata* and *H. placei*, the main cattle nematodes in the tropics.

The purpose of the present study was to apply a LMIT for evaluating IVM and MOX efficacy against well-characterized field isolates of *Cooperia* spp.

## 2. Material and methods

### 2.1. IVM resistant field isolates of Cooperia spp.

The seven field isolates of *Cooperia* spp. were obtained between June 13, 2009 and December 03, 2010 from beef cattle farms in Mato Grosso do Sul. The phenotypic evidence of IVM resistance has been characterized by FECRT, according to Coles et al. (1992) in a previous study (Feliz, 2011), as described in Table 1.

After characterization of IVM resistance, fecal samples from each farm were collected for the *in vitro* LMIT in agar gel. Nevertheless, this technique requires the use of pure cultures of parasites, thus, as four genera of the nematode (*Haemonchus*, *Cooperia*, *Trichostrongylus* and *Oesophagostomum*) were found in the same farm, isolation and production of monospecific L3 was required.

In addition to the isolates from the pre-treatment samples from the aforementioned farms, another isolate sensitive (CNPGC/UFMS) to IVM and MOX was used, which was kept at the *Centro Nacional de Pesquisa em Gado de Corte* (CNPGC – Embrapa, MS), has been cryopreserved since 2004 and has been reactivated on November 8, 2009, for the calculation of the resistance factor (RF).

### 2.2. Production of cultures of monospecific larvae

90-Day-old crossbred (Holstein-Gyr) male calves in good health condition were kept in the animal isolation sector of the *Faculdade de Medicina Veterinária e Zootecnia* (FAMEZ), in individual stalls that were cleaned daily with sodium hypochlorite and a wire brush, to help preventing helminth reinfections. Water was provided *ad libitum* and the animals were fed diets containing corn silage twice a day.

The animals were exposed to oral experimental infection with 500 L3 per kg live weight containing *Cooperia* spp., *Haemonchus* spp., *Trichostrongylus* spp. and *Oesophagostomum* spp., obtained at the aforementioned farms and identified according to morpholical descriptions of Keith (1953). Since the prepatent period of *Cooperia* spp. is shorter than that of other species, being around 14–16 days, the eggs eliminated after this period and until day 22 belonged to this genus. During this period, daily fecal cultures were produced (Roberts and O'Sullivan, 1950) for obtaining the L3. Seven days later, the L3 were extracted, their morphological identification was confirmed (Keith, 1953) and if 100% of the L3 were from *Cooperia* spp. the samples were used in the *in vitro* trial to determine the median effective concentration (EC50).

### 2.3. Chemicals

Commercial formulations of IVM (Ivomec® Solução Injetável, Merial Saúde Animal, Batch number 060/08) and MOX (Cydectin® Solução Injetável, Fort Dodge Saúde Animal, Batch number 014/08) diluted in 1% dimethyl sulfoxide (DMSO, Sigma–Aldrich D4540) were used at the following concentrations: 0.5, 1, 2, 4, 8, 16, 32 and 64 ηmol/0.5 mL distilled water.