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Characterization of moxidectin resistant *Trichostrongylus colubriformis* and *Haemonchus contortus*

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Abstract

The development of moxidectin resistance (MOX-R) in sheep parasitic gastrointestinal nematodes already carrying multiple resistances to other anthelmintic groups has made control of these strains very difficult. The anthelmintic resistance patterns of MOX-R strains of *Trichostrongylus colubriformis* and *Haemonchus contortus* were characterized to provide an insight into the remaining role of anthelmintics in the control of such strains. Homozygous MOX-R individuals of both genera were unaffected by moxidectin. For MOX-R heterozygotes a dose rate of 200 μg/kg abamectin (ABA) given orally removed 25% of *H. contortus* while 200 μg/kg MOX given orally achieved a 72% reduction. Doubling the dose rate of ABA improved the mean efficacy to 37%. Consequently, in *H. contortus*, the degree of dominance differs markedly between the two anthelmintics. A dose rate of 8mg/kg levamisole and 185 mg/kg napthalophos achieved >95% reduction in worm count of the MOX-R homozygous *H. contortus* but only 85 and 7%, respectively against the MOX-R homozygous *T. colubriformis*.

Keywords: Moxidectin; Abamectin; Napthalophos; Levamisole; Anthelmintic resistance; *Trichostrongylus colubriformis*; *Haemonchus contortus*; Sheep parasites; Goat parasites

1. Introduction

Until recently, what was known about macrocyclic lactone (ML) resistance was restricted to studies on resistance that had developed against the first generation ML, ivermectin (IVM) (Gill and Lacey, 1998). IVM resistance provided protection against IVM either as a drench or delivered in a sustained release device. In field-selected strains, IVM resistance (IVM-R) was inherited as an autosomal, major effect gene (Le Jambre et al., 2000). IVM-R provided limited side resistance to the more potent second generation ML, moxidectin (MOX) (Barnes et al., 2001). In IVM-R *Haemonchus contortus* MOX was

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equally effective against both heterozygote and homozygote worms, while in IVM-R *Ostertagia* circumcincta the homozygote resistant worms were more likely to survive MOX than the heterozygotes. As well as this potency, MOX also has a persistent activity against *Haemonchus* and *Ostertagia*. However, in this case IVM-R worms can re-establish during the period that the persistent activity prevents susceptible worms from establishing.

Now, however, MOX resistant strains of *H. contortus* are being reported in Australia (Love et al., 2003) and a MOX resistant strain of *O. circumcincta* has been reported from New Zealand (Sutherland et al., 1999). These strains can survive the initial exposure to MOX as well as the persistent activity. Therefore, during MOX exposure, MOX resistant (MOX-R) worms in contrast to the IVM-R worms continue to lay eggs through the entire period when MOX treatment is killing MOX susceptible worms.

Present drenching recommendations as well as simulation model outputs (e.g. Le Jambre et al., 1999; Barnes et al., 2001) are based on what is known about IVM resistance and not MOX resistance. Consequently, the debate on whether high potency against resident worms is or is not offset by selection during the persistent phase is biased by the lack of knowledge about MOX resistance. If, as it appears, resident MOX-R worms are unaffected by MOX, then the persistency of the drug would leave these worms unaffected by competition for at least 35 days (Shoop et al., 1997). Likewise, it needs to be determined whether MOX is more effective against the heterozygotes carrying the MOX-R gene(s) than against the homozygotes. Abamectin (ABA), a ML more potent than IVM but without the persistency of MOX, should also be tested against MOX-R homozygotes and heterozygotes. It is urgent that these questions regarding the MOX resistance phenotype be answered so that the information provided to industry by veterinary consultants on parasite control is up-to-date and of the highest quality. Consequently, when goat faeces sent to the Department of Primary Industries, Queensland (DPI, Queensland) were found to be positive for trichostrongylid eggs following treatment with MOX we decided to isolate these parasites and characterize their MOX resistance phenotype.

2. Materials and methods

2.1. Resistant strains

A goat farm where MOX was putatively failing to remove resident adult worms located on the Gold Coast hinterland was the source of the resistant strains. The drenching record for this property indicated that some age groups of goats were being drenched eight times per year with MOX. IVM drenches had not been used for the past two years when it was noticed that they were failing to control parasites. At that time MOX became the ML of choice.

Faeces sent in to the DPI, Queensland for faecal egg counts (FEC) were cultured. The third stage larvae (L3) developing from these cultures were used to infect sheep at the CSIRO McMaster Laboratory, Armidale. Once the infections became patent the sheep were dosed with 100 μg/kg MOX. There was no apparent decrease in FEC following this treatment. The eggs were then collected and cultured to produce L3. An experiment was then designed to confirm resistance in adult resident worms to MOX. Fifteen 14-month-old Merino wethers were infected with L3 raised from the sheep treated with the 100 μg/kg MOX. This was a mixed species infection but based on L3 identification each sheep received an infecting dose containing approximately 10,000 *H. contortus* and 9000 *Trichostrongylus colubriformis*. On day 21-post infection, faecal egg counts were done and the sheep allocated to groups using stratified random assignment based on FEC. The groups were: (1) control (not treated), (2) single dose MOX (=200 μg/kg moxidectin) and (3) double dose (=400 μg/kg moxidectin). The MOX was administered as a drench of oral Cydectin® (Ft Dodge Australia Ltd.). Seven days following treatment with MOX the arithmetic mean egg counts were control = 10,400, single dose = 2240 and double dose = 580 epg. On day 13 post treatment with MOX the sheep were slaughtered for worm counts as described below. The reduction in worm counts, compared to controls, was *H. contortus* 57% and 90% and *T. colubriformis* 41% and 80% for single and double dose of MOX, respectively. This confirmed that both species were resistant to MOX.

Faecal cultures were made from the sheep receiving the double dose of MOX between dosing and slaughter. These L3 were used to infect four worm