Short Communication

Detection of anthelmintic resistance on two Irish beef research farms

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BEEF and milk production systems in Ireland are largely dependent on a grass-based diet with cattle spending long periods of the year at pasture (Drennan and McGee 2009). As a result, cattle are frequently exposed to gastrointestinal nematode (GIN) challenge. Within grass-based production systems, anthelmintics are essential to optimising performance and any reduction in their efficacy may lead to reduced animal performance. Although anthelmintic resistance (AR) is more commonly reported in sheep (Sutherland and Leathwick 2011), it has been increasingly reported in cattle worldwide (Stafford and Coles 1999, Loveridge and others 2003, Soutello and others 2007, Demeler and others 2009, Edmonds and others 2010). To date, there have been no published reports of AR in cattle in Ireland.

We report the findings of a faecal egg count reduction test (FECRT) conducted at two Irish agricultural research farms (Study A and B). Two different software programmes were used to determine anthelmintic efficacy.

All animal procedures performed in this study were conducted under experimental license (B100/2869) from the Irish Department of Health and Children, in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendment of Cruelty to Animals Act 1876) Regulation 2002 and 2005.

Study A was conducted at Teagasc, Johnstown Castle, Wexford, Co. Wexford, Ireland (longitude 6°30′ W; latitude 52°17′ N; elevation 65 m above sea level (asl)). Sixty Holstein-Friesian male calves, naive to anthelmintics, were used in this study. Calves were randomised by age, weight and FEC to one of four treatment groups (n=15 per treatment) on July 4, 2013 (day 0) using the recorded calf age, live weight and FEC at day –7; (1) untreated control; (2) ivermectin injection (Ivomec Classic Injection for Cattle and Sheep 10 mg/ml, Merial Animal Health Limited); (3) levamisole injection (Levacide Injection 75 mg/ml, Norbrook Laboratories Limited) and (4) fenbendazole drench (Panacur 10 per cent W/V Oral Suspension, MSD Animal Health Ireland). Mean calf age (sd), live weight (sd) and FEC were 148 (8.4) days, 132 (16.9) kg and 407 (230) eggs per gram (EPG) at day 0, respectively. Calves were individually faecal sampled on days 0, 7 and 14 to determine their FEC.

Study B was conducted at the Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland (longitude 6°40′ W; latitude 53°30′ N; elevation 92 m asl). Twenty-four Holstein-Friesian male calves, naive to anthelmintics, were used in this study. Calves were vaccinated against dictyocaulosis (Bovilis Huskvac, MSD Animal Health Ireland) at two and six weeks before turnout on July 4, 2013. Calves were randomised by age, live weight and FEC to one of two treatment groups (n=12 per treatment) on August 12, 2013 (day 0) using the recorded calf age, live weight and FEC at day -4; (1) untreated control and (2) ivermectin injection (Ivomec Classic Injection for Cattle and Sheep 10 mg/ml, Merial Animal Health Limited). Mean calf age (sd), live weight (sd) and faecal egg count (FEC) were 252 (43.6) days, 154 (47.7) kg and 473 (322) EPG at day 0, respectively. Calves were individually faecal sampled on days 0 and 14 to determine FEC.

Individual calf FECs in both studies were determined using the McMaster method with a sensitivity of 50 EPG (Urquhart and others 1996).

Composite faecal cultures were performed (2 g of faeces per calf) on each sampling occasion to determine the composition of nematode genera in each treatment group. Cultures were incubated at 27°C for eight days and 100 L_3 larvae per culture were identified to genus level using standard identification keys (van Wyk and Mayhew 2013) on recovery. All L_3 larvae were identified when counts were less than 100. Data were analysed using the RESO FECRTv4 programme (Anonymous 1989) and the 'eggCounts' package (Torgerson and others 2014).

Cooperia was the only nematode genus recovered in post-treatment culture on day 14 in ivermectin-treated calves in both studies (Table 1). Based on the criteria for AR defined by Coles and others (1992), resistance to ivermectin was detected on both farms in the present study.

As *Cooperia* is the dose-limiting nematode for macrocyclic lactones in cattle, resistance of this genus to ivermectin is always likely to develop (Coles 2002). However, as injectable macrocyclic lactones achieve higher concentrations in abomasal mucosa than in intestinal mucosa (Lifschitz and others 2000), this may be another factor that explains their poorer efficacy towards intestinal dwelling nematodes such as *Cooperia*. On both research farms, macrocyclic lactones were used in first-grazing season calves for the previous three years with no rotation in anthelmintic classes. Such a practice may potentially encourages selection for AR-resistant nematode alleles (Urquhart and others 1996).

Although *Cooperia* species are regarded as less pathogenic compared to *Ostertagia*, and as such diagnosis of *Cooperia*-associated AR may not be as clinically significant, *Cooperia* infection can still result in production losses (Herlich 1965, Stromberg and others 2012).

Levamisole and fenbendazole demonstrated high efficacy in treating GIN challenge. However, the results of the FECRT conducted in Study A demonstrate the necessity to always sample levamisole-treated calves on day 7 post-treatment and use these FEC values for determining levamisole efficacy. This is due to the modest efficacy of levamisole against juvenile stages of common GIN (Grimshaw and others 1996), some of which may begin producing nematode eggs by day 14 post-treatment leading to misleading results for levamisole efficacy.

Both software programmes used to determine FECR demonstrated a high level of agreement in both studies (Table 2).

This is the first report of AR in cattle in Ireland. Farmers, agricultural advisers and veterinary practitioners need to be made aware of the

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TABLE 1: Nematode genera identified in faecal cultures and mean FEC of calves in treatment groups on each sampling occasion

			Study A				Study B	
Day		Treatment	Control	Ivermectin	Levamisole	Fenbendazole	Control	Ivermectin
0		Mean (sd) FEC*	463 (251.0)	373 (246.3)	367 (160.0)	423 (257.7)	440 (330.0)	508 (339.0)
	Genus	*Cooperia*	+	+	+	+	+	+
		Ostertagia	+	+	+	+	+	+
		Trichostrongylus	–	–	–	–	+	+
7		Mean (sd) FEC*	560 (600.4)	177 (116.3)	0 (0)	40 (116.8)		
	Genus	*Cooperia*	+	+	–	–		
		Ostertagia	+	+	–	–		
		Trichostrongylus	–	–	–	–		
14		Mean (sd) FEC*	500 (319.9)	253 (168.5)	30 (52.8)	3 (12.9)	245 (211.5)	154 (107.6)
	Genus	*Cooperia*	+	+	+	–	+	+
		Ostertagia	+	–	–	–	+	–
		Trichostrongylus	–	–	–	–	–	–

+/– indicates presence/absence of a genus in faecal cultures
*Denotes arithmetic mean (sd)
FEC, faecal egg count

Table 2: FECRT reduction values using the RESO programme and the 'eggCounts' package

		Study A		Study B
	Ivermectin	Levamisole	Fenbendazole	Ivermectin
Software programme				
RESO FECRTv4	49 (16 to 70)	100*	99 (94 to 100)	37 (0 to 69)
'eggCounts'	49 (35 to 63)	99.5 (97 to 100)	99 (96.4 to 99.8)	37.8 (7.2 to 60.3)

Per cent reduction (CI range)
*Unable to generate a CI
FECRT, faecal egg count reduction test

findings to ensure continual adoption of best practice in relation to the use of anthelmintics on farms, including the rotation of anthelmintics. A larger multisite study is needed to determine if the study findings are an isolated incident, or if AR is common on cattle rearing farms in Ireland.

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