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Evaluation of efficacy of toltrazuril and diclazuril in the control of subclinical eimeriosis in weaned lambs

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ABSTRACT

A field study was conducted to compare the effect of toltrazuril and diclazuril treatment against *Eimeria* infection in 142 naturally infected, weaned lambs aged 45–60 days. Lambs were divided into three groups of 48, 48 and 46 animals and treated with toltrazuril (20 mg/kg b.w.) and diclazuril (1 mg/kg b.w.). The last group of 46 lambs was left untreated as control. Oocyst faecal counts (OPG), *Eimeria* species and faeces consistency where assessed weekly from day 7 to day 63. In comparison with the control group, the OPG reduction on day 7 and 14 in toltrazuril-treated lambs was 99.1% and 97.4%, respectively, and 67% and 58% in lambs treated with diclazuril ($p < 0.005$). On day 21, the percent of OPG reduction was still significant in toltrazuril-treated lambs (76.3%, $p < 0.05$). Treatment with toltrazuril showed a decrease of *Eimeria ahsata* and *E. crandallis* oocysts at the time of maximum efficacy. The same effect was found in the diclazuril-treated group for *E. ahsata* only. No difference was found between the three groups regarding the consistency of the faeces ($p > 0.05$). Body weight gains were determined at the start and the end of the study and showed a significant increase in the toltrazuril-treated lambs ($p < 0.001$). These results suggested a greater efficacy of toltrazuril against subclinical eimeriosis in weaned lambs.

1. Introduction

Sheep eimeriosis is a protozoan enteric infection caused by parasites of the genus *Eimeria*. Several species have been described in sheep, however *Eimeria ovinoidalis*, *E. crandallis*, (Catchpole et al., 1976; Catchpole and Gregory, 1985a,b), *E. ahsata* (Mahrt and Sherrick, 1965) and *E. bakuensis* (Platzer et al., 2005) are considered the most pathogenic. Eimeriosis outbreaks mostly occur in young lambs between 4 and 8 weeks of age (Gregory et al., 1980). The infection has a great economic importance as a consequence of both clinical disease (diarrhoea) and subclinical infection and a decrease of daily weight gain and reduced performances have been reported (Alzieu et al., 1999; Le Sueur et al., 2009).

In Sardinia, eimeriosis affects 63.5% of adult sheep and up to 100% of replacement lambs particularly from January to March. Nevertheless, as most of young lambs are slaughtered at 30–40 days for human consumption, as well as in other Mediterranean regions (Saratsis et al., 2011), currently only 10–15% of female lambs are kept as replacement, weaned and treated against coccidia at 45–60 days of age, usually in February and March, when lambs show clinical symptoms (diarrhoea) or a high number of oocysts

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is found in faeces (Scala et al., 1994, 1999). In fact, an earlier treatment with effective compounds, as toltrazuril and diclazuril is at risk of drug residues in the meat.

The aim of this study was to evaluate the effect of treatment using triazinon compounds on *Eimeria* oocyst excretion, diarrhoea and weight gain in weaned lambs of 45–60 days.

2. Materials and methods

From February to April 2012 a field study was carried out in a semi-extensive farm in southern Sardinia (Italy) that raises almost 800 dairy Sarda breed sheep with a history of subclinical eimeriosis in lambs.

One hundred and forty-two female replacement lambs, ageing 45–60 days and weighting about 16 kg, were included in the study after preliminary faecal examination 7 days before starting. No anticoccidial and/or coccidiostatics drug was previously administered to sheep and no clinical sign consistent with coccidia infection was observed in the animals.

Lambs were divided into 3 groups of 48, 48 and 46 animals. Lambs were ranked per oocyst faecal counts (oocyst per gram: OPG) and for each group of 3 animals with the same OPG values, lambs were assigned randomly to one of the three treatment groups. Forty eight lambs were treated with a single oral dose of 20 mg/kg b.w. of toltrazuril oral suspension, equivalent to 0.4 ml/kg b.w. (Baycox® Bayer Animal Health, Leverkusen, Germany; TOLT group) and 48 lambs were treated with a single oral dose of 1 mg/kg b.w. of diclazuril oral suspension, equivalent to 1 ml/2.5 kg b.w. (Vecoxan® Cilang-Janssen, Beerse, Belgium; DICL group). Group C of 46 lambs was kept untreated, as control. Animals were individually identified and marked with a different colour on their back per group.

Faecal examinations were carried out on faecal samples collected from the rectum of each lamb included in the study on the treatment day (D0) and then weekly for nine follow-ups (D1 – D63: 1–9 weeks from treatment). Each faecal sample was examined by modified McMaster technique ensuring a sensitivity of 50 OPG (MAFF, 1986).

For each sampling and each treatment group, faecal cultures were prepared from a pool of positive samples using 2.5% potassium bichromate and *Eimeria* oocysts were identified by species (Eckert et al., 1995). Due to their similarity, *E. crandallis* and *E. weybridgensis* oocysts were grouped together as described by Rommel (2000). The identification of protozoa was carried out on 100 sporulated oocysts obtained from each culture. The prevalence of *Eimeria* species (number of positive findings for a given species/sum of the positive findings for all species × 100) was assessed, as described previously by Mundt et al. (2009) and Saratsis et al. (2013).

The reduction percent of OPG counts was calculated as:

$$\left[\text{M OPG C day}^{n} - \frac{\text{M T OPG day}^{n}}{\text{M OPG C day}^{n}}\right] \times 100$$

where M, arithmetic mean and n is the time of the parasitological exams; C, control; T, treated.

The clinical status of each animal (large organic functions, the state of the sensory, quality of the fur) was evaluated during sampling until the end of the study. At each faecal sampling, a score based on faeces consistency was assigned for each faecal sample according with the following criteria: (1) normal consistency; (2) soft faeces; (3) liquid; (4) haemorrhagic faeces with mucous and/or fragments of intestinal epithelium and fibrin clots. During the survey, the live weight of lambs at D0 and at D63 was evaluated.

Preliminarily, Kolmogorov–Smirnov test was applied to determine whether data were normally distributed. For data over dispersed, Mann–Whitney *U*-test was used for comparison of OPG values at each study time. Student's *t*-test was used to evaluate the differences in the average body weight gain between the three experimental groups (TOLT, DICL and C) at each study time. Percent of faecal consistency scores in the different groups were compared with Chi-square test. All statistics were carried out using Minitab and Epi Info 7 (v. 12.1).

3. Results

Kolmogorov–Smirnov test showed that data was over dispersed distributed ($p < 0.05$). As the consequence, data in Table 1 were presented as median (minimum–maximum) and for statistical comparison the Mann–Whitney *U*-test was used.

On D0, *Eimeria* oocysts were found in all the faecal samples of lambs included in the study with median values of 35,925 in TOLT group, 23,650 in DICL group and 36,400 C group, respectively. No difference in oocyst output was observed in untreated lambs (C group) until day 21, afterward OPG values decreased significantly ($p < 0.05$) to reach the minimum value on day 63 (median 8150). After 7 days post treatment (PT), oocyst output showed a significant decrease both in TOLT group and DICL group (median 150, and 3400, respectively). At this time, OPG values from TOLT group differed both from C group ($p < 0.05$) and DICL group ($p < 0.05$). On day 14, both TOLT and DICL groups still differed from the control group. The median was 400 in toltrazuril treated lambs ($p < 0.05$ *vs* C group and DICL group) and 11,325 in diclazuril treated lambs ($p < 0.05$ *vs* C group). On day 21, only lambs in TOLT group had OPG counts different from C group ($p < 0.05$). From day 28 until the end of the study (day 63) no difference was found between the two treatment groups and the control group (Table 1). Oocyst output reduction (Fig. 1) was 99.1% on day 7, 97.4% on day 14 and 76.3% on day 21, respectively, in lambs from TOLT group. From day 28 until the end of the study the decrease was very low or ineffective. In lambs from DICL group, the decrease was 64.6% on day 7, 58.5% on day 14 and 12.7% on day 21, respectively. From day 28 until the end of the study the decrease was very low or ineffective (Table 1).

Ten *Eimeria* species were identified in the faecal cultures: *E. ahsata*, *E. crandallis*/*weybridgensis*, *E. ovinoidalis*, *E. bakuensis*, *E. intricata*, *E. faurei*, *E. marsica*, *E. parva*, *E. pallida* and *E. granulosa*. In Tables 2–4 are shown the *Eimeria* species found in each group at the different times of the study. On day 0, the species found with a higher prevalence (>20%) in all groups were *E. bakuensis*, *E. crandallis*/*weybridgensis* and *E. ahsata*, while *E. ovinoidalis* and other species were occasionally identified. *E. crandallis*/*weybridgensis* and *E. bakuensis* remained relatively constant in group C, while *E. ahsata* decreased from day 21. In TOLT group, *E. bakuensis* frequency did no show any difference through the study. *E. ahsata* decrease at 4.08% and 0% on days 14 and 21, respectively, and then increased until 14.7% and 16.35% on days 35 and day 49, respectively. The frequency of *E. crandallis*/*weybridgensis* practically did no changed, but on day 14, when the percent decreased at 6.12. In Group DICL, *E. ahsata* decreased on day 7 (2.7%) and day 14 (2.87%) and from day 28 to the end of the study. *E. ahsata* and *E. bakuensis* were practically constant throughout the study.

No clinical symptom consistent with coccidiosis was found throughout the study in all the lamb groups neither difference in faecal consistency were observed (Table 5).

Mean body weights significantly increased throughout the study in all the groups of lambs ($p > 0.001$). Lambs in TOLT group had a mean body weight gain of 6578.3 ± 1374.6 g on day 63, statistically different from the lambs in control group ($p > 0.05$; Table 6). No differences were found between DICL group and the control group.