

## ANTHELMINTIC RESISTANCE IN SHEEP GASTROINTESTINAL NEMATODES IN THE NORTHWEST REGION SÃO PAULO STATE

[Resistência anti-helmíntica de nematódeos gastrintestinais de ovinos da região Noroeste do Estado de São Paulo]

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**ABSTRACT** – The occurrence of anthelmintic resistance of gastrointestinal nematodes was investigated on 11 sheep farms of the Northwest region of the State of São Paulo. The tested anthelmintics belong to the groups of the benzimidazoles, imidothiazoles, salicylanilides and macrocyclic lactones. The anthelmintic resistance was considered patent when the egg count reduction in feces was less than 90.0%, evaluated between seven and 14 days after the use of the drugs, using the fecal egg count reduction test according to World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.). The identification of the genera of gastrointestinal nematodes resistant to anthelmintics present in the properties studied was verified by larvae cultures. From 10 herds evaluated for the efficacy of moxidectin, five (50.0%) were resistant to it. Six (54.5%) of 11 herds evidenced resistance to the levamisole. Resistance to the closantel was observed in 10 (90.9%) of 11 farms examined. Resistance to the ivermectin and albendazole was detected in all farms and *Haemonchus* spp. was the most predominant nematode genus. Therefore, we report the existence of multiple anthelmintic resistance in gastrointestinal nematodes of sheep in the Northwest region of the State of São Paulo.

**Keywords:** helminth; ovine; treatment; parasite.

**RESUMO** – A ocorrência da resistência anti-helmíntica de nematódeos gastrintestinais foi avaliada em 11 rebanhos ovinos em fazendas da região Noroeste do Estado de São Paulo. Os grupos de anti-helmínticos testados foram os benzimidazóis, imidazotiazóis, salicilanilidas e lactonas macrocíclicas. A resistência anti-helmíntica foi considerada patente, quando a redução das contagens de ovos nas fezes foi inferior a 90,0%, averiguada entre sete a 14 dias após a utilização das drogas, empregando-se o teste de redução de contagem de ovos nas fezes preconizado pela World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.). A identificação dos gêneros de nematódeos gastrintestinais resistentes aos anti-helmínticos presentes nas propriedades estudadas foi verificada com a realização de coproculturas. De 10 rebanhos avaliados para a eficácia da moxidectina, cinco (50,0%) apresentaram resistência à mesma. Seis (54,5%) de 11 rebanhos evidenciaram resistência ao levamisol. Resistência ao closantel foi observada em 10 (90,9%) das 11 propriedades examinadas. Resistência a ivermectina e ao albendazol foi detectada em todas as propriedades estudadas. *Haemonchus* spp. foi o gênero de nematódeo amplamente predominante. Assim, nós relatamos a ocorrência de resistência múltipla anti-helmíntica em nematóides gastrintestinais de ovinos em fazendas da região Noroeste do Estado de São Paulo.

**Palavras-Chave:** helmintos; ovinos; tratamento; parasitos.

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## INTRODUCTION

Sheep production enables productive use of relatively small areas and proportionally smaller than beef cattle production areas and has become an interesting alternative to the farmers of the Northwest region of São Paulo State, given the increasing occupation of sugar cane in rural areas of this region. However, the herds have been composed of animals from different regions of the state and country, such as Mato Grosso, Rio Grande do Sul, Parana and Bahia. Thus, with the acquisition of more productive genomes comes also the helminths containing different sensitivity profiles to anthelmintics, which are introduced along with their hosts.

A great amount of commercial products are available for the veterinary supply market, which are indicated for treatment and control of helminth infections in ruminants. However, these products are grouped into a limited number of families of drugs according to their mechanism of action, the main ones being the benzimidazoles, imidazotiazoles, salicylanilides and the macrocyclic lactones (Almeida et al., 2010).

In all countries where sheep production is of socio-economic importance, populations of resistant helminths to different chemical groups have been detected, arousing great concern (Molento et al., 2011; Papadopoulos et al., 2012; Veríssimo et al., 2012). The diagnostic of resistance is extremely important to aid decision-making regarding parasite control programs (Fortes & Molento, 2013; Molento et al., 2013).

In Brazil, the increase in multiple drug resistance reports in the South (Cezar et al., 2010) and Southeast (Veríssimo et al., 2012) and reflects the seriousness of this problem. Gastrointestinal helminths, although causing more pronounced losses in lambs and sheep during the peripartum, can infect animals of all age groups and physiological condition, representing one of the main causes of ovine productivity loss, and being aggravated when associated with malnutrition and management failures (Amarante, 2014). The occurrence of multiple resistance alerts to an imminent failure of helminths chemical control (García et al., 2016).

Not knowing the magnitude of the problem under field conditions, this study aimed to investigate the occurrence of anthelmintic resistance in gastrointestinal nematodes in sheep herds of the Northwest region of São Paulo state, São Paulo, by implementing "in vivo" diagnosis of resistance to the main currently available drugs.

## MATERIAL AND METHODS

### Study description

Samples were collected on 11 traditionally sheep farms located at the municipality of Araçatuba, São Paulo State, Brazil, from August 2012 to September 2013. The system employed in all sheep farms was semi-intensive, and the herd composition was varied and composed of crossbreeds and specimens of the breeds were Texel, Santa Inês and Suffolk. The animals were weaned at 90 and 150 days of age. This study was approved by the Animal Experimentation Ethics Committee of Araçatuba School of Dentistry (FOA), UNESP-Univ Estadual Paulista (no. 2010-005303).

We selected eleven properties of the municipality of Araçatuba to carry out evaluation tests of the susceptibility of sheep gastrointestinal nematodes populations in relation to active principles of anthelmintics available in the market. The inclusion criteria for each of the properties in the study were the presence of weigh scales and flocks containing a number of animals exceeding 100.

They were individually identified with numbered earrings or collars and examined by the Gordon & Whitlock modified technique (Ueno & Gonçalves, 1998), using McMaster chambers for quantifications of eggs per gram of feces (EPG). Fecal samples were examined in the first 36 hours after collection. The animals with counts equal or above 200 eggs per gram of feces were selected and randomly divided into at least five groups with ten repetitions. Randomly, each group was assigned to one anthelmintic, remaining one group as control.

For the evaluation of anthelmintic resistance, the egg count reduction in feces test was utilized, according to the recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP), using the formula: OPG count reduction % =  $100 (1 - X_t / X_c)$  where  $X_t$  is the arithmetic mean of the treated group counts and  $X_c$  the average of the control group counts (Coles et al., 1992).

Groups with a minimum of ten animals were treated with Ivermectin 1% 0.2 mg/kg, subcutaneous route; Albendazole 25% 5 mg/kg, oral route; Levamisole 10% 5 mg/kg, subcutaneous route; Moxidectin 1% 0.2 mg/kg, subcutaneous route; Closantel 10mg/kg, oral route and a Control group that received no treatment. The animals were distributed so that the mean EPG was similar between the groups. The animals were weighed before treatment to adjust the correct dose according to the manufacturer recommendations.

Fecal samples were collected directly from each animal rectum using plastic gloves and the number of eggs per gram of feces was determined according to Gordon & Whitlock modified technique (Ueno & Gonçalves, 1998), sensitive to 50 eggs per gram of feces. The anthelmintic resistance was considered patent when the egg count reduction in the feces were less than 90.0%, ascertained from seven to 14 days after administration of drugs. The prevalence of gastrointestinal nematode genre resistant to anthelmintics present in the studied properties was verified by larvae culture according to Roberts and O'Sullivan technique (Ueno & Gonçalves, 1998).

## RESULTS AND DISCUSSION

Anthelmintic resistance to the main active principles available is present in flocks of this study, being *Haemonchus* spp. the widely prevalent

nematode gender in the properties after treatments. The multiple parasitic resistance in this region may have been facilitated by the commercialization of animals, since this practice spreads the resistance from one region to another.

Due to the high prevalence of gastrointestinal helminths in sheeps, especially *Haemonchus contortus*, added to their almost constant occurrence in farms located in geographic areas with favorable ecological conditions to this parasite, chemotherapy is massively employed with often indiscriminated use of drugs (Sarginson et al., 2007).

The situation of the parasitic resistance of sheep gastrointestinal nematodes recorded in the 11 examined properties are disposed in Table 1.

Table 1. Situation of anthelmintic resistance (AHR) of gastrointestinal nematodes of sheep in properties of the Northwest region of the State of São Paulo.

Active Principles	Number of Examined Properties	AHR		
		< 50.0 %	50.0 % - 90.0 %	> 90.0 %
Ivermectin	11	0	0	11
Moxidectin	10	1	4	5
Levamisole	11	2	3	6
Albendazole	11	0	0	11
Closantel	11	2	8	1

The benzimidazole anthelmintic resistance has been pointed out in most countries which explore the sheep industry. Our results show an even more serious situation that the one observed in Santa Catarina, where parasitic resistance was detected in 75% of evaluated properties (Ramos et al., 2002).

Resistance to closantel was found in 10 (90.9%) of 11 examined properties. This percentage is similar to the observed in Paraná (85.8%) and is much higher than observed in Santa Catarina (Ramos et al., 2002).

Among the anthelmintics utilized, levamisole and moxidectin were those with the best results, and yet, with resistance pointed out in 54.5% and 50.0% of the properties, respectively. Regarding to both active principles, the result to the same imidazotiazole is near of other authors in Brazil (Cezar et al., 2010; Veríssimo et al., 2012).

The parasitic resistance in this region may have been facilitated by the commercialization of animals, since this practice spreads the resistance from one region to another. Like in this study, in many farmers the management practices adopted are based on old recommendations and may not return in a good set of strategies to prevent parasite infections. Then farmers have obtained unsatisfactory results in maintaining the health and

productivity level of their enterprises (Gouveia et al., 2013).

The control of the parasitism in small ruminants remains dependent on chemotherapy which can be accompanied by complementary or alternative methods of livestock management. The recognition of anthelmintic resistance situation is a basic condition for the establishment of a rational and targeted program of control of parasitic gastroenteritis in small ruminants and testing validated for both allow the identification of more effective drugs to be used and contribute to the preservation and delay resistance development against the same.

Thus, *Haemonchus* spp. the widely prevalent nematode gender in the properties after treatments and multiple anthelmintic resistance to the main active principles available occurs in examined flocks in this study.

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