Veterinary Parasitology 332 (2024) 110301

Contents lists available at ScienceDirect

Veterinary Parasitology

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

# Effectiveness of benzimidazole treatments against *Haemonchus contortus* in sheep and goats – Do they produce similar responses?

M. Babják [a], A. Königová [a], M. Urda Dolinská [b], G. von Samson-Himmelstjerna [c], Y. Syrota [a,d], M. Komáromyová [a], M. Várady [a,*]

[a] Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, Košice 040 01, Slovakia
[b] Department of Epizootiology, Parasitology and Protection of One Health University of Veterinary Medicine and Pharmacy, Komenského 73, Košice 040 01, Slovakia
[c] Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Robert-von Ostertag Str. 7, Berlin 14163, Germany
[d] I. I. Schmalhausen Institute of Zoology NAS of Ukraine, vul. Bogdana Khmelnytskogo 15, Kyiv 01054, Ukraine

ARTICLE INFO

*Keywords:*
Sheep
Goats
Anthelmintic resistance
*in vivo* tests
Pyrosequencing

ABSTRACT

The primary aim of this study was to compare the *in vivo* responses to orally administered doses of albendazole (5 mg/kg body weight) between experimentally infected sheep and goats. Fifty-four Improved Valachian lambs and 54 Saanen goat kids were split into six groups of nine animals. The sheep and goats were infected with larvae of the gastrointestinal nematode parasite *Haemonchus contortus* containing 10, 20, 30, 40, 60, and 80 % of the isotype-1 β-tubulin gene codon 200 alleles previously shown to be associated with benzimidazole (BZ)-resistance. All groups of goats generally had higher mean eggs per gram (EPG) before treatment, which was significant ($p<0.05$) only for the group with 80 % resistance alleles. An *in vivo* faecal egg reduction test (FECRT) was used to determine the efficacy of albendazole (ABZ) eight days after treatment. Anthelmintic treatment significantly reduced the EPGs in the groups with 10, 20, and 80 % resistance alleles in sheep and with 10, 20, 30, and 40 % resistance alleles in goats. Differences in efficacy between the sheep and goats after the application of doses of ABZ recommended for sheep mostly ranged from 2 % to 10 %. The largest variation was in the group infected with worms containing 60 % resistance alleles, where the efficacy was 13 % higher in goats. Our secondary aims were to evaluate the data obtained from an *in vitro* egg hatch test (EHT) in sheep and goats and to compare these data with the results from the isotype-1 β-tubulin gene codon 200 pyrosequencing and the FECRT. The percentages of the BZ-resistance alleles were comparable with the mean hatching obtained in the EHT and were also supported by the FECRT data for all groups. The results of the *in vivo* tests should be verified in the future using *in vivo* surveys conducted in mixed breeds and infections in multiple species.

## 1. Introduction

Many studies have been published about the differences between the metabolic, immunological, and behavioural characteristics of sheep and goats (McKenna, 1984; Hoste et al., 2001; Torres-Acosta and Hoste, 2008; Hoste et al., 2010; Várady et al., 2011). One question remains: which of these factors has the greatest influence on host-parasite interactions and the effectiveness of anthelmintic treatment? One of the most important differences is the feeding behaviour of both species. Goats represent a suitable model for gaining knowledge about the interaction between the pathogenic effect of parasites and the grazing habits of the animals (Hoste et al., 2005). Sheep are typical grazing animals that prefer to graze on grass, but goats often choose to browse on various woody plants and shrubs. Goats can thus avoid most of the infectious $L_3$ larvae of gastrointestinal nematodes (GINs), which are primarily on pasture (Hoste et al., 2001). Avoiding infectious larvae, though, is also the cause of a slower onset of a fully developed immune response upon contact with GINs in goats (Pomroy et al., 1986; Hoste et al., 2008). Goat kids have a weaker ability than lambs to reduce the intensity of natural infections with GINs by immunoregulatory mechanisms (Macaldowie et al., 2003). Adult sheep are usually less infected than lambs, but the level of infection in goats is usually similar between adults and young animals (Vlassoff et al., 1999; Hoste et al., 2008). We therefore assumed that if lambs and kids were simultaneously infected

* Corresponding author.
*E-mail addresses:* babjak@saske.sk (M. Babják), konig@saske.sk (A. Königová), michaela.urda.dolinska@uvlf.sk (M.U. Dolinská), samson.georg@fu-berlin.de (G. von Samson-Himmelstjerna), syrota@saske.sk, goobar4.699@gmail.com (Y. Syrota), komaromyova@saske.sk (M. Komáromyová), varady@saske.sk (M. Várady).

https://doi.org/10.1016/j.vetpar.2024.110301
Received 6 July 2024; Received in revised form 31 August 2024; Accepted 1 September 2024
Available online 6 September 2024

with the same dose of GINs in mixed herds, the intensity of infection and the subsequent *in vivo* response to therapy would be substantially higher in lambs.

The biochemical difference between sheep and goats is another commonly discussed topic. The different rates of metabolism between these two hosts and the resulting need for a higher dose of anthelmintic for goats have been pharmacokinetically studied (McKenna and Watson, 1987; Hennessy et al. 1993a, 1993b; Sangster et al., 1991, Rolfe et al. 2013). All these variations require a specific approach by farmers and veterinarians, but creating suitable conditions for both species in small mixed farms can sometimes be difficult. The management of goat breeding on many farms thus still follows the principles used in sheep farming (Hoste et al., 2010). The emergence of anthelmintic resistance (AR) in goats is one of the most serious consequences of this practice, which in turn is a source of resistant parasites in sheep (Charles et al., 1989). The first occurrence of benzimidazole (BZ) resistance in goats was documented 50 years ago in the GIN *Trichostrongylus colubriformis* (Hotson et al., 1970). Sheep and goat numbers do not differ much worldwide (Livestock Population Trends, 2024), but goats may be less 'medicalised' or less resistance 'diagnosed' due to distribution of goats favouring developing countries compared to sheep, which are more common in developed countries. The majority of data about the pathogenic effect of parasites and host-parasite interactions in small ruminants has thus been obtained from studies carried out in sheep (Hoste et al., 2010).

Cases of AR on goat farms are currently increasing due to the continuous expansion of goat breeding (Holm et al., 2014; Babják et al., 2018, 2021a; Mickiewicz et al., 2020, 2021; Beleckė et al., 2021; Potârniche et al., 2021). These surveys were mostly carried out on larger goat farms where sheep were not present. Many smaller farms in central Europe, however, still rear sheep and goats together. Studies monitoring the *in vivo* responses of sheep and goats reared under the same conditions to an orally administered dose of a drug are nevertheless still lacking. Most studies that have compared the efficacy of drugs between both species of small ruminants have been based on the intraruminal administration of a drug and the subsequent measurement of the distribution of the active substance in plasma and abomasal fluid. The *in vivo* effectiveness of a drug can be also influenced by external factors that have a substantial and long-term effect on farm animals and can differ from the results of biochemical and *in vitro* studies.

The primary aim of this study was thus to compare the *in vivo* response to the same orally administered dose of albendazole (ABZ) in experimentally infected sheep and goats using an *in vivo* method (FECRT). Our secondary aims were to evaluate the data obtained by the *in vitro* method (EHT) in sheep and goats with identical parasitic infections and to compare the results obtained from the *in vivo* and *in vitro* tests with the frequencies of the BZ-resistance allele determined by pyrosequencing.

## 2. Material and Methods

### 2.1. Parasite isolates

The susceptible and resistant strains of the gastrointestinal parasitic nematode *H. contortus* were used for the preparation of composite infectious doses. The BZ-susceptible strain was collected as an inbred isolate of the BZ-susceptible ISE strain (Moredun nomenclature MHco3) after 15 generations of inbreeding (Roos et al., 2004). The origin of the resistant isolate was the University of Georgia, Athens, USA, where it has been passaged in goats since 2007. The presence of resistance alleles for each strain was determined by pyrosequencing, which indicated that the percentages of the codon 200 TAC resistance allele for the resistant and susceptible strains were 86 and 4 %, respectively. Infective $L_3$ larvae were collected using the Baermann technique, and different proportions of larvae were subsequently mixed to obtain composite doses containing 10, 20, 30, 40, 60, and 80 % resistance alleles.

### 2.2. Trial design

Fifty-four Improved Valachian lambs and 54 Saanen GIN naïve goat kids with an average age of 3–4 months were split into six groups of nine animals. All groups were housed for seven days in the stable for acclimatisation. All animals were subsequently infected with infectious doses consisting of 2500 $L_3$ larvae of *H. contortus*, which contained different proportions of the BZ resistance alleles. In the next phase, both species were treated by oral doses with the dose of ABZ recommended for ovines (5 mg/kg body weight (b.w.) on day (D) 35 post infection. (Albendavet 1.9 % DIVASA-FARMAVIC S.A., Barcelona, Spain). All groups were resampled eight days after treatment (D43).

### 2.3. In vivo FECRT

A modified McMaster technique (Coles et al. 1992) with a sensitivity of 50 eggs per gram (EPG) of faeces was used for the detection of strongylid eggs. The percent reduction of eggs (%FECR) after therapy was calculated using the formula of Cabaret and Berrag (2004):

$$\%FECR = (1/n)\sum(100\times(1-[Ti_2/Ti_1])$$

where $T_{i1}$ and $T_{i2}$ are pre- and post-treatment EPGs, respectively, in host $i$ from a total of $n$ hosts.

### 2.4. In vitro EHT

The EHT was performed following the protocol of Coles et al. (1992, 2006). Faecal samples for the EHT from each group were collected on D20, D28, and D35 post-infection. The samples were homogenised in tap water and stored anaerobically. Suspensions of helminth eggs for the *in vitro* tests were obtained using a filtration trough with a set of three sieves with different mesh sizes (250, 100, and 25 μm). The material from the last sieve was washed and sedimented, and the eggs were then isolated using the flotation method with a sugar solution with a specific gravity of 1.3. A stock solution of thiabendazole (TBZ) (Sigma–Aldrich, Darmstadt, Germany) was prepared by dissolving the pure compound in dimethylsulphoxide (DMSO). The final concentrations (0.05, 0.1, 0.3, 0.5, and 1 μg/ml) were prepared by adding 10 μl of each TBZ solution to 1.99 ml of a suspension of approximately 100–150 eggs/ml in water. A control (0.5 % DMSO) without anthelmintic was also included. These solutions were incubated in 24-well plates (TPP Techno PlasticProducts AG, Trasadingen, Switzerland) at 27 °C for 48 hours. The incubation was stopped by adding 10 ml of Lugol's iodine solution to each well. The test was performed with two replicates for each drug concentration and on three occasions for each isolate. The proportions of hatched and unhatched eggs were determined for each well. $ED_{50}$, which represents the concentration that prevents the hatching of 50 % of the eggs, was determined using a statistical logistic regression model.

### 2.5. Pyrosequencing assays for determining β-tubulin allele frequencies

Genomic DNA isolated from a pool of approximately 5000 *H. contortus* L3 larvae obtained from each group of sheep and goats on day 35 post-infection was used as the template for PCR for each composite dose. DNA extraction and pyrosequencing assays targeting the F200Y codon of the isotype-1 b-tubulin gene have previously been described by von Samson-Himmelstjerna et al. (2009) and Skuce et al. (2010) and were used in our study employing Hc200PySeq1 (5k – TAG AGA ACA CCG ATG AAA CAT – 3k) as the sequencing primer at 0.4 μμM.

### 2.6. Statistical analysis

The efficacy of the ABZ treatment in the groups was analysed using Student's *t*-test. Raw FECR values were first calculated for each individual in each experimental group to determine whether FECR differed

significantly between the sheep and goats. Any negative values from these calculations were then set to zero. Minor perturbations were introduced for duplicated values before the non-parametric Wilcoxon rank-sum test was conducted to avoid computational challenges. This procedure ensured the uniqueness of each value while preserving its accuracy within an acceptable range. The test results were complemented with box plots for visually comparing the FECR distributions between the two species in each group.

Two beta regression models were used, one for sheep and another for goats, to estimate the relationship between the pre- and post-treatment percentages of the resistance allele. All computational and visualisation tasks were performed in the R environment (R Core Team, 2023). Comprehensive details are available in the supplementary material (Supplement).

## 3. Results

The establishment of experimental infection was compared between the sheep and goats using pre-treatment EPGs (D35) in the groups with the same percentage of resistance alleles (Table 1). EPG was generally higher in all groups of goats before treatment, but not significantly except for the group with 80 % resistance alleles ($p<0.05$). Arithmetic means faecal egg counts recorded in all groups pre- and post-treatment are presented in Table 1. Anthelmintic treatment significantly ($p<0.05$; $p<0.001$) reduced the EPGs in the groups infected with *H. contortus* larvae showing 10, 20, and 80 % of the of the isotype-1 β-tubulin gene codon 200 BZ-resistance associated allele in sheep and with 10, 20, 30, and 40 % resistance alleles in goats. A comparison of the *in vivo* percent reduction of eggs after the ABZ treatment for both species, and the percentage of codon 200 resistance alleles from pyrosequencing, are presented in Table 2. Differences in the mean efficacy of the drug between the sheep and goats after application of the same dose mostly ranged from 2 % to 10 %.

The most noticeable variation was in the group containing 60 % resistance alleles, where the efficacy was 13 % higher in goats. Efficacy was higher in four of the sheep groups (10, 20, 40, and 80 % resistance alleles) and two of the goat groups (30 and 60 % resistance alleles). The Wilcoxon rank-sum tests across the study groups indicated that FECR did not differ significantly between the sheep and goats (Fig. 1). The percentage of codon 200 resistance alleles was in agreement with the proportions of the composite infection doses in sheep (beta regression: $\beta_{\text{pre-treatment}} \approx 4.4$, $p \ll 0.001$, pseudo-$R^2 \approx 0.96$) and goats (beta regression: $\beta_{\text{pre-treatment}} \approx 3.8$, $\ll 0.001$, pseudo-$R^2 \approx 0.92$). Table 3 compares the data obtained from the *in vitro* EHT with the pyrosequencing results. Mean hatching at a concentration 0.3 μg/ml of TBZ was comparable to the proportions of the resistant strain in the composite infectious doses for both species. $ED_{50}$ also increased in all groups simultaneously with the increasing ratio of the resistant strain in the composite doses.

**Table 1**
Arithmetic mean faecal egg counts recorded pre- and post-treatment in all experimental groups of sheep and goats.

| R[a] | Mean EPG[b] ± SD[c] | | | |
|---|---|---|---|---|
| | Sheep | | Goats | |
| | Pre-treatment | Post-treatment | Pre-treatment | Post-treatment |
| 1 (10 % R) | 5194.4 ± 2732.5 | 244.4 ± 235.0** | 8588.9 ± 6801.1 | 566.7 ± 331.7* |
| 2 (20 % R) | 2512.5 ± 1304.0 | 325.0 ± 158.1** | 4005.6 ± 3581.5 | 911.1 ± 1069.4* |
| 3 (30 % R) | 2822.2 ± 2486.5 | 1605.6 ± 1493.0 | 3733.3 ± 1926.6 | 1116.7 ± 590.7* |
| 4 (40 % R) | 3571.4 ± 2439.7 | 1750.0 ± 1138.3 | 4300.0 ± 2312.9 | 2127.8 ± 1310.2* |
| 5 (60 % R) | 1312.5 ± 574.9 | 1012.5 ± 462.8 | 9462.5 ± 11364.7 | 4181.25 ± 2739.2 |
| 6 (80 % R) | 1566.7 ± 880.7 | 722.2 ± 349.7* | 11905.6 ± 9818.1 | 6722.2 ± 6136.1 |

[a] percentage of the isotype-1 β-tubulin gene codon 200 BZ-resistance associated allele in the *Haemonchus contortus* larvae used to infect the group, [b]eggs per gram, [c]standard deviation, *reduction within group at $p<0.05$, **reduction within group at $p<0.001$.

**Table 2**
Comparison of the results of the *in vivo* faecal egg count reduction test and the percentage of isotype-1 β-tubulin gene codon 200 BZ-resistance associated allele detected by pyrosequencing from samples collected at day 35 post infection.

| R[b] | FECR[a] (%) | | Pyrosequencing - percentage of codon 200 BZ-resistance alleles | |
|---|---|---|---|---|
| | Sheep | Goats | Sheep | Goats |
| 1 (10 % R) | 95.7 | 91.3 | 7.5 | 10.5 |
| 2 (20 % R) | 86.0 | 76.1 | 14.5 | 22.8 |
| 3 (30 % R) | 52.7 | 63.3 | 23 | 39.5 |
| 4 (40 % R) | 50.6 | 48.4 | 37.5 | 40 |
| 5 (60 % R) | 23.5 | 36.5 | 45.5 | 53.5 |
| 6 (80 % R) | 46.0 | 41.4 | 72 | 74 |

[a] mean faecal egg count reduction, [b]percentage of the isotype-1 β-tubulin gene codon 200 BZ-resistance associated allele in the *Haemonchus contortus* larvae used to infect the group

## 4. Discussion

The primary aim of this study was to evaluate and compare the results of the *in vivo* FECR test between the sheep and goats treated with the same dose of ABZ. The results indicated similar *in vivo* responses to ABZ therapy, despite the well-known differences between the two species. Variation in the metabolism of ABZ between sheep and goats has been described by Hennessy et al. 1993a, where lower concentrations of ABZ-sulphoxide in plasma and abomasal fluid had to be compensated by applying higher doses of ABZ to goats. Similar studies with the same recommendation have also been published about the pharmacokinetics of oxfendazole (OFZ) and fenbendazole (FEN) (Sangster et al., 1991; Hennessy et al. 1993b). These pharmacokinetic findings indicated that orally applied ABZ should be more rapidly cleared from blood in goats than in sheep and could lead to the lower biological availability of the active substance. Goats should thus be treated orally at twice the recommended BZ dose for sheep (10 mg/kg b.w.) (Sangster et al. 1991), supporting the theory that long-term underdosing in goats would increase the incidence of AR in goats compared to sheep (Cabaret, 2000).

We anticipated that the *in vivo* response to treatment with the same orally administered dose of ABZ would differ significantly between sheep and goats. All animals in our study were treated with the dose of ABZ (5 mg/kg b.w.) recommended for small ruminants in Slovakia. The results of the *in vivo* FECR test with similar efficacies were unexpected. We hypothesised that the differences between the two host species would lead to a substantially lower egg reduction in goats after applying the same ALB dose. Not many studies have compared the *in vivo* efficacy of an orally administered drug between sheep and goats reared under the same conditions. Mwamachi et al. (1995) determined the *in vivo* efficacy of single and double doses of ivermectin (IVM), FEN, levamisole (LEV), and closantel on a mixed farm with 1120 sheep and goats. The use in goats of the dose recommended for sheep reduced efficacy in all three drugs tested (IVM, FEN, and LEV) by 73, 25, and 78 %, respectively. When 1.5-fold higher doses of LEV and IVM were applied to goats, their efficacies increased to 93 and 92 %, respectively. However, when the efficacy of single doses of all three drugs was compared between sheep and goats, differences were recorded from 4 % to 17 %, consistent with our results.

Babják et al. (2018) carried out an *in vivo* survey mapping the effectiveness of ABZ on goat farms and compared the percent reductions of strongylid eggs after the application of the recommended (5 mg/kg b.w.) and a double dose of ABZ (10 mg/kg b.w.). The results of the *in vivo* FECR test on the "resistant" farms after the application of a double dose

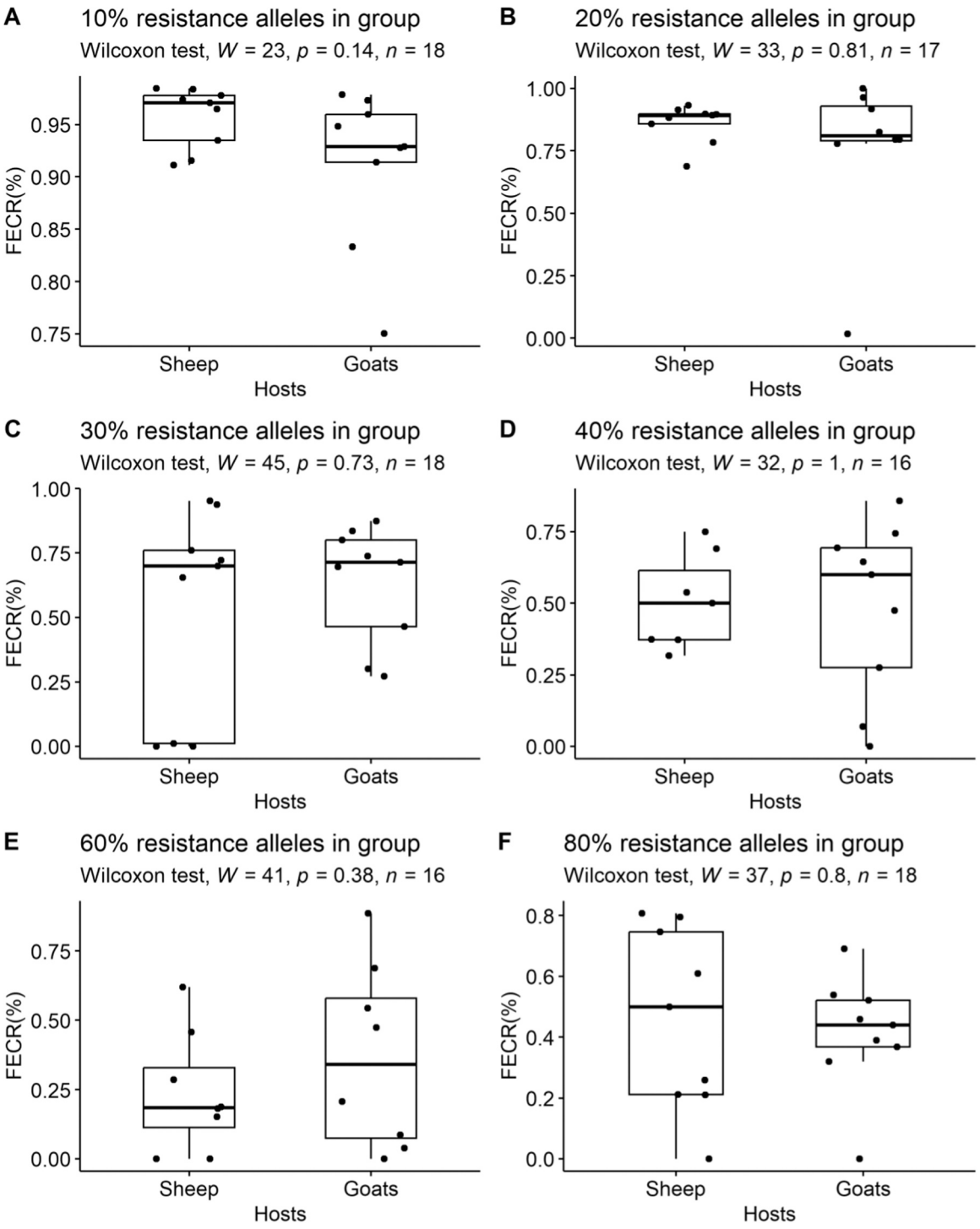


**Fig. 1.** Comparative analysis of faecal egg count reduction (FECR) between sheep and goats. Panels A-F illustrate the varying percentages of the isotype-1 β-tubulin gene codon 200 BZ-resistance associated allele in the *Haemonchus contortus* larvae used to infect the group, namely 10 %, 20 %, 30 %, 40 %, 60 %, and 80 %. The corresponding Wilcoxon test values (W and p) and the sample count, n, are provided for each panel.

of ABZ were quite different. An *in vivo* FECR test indicated an increased efficacy of approximately 20 % on most of the farms, but the other farms had the same or only a slightly increased reduction in egg count (1–5 %). These results demonstrated that dosage or interspecific differences may not strongly affect drug efficacy. The different proportion of homozygous and heterozygous resistance alleles in the parasite population could be the main factor why efficacy did not increase even after an application of a double dose or why the reduction of strongylid eggs was similar between hosts with different metabolic rates. In the current study the percentage of BZ-resistance associated the isotype-1 β-tubulin gene codon 200 allele but not the ratio of homozygous resistance alleles to heterozygotes with a resistance allele was determined.

Elard and Humbert (1999) reported that only homozygous mutant nematodes were able to survive treatment with therapeutic doses of BZs. Populations of resistant nematodes will subsequently produce resistant generations that will not respond to therapy (Martin et al., 1981) and the size of the dose of a BZ should not affect the reduction of strongylid eggs after long-term use. An analogous situation was observed in the

**Table 3**
Comparison between the values obtained from the *in vitro* egg hatch test and the percentage of isotype-1 β-tubulin gene codon 200 BZ-resistance associated allele for the composite infective doses in each group.

| | EHT[a] – mean hatching at 0.3 μg/ml TBZ | | EHT – $ED_{50}^{b}$ μg/ml TBZ[c] | |
|---|---|---|---|---|
| R[d] (%) | Sheep | Goats | Sheep | Goats |
| (10 %) | 9.91 ± 3.25 | 12.60 ± 5.30 | 0.12 ± 0.01 | 0.09 ± 0.01 |
| (20 %) | 19.08 ± 5.52 | 28.70 ± 6.60 | 0.16 ± 0.02 | 0.1 ± 0.01 |
| (30 %) | 48.83 ± 12.13 | 46.80 ± 10.60 | 0.35 ± 0.11 | 0.36 ± 0.09 |
| (40 %) | 63.00 ± 10.74 | 62.10 ± 11.10 | 0.82 ± 0.11 | 0.72 ± 0.31 |
| (60 %) | 68.83 ± 5.99 | 71.40 ± 7.60 | 1.78 ± 1.13 | 1.88 ± 0.44 |
| (80 %) | 89.33 ± 3.96 | 89.70 ± 4.10 | 28.05 ± 20.87 | 15.63 ± 12.10 |

[a] Egg hatch test, [b]concentration required to inhibit 50 % of the eggs from hatching, [c]thiabendazole, [d]percentage of the isotype-1 β-tubulin gene codon 200 BZ-resistance associated allele in the *Haemonchus contortus* larvae used to infect the group.

occurrence of multiple AR on a goat farm in Slovakia (Babják et al., 2021a), where 10 sheep shared a pasture with approximately 120 goats. The results from sheep were not included in the study, but the percent reduction in an *in vivo* FECR test was similar for both species, and the efficacy in sheep did not increase even after the application of a double dose of ABZ (Babják, unpublished data). This situation was the result of the long-term treatment of goats with therapeutic doses recommended for sheep and supports the theory that goats in mixed herds are a source of resistant parasites for sheep (Charles et al., 1989). Knowledge of the differences between the two species, however, must be applied to the management of farms with specific proportions of susceptible or heterozygous resistance alleles in the parasite population. The search for this "threshold" value could be realised by extending our study by determining the exact ratio of homozygous/heterozygous resistance alleles in each group and adding groups of sheep and goats that would be administered with double doses of ABZ. Comparing the efficacies of the drug at different proportions of homozygous and heterozygous resistance alleles in composite infectious doses and finding a proportion at which the efficacy would be the same between different doses and species would then be necessary. The species composition of the nematodes on a farm is another factor that may influence the effectiveness of a therapy. *H. contortus* was used as a model parasite in our study.

McKenna and Watson (1987) conducted a slaughter trial to compare the efficacies of OFZ, IVM, LEV, and morantel against sheep and goats experimentally infected with *H. contortus*, *T. colubriformis*, *Cooperia curticei*, and *Ostertagia* spp. They recorded similar efficacies for OFZ and IVM against all four nematode species in both hosts. The sheep and goats responded the same to the application of LEV and morantel against *H. contortus* and *C. curticei*., but these two drugs were less effective against *Ostertagia* spp. and *T. colubriformis*. In contrast, Sangster et al. (1991) described the distribution of the intraruminal administration of OFZ in sheep and goats against resistant *H. contortus* and *T. colubriformis*. Their results indicated that the efficacy against *T. colubriformis* was lower in sheep than goats but that the efficacy against *H. contortus* was similar in both hosts, in agreement with our results. Based on these data, we assumed that the reduction of strongylid eggs after therapy was not only influenced by the ratio of homozygous/heterozygous resistance alleles, but also by the species of nematode parasite in which AR had developed. Adding additional groups of both hosts that would be experimentally infected with *T. colubriformis* and *Teladorsagia circumcincta* would be necessary to monitor possible differences in the *in vivo* effectiveness of a drug in individual species of GINs.

Sangster et al. (1991) also reported differences in the distribution of a drug between intraruminal and intra-abomasal application. The way a drug is applied is another factor that affects the distribution of its active substance in the host organism. We therefore recommend a comprehensive *in vivo* study that would monitor the differences between hosts in the way an anthelmintic is administered. The results from our pyrosequencing and *in vivo* FECR test were accompanied by the data from the EHT, the most widely used *in vitro* method to detect BZ resistance. No significant differences were observed in the *in vitro* part of the study either. The sheep and goats were infected with the same species of GIN, so these data were not unexpected. The percentages of hatched eggs at the monitored concentrations were in agreement with the percentages of the codon 200 resistance alleles detected by pyrosequencing. Previous studies have monitored the hatching of eggs in experimental infections with *H. contortus* at various concentrations of BZ in EHTs and have compared the results with those from pyrosequencing (Babják et al., 2021b; Königová et al., 2021), which was a suitable early indicator of AR. Monitoring the hatching of eggs at concentrations of TBZ of 0.1 and 0.3 μg/ml in field testing for AR provides an advantage against traditional testing using a TBZ threshold of 0.1 μg/ml, where lower levels of resistance cannot be detected (Babják et al. 2018).

## 5. Conclusion

Our results showed that we could expect a similar or the same effectiveness of BZ in both species of small ruminants for monoinfections with *H. contortus* and the application of the same dose. We should therefore be careful when goats are predominantly infected with *H. contortus* and are treated with an ALB dose of 5 mg/kg b.w. This dosage was probably not an underdosage, since the estimation of the representation of the resistant and susceptible parasites in the population using *in vivo/vitro* tests was likely accurate. This *in vivo* response, however, can be similar under some conditions and influencing factors, independent of interspecific differences. An extensive *in vivo* study in mixed herds of small ruminants and infection with multiple GIN species would be necessary to clarify these factors.

## Ethics approval

All procedures were conducted following European Community guidelines (EU Directive 2010/63/EU). The experimental protocol was approved by the Ethical Committee of the Institute of Parasitology of the Slovak Academy of Sciences, following national legislation in Slovakia (G.R. 377/2012; Law 39/2007) for the care and use of research animals.

## Funding

This study was supported by funds from the Scientific Grant Agency VEGA 2/0090/22. Y. Syrota gratefully acknowledges funding support from the NextGenerationEU through the Recovery and Resilience Plan for Slovakia under project No. 09I03-03-V01-00046.

## CRediT authorship contribution statement

**G. von Samson-Himmelstjerna:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **M. Urda Dolinská:** Investigation. **M. Komáromyová:** Investigation, Formal analysis. **Y. Syrota:** Formal analysis, Data curation. **M. Babják:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **A. Königová:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Marián Várady:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2024.110301.

## References


Babják, M., Königová, A., Urda Dolinská, M., Vadlejch, J., Várady, M., 2018. Anthelmintic resistance in goat herds - *in vivo* versus *in vitro* detection. Vet. Parasitol. 254, 10–14. https://doi.org/10.1016/j.vetpar.2018.02.036.

Babják, M., Königová, A., Várady, M., 2021a. Multiple anthelmintic resistance at a goat farm in Slovakia. Helminthologia 58 (2), 173–178. https://doi.org/10.2478/helm-2021-0014.

Babják, M., Königová, A., Urda Dolinská, M., Kupčinskas, T., Vadlejch, J., von Samson-Himmelstjerna, G., Petkevičius, S., Várady, M., 2021b. Does the *in vitro* egg hatch test predict the failure of benzimidazole treatment in *Haemonchus contortus*? Parasite 28, 62. https://doi.org/10.1051/parasite/2021059.

Beleckė, A., Kupčinskas, T., Stadalienė, I., Höglund, J., Thamsborg, S.M., Stuen, S., Petkevičius, S., 2021. Anthelmintic resistance in small ruminants in the Nordic-Baltic region. Acta Vet. Scand. 63 (1), 18. https://doi.org/10.1186/s13028-021-00583-1.

Cabaret, J., 2000. Anthelmintic resistance in goats: from fiction to facts. In: Proceeding of the 7th international conference on goats Tours-Poitiers, France, 15-21 Mai 2000, pp. 793- 794.

Cabaret, J., Berrag, B., 2004. Faecal egg count reduction test for assessing anthelmintic efficacy: average versus individually based estimations. Vet. Parasitol. 121 (1-2), 105–113. https://doi.org/10.1016/j.vetpar.2004.01.020.

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P. J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A. V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44 (1-2), 35–44. https://doi.org/10.1016/0304-4017(92)90141-U.

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., Von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 136 (3-4), 167–185. https://doi.org/10.1016/j.vetpar.2005.11.019.

Elard, L., Humbert, J.F., 1999. Importance of the mutation of amino acid 200 of the isotype 1 beta-tubulin gene in the benzimidazole resistance of the small-ruminant parasite *Teladorsagia circumcincta*. Parasitol. Res. 85 (6), 452–456. https://doi.org/10.1007/s004360050577.

Hennessy, D.R., Sangster, N.C., Steel, J.W., Collins, G.H., 1993a. Comparative pharmacokinetic behaviour of albendazole in sheep and goats. Int. J. Parasitol. 23 (3), 321–325. https://doi.org/10.1016/0020-7519(93)90006-k.

Hennessy, D.R., Sangster, N.C., Steel, J.W., Collins, G.H., 1993b. Comparative kinetic disposition of oxfendazole in sheep and goats before and during infection with *Haemonchus contortus* and *Trichostrongylus colubriformis*. J. Vet. Pharmacol. Ther. 16 (3), 245–253. https://doi.org/10.1111/j.1365-2885.1993.tb00171.x.

Holm, S.A., Sörensen, C.R., Thamsborg, S.M., Enemark, H.L., 2014. Gastrointestinal nematodes and anthelmintic resistance in Danish goat herds. Parasite 21, 37. https://doi.org/10.1051/parasite/2014038.

Hoste, H., Le Frileux, Y., Pommaret, A., 2001. Distribution and repeatability of faecal egg counts and blood parameters in dairy goats naturally infected with gastrointestinal nematodes. Res. Vet. Sci. 70 (1), 57–60. https://doi.org/10.1053/rvsc.2000.0442.

Hoste, H., Torres-Acosta, J.F., Paolini, V., Aguilar-Caballero, A.J., Etter, E.M., Lefrileux, Y., Chartier, C., Broqua, C., 2005. Interactions between nutrition and gastrointestinal infections with parasitic nematodes in goats. Small Rumin. Res. 60 (1-2), 141–151. https://doi.org/10.1016/j.smallrumres.2005.06.008.

Hoste, H., Torres-Acosta, J.F., Aguilar-Caballero, A.J., 2008. Nutrition-parasite interactions in goats: is immunoregulation involved in the control of gastrointestinal nematodes? Parasite Immunol. 30 (2), 79–88. https://doi.org/10.1111/j.1365-3024.2007.00987.x.

Hoste, H., Sotiraki, S., Laundau, S.Y., Jackson, F., Beveridge, I., 2010. Goat nematode interactions: think differently. Trends Parasitol. 26 (8), 376–381. https://doi.org/10.1016/j.pt.2010.04.007.

Hotson, I.K., Campbell, N.J., Smeal, M.G., 1970. Anthelmintic resistance in *Trichostrongylus colubriformis*. Aust. Vet. J. 46 (8), 356–360. https://doi.org/10.1111/j.1751-0813.1970.tb15572.x.

Charles, T.P., Pompeu, J., Miranda, D.B., 1989. Efficacy of three broad-spectrum anthelmintics against gastrointestinal nematode infections of goats. Vet. Parasitol. 34 (1-2), 71–75. https://doi.org/10.1016/0304-4017(89)90166-0.

Königová, A., Urda Dolinská, M., Babják, M., von Samson-Himmelstjerna, G., Komáromyová, M., Várady, M., 2021. Experimental evidence for the lack of sensitivity of *in vivo* faecal egg count reduction testing for the detection of early development of benzimidazole resistance. Parasitol. Res. 120 (1), 153–159. https://doi.org/10.1007/s00436-020-06965-0.

Livestock Population Trends, 2024. [WWW Document]. URL ⟨https://livestockdata.org/resources/livestock-population-trends⟩ (accessed 8.19.24).

Macaldowie, C., Jackson, F., Huntley, J., Mackellar, A., Jackson, E., 2003. A comparison of larval development and mucosal mast cell responses in worm-naïve goat yearlings, kids and lambs undergoing primary and secondary challenge with *Teladorsagia circumcincta*. Vet. Parasitol. 114 (1), 1–13. https://doi.org/10.1016/s0304-4017(03)00110-9.

Martin, P.J., Le Jambre, L.F., Claxton, J.H., 1981. The impact of refugia on the development of thiabendazole resistance in *Haemonchus contortus*. Int. J. Parasitol. 11 (1), 35–41. ⟨https://doi:0.1016/0020-7519⟩(81)90023-0.

McKenna, P.B., 1984. Gastrointestinal parasitism and anthelmintic resistance in goats. Surveillance 11, 2–4.

McKenna, P.B., Watson, T.G., 1987. The comparative efficacy of four broad spectrum anthelmintics against some experimentally induced trichostrongylid infections in sheep and goats. N. Z. Vet. J. 35 (11), 192–195. https://doi.org/10.1080/00480169./1987.35443.

Mickiewicz, M., Czopowicz, M., Kawecka-Grochocka, E., Moroz, A., Szaluś-Jordanow, O., Várady, M., Königová, A., Spinu, M., Górski, P., Bagnicka, E., Kaba, J., 2020. The first report of multidrug resistance in gastrointestinal nematodes in goat population in Poland. BMC Vet. Res. 16 (1), 270. https://doi.org/10.1186/s12917-020-02501-5.

Mickiewicz, M., Czopowicz, M., Moroz, A., Potărniche, A.V., Szaluś-Jordanow, O., Spinu, M., Górski, P., Markowska-Daniel, I., Várady, M., Kaba, J., 2021. Prevalence of anthelmintic resistance of gastrointestinal nematodes in Polish goat herds assessed by the larval development test. BMC Vet. Res. 17 (1), 19. https://doi.org/10.1186/s12917-020-02721-9.

Mwamachi, D.M., Audho, J.O., Thorpe, W., Baker, R.L., 1995. Evidence for multiple anthelmintic resistance in sheep and goats reared under the same management in coastal Kenya. Vet. Parasitol. 60 (3-4), 303–313. https://doi.org/10.1016/0304-4017(95)00794-1.

Pomroy, W.E., Lambert, M.G., Betteridge, K., 1986. Comparison of faecal strongylate egg counts of goats and sheep on the same pasture. N. Z. Vet. J. 34 (3), 36–37. https://doi.org/10.1080/00480169.1986.35272.

Potârniche, A.V., Mickiewicz, M., Olah, D., Cerbu, C., Spînu, M., Hari, A., Györke, A., Moroz, A., Czopowicz, M., Várady, M., Kaba, J., 2021. First Report of Anthelmintic Resistance in Gastrointestinal Nematodes in Goats in Romania. Animals 11 (10), 2761. https://doi.org/10.3390/ani11102761.

R Core Team, 2023. R. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <⟨https://www.R-project.org/⟩>.

Rolfe, P., Sager, H., Schmid, V., Browning, A., Allen, B., Vanhoff, K., George, S., George, A., Nottingham, R., Potgieter, N., 2013. Efficacy of Zolvix (Monepantel) against gastrointestinal parasites and the pharmacokinetics in goats. 8th Int. Sheep Vet. Congr., : Proc. Int. Sheep Vet. Congr. 135.

Roos, M.H., Otsen, M., Hoekstra, R., Veenstra, J.G., Lenstra, J.A., 2004. Genetic analysis of inbreeding of two strains of the parasitic nematode *Haemonchus contortus*. Int. J. Parasitol. 34 (1), 109–115. https://doi.org/10.1016/j.ijpara.2003.10.002.

Sangster, N.C., Rickard, J.M., Hennessy, D.R., Steel, J.W., Collins, G.H., 1991. Disposition of oxfendazole in goats and efficacy compared with sheep. Res. Vet. Sci. 51 (3), 258–263. https://doi.org/10.1016/0034-5288(91)90074-x.

Skuce, P., Stenhouse, L., Jackson, F., Hypsa, V., Gilleard, J., 2010. Benzimidazole resistance allele haplotype diversity in United Kingdom strains of *Teladorsagia circumcincta* supports a hypothesis of multiple origins of resistance by recurrent mutation. Int. J. Parasitol. 40 (11), 1247–1255. https://doi.org/10.1016/j.ijpara.2010.03.016.

Torres-Acosta, J.F.J., Hoste, H., 2008. Alternative or improved methods to limit gastrointestinal parasitism in grazing sheep and goats. Small Rumin. Res. 77 (2), 159–173. https://doi.org/10.1016/j.smallrumres.2008.03.009.

Várady, M., Papadopoulos, E., Dolinská, M., Königová, A., 2011. Anthelmintic resistance in parasites of small ruminants: sheep versus goats. Helminthologia 48 (3), 137–144. https://doi.org/10.2478/s11687-011-0021-7.

Vlassoff, A., Bisset, S.A., McMurtry, L.W., 1999. Faecal egg counts in Angora goats following natural or experimental challenge with nematode parasites: within-flock variability and repeatabilities. Vet. Parasitol. 84 (1-2), 113–123. https://doi.org/10.1016/s0304-4017(99)00072-2.

von Samson-Himmelstjerna, G., Walsh, T.K., Donnan, A.A., Carrière, S., Jackson, F., Skuce, P.J., Rohn, K., Wolstenholme, A.J., 2009. Molecular detection of benzimidazole resistance in *Haemonchus contortus* using real-time PCR and pyrosequencing. Parasitology 136 (3), 349–358. ⟨https://doi.doi:10.1017/S003118200800543X⟩.