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ORIGINAL PAPER

Efficacy of toltrazuril (Baycox® 5% suspension) in natural infections with pathogenic *Eimeria* spp. in housed lambs

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Abstract A field study was conducted to evaluate the effect of a single oral treatment with 20 mg/kg body weight (BW) of toltrazuril (Baycox® 5% suspension)—TOL—in comparison to a single oral treatment with 1 mg/kg BW of diclazuril (Vecoxan® suspension orale, 2.5 mg/ml)—DIC—and an untreated control group (CTRL) on naturally acquired *Eimeria* infections in lambs. On a French sheep farm with a known history of coccidiosis, 75 housed lambs aged 10–14 days were randomised and allocated to one of three groups. During an observation period of 60 days after treatment, clinical (faecal consistency, BW) and parasitological parameters (oocyst excretion) were evaluated. Excretion in the negative control group started 3 days after treatment and peaked on the 31st day with a prevalence of 80%. Animals were predominantly infected with *Eimeria ovinoidalis*. Treatment with toltrazuril, but not with diclazuril, resulted in significantly reduced numbers of excreting animals. The number of excretion days and the average oocyst excretion decreased significantly in both the TOL and the DIC groups compared to the CTRL, with the TOL group showing significantly fewer excretion days and excretion intensities than the DIC group. Changes in the faecal consistency were moderate throughout the study and not significantly different between the groups. Daily weight gains were higher in the TOL group compared to the DIC and CTRL groups which did not differ. This study demonstrates the good efficacy of toltrazuril administered orally to lambs in the prepatent period in subclinical natural *Eimeria* infections in housed lambs.

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Introduction

Eimeria infections are common in sheep worldwide, and coccidiosis frequently occurs after heavy infection of susceptible animals, mostly lambs (Taylor and Catchpole 1994; Taylor 1995). Of the 15 ovine *Eimeria* species that are described, three are considered to be pathogenic, *Eimeria ovinoidalis*, *Eimeria crandallis* and *Eimeria bakuensis* (syn. *Eimeria ovina*) (Pellérdy 1974; Gregory 1990; Lindsay and Todd 1993; Taylor et al. 1995; Kaufmann 1996).

Under field conditions, natural infections with *Eimeria* oocysts usually occur in the first days following birth, and susceptibility increases in the first weeks of life (Gregory and Catchpole 1989). Depending on the species of *Eimeria*, the prepatent period varies from 2 to 3 weeks and is followed by the patent period with oocyst excretion and clinical symptoms. Clinical signs such as diarrhoea (sometimes haemorrhagic), abdominal pain, anorexia and weight loss can frequently be found in infected lambs approximately 3 weeks after birth, and they often precede oocyst shedding (Alzieu et al. 1999). Both clinical and subclinical coccidiosis (without clinical symptoms) affect animal performance, including reduced weight gain and an impaired body and carcass conformation. The risk of developing clinical coccidiosis is particularly high for housed lambs weaned early and kept on straw due to the high density of animals and the favourable conditions for

rapid sporulation and high viability of the oocysts (Berriatua et al. 1994; Yvoré et al. 1980), with lambs born later and being exposed to increasing numbers of oocysts shed by older lambs having a greater risk of developing clinical coccidiosis (Catchpole et al. 1993). Although hygienic measures can in principle reduce infection pressure, they are difficult to maintain once susceptible animals are housed together, especially in deep litter where oocysts rapidly accumulate. Therefore, control with anticoccidial drugs is frequently necessary to prevent clinical outbreaks.

The purpose of the study was to determine the effects of a single oral treatment with toltrazuril (Baycox® 5% suspension) in comparison to a single oral treatment with diclazuril (Vecoxan® suspension orale, 2.5 mg/ml) and an untreated control group on clinical and parasitological parameters in lambs infected naturally with *Eimeria* spp.

Materials and methods

Study design The study was conducted as a randomised, blinded clinical field study conducted on one sheep farm located in the centre of France (Allier) in accordance with "Good Clinical Practice" [VICH GL9 (GCP), step 7 adopted by the Committee for Medicinal Products for Veterinary Use in June 2000] and national animal welfare requirements. The effect of treatment was compared to a negative control group (untreated) and a group treated with a control product. The selected farm had a history of clinical coccidiosis which was confirmed by faecal examination prior to the study (results not shown). In all, 75 lambs, all born within five consecutive days, were included in the study. On the day of treatment, they were aged 10–14 days. Taking into account the natural occurrence of infections within the first days after birth and prepatent periods of 10–20 days for most species (Taylor et al. 1995), this time period was chosen for a metaphylactic treatment. The study started on the day of treatment (study day 0 = SD 0) and ended 60 days thereafter (SD 60). On the day of treatment, 25 lambs each were allocated to the three groups in a randomised fashion based on the date of birth, sex and the number of lambs per ewe. Group TOL was treated orally with toltrazuril, group DIC was treated orally with the control product diclazuril, and group CTRL remained untreated (negative control) to ensure the development of clinical coccidiosis due to natural infections and to evaluate the efficacy of the treatment according to the parameters specified below.

Study animals All animals (breed: Île de France) were born on a commercial farm on five consecutive days in March and identified by individual ear tags. Only healthy animals that had not been previously treated with anticoccidials or corticosteroids were included. From the day of treatment, animals in all three groups were kept with their mothers in one sheepfold under the same housing conditions. The groups consisted of 48 males (16 males in each group) and 27 females (nine females in each group).

Parameters During the study, clinical and parasitological parameters were evaluated. Faecal examinations were carried out every 3–4 days, starting 5 days before treatment until 42 days after treatment (n=14 examination days). The consistency was judged immediately after individual collection according to a faecal score (FS) key ranging from 0 (normal pellets) to 4 (with blood and/or tissue). Diarrhoea was present when the FS was >0. All faecal samples except one were also evaluated for oocyst excretion by McMaster counting and determination of the excretion of oocysts per gram of faeces (OPG) after treatment (n=13 examination days). *Eimeria* species were determined in randomly chosen samples with high OPG values on 3 days (SD 14, n=8; SD 28, n=15; SD 42, n=15) after infection. Body weights (BW) were determined on SD 0, SD 21 and SD 60.

Treatments In groups TOL and DIC, animals were treated on SD 0. Group TOL was treated with toltrazuril (Baycox® 5% suspension; Bayer Animal Health GmbH, Leverkusen, Germany) at a dose of 20 mg/kg BW per os; group DIC was treated with the control product diclazuril (Vecoxan® suspension orale, 2.5 mg/ml; Janssen-Cilag, Beerse, Belgium) at a dose of 1 mg/kg BW per os; group CTRL remained untreated as a negative control.

Statistical analysis The analysis was performed with the validated program Testimate Version 6.4 from IDV Gauting (validation of software, hardware and user according to FDA 21 CFR Part 11) and Report version 6.6 from IDV Gauting.

The tests for superiority were performed one-sided with a significance level of alpha=0.025, one-sided because the test will then prove superiority and not "any difference". All dichotomy data (i.e. yes/no-data) were tested with Fisher's exact test (2×2 tables). The quantitative oocyst excretion throughout the study was simultaneously tested with the multivariate Wilcoxon test using to the Wei–Lachin procedure. Body weight development was tested with the Wilcoxon–Mann–Whitney U test.

Results

At the beginning of the study, group compositions were not statistically different with regard to age on the day of treatment, sex, proportion of singles or twin lambs, results of physical examination or body weight (P>0.05).

Qualitative oocyst excretion The prevalence of oocyst excretion in the untreated control animals increased from SD 3 (4.2%) to SD 31 (86.4%) and remained high (<60%) until the end of the observation period. The DIC group started to excrete oocysts on SD 7 and increased to values comparable to the control on SD 35 with a maximum of 88% on the last day of observation. In the TOL group, excretion began on SD 14 and remained on low levels until SD 28 when it reached 28%. In this group, the prevalences never reached the 40% mark (Fig. 1).

In the CTRL group, all animals excreted oocysts at least once, while in the DIC group and in the TOL group, 92% and 68% of the animals shed parasites, respectively. Similarly, the percentage of excretion days during the whole examination period was 49.5% in the CTRL group, while it was reduced by about half in the DIC group and by three quarters in the TOL group (Table 1).

The statistical evaluation showed that the number of lambs that excreted oocysts at least once during the study period was significantly reduced in the TOL group compared to the CTRL (P=0.0013) and the DIC (P=0.0207) groups, while the difference between the DIC and the CTRL groups was not significant (P=0.1611). The percentage of excretion days was significantly decreased in the DIC and the TOL groups compared to the CTRL (P<0.0001 for both). The animals in the TOL group also had significantly lower percentage of excretion days compared to the DIC group (P=0.0010).

Quantitative oocyst excretion The average oocyst excretion for the duration of the study was 526×10^3 in the CTRL, while it was reduced in the DIC (21.0×10^3) and even more so in the TOL (5.20×10^3) group (Table 1). In parallel to the prevalences, the average oocyst excretion increased to values $>10^5$ OPG in the CTRL group from SD 7 and remained high during the study period. It increased in the DIC group from SD 25 and remained high on most sampling days, while in the TOL group, values were only increased once on SD 35 and otherwise remained low [see Fig. 2, values given as LN(OPG + 1)].

Table 1 Qualitative and quantitative oocyst excretion in the groups treated with diclazuril (DIC), toltrazuril (TOL) or without treatment (CTRL)

Group	Number	Excretion at least once (%)	Average excretion days in % (minimum–maximum)	Mean OPG×10^3 (Maximum OPG×10^3)
CTRL	25	100	49.5 (25.0–83.3)	526.0 (16,074)
DIC	25	92	25.3 (0.0–50.0)	21.0 (1,278)
TOL	25	68	12.8 (0.0–33.3)	5.2 (1,226)

The median OPG increased above zero from SD 21 to the end of the study in the CTRL group and from SD 35 to the end of the study in the DIC group, while it never rose above baseline in the TOL group (details not shown).

The OPG was significantly decreased in the DIC and the TOL groups compared to the CTRL (P<0.0001 for both). The animals in the TOL group also had significantly lower average OPG compared to the DIC group (P<0.0001).

The peak of oocyst shedding was high in the CTRL group between SD 7 and SD 31 (highest mean OPG: 215×10^4). The level of oocyst shedding remained low during that period for the TOL group (0.065×10^4). The diclazuril-treated lambs showed an increasing oocyst excretion from SD 21 and reached a maximum mean OPG of 8.67×10^4 in the first 31 days after treatment.

Eimeria species E. ovinoidalis was the most frequent species of coccidia identified on each evaluation date (75–80% of the samples). Other identified species included *E. crandallis* (27–50%), *Eimeria parva* (38–4%), *Eimeria faurei* (7–75%), *E. ovina* (0–40%), *Eimeria ahsata* (0–33%), *Eimeria granulosa* (0–13%), *Eimeria weybridgensis*

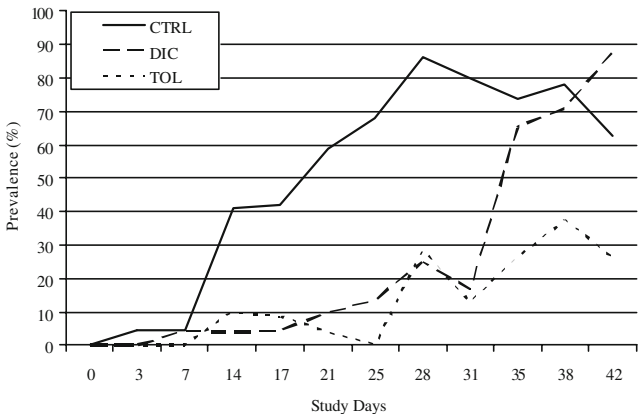

Fig. 1 Excretion prevalences during the study in the untreated control group (*CTRL*), the diclazuril-treated group (*DIC*) and the toltrazuril-treated group (*TOL*)

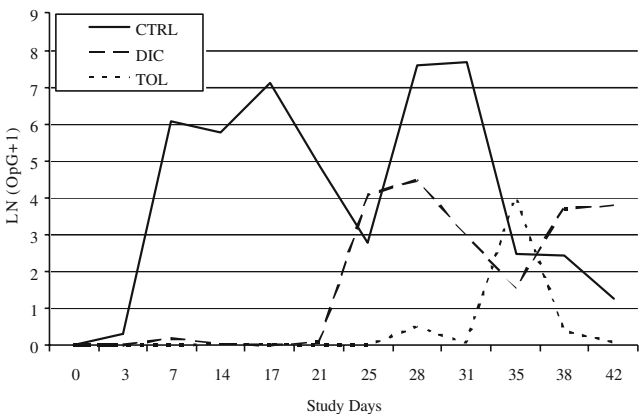

Fig. 2 Average oocysts excretion in the groups treated with diclazuril (*DIC*), toltrazuril (*TOL*) or without treatment (*CTRL*) during the course of sampling, given as LN(OPG + 1)

(0–7%) and *Eimeria pallida* (0–25%). The majority of faecal samples contained several different species (up to six). In cases of monoinfections, *E. ovinoidalis* was the main species identified.

FS and diarrhoea The average FS in all groups was low (average <0.25) until SD 25 when a gradual increase was seen; however, average FS were never higher than 0.4 in any group (Fig. 3). Only six samples had a FS>1 during the whole study period.

The percentage of animals with diarrhoea at least on one sampling day was similar in the different groups with 84% in the CTRL group and 76% in both the TOL and DIC groups. There were no statistical differences between the groups.

The percentage of diarrhoeic samples of all sampling days was 10.0% in the TOL group, 16.2% in the DIC and 14.0% in the CTRL group. The statistical analysis revealed a significant difference between TOL and DIC (P=0.0121). The maximum percentage of diarrhoea days in single animals was comparable in the TOL and CTRL groups (27% and 29% of all sampling days, respectively) and increased in the DIC group (58%). Statistical analysis revealed no significant differences in the average faecal score nor the percentage of animals with diarrhoea (P>0.025) for all cases.

Body weight development

Animals in the TOL group showed a higher average daily weight gain of 306±43.3 g compared to the DIC (277±56.0 g) and CTRL groups (279±61.5 g). The statistical analysis showed no differences between the groups.

Other parameters

None of the 75 lambs died during the study. No suspected adverse drug reaction, local or systemic, was reported for any animal treated with diclazuril or toltrazuril.

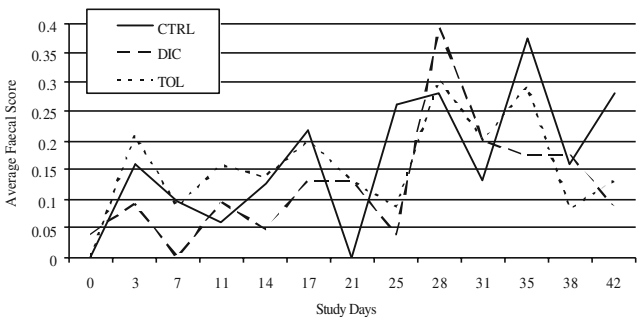

Fig. 3 Average faecal scores in the groups treated with diclazuril (*DIC*), toltrazuril (*TOL*) or without treatment (*CTRL*) during the course of sampling

Discussion

Various studies from Norway and Ireland have previously demonstrated the efficacy of an oral treatment of sheep on pasture with toltrazuril (Gjerde and Helle 1986, 1991; Taylor and Kenny 1988). This trial evaluated the efficacy of toltrazuril administered orally at 20 mg/kg BW in the prepatent period (i.e. lambs aged 12±2 days at the time of treatment) in in-door lambs, which constitutes a new metaphylactic approach to the control of coccidiosis in sheep. In in-door sheep, coccidiosis is known to affect predominantly pre-weaned and recently weaned lambs. Infection occurs frequently in the first few days following birth (Mage 2008) and can lead to oocyst excretion as soon as 2 to 3 weeks later (average duration of the prepatent period), depending on the *Eimeria* species involved. In a contaminated sheepfold, the risk of developing coccidiosis exists throughout the indoor rearing period. Gamonts and to a certain extent the late asexual stages (schizonts II) mainly contribute to the development of clinical coccidiosis in the pathogenic *Eimeria* species of sheep. Therefore, clinical symptoms frequently appear before oocysts can be detected in the faeces. In this context, the possibility to treat metaphylactically in the prepatent period can be expected to limit the incidence of clinical coccidiosis, reduce environmental contamination by the oocysts and improve the zootechnical performance of the animals.

During the study, the first lambs from the untreated control group were excreting oocysts at an age of 15±2 days (SD 3), indicating and confirming early infection after birth.

Less than 2 weeks later, more than one third of the untreated control animals were positive, and at the age of approximately 6 weeks, the prevalence of oocyst excretion reached nearly 90% of those lambs and remained high. This high prevalence indicated that the infection was progressing without significant immunological control of the parasites within the first weeks of life. Despite the massive oocyst excretion by the lambs, it was interesting to observe that the vast majority of them showed limited clinical signs (subclinical coccidiosis). This situation is common on French sheep farms and explains why the incidence of coccidiosis is probably underestimated.

Whereas oocyst shedding peaked between SD 7 and SD 31 for the untreated control group, the level of oocyst shedding remained low during that period for the lambs treated with toltrazuril (maximum mean OPG, 0.065×10^4 vs. 215×10^4 in the CTRL group). In contrast, the diclazuril-treated lambs showed increasing oocyst excretion from SD21 and reached a maximum mean OPG of 8.67×10^4 in the first 31 days after treatment.

This difference in anticoccidial efficacy between toltrazuril and diclazuril is obviously due to the different pharmacodynamic and pharmacokinetic properties. The

spectrum of activity of toltrazuril includes all intracellular stages, which has been demonstrated for *Eimeria* spp. in poultry (Haberkorn and Stoltefuss 1987). By contrast, the spectrum of activity of diclazuril against the various parasitic stages of *Eimeria* spp. of poultry is variable, e.g. diclazuril is not effective against the first schizont stages of *Eimeria maxima* and *Eimeria brunetti* (Maes et al. 1989).

In lambs, treatment-specific effects at five times the recommended dose of diclazuril (5 mg diclazuril/kg BW) in experimental infections with *E. crandallis* were noted particularly with first-generation meronts and to some extent with gamonts (Taylor et al. 2003).

The study showed not only that toltrazuril reduced the level of oocyst shedding (intensity effect) but also that it significantly reduced the average duration of oocyst excretion. Furthermore, the percentage of animals shedding oocysts (extensity effect) was reduced for a considerably longer period than diclazuril. The observations of a rise in oocyst excretion a few weeks after treatment with diclazuril are in line with published experience in lambs and also cattle. Alzieu et al. (1999) carried out studies with diclazuril (Vecoxan®) in the prophylaxis of subclinical coccidiosis in lambs kept indoors in France. Oocyst excretion increased again 14 days after treatment in the group treated once. It remained low until day 28 only in the group treated twice with diclazuril. In studies of the efficacy of diclazuril under field conditions in lambs in Austria, both the prevalence of excretion of oocysts from all species of coccidia and the prevalence of *E. ovinoidalis* were comparable to those in the untreated controls again 4 weeks after treatment (Platzer et al. 2005). Similar observations have been made in the treatment of coccidiosis in calves under practical conditions. Here too, a single treatment with toltrazuril was shown to have a considerably longer lasting effect than a single treatment with diclazuril (Mage et al. 2007; Mundt et al. 2007).

The longer-lasting anticoccidial activity of toltrazuril contributes to the reduction of oocysts in the environment, thus reducing the parasitic pressure and the risk of further heavy contamination within the herd. This is especially important in a confined environment where many oocysts can accumulate in the litter. On the performance level, a positive impact of metaphylactic treatment with toltrazuril was observed on daily weight gain, although low levels of diarrhoea indicated subclinical infections or mild symptoms in most animals.

Repeated infections over an extended period or reinfection must be expected particularly in lambs kept indoors. These infections cannot be avoided by the usual measures (Mundt and Daugschies 2007). In addition to the epidemiological aspect, the animals in a group are generally not all in the same stage of the infection since they are not the same age. Against this background, it is difficult to select the appropriate time point for treatment of a group of lambs in the prepatent period. The question of sustained efficacy of prophylaxis against coccidial infection, or in other words the frequency with which animals require treatment, is of utmost importance, and it is therefore advantageous to be able to confer protection over an extended period through effective drug prophylaxis. This is even more relevant since the protection conferred by immunity evidently develops neither fast nor lastingly.

In summary, a single metaphylactic treatment with toltrazuril significantly reduced environmental contamination with oocysts and improved weight gain in comparison to an untreated control and a matched diclazuril-treated group of animals, demonstrating the beneficial effects of this treatment in subclinical or mild coccidiosis in young lambs.

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