Veterinary Parasitology: Regional Studies and Reports 9 (2017) 25–28

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Original article

Anthelmintic resistance in grazing beef cattle in central and northeastern areas of Argentina — An update

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ARTICLE INFO

Keywords:
Anthelmintic resistance
Gastrointestinal nematodes
Grazing beef cattle
Argentina

ABSTRACT

The presence of anthelmintic resistance in Argentina has experienced a marked increase in cattle, with numerous reports showing levels of resistance of different parasite genera to different chemical groups. The aim of this study is to update comprehensively the situation of anthelmintic resistance to the different chemical groups in the most important areas of cattle production in Argentina. The study involved the determination of anthelmintic resistance in 62 cattle farms in 7 provinces using the faecal egg count reductions test. The results showed a marked increase of anthelmintic resistance compared to previous reports; the main resistant genera were *Cooperia* and *Haemonchus* to ivermectin, *Ostertagia* and *Cooperia* to ricobendazole, and *Haemonchus* to fenbendazole. There was also a distinct difference in clinical efficacies between subcutaneous ricobendazole and oral fenbendazole in favour of the latter, probably attributed to the administration route. Levamisole has showed high efficacy and broad antiparasitic spectrum. Anthelmintic resistance is widely and firmly established in grazing cattle production systems in the country; the diagnosis of resistance must be done in every particular farm in order to design a sustainable parasite control based on anthelmintics use.

1. Introduction

Anthelmintic resistance is a global threat to the sustainability of the current practices of gastrointestinal nematodes control in ruminants (Kaplan and Vidyashankar, 2012; Leathwick, 2013; Sutherland and Leathwick, 2011). Although this phenomenon in Argentina is more prevalent in small ruminants, there has been a steady increase during the last decade in cases of anthelmintic resistance in cattle (Anziani and Fiel, 2015); resistance of *Cooperia* to ivermectin was first reported in 2001 (Anziani et al., 2001; Fiel et al., 2001) but soon after reports of resistance developed by other, more pathogenic genera such as *Haemonchus* and *Ostertagia* to the very commonly used benzimidazoles also appeared (Anziani et al., 2004; Anziani and Fiel, 2004; Fiel et al., 2016; Mejía et al., 2003; Suárez and Cristel, 2007). Beef cattle production in the north and central areas of the country relies heavily on the use of anthelmintics, thus counting with information on drug efficacies is critical for the early identification of development of resistance and to avoid production loses such as those recently reported (Fazzio et al., 2011; Fiel et al., 2011a).Therefore, the aim of this study was to update comprehensively the situation of anthelmintic resistance to the different chemical classes in the most important areas of cattle production in Argentina.

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http://dx.doi.org/10.1016/j.vprsr.2017.04.003
Received 29 November 2016; Received in revised form 18 February 2017; Accepted 3 April 2017
Available online 05 April 2017

2. Materials and methods

2.1. Farms and animals

The study was conducted in 2014 and 2015, between April and September in both years, on 62 beef cattle farms located in 7 provinces of central and northeast areas of Argentina, namely: Buenos Aires (n = 25), Chaco (n = 1), Córdoba (n = 8), Corrientes (n = 10), Entre Ríos (n = 2), La Pampa (n = 7) and Santa Fe (n = 9). The selection criteria for the farms to participate in the study were: willingness of farmers to collaborate, absence of any anthelmintic treatment 60 days before first sampling, and individual faecal egg count values at first sampling of ≥ 150 eggs per gram (epg). All animals used in the study were 6–12 month-old, weaned beef calves, naturally infected with gastrointestinal nematodes. Calves were allocated to groups of 15 animals each (2014) and 10 animals each (2015), according to individual faecal egg counts. The change in group size in 2015 resulted from the fact that not all farms had enough animals with the required minimum level of faecal egg count at the time of the trial.

2.2. Anthelmintic treatments

The anthelmintics, doses and administration route used in 2014 were ivermectin (IVM), 0.2 mg/kg, subcutaneous (Rank®, MSD Animal Health); ricobendazole (RBZ), 3.75 mg/kg, subcutaneous (Axilur® PI Inyectable, MSD Animal Health); levamisole (LVM), 7.5 mg/kg, subcutaneous (Ripercol L® 7.5%, Fort Dodge). The same anthelmintics were used again in 2015, plus fenbendazole (FBZ), 5 mg/kg, oral (Axilur® Suspensión 10% Oral, MSD Animal Health), which was incorporated so as to compare the efficacies of both, oral and subcutaneous formulations of benzimidazoles. All anthelmintics were administered at the respective recommended dose rates. Ivermectin was evaluated in all 62 farms, RBZ and LVM were evaluated in 61 farms and FBZ was tested in 27 farms. All anthelmintic treatments were administered on Day 0.

2.3. Parasitological procedures and analysis

On day 0 and 14, faecal samples were taken directly from the rectum of all animals in each treatment group. Faeces were processed to estimate the nematode egg counts using a modified McMaster technique (Fiel et al., 2011b). Pooled faeces from each treatment group were cultured (Henriksen and Korsholm, 1983) in order to obtain infective larvae to identify to genera level (Niec, 1968). The faecal egg count reduction test (FECRT) was done according to McKenna (1990); the formula used for anthelmintic efficacy was: FECRT = 100 × (1 − [T2/T1]), where T1 and T2 represent the arithmetic mean of faecal egg counts on day 0 and 14, respectively. This formula was used because of the impossibility to count with a control, non-treated group in all participating farms. Anthelmintic resistance was confirmed when the FECRT demonstrated an efficacy < 90%. In those confirmed cases of resistance, the proportion of each nematode genus was compared (Cristel and Suárez, 2006) pre- and post-treatment and the larval reduction was calculated using the formula: LCRT = 100 × (1 − [LT2/LT1]), where LT1 and LT2 represent the L3 proportion of a given genus in the same treatment group on day 0 and 14, respectively.

3. Results

Anthelmintic resistance to at least one chemical class was confirmed in 59 (95.2%) out of 62 farms evaluated, while susceptibility to all tested drugs was present in 3 (4.8%) farms. Resistance to IVM and RBZ was detected in 93.5% (58/62) and 27.9% (17/61) of the farms, respectively (Table 1). Multiple resistance to IVM and RBZ was present in 26.2% (16/61) of the farms, while 7.4% (2/27) farms showed multiple resistance to IVM, RBZ and FBZ. Levamisole was the only drug to which resistance was not detected (Table 2). While the lack of efficacy of IVM was widespread amongst all provinces, the cases of resistance to RBZ were more, but not totally, confined to Córdoba and Santa Fe provinces (Table 2).

The faecal cultures at Day 0 revealed the presence (and their respective proportion, expressed in percentages) of the following nematode genera: *Cooperia* (52.1%), *Haemonchus* (26.3%), *Ostertagia* (11.1%), *Trichostrongylus* (6.5%) and *Oesophagostomum* (3.6%). The cultures post-treatment (Table 3) revealed that the predominant genus in IVM-resistant herds was *Cooperia* (100%) followed by *Haemonchus* (55.4%) and *Trichostrongylus* (32.1%). The latter genus appeared in nine

Table 1
Number of farms with FECRT < 90% and efficacy percentages of each anthelmintic tested.

Anthelmintic	Farms		Efficacy %[a]
	Numbers	%	
Ivermectin	58/62	93.5	55.58 ± 28.07 (0–88.5) *95% UCL: 62.96–95% LCL: 48.20*
Ricobendazole	17/61	27.9	73.29 ± 19.57 (13.9–88.4) *95% UCL: 83.35–95% LCL: 63.22*
Fenbendazole	2/27	7.4	80.70 ± 12.30 (72–89.4)

FECRT = faecal egg count reduction test.
95% UCL: Upper confidence interval limit (95%); 95% LCL: Lower confidence interval limit (95%). Not shown in the case of fenbendazole as there were only two farms with resistance to this anthelmintic.
[a] Mean ± standard deviation (min − max).

Table 2
Geographical distribution by province of surveyed farms with and without anthelmintic resistance.

Province	Nr. farms tested[a]	Nr. farms AR (+)					Nr. farms AR (−)
		IVM	RBZ	FBZ	LVM	RM	
Buenos Aires	25	23	3	0	0	3	2
Chaco	1	1	0	0	0	0	0
Córdoba	8	7	6	1	0	5	0
Corrientes	10	10	2	1	0	2	0
Entre Ríos	2	2	1	0	0	1	0
La Pampa	7	6	0	0	0	0	1
Santa Fe	9	9	5	0	0	5	0
Total	62	58	17	2	0	16	3
%	100	93.5	27.9	7.4	0	26.2	4.8

AR: Anthelmintic resistance, *IVM: I*vermectin, RBZ: Ricobendazole, FBZ: Fenbendazole, LVM: Levamisole, MR: Multiple resistance.
[a] Farms were tested using faecal egg count reduction test (FECRT).

Table 3
Presence of anthelmintic resistance and efficacy of each anthelmintic tested against different nematode genera, as determined by faecal cultures.

Anthelmintic	Nematode genera	AR prevalence (%)	Efficacy (%)[a]
Ivermectin	*Cooperia*	100	39.8 ± 29.9 (0–87.4)
	Haemonchus	55.4	63.5 ± 40.9 (0 − 100)
	Trichostrongylus	32.1	82.3 ± 30.6 (0–100)
	Oesophagostomum	13.6	87.7 ± 31.9 (0–100)
Ricobendazole	*Cooperia*	82.4	71.1 ± 23.4 (6.9–87.9)
	Ostertagia	76.9	63.7 ± 31.2 (0–84.3)
	Haemonchus	37.5	89.6 ± 13.9 (53.6–89.2)
Fenbendazole	*Ostertagia*	100	89[b]
	Cooperia	55.4	87.6 ± 7.6 (72–89.4)
	Haemonchus	32.1	65 ± 49.4 (30.1–100)

AR: Anthelmintic resistance.
[a] Mean ± standard deviation (min − max).
[b] Value corresponding to one (1) faecal culture.

cases of IVM-resistance, of which six of them occurred in Corrientes province. The predominant genera in those herds with resistance to RBZ were Cooperia (82.4%), Ostertagia (76.9%) and Haemonchus (37.5%), being the lowest clinical efficacy for *Ostertagia* spp. Levamisole showed high efficacy (mean general > 98%) for all nematode genera observed, except for *Ostertagia*, the efficacy levels for this genus obtained by larval reduction analysis were lower (82.3%) than for the other four genera.

4. Discussion

The widespread presence of anthelmintic resistance to at least one of the anthelmintics evaluated is much higher than previously reported (Caracostantógolo et al., 2005; Suárez and Cristel, 2007); anthelmintic resistance was present in 59% of the herds in 2005 (Caracostantógolo et al., 2005) while the present study showed a staggering 95.2%, which clearly indicates a rising dynamic of this phenomenon in the country. The strong association between IVM and *Cooperia* spp. and the high level of resistance detected for this genus in all provinces is in agreement with recent findings (Anziani and Fiel, 2015). The massive use of IVM as endectocide in Argentina for over thirty years has been, presumably, a factor in the rise of anthelmintic resistance in cattle. This is supported by market data from the Argentinean Chamber of Veterinary Products (CAPROVE) that reported that macrocyclic lactones accounted for 83% of the veterinary antiparasitic drugs sold nationwide in 2015 (Fiel and Steffan, 2016). In a study of risk factors associated to the pronounced increased of IVM-resistance, Suárez and Cristel (2014) found that treatment frequency, more specifically, > 3 treatments/year, is by far the main variable related to anthelmintic resistance; this variable ranked first above other variables considered, such as type of parasite control, i.e. empiric or actual parasitological monitoring, and refugia index. Ivermectin is also commonly used in Argentina against ticks, especially under eradication programs of *Riphicephalus (Boophilus) microplus*. A consequence of this practice would be a severe selection pressure on gastrointestinal nematodes that speeds up the development of resistance (Anziani et al., 2014). Another damning factor is that the original dissemination of local information on anthelmintic resistance in cattle production, as well as the adoption of such information by end-users, seem to have been poorly done; consequently, farmers continue using inefficient drugs with the resulting spreading of resistant alleles (Anziani and Fiel, 2015).

Regarding benzimidazoles, given that resistance to subcutaneous RBZ was detected in 27.9% (17/91) of the herds while resistance to oral FBZ was observed in just 7.4% (2/27) of the cases, it could be argued that the differences in lack of efficacy for both drugs observed in the present study are influenced by the administration route. The sharp differences in clinical efficacy between subcutaneous RBZ and oral FBZ might originate in the increased drug concentration of the orally-administered drug in the digestive tract as well as in the parasite (Álvarez et al., 2007). In a non-resistance scenario, similar anthelmintic efficacies between the two formulations would be expected (Steffan et al., 2002); however, a greater parasite-active drug contact could liken this situation to that of an increased drug dose, which would result in a higher efficacy of the benzimidazoles against nematodes with a certain degree of resistance (Hennessy et al., 1987). Similar observations have been reported for macrocyclic lactones (Lanusse et al., 2014; Lloberas et al., 2012).

The absence of anthelmintic resistance to LVM in all 61 farms concurs with the performance of this drug in Argentina (Anziani and Fiel, 2015), presumably linked to its seldom use in cattle, which is < 1% of the anthelmintics market, according to CAPROVE (Fiel and Steffan, 2016). As well, the pharmacodynamic properties of the active drug determines that the parasite is subjected to a limited exposure to it (Lanusse et al., 2013), thus having a more reduced opportunity of selecting for resistance than the macrocyclic lactones.

The most prevalent parasite genera observed in all agro-ecological regions of this study, i.e. *Cooperia*, *Haemonchus* and *Ostertagia*, followed by *Trichostrongylus* and *Oesophagostomum*, are in agreement with previous information (Anziani and Fiel, 2015), including the shift for second place observed between *Haemonchus* and *Ostertagia* in favour of the former in subtropical regions and for the latter in temperate areas (Fiel et al., 2013; Romero et al., 2013; Suárez et al., 2013). It must be pointed out that there are not significant distortions in the profiles of the regional nematode genera present throughout autumn and winter, i.e. the periods when the present survey was carried out.

Cooperia spp. was the nematode with the largest appearance in faecal cultures after IVM treatment in all farms; these levels of IVM-resistance for this genus are the highest one reported so far in the country, also coinciding with the situation in each of the provinces participating in the study. *Haemonchus* spp. and *Trichostrongylus* spp. were also identified in the farms with resistance to IVM. *Haemonchus* spp., was detected in over 50% of those farms, supporting what other authors have described previously for Argentina (Anziani et al., 2004; Fiel et al., 2016). Likewise, the existence of IVM-resistant *Trichostrongylus* spp. showed similar levels to those reached in efficacy controlled tests (Fiel et al., 2009). The high prevalence of *Cooperia* spp. and *Ostertagia* spp. resistant to RBZ is also similar to those recorded in different parts of the country (Anziani et al., 2014; Fiel et al., 2016; Mejía et al., 2003; Suárez and Cristel, 2007), with *Haemonchus* spp. showing low levels of participation. Similarly, the prevalence of the FBZ-resistant *Ostertagia* spp., *Cooperia* spp. and *Haemonchus* spp., in that order, diagnosed previously by both, FECRT and controlled efficacy tests (Fiel et al., 2016) are in tune with the results of the present study. Given that the present results evidence the ample breadth of anthelmintic resistance in the most important cattle grazing areas in the country, it is imperative not only to set up sustainable programs of parasite control based on anthelmintic use, but also that to this effect each particular livestock farm keep detailed records of the administration of all available anthelmintics.

5. Conclusion

The results from this study show a considerable rise in the presence of anthelmintic resistance in cattle in Argentina during the last decade. Based on FECRT, IVM is the drug with the highest levels of resistance, and *Cooperia* and *Haemonchus* the main genera involved. This means that, in practice, the broad spectrum of the macrocyclic lactones is very much reduced to a genus-specific level based on its high efficacy against *Ostertagia* spp.

Resistance to RBZ was observed mainly for *Ostertagia* spp. and *Cooperia* spp.; however, FBZ maintained an acceptable clinical efficacy, presumably due to its oral administration. The rising proportions of IVM-resistant and BZD-resistant *Haemonchus* spp. has a considerable risk potential. Due to its high clinical efficacy and broad spectrum, LVM should have a preferential role in future programs of parasite control involving drug rotation.

Conflict of interest statement

The authors declare no conflict of interest and disclose no financial relationship with people or organizations that could bias this work.

Acknowledgements

The authors wish to thank Gisele Bernat, Bernardino Borda, Carina Bonetti and DVM Damián Castro for their laboratory and field assistance. MSD Animal Health partially funded the study under Contract 110313.

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