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Research paper

Development of *Haemonchus contortus* resistance in sheep under suppressive or targeted selective treatment with monepantel

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ABSTRACT

This study examined the development of resistance to anthelmintics in *Haemonchus contortus* in lambs under suppressive or selective treatment regimens that included monepantel. Twenty Ile de France and 20 Santa Ines lambs were allocated to two anthelmintic treatment regimens, based on body weight and nematode faecal egg counts (FEC): targeted selective treatment (TST) or suppressive treatment, both with monepantel. Lambs of the TST group were treated individually when they presented with a packed cell volume (PCV) ≤20%. On 7 October 2016, the lambs were allocated to clean pastures, where they grazed in separated paddocks by group until late February 2017. The experimental area was contaminated with nematodes that were introduced with the experimental Ile de France and Santa Ines lambs, naturally infected with gastrointestinal nematodes. To maintain the grazing lambs in the suppressive treatment group and their pasture as free of worms as possible, these lambs were treated with anthelmintics before being allocated to their paddock and then were periodically treated with monepantel. However, the use of a suppressive treatment regimen that included monepantel over a period of 3 months resulted in the emergence of a population of resistant *H. contortus*. In the TST group, there was a rapid and progressive reduction in the efficacy of monepantel, which at the end of the experiment was only 76%. The Ile de France lambs were all treated one or more times during the experiment, whereas only two Santa Ines lambs in the TST required treatment. In conclusion, a population of *H. contortus* resistant to monepantel emerged quickly during the rainy season, even when sheep were submitted to selective treatment.

1. Introduction

Parasitism by gastrointestinal nematodes (GIN) is an important cause of economic losses in the sheep industry. Prophylaxis against GIN infections is based on anthelmintic treatment. However, lack of professional guidance and ease of access to anthelmintics have led farmers to frequently and indiscriminately use these drugs on their flocks. Therefore, parasites with resistance to most important chemical compounds (benzimidazole, macrocyclic lactone, imidazothiazoles, tetrahydropyrimidines, and salicylanilides) have emerged, resulting in a global problem that jeopardizes the control of nematode infections in ruminants (Kaplan et al., 2004; Gilleard, 2006; Amarante, 2014; Bartley et al., 2015).

A new class of anthelmintic, derived from amino-acetonitrile (AAD) and called monepantel (Kaminsky et al., 2008), was introduced into the market in 2009 and is considered an important alternative treatment when there is multiple resistance to other classes of antiparasitics used to treat sheep (Ciuffa et al., 2013). In 2013, two nematode species (*Teladorsagia circumcincta* and *Trichostrongylus colubriformis*) resistant to monepantel were first reported in New Zealand (Scott et al., 2013). Since then, resistance in *Haemonchus contortus* has been reported in Uruguay (Mederos et al., 2014), the Netherlands (Van den Brom et al., 2015), Australia (Sales and Love, 2016; Lamb et al., 2017), and Brazil (Martins et al., 2017).

The mechanisms that underlie the emergence of resistance are complex and may include mutations in the genomes of parasites (Rufener et al., 2013). In *Caenorhabditis elegans* resistant to monepantel, a spontaneous mutation in the *acr-23* allele was identified, whereas in *H. contortus*, a mutation was found to occur in *Hco-mptl-1* and *Hco-des-2H* (Rufener et al., 2009; Bagnall et al., 2017).

Frequent treatment is considered one of the main risk factors for the development of anthelmintic resistance, especially when the anthelmintic is administered to all of the sheep in a flock. Resistant parasites, which survive treatment, are selected and reproduce, resulting in a

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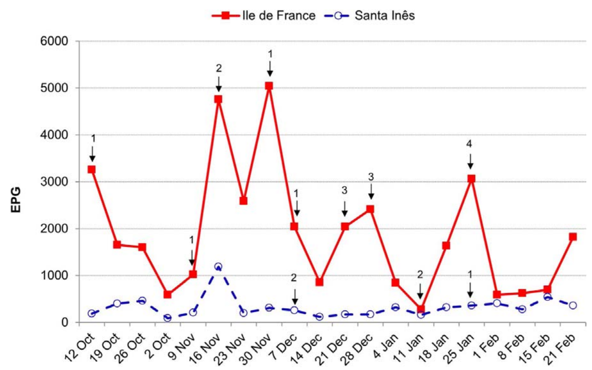

Fig. 1. Average of eggs of strongyles per gram of faeces (EPG) of lambs under targeted selective treatment with monepantel. Arrows indicate the number of animals treated on each occasion owing to PCV ≤ 20%.

progressive increase in the frequency of genes in the population that confer resistance (Besier, 2012; Falzon et al., 2014). To increase the size of the worm population in refugia, targeted selective treatment (TST) has emerged as an alternative treatment regimen that can be used to delay the onset of anthelmintic resistance (Besier, 2012).

From October 2016 to February 2017 (the rainy season), in an experiment designed to determine the productive performance of lambs, a decrease in the efficacy of monepantel was observed. Thus, in the present study, we aimed to describe the development of *H. contortus* resistance in lambs naturally infected by GIN and submitted to suppressive or selective treatment with monepantel.

2. Material and methods

2.1. Animals and pasture

All the procedures involving animals in this study are in accordance to international ethical standards and were approved by the Animal Use Ethics Committee of the FMVZ/Unesp (47/2016).

Twenty Ile de France and 20 Santa Ines 3-month-old uncastrated male lambs were purchased from farms located in São Manuel and Vitoriana, respectively, in São Paulo state, Brazil. The lambs were raised indoors at their farms of origin. The Santa Ines and Ile de France lambs arrived in the experimental area of the University on September 29 and 30, 2016, respectively, where they were housed until the beginning of the experiment. On the day of arrival, blood and faecal samples were collected for haematological and parasitological examinations. The Ile de France and Santa Ines lambs presented averages of 435 (range of 0–1100) eggs per gram (EPG) and 1553 (0–7300) EPG, respectively. Based on faecal culture, Santa Ines and Ile de France lambs were found to harbour *Haemonchus* spp. (63% and 86%, respectively) and *Trichostrongylus* spp. (37% and 14%, respectively) infections. All lambs received a clostridiosis vaccine (Poli-Star®, Vallée), with a booster dose administered 2 months later, and toltrazuril (14 mg/kg, Farmacox®, Farmabase Animal Health) to prevent coccidiosis.

The experiment was in a 2 × 2 factorial, with two anthelmintic treatment regimens (TST or suppressive) and two sheep breeds (Santa Ines and Ile de France). The animals of each breed were allocated to two groups balanced as far as possible for body weight and nematode faecal egg counts (FEC). The Ile de France showed average of 300 EPG (range 0–1100 EPG) and the Santa Ines average of 950 (0–7300 EPG) in the Group under suppressive treatment, while in the TST group, the Ile de France presented average of 300 (0–1100 EPG) and the Santa Ines 600 (0–6700 EPG).

At the beginning of the experiment, one Santa Ines and one Ile de France lamb died. For this reason, the experimental groups had the following number of animals:

- Group of lambs with low exposure to GIN that received suppressive treatment: 10 Ile de France and 9 Santa Ines lambs maintained in the same paddock.
- Group of high exposure to GIN that received TST: 9 Ile de France and 10 Santa Ines lambs maintained in the same paddock.

On 7 October 2016, the lambs were allocated in groups to separate paddocks in a pasture of *Cynodon* spp. and *Urochloa decumbens* that was maintained without ruminants from 1 March 2016 until the beginning of the present experiment. At the study site, nematode infective larvae were recovered for a maximum of 3–4 months after pasture contamination (Carneiro and Amarante, 2008). Because the pasture

Table 1
Faecal egg count reduction (FECR) in lambs under target selective treatment with monepantel (high-exposure group). Date of the treatment, number of eggs of strongyles per gram of faeces (EPG) on the day of the treatment (pre-treatment) and 14 days post-treatment.

Month	Lamb identification	Date	EPG		FECR (%)
			Pre-treatment	14 days post-treatment	
October	Ile de France – 33	12-Oct	15400	0	100%
November	Ile de France – 33	09-Nov	2700	400	98% (100; 91)
	Ile de France – 34	16-Nov	6700	0	
	Ile de France – 38	16-Nov	6200	100	
	Ile de France – 40	30-Nov	15500	0	
December	Santa Ines – 22	07-Dec	1100	0	83% (98; 0)
	Santa Ines – 29	07-Dec	200	0	
	Ile de France – 33	07-Dec	4200	0	
	Ile de France – 35	07-Dec	7600	0	
	Ile de France – 31	21-Dec	1800	0	
	Ile de France – 38	21-Dec	400	4800	
	Ile de France – 34	28-Dec	10100	300	
	Ile de France – 36	28-Dec	4500	100	
January	Ile de France – 31	11-Jan	100	0	76% (95; 0)
	Ile de France – 39	11-Jan	300	3800	
	Ile de France – 33	25-Jan	6300	600	
	Ile de France – 34	25-Jan	4400	400	
	Ile de France – 35	25-Jan	8000	400	
	Ile de France – 40	25-Jan	1900	100	
	Santa Ines – 22	25-Jan	1500	0	

FECR (%) in each month = 100 × (1–post-treatment mean EPG/pre-treatment mean EPG). Upper and lower confidence interval limits (95%) are in parentheses.