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Multiple anthelmintic resistance at a goat farm in Slovakia

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Summary

Cases of parasite resistance to the main classes of anthelmintics are increasingly reported from small ruminants at farms in Europe. We visited a goat farm in Slovakia in November 2019 with suspected parasite problems that reduced productivity and performed an *in vivo* faecal egg count reduction test (FECRT) and an *in vitro* larval development test (LDT) for all three main classes of anthelmintics. The lowest efficacy (60 %) detected by FECRT was for ivermectin (IVM). Benzimidazole (BZ) efficacy ranged between 80.3 and 86.5 %, and levamisole (LEV) efficacy was 94 %. The results from the *in vivo* FECRT test were confirmed by the *in vitro* LDT. Minimum inhibitory concentration (MIC) 173.6 ng/ml for IVM several times exceeded the recommended threshold of 21.6 ng/ml. Mean LD_{50} for BZ was equal to the threshold concentration, but the other threshold criteria indicated a low level of resistance in the population. The LDT did not indicate the presence of resistance only for LEV. The MIC 1.0 μg/ml for LEV was evaluated as susceptible with respect to species composition. *Teladorsagia* was the dominant genus after treatment with BZ, IVM, and LEV. *Haemonchus contortus* was identified after treatment with BZ and LEV.

Keywords: Small ruminants; anthelmintic resistance; ivermectin; benzimidazole; *Teladorsagia* spp.; *Haemonchus contortus*

Introduction

Anthelmintic resistance (AR) to the main classes of anthelmintics is rapidly increasing in small ruminants at farms throughout the world. Multidrug resistance is common on sheep farms in humid tropical areas of Central and South America where was reported lack of *in vivo* efficacy for recommended dosages of ivermectin, albendazole and levamisole (Herrera-Manzanilla *et al*., 2017; Chaparro *et al*., 2017) or reduced effectiveness of macrocyclic lactones and benzimidazoles (Santiago-Figureoa *et al*., 2019). In Europe, multidrug resistance has been detected in *Haemonchus contortus* in The Netherlands (Van den Brom *et al*., 2013) and France (Cazajous *et al*., 2018) and in *Teladorsagia circumcincta* in Ireland (Keegan *et al*., 2015). Information has been lacking in recent decades about the prevalence of AR in goat herds, because most surveys have focused on sheep. A comparison of parasitism with gastrointestinal nematodes (GINs) found that only 20 – 25 % of studies included goats (Hoste *et al*., 2010). Cases of AR at goat farms in Europe have been reported from France (Paraud *et al*., 2009), Italy (Cringoli *et al.,* 2007; Zanzani *et al*., 2014), Demark (Holm *et al*., 2014), and Poland (Mickiewicz *et al*., 2017; 2019). Despite these reports, more surveys need to be conducted at goat farms. The owner of the farm in our study suspected poor efficacy of benzimidazoles (BZs) due to low weight gains and high morbidity of yearlings. The main goal of this study was thus to test the presence of BZ resistance on this farm. Two additional an-

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thelmintics, ivermectin (IVM) and levamisole (LEV) were included to test their efficacies. Another objective was to morphologically identify species of nematodes from larval cultures before and after treatment.

Materials and Methods

Study design

The farm was situated in the lowlands of the southeastern part of Slovakia. The herd consisted of three adult males, 86 adult females, and 31 kids of White and Brown short-haired goats. The animals were grazed from March to November on a 28-ha pasture. Ten sheep shared the pasture with the goats. We collected faecal samples from 50 goats with similar ages selected from the herd in November 2019. The number of eggs per gram (EPG) of faeces was determined by flotation using a sugar solution with specific gravity 1.28 according modified McMaster technique with a sensitivity of 50 EPG (Coles *et al*., 1992). Twenty-one goats with EPGs >500 were subsequently split into three groups of seven animals. On day 0, group 1 was treated with 5 mg/kg body weight (bw) of albendazole, the recommended dose for small ruminants but specific for sheep (Albendavet, DIVASA-FARMAVIC S.A., Barcelona, Spain), group 2 was treated with IVM at a dose of 200 µg/kg bw (Ivomec, MERIAL, Lyon, France), and group 3 was treated with LEV at a dose of 5 mg/kg bw (Ripercol Drench, Elanco, Greenfield, USA). Eggs were counted 0 and 10 d after treatment.

Faecal egg count reduction test (FECRT)

The FECRT was conducted following the recommendations of the World Association for the Advancement of Veterinary Parasitology (Coles *et al*., 1992, 2006). Percent egg reduction (%FECR) was calculated using two methods: %FECR = $(1/n)\sum(100\times(1-[T_{i2}/T_{i1}])$, where T_{i1} and T_{i2} are pre- and post-treatment EPGs, respectively, in host i from a total of n hosts (Cabaret & Berrag, 2004), and %FECR = $100\times(1-[T_2/T_1])$, where T_1 and T_2 are the arithmetic means of the treated group before and after treatment, respectively, with no control group (Kochapakdee *et al*., 1995). The parasite population was considered resistant if %FECR was <95 % (McKenna, 1990).

Larval development test (LDT)

The *in vitro* LDT was used as described by Hubert and Kerboeuf (1992), with modifications as described by Várady *et al*. (1996). The test for each group was performed with two replicates. Helminth eggs recovered from the faeces were incubated for 7 d in 96-well microtitre plates containing culture medium (yeast extract with Earle's Balanced Salt Solution and a physiological salt solution) in aqueous solutions at various concentrations of thiabendazole (TBZ) (0.0006 – 1.28 µg/ml), IVM aglycone (0.084 – 173.6 ng/ml), and LEV (0.020 – 32 µg/ml). The test was performed on two occasions before treatment. The concentrations of TBZ, needed to inhibit development to third-stage larvae (L_3) by 50 % (LD_{50}) and 99 % (LD_{99}) were determined using a logistic regression model (Dobson *et al*., 1987). We determined whether a parasite population was resistant to BZ based on threshold parameters such as LD_{50} (0.02 µg/ml), the minimum concentration required to completely inhibit the development of larvae to the L_3 stage (MIC), and LD_{99} (0.03 µg/ml) (Coles *et al*., 2006; Várady *et al*., 2006). IVM resistance have been confirmed using the MIC threshold of 21.6 ng/ml IVM aglycone by Dolinská *et al*. (2014). LEV resistance was assessed on the basis of discriminating doses of 0.5 µg/ml established by Coles *et al*., 2006, 1.0 µg/ml (Coles *et al*., 1988) and 2.5 µg/ml (Taylor, 1989). L_3 larvae of genera from the nematode family Trichostrongylidae were harvested using the Baermann technique, and 100 randomly selected L_3 were identified before treatment. One hundred L_3 larvae were identified from each treatment group 10 d after treatment as described by Van Wyk & Mayhew (2013).

Ethical Approval and/or Informed Consent

All procedures performed in this study were in accordance with the ethical standards of the Ethics Committee on 20-April-2015, meets the requirements of the Ethics Committee of the Institute of Parasitology of the Slovak Academy of Sciences in accordance with the national legislation in Slovakia - Animal Welfare Act No. 23/2009 and was approved on 1-January-2016.

Results

FECRT

The results of the FECRT are presented in Table 1. EPGs of strongyle-type eggs at day 0 ranged from 550 to 4850. Low numbers of *Nematodirus* and *Trichuris* eggs were also detected. Only strongylid eggs were detected 10 d after treatment in all groups. The FECRT results indicated close agreement between both methods of calculation. The lowest efficacy was recorded in the IVM group, with reductions of 59 and 60.4 % using the Cabaret & Berrag (2004) and Kochapakdee *et al*. (1995) methods, respectively. The

Table 1. Results of the *in vivo* FECRT: percent reduction 10 days after treatment.

Method of calculation	FECR (%)		
	BZ group	IVM group	LEV group
Cabaret and Berrag (2004)	80.3	59.0	94.2
Kochapakdee (1995)	86.5	60.4	94.0

FECRT, faecal egg count reduction test; BZ, benzimidazole; IVM, ivermectin; LEV, levamisole

Table 2. Values of LD_{50} and MIC from the LDT for each group of goats.

LD_{50} ± SD BZ group (μg/ml)	MIC IVM group (ng/ml)	MIC LEV group (μg/ml)
0.020 ± 0.0007	173.6	1.0

LDT, larval development test; SD, standard deviation; LD_{50}, concentration that inhibits development to the L_3 stage by 50%; MIC, minimum inhibitory concentration; BZ, benzimidazole; IVM, ivermectin; LEV, levamisole

BZ group had efficacies of 80.3 and 86.5 %, and the LEV group had efficacies of 94.0 and 94.2 %, using the Cabaret & Berrag (2004) and Kochapakdee *et al*. (1995) methods, respectively.

LDT

The *in vitro* LDT was performed to confirm the results of the *in vivo* FECRT. Mean LD_{50} for all three groups of goats is presented in Table 2. Mean LD_{50} was 0.02 μg/ml TBZ, identical to the threshold concentration. The high minimum inhibitory concentration (MIC) values in both assays (1.28 μg/ml TBZ) and LD_{99} (0.104±0.098 μg/ml TBZ), however, indicated a low (approximately 10 – 15 %) level of BZ resistance in the population. MIC values for IVM aglycone (173.6 ng/ml) were several times higher than the discriminating dose of 21.6 ng/ml indicating a high level of IVM resistance at farm. The MIC 1.0 μg/ml for LEV group was in agreement with the results of FECRT and parasite population was considered as susceptible.

Morphological differentiation of L_3 larvae

Morphological identification of L_3 larvae before treatment indicated the presence of *H. contortus*, *Teladorsagia*, *Trichostrongylus*, and *Oesophagostomum*/*Chabertia ovina* (Table 3). *Teladorsagia* was the only genus identified in the IVM group 10 d after treatment. *Teladorsagia* was also the dominant genus in the LEV and BZ groups. *H. contortus* was also identified in both groups.

Discussion

We confirmed the presence of a multidrug-resistant strain of *Teladorsagia* and a BZ-resistant strain of *H. contortus*. The efficacy of the FECRT was lowest (approximately 60 %) in the IVM group, which was also confirmed by LDT, despite the suspicion of the ineffectiveness of BZs. This low efficacy was probably the consequence of frequent use and incorrect dosing for many years and can be expected on most goat farms where similar approaches of farm management have been applied. Nationwide data on the prevalence of IVM resistance in goat herds in Slovakia, however, is still lacking. IVM has been used for decades to prevent and treat ectoparasites and may lead to selection pressure on gastrointestinal parasites that can in turn lead to the development of AR at farms (Cazajous *et al*., 2018).

The first national survey of goat farms, which focused only on BZs, was not carried out until 2014 – 2016 and detected AR on 30 farms (Babják *et al*., 2018), long after multidrug-resistant strains were detected in goats imported from New Zealand (Várady *et al*., 1993). A similar national survey would also be desirable for macrocyclic lactones (MLs) due to the critical situation of the prevalence of BZ resistance. Our results for the IVM group support this statement. Egg output in the *in vivo* FECRT after treatment and MIC 1.0μg/ml from LDT indicated the presence of a sensitive GIN population, perhaps because LEV-based products have not been available on the local market for more than two decades and have therefore not been used. The presence of almost 10 % L_3 larvae of *H. contortus* in the larval cultures for the LEV group after therapy however, indicates the continued monitoring of efficacy. Discriminating dose of 0.5 μg/ml according to Coles *et al*. (2006) was exceeded, but due to the presence of *H. contortus* larvae after therapy as well as in the LDT at concentration of 0.5 μg/ml, a threshold used by Taylor *et al*. (1989) 2.5 μg/ml has been applied. The population would be assessed as susceptible even if we used the threshold 1.0 μg/ml established by Coles *et al*. (1988) for *Trichostrongylus colubriformis*. Despite these differences in thresholds for each species, the value of 1.0 μg/ml can be considered as a borderline between susceptible and resistant population, and it is necessary to control

Table 3. Results of the morphological identification of L_3 larvae before and after treatment.

Before treatment (%)		After treatment (%)			
Nematode	%	Nematode	BZ group	IVM group	LEV group
Teladorsagia	45	*Teladorsagia*	79	100	91
Haemonchus contortus	17	*Haemonchus contortus*	21	–	9
Oesophagostomum/*Chabertia ovina*	19	*Oesophagostomum*/*Chabertia ovina*	–	–	–
Trichostrongylus	19	*Trichostrongylus*	–	–	–

BZ, benzimidazole; IVM, ivermectin; LEV, levamisole the effectiveness of LEV in the future and use the correct dosing of drug in therapy.

An effective anthelmintic strategy needs the administration of the correct dose of drug tailored to the metabolic and physiological characteristics of each host species. Underdosing is one of the most important factors responsible for accelerating the development of AR (Torres-Acosta & Hoste, 2008). A survey of goat farms conducted in northern Italy found that recommended doses for sheep were applied at six of eight farms tested, and only three farms had an adequate efficacy of ML (Lambertz *et al*., 2019). Eprinomectin (EPM) is currently the only anthelmintic with no milk-withdrawal period authorized for goats. The development of an optimal pour-on dose of EPM for dairy goats is currently being discussed due to differences in reduction between lactating and dry goats and to strong individual variability with the pour-on formulation (Rostang *et al*., 2020). The importance of correct dosing was demonstrated by a case in Poland, where EPM resistance developed after two years of use (Mickiewicz *et al*., 2019). Murri *et al*. (2014) conducted a survey of 43 goat farms in the canton of Berne, Switzerland, where 39 farms had faecal egg reductions <90 % after the application of the recommended dose of EPM (1 mg/kg bw). More clinical studies are needed to confirm the recommended dose of EPM in goats, especially in countries where similar studies have not been yet performed. A reduced efficacy of EPM can be also expected at Slovak farms, because its mechanism of action is the same as for other MLs.

Compared to sheep, goats require a different approach in drug dosing due to higher metabolic rate. Most of anthelmintics commonly applied on farms still do not have a licensed dose for use in goats (Várady *et al.,* 2011). Differences in drug bioavailability between sheep and goats were demonstrated by Sharma *et al*. (2014), where treatment with the same doses of LEV and fenbendazole reduced faecal egg counts by 100 % in sheep but only by 82.60 and 78.87 %, respectively, in goats. Using double doses of anthelmintics would be appropriate in goats from the point view of farm management and the prevention of the development and spread of AR. Cringoli *et al*. (2004) observed significant differences in percentage egg reduction between single (0.5 mg /kg bw) and double dose (1 mg/kg bw) of EPM in naturally infected goats. The survey of 30 goat farms in Slovakia by Babják *et al*. (2018), however, found that the use of a double dose in the FECRT could underestimate the presence of resistant strains. The dose recommended for sheep for the diagnosis of AR using the *in vivo* FECRT would therefore be more suitable, which could also indicate the low level of AR at goat farms and for this reason was this approach also applied in the current study.

Faecal examination before treatment and subsequent selective therapy is another approach that is still not applied in many countries. Valcárcel *et al*. (2015) applied three systems of targeted selective treatment (egg output, clinical signs, and criteria of live weight) for two consecutive years to reduce the frequency of treatment from 37.95 to 100 % without affecting health or production. The approaches of targeted selective therapies are costly but can prevent the development of resistance, which can cause greater production and financial losses. The application of anthelmintics at many farms is often used as the first solution without prior clinical or laboratory diagnosis. Factors such as underdosing, poor estimation of weight, and ignoring differences between sheep and goats were also reported by Mickiewicz *et al*. (2017) for the first case of BZ resistance in goats in Poland. BZs and MLs in our study were frequently applied more than twice a year. The owner also did not quarantine newly purchased animals, which could serve as sources of resistant parasites, as has been described in several studies (Várady *et al*., 1993; Himonas & Papadopoulos, 1994; Requejo-Fernandez *et al*., 1997). All new animals in our study were from Slovakia, so we can expect the presence of multidrug-resistant parasites on several other farms if the animals were sources of AR parasites. This case is an example of how the most common faults in farm management can lead to the development of resistance to multiple anthelmintics.

Conflict of Interest

Authors state no conflict of interest.

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