Veterinary Parasitology 327 (2024) 110133

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Metaphylactic strategies using toltrazuril against coccidiosis in goat kids

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ARTICLE INFO

Keywords:
Eimeria infections
Control
Triazines
Majorera goat

ABSTRACT

Goat coccidiosis compromises animal welfare, reduces productivity and may cause mortality and delayed growth rates in goat kids around the weaning period worldwide. This field study was conducted to evaluate the efficacy of metaphylactic treatments with two doses of toltrazuril (20 or 40 mg/kg body weight – BW, p. o.), at different timing, in kids naturally infected with *Eimeria* spp. A total of 97 healthy goat kids (Majorera milk aptitude breed) were divided into five groups, depending on the age of treatment (2 or 7 weeks). One group remained untreated as a negative control until the end of the study. Faecal oocyst shedding, faecal consistency, and body weight of the animals were monitored at day 0 and at weekly intervals. Counts of oocysts per gram of faeces (OPG) were determined by a modified McMaster technique. Morphometric identification of *Eimeria* species was carried out on individual faecal samples from each experimental group after oocyst sporulation. Goat kids treated at two weeks of age maintained OPG values close to zero during the 5 weeks post-treatment and, overall, had lower faecal oocyst counts than untreated control animals. No significant differences were observed between the two doses of toltrazuril used in two-week-old treated animals. By contrast, when treatment was carried out at seven weeks of age, the dose of 40 mg/kg BW of toltrazuril reduced oocyst levels for longer and to a greater extent than the 20 mg/kg dose. Irrespectively of the treatment and dose, toltrazuril delayed the appearance of pathogenic *Eimeria* species, i. e. *Eimeria ninakohlyakimovae* and *Eimeria arloingi.* As a whole, *Eimeria christenseni*, with a rather moderate pathogenicity, was highly predominant throughout the study period, including the untreated control group, which was probably the reason why clinical signs of coccidiosis were barely observed throughout the experiment. Under these circumstances, the positive effect of toltrazuril on body weight condition observed in some treated groups was difficult to correlate to the timing and doses. Metaphylactic treatments with 20 mg/kg BW toltrazuril given at two weeks of age are sufficient to control oocyst excretion in goat kids; whereas if administered later in 7-week-old animals, thereby coinciding with the frequently observed peak of oocyst elimination in goat kids under field conditions, a higher dose might be advisable to prevent environmental contamination with infectious oocysts.

1. Introduction

The genus *Eimeria*, a host-specific obligate intestinal apicomplexan protozoan, is responsible for coccidiosis in many vertebrate hosts worldwide (Chapman et al., 2013). Coccidiosis in sheep and goat compromises animal welfare, reduces productivity and may cause mortality, especially in young animals during their first weeks of life (Foreyt, 1990; Chartier and Paraud, 2012). In goat kids, the intensity of *Eimeria* oocyst excretion usually reaches peak values around the weaning period, i. e. between 6–10 weeks of age (Ruiz et al., 2006; Chartier and Paraud, 2012). The main clinical sign of eimeriosis in goat kids is non-haemorrhagic diarrhoea, characterized by watery faeces with clumps of mucus and colour changes from brown to yellow (Koudela and Boková, 1998). Therefore, these animals show dehydration, reduced feed intake, weight loss, a decreased appetite and even death in cases of severe infections (Yvoré et al., 1980; Bidorff et al., 1986). In addition, subclinical coccidiosis can cause impairment of growth and poor weight gain from gut damage, which also leads to significant economic losses

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https://doi.org/10.1016/j.vetpar.2024.110133
Received 21 November 2023; Received in revised form 16 January 2024; Accepted 16 January 2024
Available online 19 January 2024

(Foreyt, 1990; Ruiz et al., 2006; Silva et al., 2017).

Several monoxenous *Eimeria* species have been described in sheep and goat worldwide. Of the at least 13 *Eimeria* species described in goats, the most prevalent species are *E. ninakohlyakimovae*, *E. arloingi* and *E. alijevi* (Lima, 1980; Yvoré et al., 1981; Vercruysse, 1982; Faizal and Rajapakse, 2001; Ruiz et al., 2006), amongst which *E. ninakohlyakimovae* and *E. arloingi* are the most pathogenic ones (Levine, 1985; Yvoré et al., 1985; Koudela and Boková, 1998; Silva et al., 2017). In the field, infections with multiple *Eimeria* species are common in goats (Ruiz et al., 2006). Pathogenic effects of infections with different *Eimeria* species might be aggravated due to extended damage of the intestines since different species reside in different intestinal sections. In addition, longer-lasting clinical effects might be observed due to different reproductive patterns and prepatent and patent periods of different *Eimeria* species (Craig, 1986).

The control of eimeriosis in ruminants aims to reduce mortality and morbidity, as well as to ensure animal weight gains, thus resulting in economic benefits for farmers (Foreyt, 1990). Triazine derivatives such as diclazuril and toltrazuril have a significant efficacy against *Eimeria* species since these compounds can early reduce oocyst excretion. Accordingly, several studies have confirmed the efficacy of diclazuril and toltrazuril on sheep coccidiosis (Gjerde and Helle, 1986, 1991; Taylor and Kenny, 1988; Stafford et al., 1994; Alzieu et al., 1999; Platzer et al., 2005; Dittmar et al., 2009; Le Sueur et al., 2009; Mundt et al., 2009; Taylor et al., 2011; Diaferia et al., 2013; Saratsis et al., 2013; Scala et al., 2014; Pérez-Fonseca et al., 2016; de Souza Rodrigues et al., 2016, 2017). By contrast, these compounds have been much less investigated in caprine coccidiosis (McKenna, 1988; Chartier et al., 1992; Šlosárková et al., 1998; Balicka-Ramisz, 1999; Ruiz et al., 2012; Iqbal et al., 2013a, b; Nunes et al., 2015; Awasthi et al., 2022).

Toltrazuril is an anticoccidial drug widely used in chicken, turkeys, piglets, calves and lambs (EMEA/CVMP/278616/ 2005; Karembe et al., 2021). This compound acts against asexual and sexual intracellular stages of *Eimeria* life cycle in poultry (i. e. schizogony and gamogony) (Haberkorn, 1987; Harder and Haberkorn, 1989), especially affecting parasite mitochondria, the endoplasmatic reticulum and hampering nuclear division not only in schizonts (meronts) but also microgamonts (Mehlhorn et al., 1984; Mehlhorn, 2008). Previous studies in goats have shown the clinical efficacy of ponazuril, a metabolite of toltrazuril, in decreasing faecal *Eimeria* oocyst counts (Gibbons et al., 2016) and on how this compound is absorbed after a single oral dose application (Love et al., 2015). In addition, a higher dose rate of toltrazuril should be administrated in goats compared to the dose rate for sheep or cattle (15–20 mg / kg BW) (Gjerde and Helle, 1986; Chartier et al., 1992; Epe et al., 2005). The persistent efficacy of toltrazuril is generally long but variable according to target species and literature (i. e. > 80%, 9 days post-treatment (p. t.) in piglets, 3–4 weeks p. t. in calves or more than 9 weeks p. t. in lambs (Mundt et al., 2007; Diaferia et al., 2013; Zapa et al., 2022). Basic pharmacological information is essential to establish the dose rate and then determine the efficacy of different control programs for goat coccidiosis in arid and semi-arid zones, where this ruminant species represents one of the most important economic resources for farmers (Abo-Shehada and Abo-Farieha, 2003; Ruiz et al., 2006). Therefore, the aim of this study was to evaluate the clinical and parasitological outcomes of different metaphylactic treatments with toltrazuril in goat kids naturally infected with *Eimeria* spp.

2. Materials and methods

2.1. Study farm

This study was carried out in a semi-extensive goat farm with common clinical manifestations of moderate to severe naturally acquired coccidiosis occurring during and after the weaning period. This farm is located in San Bartolomé de Tirajana (27°47'29.4"N 15°31'15.0"W) in the south of Gran Canaria Island (Spain). In this arid region, the temperature ranges between 15 °C in winter and 28 °C in summer with an average of 21 °C, and the monthly rainfall ranges from zero in summer to 60 mm in winter (https://www.climatestotravel.com/climate/canary-islands/gran-canaria; accessed Dec 5, 2023). These climatic conditions have contributed to the development of goat industry in this area. In fact, this region holds 57% of the goat population of Gran Canaria. Animals are either kept in medium-sized farms (around 300 animals) or large farms (≥ 1000 animals). The farm chosen as study site housed approximately 5000 goats (Majorera breed with milk aptitude) during the trial. This farm has three breeding seasons, late winter to early spring (February to March), early summer (June) or autumn (October-November).

2.2. Animals

This trial was performed with a total of 97 healthy Majorera goat kids ($n = 97$) either in their second or seventh week of age at the beginning of the study, and all naturally exposed to *Eimeria* spp.-oocysts within farm premises. All animals were born on the study farm itself and belonged to the same farrowing period (early spring). They were separated from their mother immediately after birth and, after 2–3 days of colostrum administration, and fed with special artificial milk (Bacilactol®, CAPISA, Canary Islands, Spain) for lactating goat kids from an automated feeding system. Water, hay, and supplementation with starter concentrate for weaning kids (CAPISA) were available during the whole experimental setting. No animal had received any medication before the study. Efficacy of metaphylactic treatments with two doses of toltrazuril (Baycox® 50 mg/ml suspension, Bayer Animal Health GmbH, Germany) were assessed in these goat kids, as described below.

2.3. Study design

Goat kids were randomly assigned (by birth weight) to five groups and correspondingly identified by an ear tag attached to a chain around their necks. To not alter the management conditions on the farm, the animals included in the experiment shared the same facilities as the kids born in the same kidding period (approximately, 300 goat kids in total). All five experimental groups were run in parallel. At two weeks of age, groups 1 ($n = 21$) and 2 ($n = 21$) received 20 mg or 40 mg toltrazuril (Baycox®)/kg body weight (BW) p. o. once, respectively. At seven weeks of age, group 3 ($n = 23$) and 4 ($n = 22$) were treated once with 20 mg or 40 mg/kg BW p. o. of this drug, respectively. Finally, group 5 ($n = 10$) remained untreated as a negative control until twelve weeks of age. For the presentation of the results and the discussion the experimental groups may be referred to as: group 1 (G1): "low dose early treatment"; group 2 (G2): "high dose early treatment"; group 3 (G3): "low dose late treatment"; group 4 (G4): "high dose late treatment"; and group 5 (G5): "control".

Counts of oocysts per gram of faeces (OPG), BW, faecal consistency and soiling of the anal region were monitored just before treatment and at weekly intervals for 11 (groups 1–2 and 5) and 6 (groups 3 and 4) weeks post-treatment, respectively. Individual faecal samples were taken manually, carrying gloves, from the rectum.

All animal procedures were conducted in strict accordance with national ethics, the current European Animal Welfare Legislation (ART13TFEU) and by institutional review board-approved protocols (project OEBA-ULPGC 16/2018 authorized on 30/11/2018, administrative decision 902/2018, Department of Agriculture and Livestock, Government of the Canary Islands).

2.4. Parasitological analysis

Oocysts per gram of faeces (OPG) were determined by a modified McMaster technique (Thienpont et al., 1979). The lower detection limit was 50 OPG. In the case of very high oocyst counts, dilutions of the faecal suspension by 10 or 100 times were performed to enable proper counting.

Morphological identification of *Eimeria* species was carried out over 20 oocysts per animal and the relative proportion of all the species found for each experimental group was determined. For sporulation, faecal material was individually incubated in a 2% potassium dichromate solution at room temperature (RT) for at least one week under constant oxygenation (Hermosilla et al., 2002). Sporulated oocysts were then concentrated by flotation in saturated sodium chloride solution and identified at 400 × magnification applying a calibrated eyepiece according to keys previously reported (Levine and Ivens, 1986; Alyousif et al., 1992; Soe and Pomroy, 1992).

2.5. Clinical and productive determinations

The faecal consistency was scored on a scale from one to five (1, normal consistency; 2, soft unformed; 3, semiliquid; 4, watery diarrhoea; 5, diarrhoea with blood and/or intestinal mucosal tissue). A score ≥ 3 was considered as diarrhoea. Soiling of the anal region was recorded as no soiling (score = 1), dry soiling (score = 2) and humid soiling (score = 3). Other clinical features, such as respiratory signs or the presence of contagious ecthyma, as well as the BW were also recorded at weekly intervals.

2.6. Statistical analysis

Data were analysed descriptively and statistically. For the analysis, faecal oocyst counts, expressed as OPG, were logarithmically transformed, and added by 1 (log [OPG+1]) to obtain normal distributions (Kolmogorov–Smirnov's normality test). The relative reduction of oocyst excretion was calculated based on the formula $C - T/C \times 100$, with C representing the mean OPG values of the untreated control group (group 5) and T representing the mean OPG values of the treated groups (groups 1–4) (Saratsis et al., 2013). An estimate of the overall treatment efficacy for each group was calculated on the basis of the area under the curve (AUC). The estimation of the BW gains over time was expressed as growth rate (ln weight 2 −ln weight 1)/t × 100), with t representing the number of days between the sampling time points 1 and 2.

The average of faecal consistency score, body weight and log (OPG+1) were calculated and then compared among the different groups by one-way ANOVA with pairwise post-hoc comparisons by the Tukey test, while differences between *Eimeria* species in each group were analyzed by non-parametric Chi-square test. Finally, the Pearson correlation test was used for the analysis of the association between parasitological and productive parameters (BW) at all sampling times (weeks 1–8). Analyses were carried out by the statistical software SigmaPlot 14.5 (Systat Software Inc., Germany) and the differences were considered significant at $P < 0.05$.

3. Results

3.1. Faecal oocyst excretion

The differences in OPG (oocysts per gram of faeces) over time in the five experimental groups are shown in Fig. 1. In the non-treated goat kids (control group), *Eimeria* oocysts were first detected at two weeks of age, with peak values at week 7 (1.37 $\times 10^6$ OPG). From this week onwards, OPG counts progressively dropped until the end of the trial (week 13 of age), with around 60 thousand OPG counts being recorded.

OPG values of the untreated control group (group 5) were significantly higher at weeks 5, 6, 7, 10 and 11 of the experiment ($P < 0.05$ – $P < 0.001$) (Fig. 1A) when compared to those of goat kids treated with 20 mg/kg toltrazuril at two week of age (group 1). Early treatment with a higher dose of toltrazuril also reduced OPG counts in relation to control group 5, with significant differences being recorded at weeks 5, 6, 7, 8 and 10 ($P < 0.05$ – $P < 0.001$) and almost statistical differences in week 11 of the experiment ($P = 0.065$) (Fig. 1A). OPG values

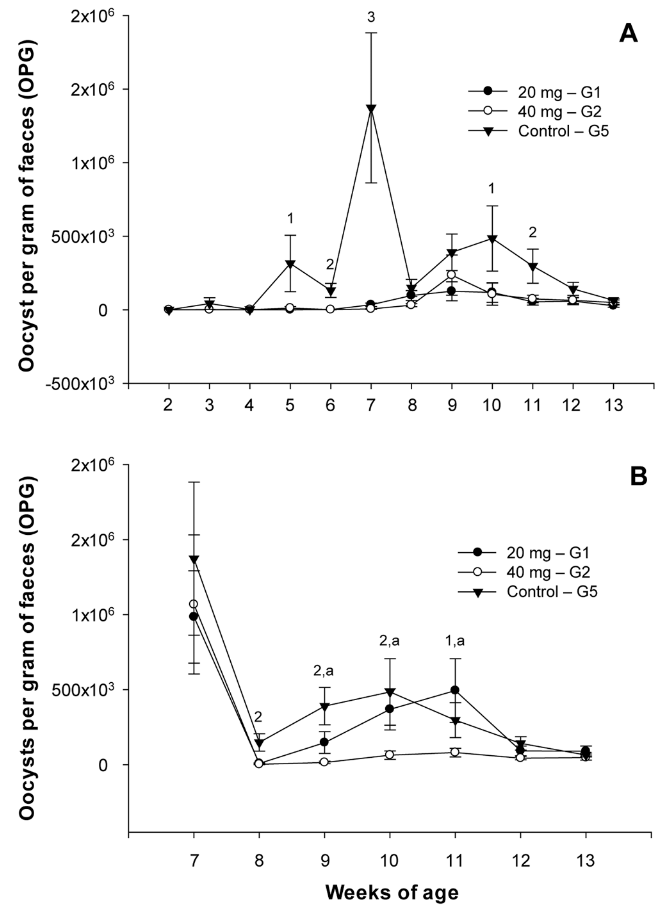

Fig. 1. *Eimeria* oocyst counts per gram of faeces in goat kids naturally infected with *Eimeria* spp. and treated with toltrazuril. Two doses (20 and 40 mg/kg BW, p. o.) were applied either at two week of age (groups G1-G2; early treatment) (A) or at seven weeks of age (groups G3-G4; late treatment) (B). Untreated control; non-treated animals (group 5). The data are expressed as means ± SEM. (1, 2, 3) $P < 0.05$ - $P < 0.01$ - $P < 0.001$, G1-G4 vs G5; (a) $P < 0.05$, G3 vs G4.

throughout the observation period did not different statistically between the low and high dose groups treated at two weeks of age.

The treatment of 7-week-old goat kids with both doses of toltrazuril significantly reduced faecal oocyst counts one week after treatment when compared to untreated control group 5 ($P < 0.01$) (Fig. 1B). In the following sampling times, the 40 mg/kg dose (group 4) maintained OPG values significantly reduced in week 9 and 10 ($P < 0.01$), while no differences were observed with respect to control group 5 in the remaining weeks of the experiment. By contrast, goat kids treated with 20 mg/kg toltrazuril at 7 weeks of age registered OPG counts similar to non-treated controls in weeks 9, 10, 11, 12 and 13 of the experiment. In addition, when comparing the two treatment doses, faecal oocyst counts were found to be significantly lower in goat kids receiving 40 mg/kg (group 4) from weeks 9 to 11 ($P < 0.05$).

Overall, OPG values showed high individual variations within groups. Individual maximum oocyst excretion was recorded in control group 5 at week seven of the experiment (8.6 $\times 10^6$ OPG) coinciding with mean peak values found in this group. In groups 1 and 2, the individual maximum excretion was recorded in week 5 after treatment (week 7 of life) (1.2 $\times 10^6$ OPG and 2.12 $\times 10^6$ OPG, respectively), also when mean OPG values were higher in these two experimental groups (Fig. 1A). A similar result was detected in groups 3 and 4, where the individual maximum excretion was recorded at mean peak OPG values (Fig. 1B), four weeks after treatment (week 11 of life) (4.1 $\times 10^6$ OPG and 4.1 $\times 10^5$ OPG, respectively). On the days of maximum OPGs, the animals in group 4 had the lowest values.

3.2. Treatment efficacy

The mean oocyst reduction over time in two-week-old (groups 1–2) and in 7-week-old goat kids (groups 3–4) is shown in Table 1. In groups 1 and 2, the mean oocyst reduction was higher than 90% from week 1 to week 5 after treatment (seven weeks of age), thereafter dropping to values ranging from 81.7% to 23.2%. No differences were observed between the efficacy values of the two toltrazuril doses used, except at week 5 post-treatment (p. t.), when the group receiving 40 mg/kg (group 2) had a significantly higher oocyst reduction than group 1 ($P < 0.05$). The administration of 40 mg/kg of toltrazuril in 7-week-old goat kids resulted in oocyst reduction > 85% one and two weeks after treatment. This difference to the control group gradually became smaller towards the end of the observation period (6 weeks after treatment). In the group with the low dose at 7 weeks of age, an oocyst reduction > 60% was only seen in the first week after treatment, whereas the effect in the following weeks was low or absent (Table 1). On this occasion, only at week 2 post-treatment goat kids treated with 40 mg/kg BW (group 4) had a significantly higher oocyst reduction than animals treated with single dose (group 3) ($P < 0.01$).

When treatment efficacy was estimated on the basis of area under the curve (AUC) (Table 1) in early treatment, an oocyst reduction of 74% and 49% were recorded in groups 1 and 2, respectively, without significant differences. By contrast, late treatment resulted in 24% and 65% oocyst reductions when using 20 mg/kg and 40/mg kg, respectively, and the differences between groups were significant ($P < 0.05$). This estimation could constitute an approximation to understand the average oocyst contamination of the environment, which has unquestionable epidemiological implications (Ruiz et al., 2014; Joachim et al., 2018).

3.3. Body weight (BW) and growth rates

Goat kids treated with 20 mg/kg BW p. o. of toltrazuril at two-weeks of age (group 1) showed increased BW from week 4 p. t. until the end of the study when compared to non-treated animals (group 5), with significant differences being recorded at weeks 5, 6, 7 and 8 p. t. ($P < 0.05$ – $P < 0.01$) (Table 2). By contrast, treatment with 40 mg/kg of toltrazuril showed higher body weight than controls only from week 7 p. t. onwards, and significant differences could not be demonstrated. Moreover, significant differences were found between group 1 and 2 at weeks 5, 6, 7 and 8 p. t. ($P < 0.05$ – $P < 0.01$).

Seven-week-old goat kids treated with toltrazuril at 20 mg/kg BW resulted in increased body weight from week 2 p. t. until the end of the experiment ($P < 0.05$ in weeks 2 and 3 p. t.) in comparison to control group. On the other hand, treatment with 40 mg/kg (group 4), only revealed in increased BW in week 2 (eight weeks of age) and 4 p. t. (ten weeks of age), without significant differences.

Accordingly, the overall growth rate at the end of the study was significantly higher in goat kids treated with 20 mg/kg BW at two (group 1) ($P < 0.05$) or seven weeks of age (group 3) ($P < 0.001$), whereas no differences between groups treated with the higher dose and non-treated animals could be demonstrated (Fig. 2A, B).

3.4. Faecal score and soiling

A similar faecal consistency pattern was observed over time in untreated (control group 5) and toltrazuril treated goat kids at two (groups 1–2) or seven weeks of age (groups 3–4) (date not shown). Goat kids treated at week 2 of age (groups 1–2) and control (group 5) had an overall faecal score between one and two, indicating normal to soft unformed faecal consistency, in 95% of the samples analysed from week one to week twelve after treatment. Only one animal had diarrhoea with blood and/or fragments of intestinal mucosa (faecal score = 5) (group 1, two weeks p. t.). Goat kids treated at 7 weeks of age showed a global faecal score of 1–2 in 91% (group 3) and 85% (group 4) of the faecal samples analysed, slightly lower than in the corresponding untreated animals (100%). Semiliquid faeces (faecal score = 3) and watery diarrhoea (faecal score = 4) were observed in 8% and 15% of the samples analysed in group 3 and 4, respectively. Statistical analysis revealed no significant differences between groups in the average faecal score.

Soiling of the anal region was recorded in treated and non-treated kids from the beginning until the end of the experiment (data not shown). From week one to week six after treatment, a mean soiling score of ≤ 2, indicating no soiling (score = 1) or dry soiling (score = 2), was observed in both non-treated and treated goat kids at two weeks of age, while no soiling was detected afterwards. Groups 1, 2 and 5 showed dry soiling in 23.7%, 27.3% and 28.6% of the samples analysed, respectively. On the other hand, most of the kids treated with toltrazuril at 7 weeks of age and the corresponding untreated control group showed no soiling over time. In this case, groups 3, 4 and 5 had dry soiling in 15.7%, 13.5% and 8.7% of the samples analysed, respectively. Overall, no significant differences were recorded between groups at any time of treatment.

No animals died during this study and no adverse effects were reported from treatment with the two different doses of toltrazuril employed (i. e. 20 mg/kg BW and 40 mg/kg BW).

3.5. *Eimeria* species

A total of 10 *Eimeria* species were identified in all groups, at least once, from the beginning to the end of the trial. Overall, the predominant species of coccidia were *Eimeria christenseni* (67.3%), *E. arloingi* (21.9%) and *E. ninakholyakimovae* (5.6%). Minor species included *E. jolchijevi*, *E. hirci*, *E. aspheronica*, *E. caprina*, *E. caprovina*, *E. parva* and *E. pallida*, with specific frequency of 0.3 – 11.4% on different time points of sampling. All these minor *Eimeria* species were named as "other species" and had a global frequency of 5.2% in the experiment (Table 3).

In groups 1 and 2, frequencies of the different *Eimeria* species significantly varied with time ($P < 0.05$ - $P < 0.001$) (Table 3). In both groups, the frequency of *E. christenseni* was high until week 4 p. t. (> 80%) and decreased thereafter to mean values of about 50%. By contrast, the percentage of this *Eimeria* species in the corresponding

Table 1
Eimeria oocyst reduction (%) and Area Under the Curve (AUC)* (%) in goat kids naturally infected with *Eimeria* spp. and treated with two different doses (20 and 40 mg/kg B. W.) of toltrazuril at two- or seven weeks of age (W)* *.

	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	AUC
20 mg / 2 weeks	99.9	90.9	99.9	98.9	97.5	34.7	67.7	75.8	81.7	59.1	56.7	74.4
40 mg / 2 weeks	96.7	91.7	96.6	99.4	99.6[a]	78.8	39.7	78.2	75.4	55.2	23.2	49.2
20 mg / 7 weeks						62.4	23.9	-66.4	35.2	-37.7	94.7	24.5
40 mg / 7 weeks						96.2	86.9[b]	72.8	69.5	25.9	98.3	65.0

(*) Area Under the Curve (AUC) for the 4 treated groups.
(**) Group 1 (20 mg / 2 weeks): Two-week of age, treated with 20 mg/kg B.W. (early treatment – low dose); Group 2 (40 mg / 2 weeks): Two-week of age, treated with 40 mg/kg B.W. (early treatment – high dose); Group 3 (20 mg / 7 weeks): Seven-weeks of age, treated with 20 mg/kg B.W. (late treatment – low dose); Group 4 (40 mg / 7 weeks): Seven-weeks of age, treated with 40 mg/kg B.W. (late treatment – high dose); (a) $P < 0.05$ Group 1 vs Group 2; (b) $P < 0.01$ Group 3 vs Group 4.

Table 2
Body weight in goat kids naturally infected with *Eimeria* spp. and treated with two different doses (20 and 40 mg/kg B.W.) of toltrazuril at two- or seven weeks of age (W), and non-treated control animals* .

	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13
Two-weeks												
20 mg / 2 weeks	3.9 ± 0.1	4.3 ± 0.1	4.7 ± 0.2	5.6 ± 0.2	6.3 ± 0.2	7.1 ± 0.2[1, a]	8.1 ± 0.2[1, a]	9.3 ± 0.3[1, a]	10.6 ± 0.3[1, a]	10.9 ± 0.4	12.1 ± 0.3	12.7 ± 0.4
40 mg / 2 weeks	4.1 ± 0.1	4.3 ± 0.2	4.8 ± 0.1	5.4 ± 0.2	5.9 ± 0.2	6.4 ± 0.2	7.1 ± 0.2	8.3 ± 0.3	9.7 ± 0.3	10.1 ± 0.5	11.0 ± 0.4	11.9 ± 0.5
Control	3.9 ± 0.1	4.3 ± 0.1	4.9 ± 0.1	5.3 ± 0.2	5.9 ± 0.2	6.5 ± 0.2	7.2 ± 0.3	7.8 ± 0.3	8.8 ± 0.4	10.1 ± 0.4	10.8 ± 0.7	11.4 ± 0.6
Seven-weeks												
20 mg / 7 weeks						7.1 ± 0.3	7.8 ± 0.4	8.7 ± 0.4[2]	10.3 ± 0.4[2]	11.0 ± 0.5	11.7 ± 0.4	11.9 ± 0.5
40 mg / 7 weeks						6.9 ± 0.2	7.4 ± 0.3	8.5 ± 0.3	9.1 ± 0.4	10.7 ± 0.4	10.5 ± 0.4	11.1 ± 0.6
Control						6.5 ± 0.2	7.2 ± 0.3	7.8 ± 0.3	8.8 ± 0.4	10.1 ± 0.4	10.8 ± 0.7	11.4 ± 0.6

(*) The data are expressed as means (Kg) ± SEM. Group 1 (20 mg / 2 weeks): Two-week of age, treated with 20 mg/kg B.W. (early treatment – low dose); Group 2 (40 mg / 2 weeks): Two-week of age, treated with 40 mg/kg B.W. (early treatment – high dose); Group 3 (20 mg / 7 weeks): Seven-weeks of age, treated with 20 mg/kg B.W. (late treatment – low dose); Group 4 (40 mg / 7 weeks): Seven-weeks of age, treated with 40 mg/kg B.W. (late treatment – high dose); Group 5: Non-treated control animals. (1) P < 0.05 – P < 0.01 Group 1 vs Group 5 (non-treated animals); (2) P < 0.05 Group 3 vs Group 5; (a) P < 0.05 – P < 0.01 Group 1 vs Group 2.

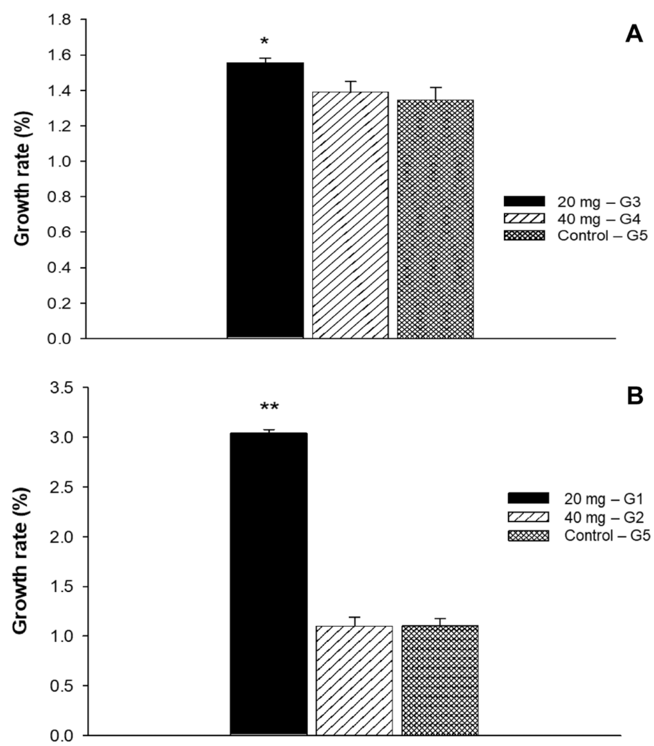

Fig. 2. Growth rates (ln weight 2 −ln weight 1)/t × 100) in goat kids naturally infected with *Eimeria* spp. and treated with two different doses (20 and 40 mg/kg BW, p. o.) of toltrazuril at two weeks of age (groups G1-G2; early treatment) (A) or at seven weeks of age (groups 3–4; late treatment) (B). Global growth rates are represented: days 0 to 84 post-treatment (groups 1 and 2), and days 0–42 post-treatment (groups G3 and G4). Untreated control; non-treated animals (group 5). (*) $P < 0.05$; (**) $P < 0.001$.

untreated control group was more stable during the whole sampling period and had mean values of about 70%. Conversely, frequencies of *E. arloingi* in treated animals were lower than 15% during the first 4 weeks p. t. and then progressively increased, with global mean values of approximately 35%. Compared to treated groups, control animals had relatively high percentages of *E. arloingi* the first two weeks p. t. and lower rates afterwards, around an overall mean value of 15%. Finally, the global frequency of *E. ninakohlyakimovae* was approximately the same in both treated and controls animals, although the untreated control group had higher rates at the beginning of the experiment (Table 3).

Goat kids treated at 7 weeks of age, either with 20 or 40 mg/kg BW of toltrazuril showed approximately the same percentage of *E. christenseni* as control animals, with overall rates of around 80% (Table 3). Global rates did not differ between control and treated groups concerning *E. arloingi* (about 10%). However, in contrast to controls, the frequency of this *Eimeria* species in treated animals was close to zero during the first 3 weeks p. t., showing an increase thereafter. A similar pattern was recorded for *E. ninakohlyakimovae*, but in this case the overall proportion of this *Eimeria* species was around 5% (Table 3). Based on these results, significant differences between the frequencies of the *Eimeria* species monitored during the second phase of the study were also found ($P < 0.05$ - $P < 0.001$).

3.6. Correlation analysis

The analysis of correlation among clinical parameters revealed a strong positive correlation ($P < 0.001$) between faecal score and soiling, while no correlation was observed with other clinical features such as the presence of respiratory signs or contagious ecthyma. On the other hand, both faecal score and soiling were negatively correlated with the BW of the animals ($P < 0.001$ and $P < 0.05$, respectively).

Concerning parasitological parameters, no significant correlations were found between OPG counts and both clinical and productive data. In contrast, Log transformed OPG values were negatively correlated with faecal score ($P < 0.001$), soiling ($P < 0.05$) and the BW of goat kids ($P < 0.001$).

4. Discussion

Anticoccidial compounds aimed to control caprine coccidiosis, a costly and neglected parasitic disease of goat industry, when compared to chicken coccidiosis. Caprine coccidiosis is caused by protozoan parasites of the genus *Eimeria* and controlled by the usage of either ionophores or synthetic compounds such as toltrazuril and diclazuril (Noack et al., 2019). The timing of appropriate anticoccidial treatment depends mainly on the farm history, farm management/hygiene and on the rearing system. Importantly, treatment should be done during the prepatent period to ensure that goat kids will not suffer clinical coccidiosis but should have certain contact to different stages of the parasite in order to generate protective immunity to homologous *Eimeria* re-infections (Matos et al., 2017, 2018).

Based on the prepatent periods of pathogenic *Eimeria* species in

Table 3
Percentage of the *Eimeria* species found in faeces in goat kids (two- or seven-weeks of age) naturally infected with *Eimeria* spp. and treated with toltrazuril (20 or 40 mg/kg B.W., and non-treated control group*.

	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13
20 mg / 2 weeks												
E. christenseni	99	100	100	83	94	25	46	45	48	55	52	61
E. arloingi	0	0	0	6	5	67	36	36	41	21	28	31
E. ninakohlyakimovae	0	0	0	0	1	4	6	7	8	11	5	4
Other spp.	1	0	0	11	0	4	12	11	3	13	15	4
	(0.03 ± 0.03)	(0.04 ± 0.02)	(0.13 ± 0.05)	(0.36 ± 0.2)	(14.2 ± 6.8)	(33.8 ± 11.7)	(96.7 ± 33.8)	(126.0 ± 64.6)	(117.6 ± 67.3)	(54.2 ± 22.2)	(58.1 ± 23.9)	(27.7 ± 10.0)
40 mg / 2 weeks												
E. christenseni	98	92	100	83	90	86	48	18	50	42	57	32
E. arloingi	4	4	0	15	8	11	41	69	26	43	33	49
E. ninakohlyakimovae	0	0	0	3	0	1	7	10	12	8	5	11
Other spp.	2	4	0	0	2	2	4	3	12	7	4	7
	(0.05 ± 0.08)	(1.37 ± 1.14)	(0.12 ± 0.04)	(10.8 ± 11.0)	(0.72 ± 0.35)	(5.6 ± 2.9)	(31.4 ± 10.8)	(235.3 ± 136.3)	(105.7 ± 75.3)	(73.0 ± 25.8)	(63.6 ± 21.0)	(49.1 ± 16.7)
20 mg / 7 weeks												
E. christenseni							70	100	98	95	69	67
E. arloingi							16	0	1	4	20	19
E. ninakohlyakimovae							10	0	0	0	4	6
Other spp.							4	0	0	1	6	7
							(7.8 ± 3.5)	(146.8 ± 72.5)	(369.0 ± 137.6)	(493.7 ± 212.9)	(92.0 ± 32.6)	(89.6 ± 34.9)
20 mg / 7 weeks												
E. christenseni							69	100	100	93	88	75
E. arloingi							15	0	0	0	9	18
E. ninakohlyakimovae							6	0	0	0	1	3
Other spp.							10	0	0	7	1	5
							(2.4 ± 2.2)	(14.7 ± 8.3)	(63.5 ± 28.3)	(80.8 ± 290.0)	(43.3 ± 10.8)	(47.4 ± 17.0)
Control												
E. christenseni	98	97	45	64	72	73	70	89	80	80	86	73
E. arloingi	1	3	48	16	25	16	16	8	10	8	7	18
E. ninakohlyakimovae	0	0	2	14	3	7	9	2	6	2	5	6
Other spp.	1	0	5	5	1	4	5	1	4	9	2	3
	(1.43 ± 0.95)	(41.9 ± 39.7)	(1.5 ± 1.2)	(314.6 ± 192.1)	(131.0 ± 47.7)	(1373.3 ± 509.9)	(148.0 ± 58.1)	(390.2 ± 124.4)	(484.9 ± 222.2)	(296.76 ± 116.1)	(142.0 ± 44.8)	(64.05 ± 13.4)

(*) Other spp. included: E. jolchijevi, E. hirci, E. aspheronica, E. caprina, E. caprovina, E. parva and E. pallida. W: weeks of age. Group 1 (20 mg / 2 weeks): Two-week of age, treated with 20 mg/kg B.W. (early treatment – low dose); Group 2 (40 mg / 2 weeks): Two-week of age, treated with 40 mg/kg B.W. (early treatment – high dose); Group 3 (20 mg / 7 weeks): Seven-weeks of age, treated with 20 mg/kg B.W. (late treatment – low dose); Group 4 (20 mg / 2 weeks): Seven-weeks of age, treated with 40 mg/kg B.W. (late treatment – high dose); Group 5 (control): Non-treated control animals. Mean OPG values ± SEM (x 103) are indicated in parentheses.

sheep (Taylor et al., 1995), most of the studies evaluating the efficacy of toltrazuril against coccidiosis in lambs have been launched as metaphylactic treatment from 10–20 days of life onwards, and usually after turnout (Gjerde and Helle, 1986, 1991; Taylor and Kenny, 1988; Stafford et al., 1994; Platzer et al., 2005; Dittmar et al., 2009; Le Sueur et al., 2009; Mundt et al., 2009; Diaferia et al., 2013; Saratsis et al., 2013; Scala et al., 2014; Pérez-Fonseca et al., 2016; de Souza Rodrigues et al., 2016, 2017). The studies using toltrazuril in goats as metaphylactic treatment against coccidiosis are more limited (Chartier et al., 1992; Šlosárková et al., 1998; Balicka-Ramisz, 1999; Iqbal et al., 2013a, 2013b; Nunes et al., 2015) and, in some cases, the suggested timing of treatment is beyond the critical period for clinically manifest coccidiosis or even not available. In the present study, two different time points were evaluated to investigate the efficacy of toltrazuril in goat rearing systems. Based on our observations of ongoing research (data not shown) indicating that faecal oocyst counts higher than 1×10^5 OPG can be already found in one-month goat kids, a first batch of animals were treated at 15 days of life (prompt treatment) as described for lambs by Le Sueur et al. (2009). The second timing for treatment was established at seven weeks of age, approximately two weeks before the onset of weaning, which is considered the most critical period for coccidiosis outbreaks, and when non-immune goat kids are more susceptible to *Eimeria* infections (Ruiz et al., 2006; Chartier and Paraud, 2012; Matos et al., 2018). The results of the present study confirm the efficacy of toltrazuril in goat kids against caprine coccidiosis given at two time points. Treatment at 2 weeks of life maintained faecal oocyst excretion at zero for five consecutive weeks and only started to increase from week 8 of life. By this anticoccidial treatment, the animals did not experience the peak of oocyst elimination that occurred at week 7 of life in the untreated control animals and, accordingly, the intestinal damage caused by the release of the oocysts would have diminished. Interestingly, subsequent levels of infection in treated goat kids were mild compared to controls, indicating that previous contact with the parasite may have allowed the development of a protective immune response as reported for other ruminant species (Matos et al., 2018). This is important to emphasize because inappropriate timing of treatment (i. e. before animals have had enough contact to the parasite) may result in severe clinical coccidiosis in goat kids, coinciding with the stressful weaning period. In the farm where the experiment was done, weaning usually takes place from two months of life onwards and, apart from the change of feeding, generally involves the allocation of the animals into different pens, all this causing stress and, probably, weakening of the immune system. Accordingly, untreated control animals had another peak of oocyst excretion at week 9–11 of life. This second peak was considerably lower than the previous one, recorded at week 7 of life, indicating that some immunity against *Eimeria* infections had been mounted. Long-lasting effect of toltrazuril (10–11 weeks) has been previously observed in goat kids treated with toltrazuril at different ages (2, 3, 4 and 5 weeks of age) (Nunes et al., 2015) and other host species (Karembe et al., 2021). However, in contrast to our results, OPG counts close to zero were only observed one week after treatment in this study (Nunes et al., 2015). Additionally, here we further probed the efficacy of toltrazuril in goat kids given at week 7 of life in order to prevent weaning-associated coccidiosis, although with differences according to the dose employed as it will be discussed below. The efficacy was lower than at 2-week treatment both in terms of percentage of OPG reduction and persistence of the effect, but comparable with previous data on the use of toltrazuril in older goat kids: 1–3 months old (Iqbal et al., 2013a), 3–6 months old (Awasthi et al., 2022), 4–6 months old (Chartier et al., 1992).

Although, there are limited published pharmacokinetic data evaluating toltrazuril in small ruminants (Stock et al., 2018), a previous study has shown that toltrazuril sulfone is absorbed equally well following a single oral dose in weaning goats as it has been described in cattle (Love et al., 2015). In fact, in pregnant and non-pregnant goats, toltrazuril is well absorbed after a single oral dose (Elazab et al., 2021), similarly to what occurs in pregnant and non-pregnant ewes (Al-Qadri et al., 2020). The pharmacokinetic of antiparasitic drugs, in this case a synthetic anticoccidial compound, it is essential not only to ensure the expected effect but also to avoid the appearance of resistances associated to underdosing (Escudero et al., 1999). Consistently, this factor in regular coccidiosis control was referred as a plausible cause of the first report of *Eimeria* spp. resistance development against toltrazuril reported in sheep herds of Norway (Odden et al., 2017, 2018). To address the underdose issue, two different doses (i. e. 20 or 40 mg/kg BW, p. o.) were assessed in the present study at both treatment times based on a previous study showing that a higher dose rate of toltrazuril should be administrated compared to the dose rate for sheep or cattle (Chartier et al., 1992). As pointed out above, the efficacy of both doses was approximately the same when treatment was performed at 2 weeks of age. By contrast, in therapeutic treatment at week 7 of life important differences were identified on the effectiveness of the two doses. The higher dose was much more effective than the commonly 20 mg/kg BW used for lambs and maintained close to zero the levels of OPG during the first 4 weeks post-treatment. This is in agreement to previous data reported by Chartier et al. (1992), who described an oocyst reduction during 1 week after treatment with 20 mg/kg BW in 4–6 months old goat kids, while the reduction was extended to 3 weeks when using a higher dose. A similar finding was also reported for the other triazine diclazuril (Ruiz et al., 2012), although in this study the differences between the two doses were not so significant. The relatively poor effect of treatment with 20 mg/kg BW in older goat kids found here differs from other studies using the same dose in goat kids of approximately the same age (Iqbal et al., 2013a) or even in older animals (Chartier et al., 1992; Awasthi et al., 2022). Based on the current study, we cannot determine the reason for those differences, except that the pharmacokinetic of the drug might differ depending on the goat breed employed. Although the same hypothesis may be applied to age-dependent effects of toltrazuril observed here, further investigations must be addressed in this direction to prove the inter-assay repeatability of the results.

In this study, moderate significant differences in BW gain were found between untreated goat kids and those metaphylactically treated with toltrazuril. When overall growth rates were evaluated, animals treated either at 2 weeks or 7 weeks after birth with 20 mg/kg BW of toltrazuril were those which had higher growth rates compared to control group. This is in agreement to previous studies showing the efficacy of toltrazuril (20 mg/kg) in body weight gains 1- or 4- weeks after treatment (Iqbal et al., 2013a, b). No benefit on growth performance was found in kids treated with 40 mg/kg BW of toltrazuril as shown before by Chartier et al. (1992). In addition, toltrazuril given at 20 mg/kg BW had better effects on improving BW in 7-week-old treated animals, which is inconsistent to the higher treatment efficacy, in terms of OPG reduction, recorded in goat kids treated at the younger age. In addition to considering that increasing the dose rate may not be only beneficial, the low rate of clinical signs found here during most of the experiment, particularly in control animals, may be the explanation for the lack of correlation between OPG reduction and BW performance when evaluating the dose-dependent effects of toltrazuril. In fact, the percentage of animals with diarrhea and soiling was rather low. As for soiling, the percentage of animals showing this clinical sign was higher in the early phase of the experiment and no differences were found between treated and untreated animals despite differences on OPG counts, so other factors (e.g., feeding disorders or other gastrointestinal infections) could be involved in early gastrointestinal disturbances. In agreement, several studies have hypothesized that the improvement of BW under moderate levels of *Eimeria* infection might have been inconsistent (Yvoré et al., 1985; Chartier et al., 1992). On another hand, variable effect on growth performance have been also shown in lambs according to time of treatment; for instance, a single dose of toltrazuril (20 mg/ kg BW) increased weight gains in lambs after weaning (Saratsis et al., 2013), while no effect was found when treatment was given at 10–14 days (Le Sueur et al., 2009), 11 days, 18 days (Saratsis et al., 2013) or even 25 days of age (de Souza Rodrigues et al., 2017). As previously suggested (Iqbal et al., 2013a, b) older kids may have a better ability to improve weight gains upon treatment with toltrazuril due to the development of a higher protective immunity against *Eimeria* infections (Chartier and Paraud, 2012).

In the present study, to identify the presence of pathogenic *Eimeria* species which may be responsible for clinical coccidiosis, and to evaluate the kinetics of these species after treatment with toltrazuril, *Eimeria* oocysts identification was performed at all sampling time points. Throughout this trial, a total of 10 *Eimeria* species were identified at least once, in each of the five experimental groups. *Eimeria christenseni* and *E. arloingi* were the most frequent species. The strongly pathogenic *E. ninakohlyakimovae* was found in moderate frequency, but still high compared to the remaining species found in the study and named as "other species". These results are in agreement with those reported in previous studies on goat coccidiosis performed in Gran Canaria (Ruiz et al., 2006, 2012), with slight variation in species repertoire when compared to other publications worldwide (Chartier et al., 1992; Balicka-Ramisz, 1999; Young et al., 2011; Chartier and Paraud, 2012; Iqbal et al., 2013b; Gibbons et al., 2016; Keeton and Navarre, 2018).

The kinetics of the three most prevalent *Eimeria* species differed after differed after both early and late treatment. *Eimeria arloingi* and *E. ninakohlyakimovae* had a significantly lower frequency than untreated animals for up to 4 weeks post-treatment. Slight differences were observed depending to the dose of toltrazuril employed, with animals treated with 40 mg/kg dose of toltrazuril at two weeks of age being those which showed a greater delay in the appearance of pathogenic species (around five weeks post-treatment). In contrast, untreated control animals already had moderate frequencies for *E. arlongi* and *E. ninakohlyakimovae* already at week 3 to 5, which could lead to earlier onset of clinical signs of coccidiosis due to the pathogenicity of these *Eimeria* species in goats (Yvoré et al., 1985; Koudela and Boková, 1998).

Eimeria christenseni was the predominant *Eimeria* species in untreated animals throughout the experiment, and only slightly decreased when the frequency of *E. arloingi* and *E. ninakohlyakimovae* increased. In general, the predominance of *E. christenseni,* considered as a species moderate pathogenicity (Taylor and Catchpole, 1994), would explain why clinical signs of coccidiosis were not evident in any of the experimental groups. Conversely, the frequency of *E. christenseni* was particularly high in treated groups during the first weeks after treatment, in agreement to previous studies in goat kids (with no age reference) treated with toltrazuril (20 mg/kg BW) (Balicka-Ramisz, 1999). In this trial, the decrease in the frequency of *E. arloingi*, particularly, and of *E. ninakohlyakimovae* one week after treatment was more evident than that found for *E. christenseni* (Balicka-Ramisz, 1999). As for *E. ninakholyakimovae,* the frequency in oocyst reduction was dose dependent in goat kids of 4–6 months old, being maintained for 7 days (20 mg/kg BW) and between 14 and 21 days (40 mg/kg BW) after treatment when compared with the control (Chartier et al., 1992). No difference was observed between 20 mg/kg BW and 40 mg/kg BW in our study, probably because this trial was conducted with younger goat kids. Overall, data on speciation hint that toltrazuril is more effective against pathogenic species, as suggested elsewhere (Torres et al., 2021), although single-species investigations should be undertaken to ultimately determine this hypothesis.

Based on the results presented here it can be concluded that toltrazuril is highly effective in reducing faecal OPG counts, in particular for pathogenic *Eimeria* species, with the treatment efficacy being more evident in goat kids treated at earlier timing. Although timely treatment could indeed be considered a key factor, a higher dose of toltrazuril (40 mg/kg) seems to be more effective in terms of OPG reductions in animals treated at older ages; however, the corresponding association to growth performance was not clear, probably because clinical signs of coccidiosis were only occasionally recorded during the whole investigation period. This study provides further input for a more rational use of anticoccidials in goats, in this case toltrazuril, which has not yet been registered for use in this host species.

CRediT authorship contribution statement

Guedes Aránzazu C.: Investigation, Formal analysis, Writing - Original Draft. **Conde-Felipe Magnolia:** Investigation, Writing - Original Draft, Visualization. **Barba Emilio:** Investigation, Formal analysis. **Molina José Manuel:** Investigation, Methodology, Writing - Review & Editing. **Muñoz María del Carmen:** Investigation. Ferrer Otilia: Investigation. **Martín Sergio:** Investigation, Resources. **Hermosilla Carlos:** Writing - Review & Editing. **Taubert Anja:** Writing - Review & Editing. **Ruiz Antonio:** Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

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.

Acknowledgments

This work was supported by the Agencia Canaria de Investigación, Innovación y Ciencias de la Información (ProID2017010039). We sincerely thank the participating farmer to give us the permission to conduct the experiment and to the staff for helping in the management of the animals during treatment and sampling.

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