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Resistance of gastrointestinal nematodes to the most commonly used anthelmintics in sheep, cattle and horses in Spain

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ABSTRACT

The objective of this study was to evaluate the status of anthelmintic resistance (AR) in ruminants and horses in Spain. The efficacy of commonly used macrocyclic lactones (MLs) – ivermectin (IVM) and moxidectin (MOX) – was measured in sheep, cattle and horses. In addition, albendazole (ABZ) and levamisole (LEV) were evaluated in sheep and oxibendazole (OXI) and pyrantel (PYR) in horses. Efficacy was evaluated based on the difference between the arithmetic mean pre- and post-treatment faecal egg count (in cattle and horses), or compared to an untreated control group (in sheep). AR was present when the percentage reduction in egg count was <95% and the lower 95% confidence interval (CI) was <90%; if only one of these two criteria was met, the finding was recorded as suspected AR (SAR). In horses, AR–PYR and OXI was considered when the percentage reduction in egg count was ≤90% and the lower 95% CI was ≤80%. For each animal species, at least 10 study sites were selected.

AR to at least one of the drugs was detected in all 10 sheep flocks; the main parasite identified after treatment was *Teladorsagia circumcincta*. Moreover, in 5 flocks multidrug resistance was identified, on 4 farms to drugs from different families, on one farm to both MOX and IVM and on another farm to all drugs tested. In cattle, the efficacy of both MOX and IVM was 100% on 4 and 3 farms, respectively, and therefore 60% of these farms were considered to have AR or SAR to both MLs. The most frequent parasite identified after treatment was *Trichostrongylus* spp., although *Ostertagia ostertagi* was also identified after treatment on one farm. In contrast to ruminants, the 4 drugs evaluated in horses were highly efficacious against strongyles, with efficacies for the MLs and OXI between 95 and 100% and between 94 and 100% for PYR, although 3 herds were SAR against PYR.

In conclusion, AR to at least one of the commonly used drugs was identified on all sheep flocks investigated in the northwest of Spain. The occurrence of AR to MLs in cattle was higher than expected but consistent with what was observed in sheep. In horses, all currently used drugs were confirmed as effective against strongyles.

1. Introduction

Gastrointestinal nematodes (GIN) are the most common parasites causing disease in grazing ruminants and horses worldwide. The impact of GIN infection in these animal species is linked to the clinical signs associated with infection, but also to subclinical economic losses related with decreased growth and milk production and the costs of anthelmintic treatments.

Nowadays the prevalence of GIN infections is increasing again, not only as a consequence of global warming or environmental changes, but also by the development of anthelmintic resistance (AR) (Martínez-Valladares et al., 2013a). AR has emerged as a result of the frequent use of anthelmintics to control GIN infections, and farm management practices that provide insufficient refugia. Recently two anthelmintics, monepantel (Kaminsky et al., 2011) and derquantel (Little et al., 2010), have been introduced for the treatment of sheep with GIN. However, there are already some reports on AR against monepantel in New Zealand, The Netherlands and Uruguay (Scott et al., 2013; Dobson et al., 2014; Mederos et al., 2014), which indicates that developing new molecules is only a

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Table 1
Sheep farms (S01–10): arithmetic mean strongyle faecal egg count (FEC) and larval identification in the control animals at day 14 post-treatment.

Farm	FEC (range)	Larval identification in%			
		Haem	Tela	Tricho	Other species
S01	290 (105–765)	4	90	2	4 Bu
S02	41 (0–150)	0	98	2	0
S03	228 (0–1170)	0	98	2	0
S04	1491 (675–3540)	3	32	3	52 Bu + 7 Cha + 3 Nem
S05	157 (30–750)	0	94	6	0
S06	95 (0–510)	0	54	5	2 Bu + 39 Cha
S07	188 (30–480)	0	98	0	2 Cha
S08	32 (0–120)	0	90	10	0
S09	195 (0–720)	0	87	13	0
S10	228 (0–750)	0	100	0	0

Haem: *Haemonchus* sp.; Tela: *Teladorsagia* spp.; Tricho: *Trichostrongylus* spp.; Nem: *Nematodirus*; Cha: *Chabertia*; Bu: *Bunostomum*.

temporary solution. Sustainable use and integrating new molecules in well-thought out management programs are the path forward.

In order to do so, a better understanding of the occurrence of AR is needed. The worldwide occurrence of AR in sheep, cattle and horses has been already reviewed before (Sutherland and Leathwick, 2011; Ballweber and Baeten, 2012; Papadopoulos et al., 2012; Torres-Acosta et al., 2012; Matthews, 2014; Peregrine et al., 2014). Next to AR, the increasing number of reports on multidrug resistance (MDR) to the most commonly used anthelmintic families – benzimidazoles (BZ), macrocyclic lactones (ML), including moxidectin (MOX), in sheep, cattle and horses and also imidazothiazoles in sheep and the tetrahydropyrimidine pyrantel (PYR) in horses, causes concern. MDR has been described in sheep flocks from UK (Sargison et al., 2001), Spain (Martínez-Valladares et al., 2012, 2013b) or Brazil (Almeida et al., 2010; Cezar et al., 2010), in cattle herds in the US (Gasbarre et al., 2009) or in horses in Brazil (Canaver et al., 2013), among others.

In the current study, the present status of AR to the most used drugs is evaluated in sheep and cattle, in the northwest of Spain, and in horses in different regions of the country.

2. Materials and methods

2.1. Selection of study locations and screening

The study was conducted from October 2011 until November 2012, and included adult sheep flocks and beef cattle herds located in the northwest of Spain as well as herds of horses in northeast ($n = 2$), central ($n = 6$) and south of Spain ($n = 3$).

The sheep flocks were selected based on a history of frequent deworming in previous years, and reduction in production parameters despite treatment. However, cattle and horse herds were randomly selected.

At the beginning of the study, individual faecal samples, directly collected from the rectum, were randomly taken in each flock or herd to select those flocks or herds with the highest faecal egg count (FEC). Individual faecal samples were analysed using a modified McMaster technique (MAFF, 1986) with a sensitivity of 15 eggs per gram (epg) in sheep, 12.5 epg in cattle and 10 epg in horses. Therefore, according to the animal species each individual McMaster was carried out mixing 3 g of sheep faeces, 4 g of caw faeces or 5 g of horse faeces with 42, 46 or 45 ml of water, respectively. Then, all eggs present in 1 ml of the mixture were counted for the FEC.

All animals involved in the study were naturally infected with GIN.

2.2. In vivo detection of AR in GIN

A randomized complete block design was used for each flock or herd, with the individual animal as the experimental unit. Blocking was based on the pre-treatment individual FEC. FEC was determined using a modified McMaster technique for each animal species as previously described. In each flock or herd, suitable animals were ranked according to their pre-treatment strongyle FEC into groups; animals with the highest strongyle FEC were included first in the study.

The study in sheep flocks was performed on 10 farms with animals divided into 5 groups of 10 animals each. In addition to the untreated control group, there were four treatment groups. Animals were either treated with the oral formulations of albendazole (ABZ; Sinvermin ovino® at 3.75–4.75 mg/kg bodyweight; Syva) and levamisole (LEV; Endex® at a dose rate of 7.5 mg/kg; Novartis) or with the injectable formulations of MOX (Cydectin®, injectable solution 1% at 0.2 mg/kg bodyweight; Zoetis) and IVM (Ivomec®, at subcutaneous injection at 0.2 mg/kg bodyweight; Merial).

A total of 10 cattle herds were included in the study. In each herd animals were distributed between 2 groups with a target number of 10 animals. Animals were treated by subcutaneous injection with injectable formulations of MOX (Cydectin®, injectable solution 1% at 0.2 mg/kg bodyweight; Zoetis) or IVM (Ivomec®, 1% injection at 0.2 mg/kg bodyweight; Merial).

The study also included 11 herds of horses. In each herd, horses were distributed over 4 groups with a target number of 4–7 animals per herd, depending on the availability of GIN positive horses. The arithmetic mean FEC in all treatment groups on each herd had to be above the minimal threshold of 100 epg. Animals were either treated with oral formulations of MOX (Equest® at 0.4 mg/kg bodyweight; Zoetis) or IVM (Eqvalan®, at 0.2 mg/kg bodyweight; Merial). If enough animals were available, the remaining animals were allocated to additional treatment groups and treated with oxibendazole (OXI; EQ VERM®, at 10 mg/kg bodyweight; MSD) or PYR (Strongid®, at 19.0 mg/kg bodyweight; Zoetis).

In each sheep flock, larval identification after treatment was performed in all groups based on a bulk sample of faeces. In cattle herds, larval identification was performed before and after treatment based on the bulk sample. The morphological characteristics of at least 100 third-stage (L3) larvae per bulk sample were recognized following MAFF's keys (1986) for larval identification.

2.3. Statistical analysis

In sheep, the percentage reduction in the arithmetic mean strongyle FEC relative to the untreated control group was done based on the arithmetic mean of the FEC data collected on Day 14: (FEC control – FEC treated)/FEC control. In cattle and horses, the percentage reduction in the arithmetic mean strongyle FEC on Day 14 (after) relative to Day 0 (before): (FECD0 – FECD14)/FECD0. A 95% confidence interval (CI) around the efficacy was calculated using bootstrap analysis with 1000 iterations (The R Foundation for Statistical Computing, version 2.10.0). The AR status per treat-