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EFFICACY OF ANTHELMINTIC MEDICATION AGAINST GASTROINTESTINAL NEMATODES IN ROMANIAN GOATS

EFICACITATEA MEDICAȚIEI ANTIHELMINTICE ÎMPOTRIVA NEMATODELOR GASTROINTESTINALE A CAPRINELOR DIN ROMÂNIA

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ABSTRACT | REZUMAT

Because of the misbelief that sheep and goats share common gastrointestinal parasites, have similar immune reactions and require the same antiparasitic control programs, goat production is currently facing substantial economic losses. This study aimed to determine the efficacy of the most commonly used anthelmintic drugs for the control and treatment of gastrointestinal nematodes (GIN) in Romanian goat farms. In the period from October 2017 to May 2018, a total number of 30 goats were randomly selected, and faecal samples were collected for further *in vitro* and *in vivo* study. Faecal egg count reduction test (FECRT) was used to evaluate the efficacy of ivermectin (IVM). Egg hatching assay (EHA) and Larval development assay (LDA) were used to evaluate the efficacy of the following benzimidazoles (BZs): albendazole (ABZ), mebendazole (MBZ), fenbendazole (FBZ), and thiabendazole (TBZ). The mean percentage of EPG reduction was 91.31% for IVM, with a lower confidence limit of less than 90% and upper confidence limit >95%, revealing a susceptibility of IVM resistance in the studied population. In all in vitro tests BZs efficacy was high. In EHA the mean egg hatching percentage varying between 13.89 and 25.28%, values lower than the accepted maximum of 50%. According to EHA results, the lowest risk to induce AR was registered for ABZ (Y = -461.26) and MBZ (Y = -451.31). However, LDA showed that ABZ has a higher risk to induce AR in *Trichostrongylidae* larvae, followed by TBZ.

Keywords: Goat nematodes, *Trichostrongylidae*, benzimidazoles, ivermectin

În prezent, datorită părerii greșite conform căreia caprinele și ovinele au aceeași populație de paraziți intestinali, prezintă reacții imune similare și necesită aceleași programe de control antiparazitar, crescătoriile de caprine suferă pierderi economice importante. Scopul acestui studiu a fost de a determina eficacitatea preparatelor antihelmintice cel mai frecvent utilizate în controlul și tratamentul nematodelor gastrointestinale (GIN) în fermele de caprine din România. În perioada octombrie 2017 - mai 2018, un număr total de 30 de capre au fost selectate randomizat, iar fecalele au fost colectate pentru studii *in vitro* și *in vivo* ulterioare. Testul reducerii ouălor din fecale (FECRT) a fost utilizat pentru evaluarea eficacității ivermectinei (IVM). Testul de eclozionare a ouălor (EHA) și testul de dezvoltare larvară (LDA) au fost utilizate în evaluarea eficacității următorilor benzimidazoli (BZs): albendazol (ABZ), mebendazol (MBZ), fenbendazol (FBZ) și tiabendazol (TBZ). Procentul mediu de reducere al ouălor per gram (OPG) a fost de 91,31% pentru IVM, cu limita inferioară a intervalului de încredere mai mică de 90%, iar limita superioară mai mare de 95%, arătând o tendință de rezistență la IVM a populației de caprine studiate. În toate testele *in vitro* eficacitatea BZs a fost ridicată. La EHA procentul mediu de eclozionare a variat între 13,89 și 25,28%, valori mai scăzute în comparație cu valoarea maximă acceptată de 50%. Conform rezultatelor obținute la EHA, un risc scăzut de inducere a rezistenței a fost înregistrat pentru ABZ (Y = -461,26) și MBZ (Y = -451,31). Cu toate acestea, LDA a arătat că riscul de inducere a rezistenței antihelmintice la larvele de *Trichostrongylidae* este cel mai înalt pentru ABZ, urmat de TBZ.

Cuvinte cheie: Nematodele caprinelor, *Trichostrongylidae*, benzimidazoli, ivermectină

According to the Food and Agriculture Organization of the United Nations, in 2017, the world goat population reached 1.03 billion, from which: Asia 53.3%, Africa 40.8%, America 3.6%, Europe 1.9%, and Oceania 0.4%. The total number of goats in Romania was around 1.48 million, goat and sheep farming being an important source of income especially in the Centre Region (11). However, nowadays farmers are facing substantial economic losses, and one of the reasons is gastrointestinal nematode (GIN) infections (14).

GIN infections affect sheep and goats worldwide, causing both disease and production losses (17).

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It is believed that each year, worldwide, tens of billions of dollars are spent on control and treatment of GIN in both cattle and small ruminants (22). The most common found nematode genera in goats are the *Teledorsagia*, *Haemonchus*, *Trichostrongylus*, *Nematodirus*, *Strongyloides, Oesophagostomum,* and *Chabertia*, the last two being usually a part of mixed burden (3, 8, 9, 10, 14). Classes of anthelmintics used worldwide for GIN infections in both sheep and goats are benzimidazoles (BZs), imidazothiazoles, tetrahydropyrimidines, macrocyclic lactones (MLs), aminoacetonitrile derivatives (AADs), and spiroindoles (1).

For a long period, it was believed that sheep and goats share common GIN, have a similar immune response, regulatory mechanisms, hepatic metabolism and react the same to anthelmintic drugs (20). As a consequence of this misconception, the same control programs were applied to both species (14). Although existing anthelmintic drugs could provide adequate control of GIN, their frequent administration, inadequate dosage, and misuse have led to the rapid development of anthelmintic resistance (AR) worldwide (4, 19). AR to both BZs and levamisole in goat GIN has been reported in France (5, 6), Denmark (13, 21), Italy (23), Austria (12) and Poland (18).

The present study aimed to examine *in vitro* efficacy of the most commonly used BZs: albendazole (ABZ), mebendazole (MBZ), febendazole (FBZ), and thiabendazole (TBZ) and *in vivo* efficacy of ivermectin (IVM) which represents the class of MLs.

MATERIALS AND METHODS

Study design

The study was conducted from October 2017 to May 2018, using GIN populations from a herd composed of 290 Romanian Carpatina goats. The farm was located in the village of Poiana Aiudului, Alba Country, Centre Region of Romania. The herd grazes throughout most of the year using the pastures around the farm. In 2013 the farm had a history of unsuccessful treatment with FBZ and since then, IVM was used 3-4 times / year for treatment and prevention of both GIN and psoroptic mange. All these treatments have been made by the owner without supervision or medical recommendation. The last anthelmintic treatment was carried out in December 2017. A group of 30 goats (10.34% of the herd), between 3 - 5 years old, was randomly selected for further *in vitro* and *in vivo* testing.

Faecal Egg Count Reduction Test (FECRT)

The efficacy of IVM was studied using 30 randomly selected goats, which did not receive anthelmintic treatment for 20 weeks before the test. Individual faecal samples were collected on day 0 (before) and day 14 after the administration of IVM. Faecal samples with the approximate weight of 10 g were collected directly from the rectum and stored separately in plastic tubes filled with water, at about 20 °C in a dark place. Each goat was weighted and after that ivermectin was administered subcutaneously at a dosage of 0.2 mg/kg body weight. Faecal egg counts (FEC) were performed using the McMaster technique. The egg per gram (EPG) of faeces was calculated using the following formula:

EPG = no. eggs from the first and second chamber x 50

FECRT was calculated as follows:

FECRT = (EPG pre-treatment – EPG post-treatment) / EPG pre-treatment x 100

The upper (UCL) and lower (LCL) limits for the confidence limit (CI) were also calculated for the statistical analysis of FECRT results.

Egg Hatch Assay (EHA)

Faecal samples were collected at the end of October, before anthelmintic treatment, from 30 goats and mixed, obtaining a pooled faecal sample. EHA was performed to evaluate the efficacy of ABZ, MBZ, FBZ, and TBZ, using the method described by Le Jambre (1978), but with minor modifications (15). Briefly, for each drug, 11 different wells were used, together with two negative controls. In each of 11 wells were added 790 µl of distilled water, 200 µl of egg suspension, and 10 µl of prepared drug dilutions. Final dilutions of ABZ, MBZ, FBZ, and TBZ in wells being 5.0, 2.5, 1.25, 0.62, 0.31, 0.15, 0.078, 0.039, 0.019, 0.0098, and 0.0049 µg/ml. For the control solution M_1, 800 µl of distilled water and 200 µl of egg suspension were used, solution M_2 was obtained by mixing 10 µl of HCl, 790 µl of distilled water and 200 µl of egg suspension. The number of eggs contained in 200µl of egg suspension was around 500 parasite eggs. Plates were incubated at 26-27 °C for 24 hours. To stop the incubation, 10 µl of Lugol's iodine solution was added to all the wells. The percent of hatched and developed eggs was calculated using the following formula (7, 15):

Egg hatching % = [(Embryonated eggs + Larval stage L_1)] / [(Unembryonated eggs + (Embryonated eggs + Larval stage L_1)] x 100

Larval Development Assay (LDA)

LDA was performed to test larvicidal effects of ABZ, MBZ, FBZ, and TBZ, on *Trichostrongylidae* eggs. The dilution protocol used in LDA was similar to the one described previously for EHA. The final concentrations of stock solutions ranging from 5.0 to 0.0098 µg/ml. For the first 24 hours, the mix of distilled water and egg suspension (990 µl) was incubated at 26 °C. After that, 10 µl of drug dilutions were added and the incubation continued for another 10 days. For M_1, 10 µl

of distilled water and for M_2, 10 µl of HCl were added. The percent of larval development (LD %) was calculated to determine the minimum inhibitory concentration (MIC), using the following formula:

LD % = [L_3 larvae / (Unembryonated eggs + L_3 larvae)] x 100

RESULTS AND DISCUSSIONS

FECRT

The mean arithmetic EPGs before treatment was 1150, ranging between 350 to 3200 EPG. Fourteen days after treatment, the mean arithmetic EPGs was significantly lower than on-day 0, being equal to 100 EPG, with variations between 0 and 500 EPG. Statistical analyses of data obtained from FECRT showed a FECR% value of 91.31%, with LCL of CI equal to 17% and the UCL of CI being 99%. Taking into consideration that the FECR% is higher than 90% and lower than 95%, but the UCL is higher than 95%, we can say that the *Trichostrongylidae* population of the tested farm is susceptible to AR phenomena (16). This can be due to the exclusive usage of IVM as an anthelmintic agent during the last 3 years.

EHA

When analysing the EH% for ABZ, FBZ, MBZ, and TBZ in comparison with reference concentration, it can be observed that all of the tested substances have EH% values lower than 50% (the accepted threshold). It can be concluded that all tested BZs have a relatively high efficacy on *Trichostrongylidae* eggs, the strongest efficacy been recorded for ABZ and MBZ.

LD50 showed that from all tested molecules, the most effective one was FBZ, with CL50 equal to -0.525 µg/ml, followed by MBZ and ABZ. The CL 50 value for TBZ was 2.5849 µg/ml, but it cannot be taken into consideration, since TBZ needs to be in contact with parasitic forms longer than the other tested drugs to manifest the desirable effect. Less susceptible to induce AR in studied GIN population, due to regression line equation results, are considered to be ABZ (Y = -461.26) and MBZ (Y = -451.31).

LDA

In ABZ, Y = 531.02, meaning that ABZ has a higher risk to induce AR in the *Trichostrongylidae* larvae of the studied population. In MBZ, MIC value was 0.0489 µg/ml with a mean LD% of 2.36% and Y = -459.77. Similar to MBZ results were obtained with TBZ. MBZ showed a low risk to induce AR, followed by TBZ (medium risk), in comparison with FBZ and ABZ (Table 1). The results of EHA and LDA showed that the efficacy of BZs in the studied GIN population is medium to high, with a low risk for ABZ and MBZ to induce anthelmintic resistance. However, as a result of continuous administration of IVM during 3 years, the mean % of egg reduction is <95%, creating a risk of installation of AR, and the owner being advised to change the ant-helmintic medication.

The potential risk of AR installation in Romanian goats as well as already reported cases from other European countries, under-dosage being one of them. For a long period and even nowadays, goats and sheep were believed to require the same dosage of anthelmintic drugs, being classified in product labels as "small ruminants" or "sheep and goats". However, recent studies have shown that goats eliminate BZs faster than sheep, and as consequence require a higher dosage of BZs (2). Other factors causing AR resistance

Table 1

LDA results for benzimidazoles

Drug dilution (µg/ml)	ABZ		FBZ		MBZ		TBZ	
	LD%	RLE	LD%	RLE	LD%	RLE	LD%	RLE
5.0000	0.00	531.02	0.00	35.69	0.00	-459.77	0.00	-459.77
2.5000	0.00	280.60	0.00	26.46	0.00	-227.61	0.00	-227.61
1.2500	0.00	155.39	50.00	21.85	0.00	-111.53	0.00	-111.53
0.6250	16.67	92.79	0.00	19.54	0.00	-53.49	0.00	-53.49
0.3125	0.00	61.49	0.00	18.39	0.00	-24.47	0.00	-24.47
0.1563	100.00	45.84	0.00	17.81	0.00	-9.97	0.00	-9.97
0.0781	0.00	38.01	100.00	17.52	0.00	-2.71	0.00	-2.71
0.0391	0.00	34.10	0.00	17.38	0.00	0.91	0.00	0.91
0.0195	100.00	32.14	0.00	17.30	0.00	2.73	0.00	2.73
0.0098	0.00	31.17	0.00	17.27	0.00	3.64	0.00	3.64
Mean M1+M2	25.00	30.18	25.37	17.23	25.93	4.55	25.93	4.55
	MIC (µg/ml)		MIC (µg/ml)		MIC (µg/ml)		MIC (µg/ml)	
	-0.2951		-4.3289		0.0489		0.0489	

RLE - Regression line equation; MIC - Minimum inhibitory concentration; LDA - Larval development assay

are the administration of anthelmintic medication without knowing the weight of the animal and frequent administration of the same medication.

CONCLUSIONS

To control the development of AR, animal owners and veterinary practitioners should be advised to regularly test the efficacy of anthelmintic drugs they intend to use, to rotate the medication used, to monitor EPG of faeces, and the manifestation of clinical signs like anaemia, anorexia or decrease in production.

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