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Research paper

Adaptation of a 96-well plate larval migration inhibition test for measuring the sensitivity of cyathostomins to macrocyclic lactone anthelmintics

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ABSTRACT

The use of macrocyclic lactone drugs for control of equine cyathostomins is threatened by increasing levels of resistance. Detection of changes in drug sensitivity is important for effective and sustainable management of cyathostomins, however, at present such detection relies on the use of the faecal egg count reduction test, which is known to be an insensitive method. The present study therefore aimed to examine the use of a 96-well plate larval migration inhibition test for detection of resistance to macrocyclic lactone drugs in cyathostomins. We optimised conditions for migration of larvae, and examined the effects of larval storage time on drug dose responses. The modified test was able to define the sensitivity of cyathostomin isolates to ivermectin and eprinomectin in terms of dose response curves, and IC_{50} and IC_{95} values. The IC_{95} showed much greater consistency than the IC_{50} with larvae that had been stored for different periods prior to the test. Comparisons between two isolates, which had both been defined previously as susceptible using faecal egg count reduction tests, showed more variation at the IC_{50} compared to the IC_{95}. Limitations of the test included the degree of variation in control-well migration despite optimisation of migration incubation conditions, and the need to incorporate a method to determine the species composition of the larval populations to account for possible species differences in drug sensitivity among cyathostomins. Validation of the technique on reference susceptible and resistant isolates of known species composition is still required.

1. Introduction

Cyathostomins are the most important target for equine worm control strategies worldwide. Management of these ubiquitous nematodes, however, has been hampered by the development of anthelmintic resistance over the last 40–50 years to the extent that resistance is now one of the biggest challenges facing horse owners and managers (Matthews, 2014). Resistance to tetrahydropyrimidine anthelmintics in cyathostomins has been reported widely, while resistance to benzimidazole (BZ) drugs is also considered highly prevalent (Kaplan, 2002). Hence, the macrocyclic lactone (ML) drugs have become the cornerstone of worm control programs worldwide. Unfortunately, the sustainability of this use of ML drugs is threatened by reports of drastically shortened egg reappearance periods (ERPs) (von Samson-Himmelstjerna et al., 2007; Dudeney et al., 2008; Lyons et al., 2008; Molento et al., 2008; Lyons and Tolliver, 2013) due to reduced efficacy against luminal L4 stages (Lyons et al., 2009; Lyons et al., 2010; Lyons and Tolliver, 2013), and efficacies of < 95% as determined with faecal egg count reduction tests (FECRTs) (Traversa et al., 2009; Canever et al., 2013; Bishop et al., 2014; Relf et al., 2014).

An important component of combating increasing levels of ML resistance is the use of sensitive resistance-detection tools, allowing the earliest possible detection of the emergence of resistance on a property. However, an adequate test has yet to be developed for the horse industry. The gold standard FECRT has many limitations as it is applied to horses (Kaplan, 2002) and is only thought to be reliable when at least one quarter of the nematode population are resistant (Martin et al., 1989). At this level of sensitivity, significant levels of resistance are theoretically able to develop undetected at the farm level. Once resistance is detected by the FECRT at 14 days post-treatment, very little can be done to revert the cyathostomin population towards sensitivity (Jackson and Coop, 2000; Leathwick et al., 2001) and the drug thereafter is of limited use. Until important genetic breakthroughs are made elucidating the genes involved in ML resistance which may lead to a suitable molecular test (Kotze et al., 2014), efforts may be best placed in the adaptation of *in-vitro* methods that have shown promise in measuring differences in drug sensitivity in ruminant nematodes (Coles et al., 2006; Kotze et al., 2006; Demeler et al., 2010b).

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Table 1
Comparison of larval migration inhibition test protocols used to test the sensitivity of a range of nematode species to various anthelmintic drugs.

Nematode species	Exsheathed	Plate Format (well number)	Mesh pore size (um)	Agar used	Drugs tested	Drug exposure phase			Migration phase			Reference
						Temp (°C)	time (h)	light/dark	Temp (°C)	time (h)	light/dark	
CYA	Y		40	N	IVM	37	2	dark	37	1	dark	Van Doorn et al. (2010)
CYA	Y	24	25	N	MOX IVM EPM ABM	21	2	dark	37	45 min	dark	Rendon (2012)
CYA	Y	24	25	N	IVM	26	2	dark	26	2	dark	McArthur et al. (2015)
CYA	Y	24	25	N	Plant extracts	26	24, 18		26	2		Peachey et al. (2015, 2016)
H. con	Y	Petrie dish: 10 reps each for control and IVM	38	Y (1.4%)	IVM	28	6	dark	28	18	light	d'Assonville et al. (1996)
H. con	Y	Vials/cylinders		Y (1.4%)	IVM MOX	27	6		27	18	light	(Molento and Prichard, 2001)
H. con	N	96	20/40	N	IVM	28	24		28	24		(Schutjens, 2014)
H. con	N	96	20	Y (0.1%)	IVM LEV TBZ	27	48		27	24	light	Raza et al. (2015)
T. col	Y	48	20	N	LEV	22	2		22	16		Wagland (1992)
T. col	Y	48	20		LEV MOR PIP	room	14–18					Rabel et al. (1994)
T. col	Y	48	20		Condensed tannins	37	2		room	16–18		Molan et al. (2000)
Cooperia spp	Y	24	50 + 38	Y (1.4%)	IVM MOX	27	6		27	18	light	(Almeida et al., 2013)
C. onc	N	24	28	Y	IVM	28	24	dark	28	24		(El-Abdellati et al., 2010)
H. con *T. col* *T. circ*	N	96	20	Y (0.125%)	IVM EPM ABM	27	24		27	48	light	Kotze et al. (2006)
O. ost	N	24	25–30	Y (1.5–2%)	IVM	28	24	dark	28	24	light	Demeler et al. (2005, 2010a,b, 2012, 2013)
C. onc			(28)		LEV TBZ							

Nematode species: CYA = cyathostomins; T. col = *Trichostrongylus colubriformis*; H. con = *Haemonchus contortus*; T. circ = *Teladorsagia circumcincta*; O. ost = *Ostertagia osteragi*; C. onc = *Cooperia onchophora*. Drugs tested: IVM = ivermectin; MOX = moxidectin; EPM = eprinomectin; ABM = abamectin; LEV = levamisole; MOR = morantel; PIP = piperazine; TBZ = thiabendazole. Y = yes; N = no. Blank field indicates no description was provided of the particular condition.