

Anthelmintic resistance of gastrointestinal nematodes in sheep to monepantel treatment in central region of Rio Grande do Sul, Brazil¹

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ABSTRACT- Ramos F., Portella L.P., Rodrigues F.S., Reginato C.Z, Cezar A.S., Sangioni L.A. & Vogel F.S. 2018. **Anthelmintic resistance of gastrointestinal nematodes in sheep to monepantel treatment in central region of Rio Grande do Sul, Brazil.** *Pesquisa Veterinária Brasileira* 38(1):48-52. Departamento de Medicina Veterinária Preventiva, Centro de Ciências Rurais, Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil. E-mail: fernandaramos_7@yahoo.com.br

Given the numerous reports of anthelmintic resistance of sheep nematodes to different anthelmintic compounds, this study aimed to evaluate the resistance status of gastrointestinal nematodes from naturally infected sheep to monepantel in the state of Rio Grande do Sul. Four farms that present extensive raising system and absence of anthelmintic treatment for 60 days were selected for the study. Lambs that present counts of eggs per gram of feces (EPG) ≥ 200 (sensitivity of 50 EPG) one day (D-1) before the treatment were selected for the study and randomly separated into two groups, a control group and an experimental group treated with monepantel. Feces were collected 9 days after the treatment (D+9) for EPG counts and fecal culture. The monepantel was 100% effective only on 2. The efficacy found on farm 1, 3, and 4 were 2.82%, 25.8%, and 78.4%, respectively. There were no viable larvae post-treatment at farm 2, but the genera *Haemonchus*, *Trichostrongylus*, *Cooperia*, and *Strongyloides* were resistant to it at the other farms. This study shows the presence of parasites resistant to the treatment with monepantel, pointing to the importance of monitoring its efficacy in sheep flocks of Rio Grande do Sul, Brazil.

INDEX TERMS: Anthelmintic resistance, gastrointestinal nematodes, sheep, monepantel, small ruminants, efficacy.

RESUMO.- [Resistência anti-helmíntica de nematódeos gastrointestinais de ovinos ao monepantel na região central do Rio Grande do Sul.] Devido aos numerosos relatos de resistência anti-helmíntica de nematódeos gastrointestinais de ovinos a diferentes compostos, este estudo objetivou avaliar o status da resistência de nematódeos gastrointestinais de ovinos naturalmente infectados ao monepantel no estado do Rio Grande do Sul. Quatro fazendas que apresentam sistema extensivo de criação e ausência de

tratamento anti-helmíntico por 60 dias foram selecionados para o estudo. Animais que apresentassem as contagens de ovos por grama de fezes (OPG) ≥ 200 (sensibilidade de 50 OPG) um dia (D-1) antes do tratamento foram selecionados para o estudo e separados em dois grupos, um grupo controle e um grupo experimental tratado com monepantel. Fezes foram coletadas nove dias após o tratamento (D + 9) para realização do OPG e cultura fecal. O monepantel foi 100% eficaz apenas na propriedade 2. A eficácia encontrada nas propriedades 1, 3 e 4 foi 2,82%, 25,8% e 78,4%, respectivamente. Não houveram larvas viáveis após o tratamento nas propriedades 2, porém os gêneros *Haemonchus*, *Trichostrongylus*, *Cooperia* e *Strongyloides* demonstraram resistência a este nas demais propriedades. Este estudo mostra a presença de parasitas resistentes ao tratamento com monepantel, apontando para a importância de monitorar a sua eficácia em rebanhos de ovinos do Rio Grande do Sul, Brasil.

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TERMOS DE INDEXAÇÃO: Resistência anti-helmíntica, nematódeos gastrointestinais, ovinos, monepantel, pequenos ruminantes, eficácia.

INTRODUCTION

Among the diseases that affect small ruminants and result on decrease of productivity, parasitic infections caused by gastrointestinal nematodes are the major concern and obstacle faced by sheep farmers (Sczesny-Moraes et al. 2010). These parasites are associated with low body development, decrease in reproductive indexes and increase in the mortality rate in the flock.

This nematodes have developed resistance to the most common drugs available (benzimidazoles and macrocyclic lactones) which is recognized by the increase number of treatments needed to control them in small ruminants (Waller et al. 1996, Wolstenholme et al. 2004). Different studies have reported this lack of efficacy of different chemical groups, such reports include the studies of Cezar et al. (2010), Borges et al. (2015), and Bichuette et al. (2015) in the states of Rio Grande do Sul, Bahia, and São Paulo respectively.

In the year 2012, it was launched in Brazil the monepantel (Zolvix®), a molecule from amino-acetonitrile derivatives (AADs) chemical group. Despite this being a new molecule, in recent years, it has been notice parasitic resistance to this drug in several regions worldwide, including Brazil, (Scott et al. 2013, Mederos et al. 2014, Cintra et al. 2015, Van den Brom et al. 2015, Sales & Love, 2016). In view of these reports, the present study aimed to evaluate the resistance status of gastrointestinal nematodes from naturally infected sheep to monepantel at different farms in the state of Rio Grande do Sul, Brazil.

MATERIALS AND METHODS

Farms and animals. This study was conducted at four farms located in the central region of Rio Grande do Sul. Farm 1 is located in the county of São Pedro do Sul (29° 41' 14,88"S, 54° 26' 34,06" W), farm 2 in the county of São Gabriel (29°59'12.5"S 54°25'47.6"W) and farms 3 and 4 in the county of São Martinho da Serra (29° 32' 03.0"S, 53°51'24.04"W). The flocks were selected based on location and previous consent by farmers. All farms perform the breeding of cattle, horses, and sheep in an extensive raising system. The flock at farm 1 is composed of about 70 Texel- Ile de France cross breeding; at farm 2, the flock is composed of 300 Corriedale animals; farm 3 perform the breeding of 1000 Texel and Suffolk sheep and at farm 4 the flock is composed of 250 Texel and Texel-Crioula cross breeding. All farms make the control of gastrointestinal parasites based on the result of efficacy tests witch are perform, at least, once a year. Results obtained through previous efficacy tests show that different anthelmintic compounds, such as ivermectin, doramectin, moxidectin, nitroxinil, disophenol, levamisole, albendazole, fenbendazole, and closantel are no longer effective for the control of the gastrointestinal nematodes on all these flocks, been the genera *Haemonchus*, *Cooperia*, and *Trichostrongylus* the most resistant to treatment with this drugs. Because of these resistance problems faced by all the farms, all of them already had a historic of monepantel (Zolvix®) use prior the study start.

At all farms, the treatment with anthelmintics is made in intervals of 30 days or less in the hot season (spring and summer), or when the animals shows clinical signs suggestive of parasitosis

like apathy, weight loss, submandibular edema, paleness of mucous membranes, and an increase in the egg count per gram of feces (EPG). Selective treatment is performed only at farm 1 according with the famacha score (Molento et al. 2004).

Experimental groups and animals treatment. Fecal samples from naturally infected animals of both genders, which were approximately six months of age, were collected directly from the rectum of each animal at all farms. This animals have not received treatment with anthelmintic at least 60 days before the experimental period.

Fecal samples were collected one day before (D-1) and nine days (D+9) after treatment began, a practice adopted from previous studies conducted by Mederos et al. (2014) and Van den Brom et al. (2015). All samples were stored in individual plastic bags and refrigerated until processing, which was done immediately after all samples were collected.

The count of eggs per gram of feces (EPG) was performed using a modified McMaster technique, with a sensitivity of 50 EPG. Therefore, 2 g of homogenized feces were mixed with 28 ml of a saturated solution, filtered, and transferred to a McMaster chamber for microscopic identification and for calculating the EPG. The animals that presented EPG ≥ 200 on D-1 were selected and divided into two groups forming randomized blocks. At all farms, one control group was used to monitor the natural changes of EPG throughout the experimental period (Lyndal-Murphy et al. 2014, Neves et al. 2014) been 10 lambs on farm 1, 12 on farm 2, 17 on farm 3 and 10 on farm 4. Other group treated with monepantel (Zolvix®, Elanco) was formed with the same number of animals of the control group in each farm. These animals received this compound at its recommended dosage of 2.5mg/kg. All animals in the treated group were weighed, and they received monepantel orally with a syringe at a dose corresponding to their body weight, whereas the control group did not receive any type of treatment.

Ethics statement. The use of animals was approved by the Committee of Ethics in Animal Experimentation of the Federal University of Santa Maria, under protocol no. 8088190815.

Analysis and interpretation of results. As recommended by Neves et al. (2014), the pre and post-treatment EPG counts of the treated group were used to determine the efficacy of the monepantel treatment. For this purpose, we used the approach described by Torgerson et al. (2014), which is available at <<http://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/>>.

The efficacy of treatment, according to the genera of parasites identified in the culture of larvae from days D-1 and D+9, was determined using the formula: $PR=100x (PER_{initial} - PER_{final} / PER_{initial})$. In this equation, PR is the percentage of reduction by genera, $PER_{initial}$ and PER_{final} are the percentages of each gender one day before (D-1) and 9 days (D+9) after treatment began, respectively (Coles et al. 1992, 2006).

The status of anthelmintic resistance was interpreted according to the recommendations of Lyndal-Murphy et al. (2014), based on the guide of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al. 1992), which analyses the reduction percentage of the EPG and the upper limits (UL) and lower limits (LL) of the confidence interval (CI) to 95%. Thus, the treatment was classified as: effective (percentage of reduction of EPG and UL 95% equal or superior to 95% and 95% LL above 90%); ineffective (percentage reduction and UL95% below 95% and LL95% below 90%); or inconclusive (none of the previous criteria filled).

RESULTS

The arithmetic means, maximum and minimum EPG, and EPG reduction percentage of groups treated with monepantel are present in Table 1. By the observation of this table, it is possible to check that there was no efficacy of

Table 1. Results of eggs per gram of feces (EPG): arithmetic mean, maximum and minimum values of EPG and percentage reduction of EPG with upper and lower values of the confidence interval (CI) at 95%, before (D-1) and after (D+9) monepantel (Zolvix®) treatment, of lambs naturally infected from different farms in the state of Rio Grande do Sul, Brazil

	Day-1		Day + 9		
	Mean	EPG min-max	Mean	EPG min-max	EPG reduction (95% CI)
Farm 1					
Control (n=10)	531.2	250-1100	1418.7	0-5400	NA
Monepantel (n=10)	533.3	200-1400	777.7	0-1900	2.82(0.001- 12.2)
Farm 2					
Control (n=12)	2318.1	300-5700	2859.0	450-6000	NA
Monepantel (n=12)	3427.2	200-5200	0	0-0	99.9 (99.5-100)
Farm 3					
Control (n=17)	1006.3	200-5500	1629.4	0-10400	NA
Monepantel (n=17)	10835	2000-24500	7970.6	2600-16600	25.8 (20.1- 30.7)
Farm 4					
Control (n=10)	536.3	200-1900	1590.1	400-5200	NA
Monepantel (n=10)	1170	200-5700	240	0-2300	78.4(68.5-83.7)

NA = not apply, CI = confidence interval.

Table 2. Proportions of the genera of gastrointestinal nematodes identified before the treatment (D-1) with monepantel (Zolvix®) in the feces of naturally infected lambs from four farms in the central region of the state of Rio Grande do Sul, Brazil

Farm	Genera of the gastrointestinal nematodes found one day before the treatments (%)					
	<i>Cooperia</i> spp.	<i>Haemonchus</i> spp.	<i>Trichostrongylus</i> spp.	<i>Ostertagia</i> spp.	<i>Oesophagostomum</i> spp.	<i>Strongyloides</i> spp.
1	25.5	54.9	9.8			9.8
2	18	72	8	2		
3	16	46	38			
4		32	54	2		

Table 3. Proportions of the genera identified after the treatment (D+9) with monepantel (Zolvix®) in the feces of naturally infected lambs from four farms in the state of Rio Grande do Sul and respective efficacy of the treatment for each genera

Farm	Genera of the gastrointestinal nematodes found nine days after the treatments (%) and efficacy of the treatment per genus					
	<i>Cooperia</i> spp.	<i>Haemonchus</i> spp.	<i>Trichostrongylus</i> spp.	<i>Ostertagia</i> spp.	<i>Oesophagostomum</i> spp.	<i>Strongyloides</i> spp.
1	19.6 (23.1%)	23.4 (57.1%)	8(18.3%)		0(100%)	49(0%)
2	0 (100%)	0 (100%)	0(100%)	0(100%)		
3	8(50%)	15 (30.4%)	77(0%)			
4	14(0%)	62(0%)	24(55.50%)	0(100%)		

the treatment with monepantel (Zolvix®) at tree of the four (3/4) farms studied. It is also possible to see that there was a natural increase of EPG of the control groups at all farms, showing that there was no environmental factor associated to the decrease of EPG values in the treated groups during the experimental period. The genera of parasites identified before the treatment of the animals (D-1) are present in Table 2. Nine days after the use of this compound (D+9), there was no significative change in the proportion of the genera of nematodes identified in the control group of all farms (data not show). However, some changes appear in the treated groups, pointing to different levels of resistance of the nematode population present at each farm (Table 3).

It was possible to ascertain on D+9 that some animals of the treated groups at all farms present suggestive clinical signs of persistent infection by gastrointestinal nematodes. Those signs include apathy, lethargy, and anemia of mucous membranes, pointing to a treatment inefficacy.

DISCUSSION

The resistance of gastrointestinal nematodes in small ruminants to anthelmintic treatment can be observed in several

regions of the world, including in Brazil (Duarte et al. 2012, McMahon et al. 2013, Martínez-Valladares et al. 2015). Little et al. (2010) have argued that the treatment of the animals with effective anthelmintic therapies, used in a strategic way and in conjunction with other control practices, is the best option for producers. However, studies have shown that parasite resistance has gradually increased. The continuing lack of information concerning the treatment of parasite-infected animals has led to the inefficacy of the compounds belonging to the three major classes of broad-spectrum anthelmintic treatments (Sutherland et al. 2008). As a reflex of this farm and flock owners have suffered serious consequences, including, in some cases, the dissolution of their flocks and properties (Blake & Coles 2007).

The drug monepantel belongs to a group of amino-acetonitrile derivatives (AADs), which represent a new class of anthelmintic therapy. This compound was launched in the year of 2009, reaching the Brazilian market in 2012, and consequently providing a new alternative treatment for farmers (Hosking et al. 2008). However, similar to the studies of Mederos et al. (2014) and Scott et al. (2013) in Uruguay and New Zealand respectively, the parasitic popu-

lations identified in this studied showed resistance to monepantel only four years after the product became available in the market.

After seventeen applications of this anthelmintic treatment over the course of two years, Scott et al. (2013) observed strains of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* resistant to the treatment with monepantel in goats and sheep. However, the results obtained in our study suggest that the appearance of resistant strains can occur after fewer generations than observed in the study performed by Scott et al. (2013). The nematode populations resistant to monepantel (Zolvix®) treatment observed in this study, were detected after only four, three and five applications of this principle in its therapeutic dosage, at farms 1, 3, and 4, respectively. These data are similar to those obtained by Cintra et al. (2015), who found the selection of strains of *T. colubriformis* following the use of monepantel in a non-suppressive treatment and involving the selective treatment of animals over a period of just five months.

One of the reasons for the apparent resistance of these gastrointestinal nematodes populations to monepantel, may be due to the low presence of larvae in refuge, or in other words, the larvae that had no exposure to the anthelmintic treatments. The lack of selective treatments, presence of few animals naturally resistant to parasitism, and the treatments that were made in a suppressive form, could explain the anthelmintic resistance observed at farms 1, 3, and 4 (Kenyon et al. 2009). This practice leads to rapid selection of the nematode populations because, even if a small parcel survive an effective treatment, this will be the unique stock of larvae available for the reinfection of flock (Busin et al. 2013). However, Bartley et al. (2015) argue that aspects such as prior exposure to anthelmintic compounds; inherent sensitivity to compounds in functional dose-limiting species; the initial frequency of potential genes for resistance within a population; the nature of the genetic heritage (dominant/recessive), and the impact of non-specific mechanisms of resistance on the survivability of certain isolated individuals, hinder the estimates of what may occur within certain parasitic populations after the anthelmintic treatment is administered to the animals.

Combinations of anthelmintics compounds could restore the efficacy of the treatments on the studied farms, as was demonstrated in a study conducted by Cezar et al. (2011). However, similar to conventional treatments, the long-term use of such combinations can become ineffective as the nematode populations are resistant to all classes of available drugs (Cezar et al. 2010). Regardless, alternative methods of control must be integrated to productive systems. These new methods should be more effective than combined allopathic treatments and help to slow the development of resistance (Waller 2006, Torres-Acosta & Hoste 2008).

The results of this study, summed up to the historic anthelmintic resistance to other principles at all farms, confirm the need for the establishment of control programs based on clinical and epidemiological criteria, and the need for frequent monitoring of the efficacy of such tre-

atments (Cezar et al. 2010). In this context, according to the recommendations of Waller (2006) and Molento et al. (2013), alternative methods that lead to a reduction of the frequency of treatments should be adopted. Such methods could include the selection of less susceptible animals and the alternation between agricultural and livestock farming activities. This may slow down the development of parasite resistance and increase the lamb's development.

CONCLUSIONS

The resistance of gastrointestinal nematodes in sheep has gradually worsened after the compound monepantel launch on the Brazilian market. The compound has been shown to be ineffective at three of the four studied farms in the state of Rio Grande do Sul.

Studies which aim to understand the molecular mechanisms for the development of resistant parasite populations to the monepantel treatment are necessary, in order to develop strategies to delay the appearance of new cases of resistance to this compound and increase its efficiency.

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