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Control of Coccidiosis due to *Eimeria bovis* and *Eimeria zuernii* in Calves with Toltrazuril under Field Conditions in Comparison with Diclazuril and Untreated Controls

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Abstract

The efficacy of preventive treatment with toltrazuril against natural infections with *Eimeria bovis* and/or *Eimeria zuernii* in calves was investigated in comparison with diclazuril and untreated controls. The study was conducted as a multi-centred, blinded, controlled and randomised field study, with 164 calves at four centres (farms) in northern, eastern and southern Germany. All participating farms had a known history of coccidiosis. Animals were randomised to treatment 14 days after stabling in the respective facility: group I (57 animals) received 15 mg toltrazuril per kg body weight, group II (54 animals) 1 mg diclazuril per kg body weight and group III (53 animals) served as sham-treated controls. The assessment of efficacy was based on oocyst excretion of *E. bovis* and *E. zuernii* (opg) throughout the study (three to four times per week; study duration 57 days).

Coccidiosis due to *E. bovis* and/or *E. zuernii* occurred in all centres. However, the extent and course over time were variable. The duration and rate of oocyst excretion of *E. bovis* or *E. zuernii* were significantly lower in the toltrazuril-treated group compared to groups II and III. It is concluded that a single prophylactic treatment with toltrazuril on day 14 after stabling provides effective and sustainable control of stable coccidiosis under various field conditions.

Introduction

Bovine coccidiosis is an important disease occuring mainly in worldwide calf-rearing and often induces massive diarrhoea (Ernst and Benz 1986). Infections, especially with *Eimeria bovis* and *Eimeria zuernii*, which are the main coccidial pathogens in housed cattle (Marshall *et al.* 1998, Daugschies and Najdrowski 2005), may lead to considerable intestinal damage and subsequent economic losses (Fitzgerald 1980). Decreased weight gain, exhaustion and frequent secondary bacterial infections typically occur as secondary complications in severely affected calves (Ernst and Benz 1986). Effective treatment of infection with pathogenic *Eimeria* spp. is therefore required. Unfortunately, therapeutic treatment of the disease must generally be considered as insufficient due to the intestinal lesions caused by early stages of the parasites (Daugschies and Najdrowski 2005), which will result in diarrhoea. Thus, the treatment of choice has to be a preventive treatment before onset of clinical disease and oocyst excretion.

In several previous investigations, the preventive anticoccidial effect of toltrazuril was demonstrated against infections with both *E. bovis* (Mundt *et al.* 2003, Mundt *et al.* 2005a) and *E. zuernii* (Staschen 2004, Mundt *et al.* 2005a, Mundt *et al.* 2005b) in calves.

The intention of the present study was to investigate the efficacy of preventive treatment with toltrazuril against natural infections with pathogenic *Eimeria* spp. in calves, compared with diclazuril and sham-treated controls. Treatment was administered two weeks after stabling based on the assumption that calves were exposed to infection from the beginning of the stabling period.

Material and methods

The study was designed as a controlled, randomised, blinded, multi-centred field study according to the standards of Good Clinical Practice. It was conducted at four study sites (centres I–IV) located in different regions of Germany. The timing of the trial overlapped partly in the study centres. The duration of each study cycle was 57 days [study day (SD) 0 to SD 56]. SD 0 was the day of stabling. Data on the individual study centres, feeding and housing conditions are given in Tab. 1. A total of 164 calves, including 52 dairy heifer calves (Holstein Friesian) and 112 bull calves (Holstein Friesian, Red Pied Friesian, Angler, Simmental and [Hereford] Mix) were included in the study. Between 24 and 49 animals were included in each cycle and these were divided into three treatment groups (I. toltrazuril; II. diclazuril; III. sham-treated controls) containing the same number of animals each (±1–2 if the number of animals was uneven).

The calves were aged between nine and 80 days at the beginning of the trial. They were transferred to pens on SD 0 in which coccidiosis had been reported in animals previously reared there. The health status of all the animals was good to acceptable at the time of stabling. In centres I and II the calves were bought from commercial breeders. In centres III and IV the calves were born and kept on site prior to the study, although in other areas. In both cases the calves were moved to the contaminated part of the respective farms on SD 0 and housed. On SD 14 the animals were weighed and assigned to treatment groups in increasing order of body weight using a randomisation list. They received the following treatment once by the oral route:

I. 15 mg toltrazuril per kg body weight;
II. 1 mg diclazuril per kg body weight;
III. sham-treatment with water.

Study centre	Group	No. of calves	No. of calves in total	Housing conditions and feeding
C I (Bavaria)	Toltrazuril Diclazuril untreated control	8 8 8	24	Loose housing with deep bedding; milk replacer twice a day, corn silage and concentrates
C II (Schleswig-Holstein)	Toltrazuril Diclazuril untreated control	14 14 14	42	Loose housing with deep bedding, feeding area with slatted floor; grass silage, wheat, corn silage, automatic feeder for milk replacer
C III (Saxony)	Toltrazuril Diclazuril untreated control	17 16 16	49	Loose housing with straw bedding; automatic feeder for milk replacer; concentrates twice a day, corn silage, hay *ad libitum*
C IV (Brandenburg)	Toltrazuril Diclazuril untreated control	18 16 15	49	Loose housing with straw bedding; automatic feeder for milk replacer, concentrates twice a day, hay *ad libitum*

Tab. 1 Experimental design

The medications used were Baycox®, Bayer AG, Leverkusen, Germany (5% toltrazuril suspension) and Vecoxan®, Janssen Pharmaceuticals, (0.25% diclazuril suspension). All animals were subjected to daily health checks and the assessment of body weight throughout the study period. The study period for each cycle ended six weeks after treatment.

Rectal faecal samples were obtained by stimulating the anus, faecal consistency was scored (0 = normal to pasty; 1 = semi-liquid; 2 = liquid to watery; 3 = watery with blood and/or tissue) and samples were sent to the laboratory for parasitological analysis. Oocyst excretion was determined quantitatively three to four times per week using the McMaster method (Thienpont *et al.* 1990), modified by using 4 g of fresh faeces per 60 ml of saturated NaCl solution and mixing the suspension with a magnetic stirrer for about two minutes at the highest setting before transferring it to the McMaster slide. The quantitative results obtained were documented as absolute opg (oocysts per gram faeces) values. The faecal oocysts counts were documented separately for *E. bovis*, *E. zuernii* and for the sum of all *Eimeria* spp. oocysts counted.

Statistical methods

The statistical analyses were performed as efficacy analyses for the investigational parameter of oocyst excretion. The whole study duration was divided into three time periods for evaluation purposes. The first period comprised SD 0 to SD 14, and as the pretreatment period was excluded from any efficacy analyses, the remaining 42 study days were divided into two spans of 21 study days each (SD 15–35; SD 36–56), corresponding to the proposed lifecycles of the pathogenic *Eimeria* species investigated. With respect to the extent of oocyst excretion (primary criterion: days with opg > 0), efficacy was measured by a cascade of Wilcoxon-Mann-Whitney one-sided tests of superiority or non-inferiority. The outline of the statistical hypothesis cascade was as follows:

1. Treatment to end of study – toltrazuril vs. untreated control – test of superiority
2. Treatment to end of study – toltrazuril vs. diclazuril – test of non-inferiority
3. Treatment to SD 35 – toltrazuril vs. untreated control – test of superiority
4. Treatment to SD 35 – toltrazuril vs. diclazuril – test of non-inferiority
5. SD 35 to end of study – toltrazuril vs. untreated control – test of superiority
6. SD 35 to end of study – toltrazuril vs. diclazuril – test of non-inferiority
7. Treatment to end of study – toltrazuril vs. diclazuril – test of superiority
8. Treatment to SD 35 – toltrazuril vs. diclazuril – test of superiority
9. SD 35 to end of study – toltrazuril vs. diclazuril – test of superiority

The hypothesis 1–9 were first tested for *E. bovis* and *E. zuernii* together, then for *E. bovis* and lastly, for *E. zuernii*. Consequently, the a priori ordered hypothesis scheme contained 27 single hypotheses. Non-inferiority was defined as a MW measure of 0.36, superiority as 0.5. The experiment-wise multiple-level alpha was defined as alpha = 0.025 one-sided (as required for confirmatory studies). The primary hypothesis tests were performed according to the principle of a priori ordered hypotheses (Maurer *et al.* 1995).

The body weights and faecal scores were also analysed with the Wilcoxon-Mann-Whitney-U Test in a descriptive manner.

The confirmatory analysis was performed using the ITT population (intention to treat).

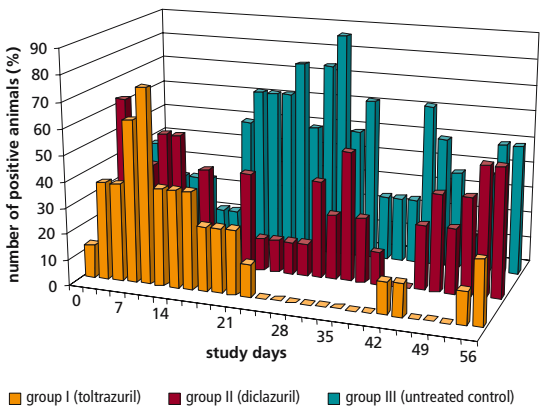
Results

Coccidial infections caused by *E. bovis* and/or *E. zuernii* developed in all study cycles. However, the extent of the infection and its course over time varied between the centres.

Diarrhoea occurred at all centres and in all groups; there were no significant differences.

At centres I–III, where the coccidial infection developed as expected, faecal scores >1 (diarrhoea) were recorded during the study period after treatment (SD 15–56) in 33.3% (group I), 47.4% (group II) and 44.7% (group III) of the animals (Tab. 2). The figures if centre IV is included (where oocysts were excreted at an unexpectedly late stage) are 38.6%, 41.5% and 38.9%. Haemorrhagic diarrhoea (faecal score 3) was not observed. At centre II, rotaviruses and *E. coli* were identified as a cause of diarrhoea. Intercurrent disorders, primarily respiratory infections, occurred in all groups during the study, particularly at centre III. There were no differences in body weight development (data not shown).

The average extent of *E. bovis* and *E. zuernii* oocyst excretion for centres I–IV is shown in Fig. 1 to 5. The oocyst excretion developed as expected at centres I–III; the calves in the control group started to excrete oocysts from about three weeks after



% of positive animals centre I

study day	0	2	4	7	9	11	14	16	18	20	22	24	26
group I	12.5	37.5	37.5	62.5	75.0	37.5	37.5	37.5	25.0	25.0	25.0	12.5	0.0
group II	62.5	50.0	37.5	50.0	50.0	12.5	37.5	0.0	0.0	37.5	12.5	12.5	12.5
group III	37.5	37.5	25.0	25.0	25.0	12.5	12.5	50.0	62.5	62.5	62.5	75.0	50.0

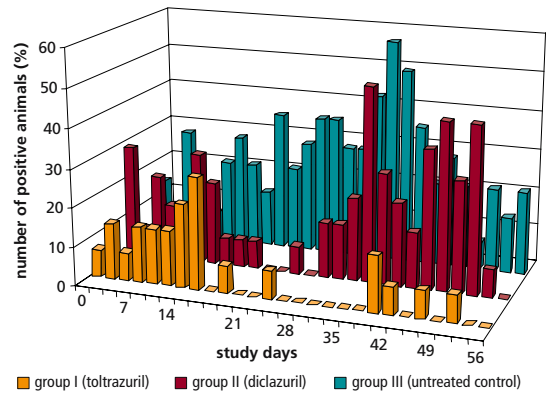
(cont.)

study day	28	30	32	34	35	37	39	42	44	46	49	51	53	56
group I	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.5	12.5	0.0	0.0	0.0	12.5	25.0
group II	12.5	37.5	25.0	50.0	25.0	12.5	0.0	0.0	25.0	37.5	25.0	37.5	50.0	50.0
group III	75.0	87.5	50.0	62.5	25.0	25.0	25.0	62.5	50.0	37.5	12.5	37.5	50.0	50.0

Fig. 1 Extensity (%) of *E. bovis* and *E. zuernii oocyst* excretion (centre I)

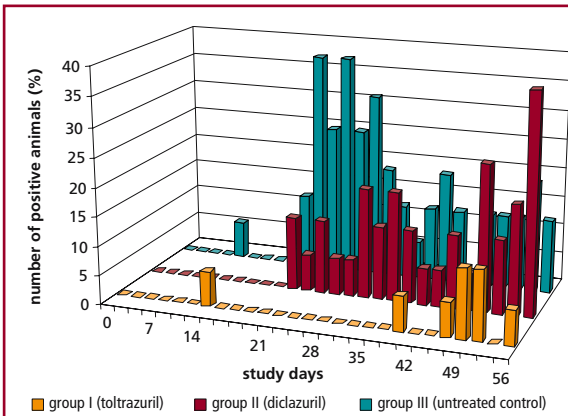
Group I (toltrazuril; n = 39)			
Days	**SD 15–35**	**SD 36–56**	**SD 15–56**
0	69.2	89.7	66.7
1	10.3	7.7	7.7
2	10.3	2.6	12.8
3	7.7	0	7.7
4	2.6	0	5.1
5	0	0	0
6	0	0	0
Group II (diclazuril; n = 38)			
Days	**SD 15–35**	**SD 36–56**	**SD 15–56**
0	57.9	81.6	52.6
1	15.8	10.5	18.4
2	13.2	7.9	10.5
3	7.9	0	5.3
4	2.6	0	7.9
5	2.6	0	2.6
6	0	0	2.6
Group III (untreated control; n = 38)			
Days	**SD 15–35**	**SD 36–56**	**SD 15–56**
0	57.9	92.1	55.3
1	13.2	7.9	15.8
2	15.8	0	10.5
3	2.6	0	7.9
4	2.6	0	2.6
5	5.3	0	5.3
6	2.6	0	2.6

Tab. 2 Number of days with faecal score > 1 (frequency count, %; centres I–III)



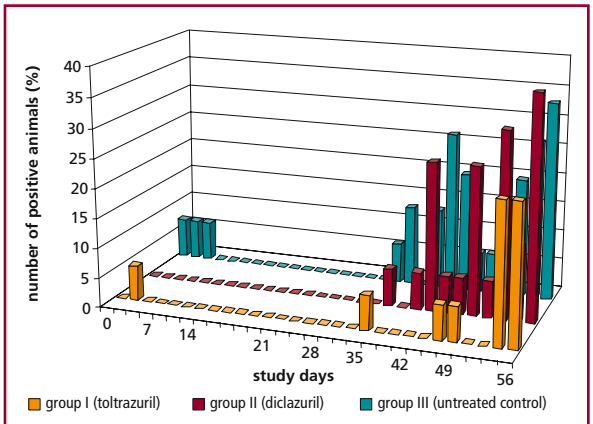
% of positive animals centre II														
study day	0	2	4	7	9	11	14	16	18	20	22	24	26	
group I	7.1	14.3	7.1	14.3	14.3	14.3	21.4	28.6	0.0	7.1	0.0	0.0	7.1	
group II	28.6	7.1	21.4	14.3	14.3	28.6	21.4	7.1	7.1	7.1	0.0	0.0	7.1	
group III	14.3	0.0	28.6	14.3	7.1	21.4	28.6	21.4	14.3	35.7	21.4	28.6	35.7	
(cont.)														
study day	28	30	32	34	35	37	39	42	44	46	49	51	53	56
group I	0.0	0.0	0.0	0.0	0.0	0.0	14.3	7.1	0.0	7.1	0.0	7.1	0.0	0.0
group II	0.0	14.3	14.3	21.4	50.0	28.6	21.4	14.3	35.7	42.9	28.6	42.9	7.1	0.0
group III	35.7	28.6	28.6	42.9	57.1	50.0	35.7	21.4	28.6	21.4	7.1	21.4	14.3	21.4

Fig. 2 Extensity (%) of *E. bovis* and *E. zuernii oocyst* excretion (centre II)



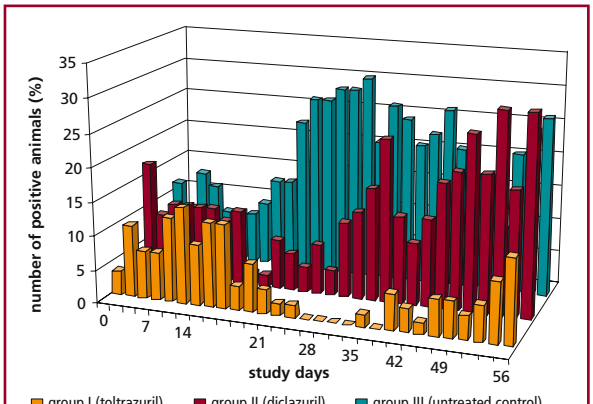
% of positive animals centre III														
study day	0	2	4	7	9	11	14	16	18	20	22	24	26	
group I	0.0	0.0	0.0	0.0	0.0	0.0	5.9	0.0	0.0	0.0	0.0	0.0	0.0	
group II	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.5	6.3	12.5	
group III	0.0	0.0	0.0	0.0	6.3	0.0	0.0	0.0	0.0	12.5	37.5	25.0	37.5	
(cont.)														
study day	28	30	32	34	35	37	39	42	44	46	49	51	53	56
group I	0.0	0.0	0.0	0.0	0.0	0.0	5.9	0.0	0.0	5.9	11.8	11.8	0.0	5.9
group II	6.3	6.3	18.8	12.5	18.8	12.5	6.3	6.3	12.5	6.3	25.0	12.5	18.8	37.5
group III	25.0	31.3	18.8	12.5	6.3	12.5	18.8	12.5	0.0	12.5	12.5	12.5	18.8	12.5

Fig. 3 Extensity (%) of *E. bovis* and *E. zuernii oocyst* excretion (centre III)



% of positive animals centre IV														
study day	0	2	4	6	8	10	12	14	16	18	20	22	24	26
group I	0.0	5.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
group II	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
group III	6.7	6.7	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
(cont.)														
study day	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56
group I	0.0	0.0	0.0	0.0	5.9	0.0	0.0	0.0	0.0	5.9	5.9	0.0	0.0	23.5	23.5
group II	0.0	0.0	0.0	0.0	6.3	0.0	6.3	25.0	6.3	6.3	25.0	6.3	31.3	12.5	37.5
group III	0.0	0.0	0.0	6.7	13.3	0.0	13.3	26.7	20.0	6.7	6.7	6.7	20.0	26.7	33.3

Fig. 4 Extensity (%) of *E. bovis* and *E. zuernii oocyst* excretion (centre IV)



% of positive animals centres I–IV														
study day	0	2	4	6	8	10	12	14	16	18	20	22	24	26
group I	3.6	10.7	7.1	7.1	12.5	14.3	8.9	12.5	12.5	3.6	7.1	3.6	1.8	1.8
group II	17.0	9.3	11.1	11.1	11.1	11.1	9.4	11.1	1.9	1.9	7.4	5.6	3.7	7.4
group II	11.3	7.5	13.2	11.3	7.5	7.5	7.5	9.4	13.2	13.2	22.6	26.4	26.4	28.3
(cont.)														
study day	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56
group I	0.0	0.0	0.0	0.0	1.8	0.0	5.4	3.6	1.8	5.4	5.4	3.6	5.4	8.9	12.5
group II	3.7	11.1	13.0	16.7	24.1	13.0	9.3	13.0	18.5	20.4	25.9	20.4	29.6	18.5	29.6
group III	28.3	30.2	20.8	26.4	24.5	20.8	22.6	26.4	20.8	17.0	17.0	9.4	20.8	24.5	26.4

Fig. 5 Extensity (%) of *E. bovis* and *E. zuernii oocyst* excretion (centres I–IV)

stabling. In addition, animals at centres I and II were already excreting in the first three weeks. The calves at centre IV did not start to excrete oocysts until about five weeks after stabling. The extent of *E. bovis* and *E. zuernii* excretion post treatment was up to 28.6% in group I (toltrazuril), up to 50% in group II (diclazuril) and up to 87.5% in group III (untreated control). Fig. 5 shows that the average

Toltrazuril vs. untreated control				
Period	***Eimeria species***	**Centres I–III**	**Centre IV**	**Centres I–IV**
Day 15–35	*E. bovis*	**	n.s.	**
	E. zuernii	**	**	**
	E. bovis + *E. zuernii*	**	n.s.	**
Day 36–56	*E. bovis*	**	**	**
	E. zuernii	**	n.s.	**
	E. bovis + *E. zuernii*	**	*	**
Day 15–56	*E. bovis*	**	**	**
	E. zuernii	**	n.s.	**
	E. bovis + *E. zuernii*	**	**	**
Toltrazuril vs. Diclazuril				
Period	***Eimeria species***	**Centres I–III**	**Centre IV**	**Centres I–IV**
Day 15–35	*E. bovis*	**	**	**
	E. zuernii	*	**	n.s.
	E. bovis + *E. zuernii*	**	**	**
Day 36–56	*E. bovis*	*	**	**
	E. zuernii	**	n.s.	**
	E. bovis + *E. zuernii*	**	n.s.	**
Day 15–56	*E. bovis*	**	**	**
	E. zuernii	**	n.s.	**
	E. bovis + *E. zuernii*	**	n.s.	**

Tab. 3 Statistical evaluation of the extend of oocyst excretion: p-values (Wilcoxon-Mann-Whitney U test, one sided)
** P < 0.010; * P < 0.025; n.s. not significant

extensity of pathogenic oocyst excretion after treatment in all centres varied from 0 to 12.5%, in group I (toltrazuril), from 1.9 to 29.6% in group II (diclazuril) and from 9.4 to 30.2% in group III (untreated control). The individual *Eimeria* spp. showed comparable behaviour with respect both to the course over time and to inter-group differences (see statistical evaluation, Tab. 3).

For the statistical analysis of oocyst excretion post treatment, the study period after treatment (SD 14) was divided into two periods of 21 days each, in accordance with the proposed life cycle of the pathogens (SD 15–35; SD 36–56; see statistical methods). The results of 54 statistical calculations are shown in Tab. 3. For centres I–III the statistical calculation shows significant superiority for both study periods (SD 15–35 and SD 36–56) and also for the total study duration post treatment between group I (toltrazuril) and group III (untreated control), as well as group II (diclazuril). The differences in centre IV were less distinct (unexpectedly late development of oocyst excretion). The data underlying the statistical evaluation of both pathogenic *Eimeria* spp. over the whole post treatment period (centres I–IV) are presented graphically in Fig. 6, too.

The intensity of *E. bovis* and *E. zuernii* oocyst excretion after treatment ranged from a minimum of 0 to a maximum of 4,450 opg, with an arithmetic mean of 13.1 in group I (toltrazuril), from 0 to 7,300 opg with a mean of 58.5 in group II (diclazuril), and from 0 to 45,500 opg with a mean of 318 in group III (untreated control) .

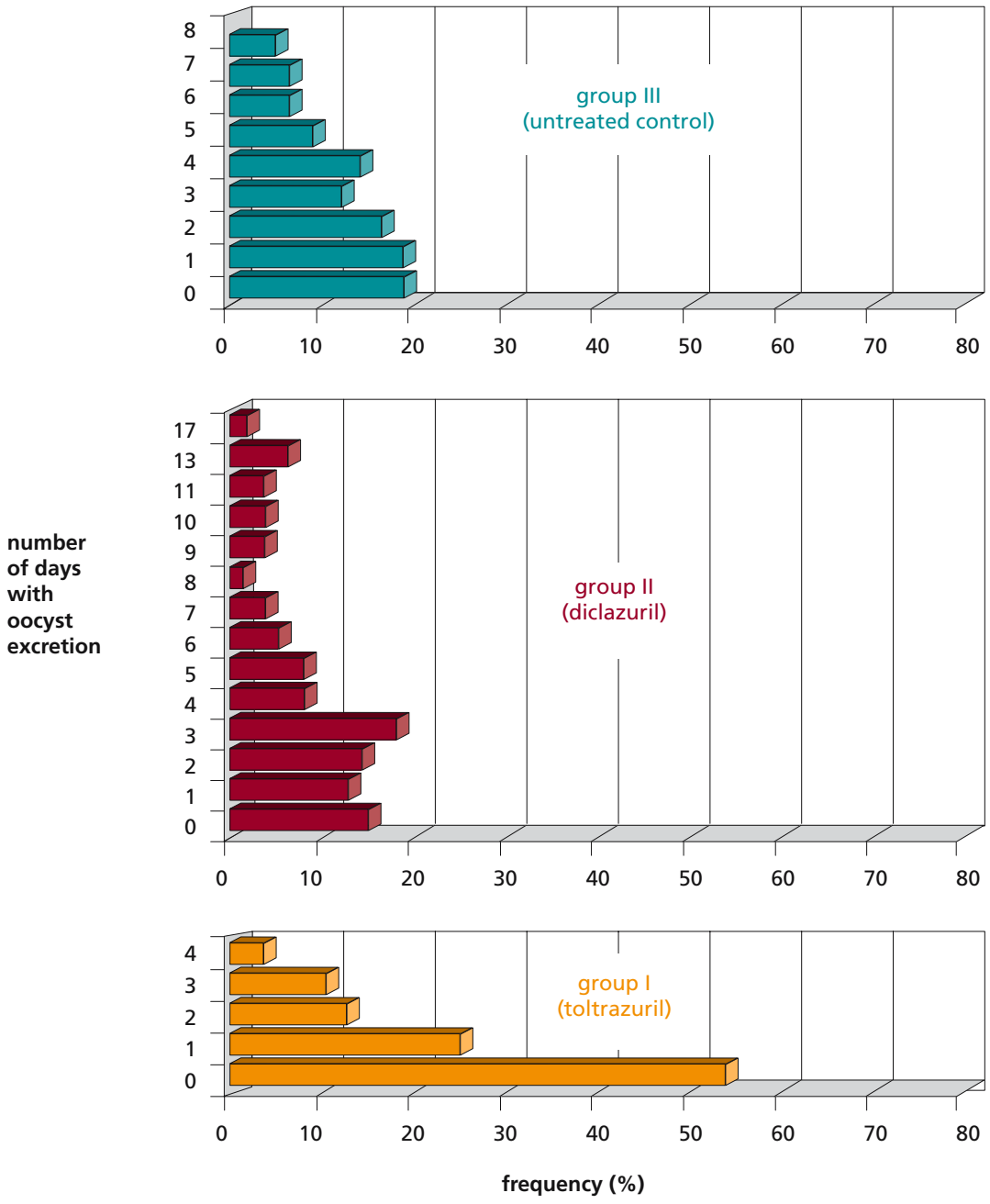


Fig. 6 Average number of days with *E. bovis* and *E. zuernii* oocyst excretion (frequency, %) post treatment (SD 15–56) in centres I–IV

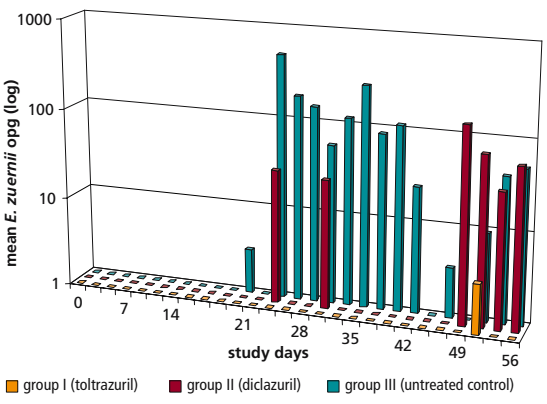


Fig. 7 Mean *E. zuernii oocyst* excretion centre III

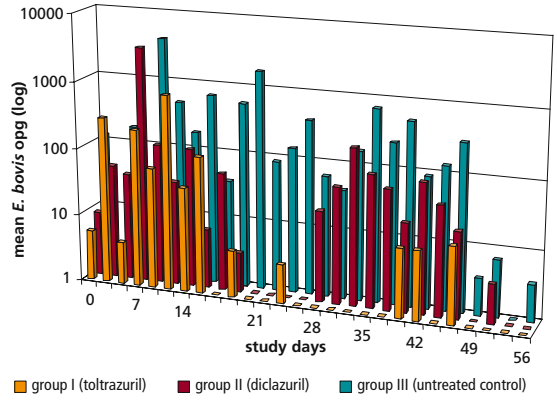


Fig. 9 Mean *E. bovis oocyst* excretion centre II

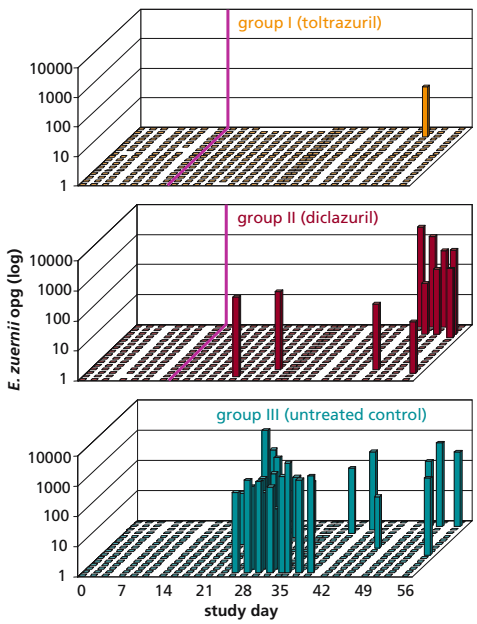


Fig. 8 Individual *E. zuernii oocyst* excretion centre III

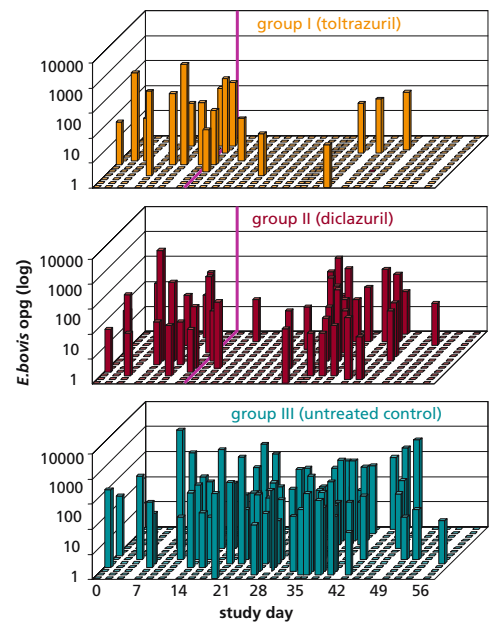


Fig. 10 Individual *E. bovis oocyst* excretion centre II

The following examples of excretion of *E. bovis* and *E. zuernii* oocysts illustrate the results obtained in the course of the study and the distribution between the groups. Centre III serves as an example of coccidial infections when animals are exposed typically from the time of stabling. Oocyst excretion is observed from about three weeks after stabling onward. Fig. 7 shows the mean *E. zuernii* oocyst excretion, Fig. 8 shows the corresponding excretion of the individual animals within the three groups. The un-

treated control group excreted oocysts in a typical time of about three weeks after stabling. The calves in the diclazuril group shed considerably fewer oocysts than the untreated controls. Excretion increased towards the end of the study. There was no oocyst excretion in the toltrazuril group at any time during the study period, except for one instance of individual oocyst excretion (study day 51; 50 *E. zuernii* opg).

Centre II serves as an example of oocyst excretion already during the first weeks after stabling. Animals at centre II were already infected when they were stabled. Fig. 9 and 10 show the *E. bovis* excretion in the three groups (mean and individual data). The untreated calves shed oocysts over more or less the whole study period, whereas the animals in group II (diclazuril) showed little excretion for about two weeks after treatment. Most of the calves started shedding oocysts again thereafter. In group I (toltrazuril) there was no observable oocyst excretion throughout the study period except in five animals, which shed small amounts of *E. bovis* oocysts (SD 20, one animal, 50 opg; SD 26, one animal, 50 opg; SD 39 and 40, one animal, 100 and 150 opg respectively; SD 46, one animal, 200 opg).

Discussion

Bovine coccidiosis is a widespread infection (Ernst and Benz 1986, Marshall *et al.* 1998) which can cause sub-clinical to acute diarrhoea (Ernst and Benz 1986). The pathogens involved in stable coccidiosis are *E. bovis* and *E. zuernii* (Daugschies and Najdrowski 2005). The present study investigated the anticoccidial efficacy of toltrazuril, in comparison with diclazuril as a drug control. Both drugs were used preventively (14 days after the animals had been housed and thus 14 days after the assumed time of infection) since the nature of the disease – which produces early intestinal lesions during the pre-patent period (Mundt *et al.* 2003, Mundt *et al.* 2005b, Stockdale 1977) – suggests that this approach is likely to be the most successful (Mundt *et al.* 2003, Staschen 2004). A sham-treated control group was included at each centre so that the development of coccidiosis in each herd could be observed. Prior investigations had shown that coccidiosis was present on all the farms included.

A multi-centre approach was chosen so that the results of the study would be applicable to various field conditions, such as variable pressure of infection and different forms of husbandry and management. Coccidiosis developed in the untreated groups at all four centres during the study, manifesting primarily in the form of considerable excretion of *E. bovis* and *E. zuernii* oocysts. There was no clear correlation between diarrhoea and coccidiosis, or the distinction cannot be made clearly, since the consistency of the faeces is affected by other factors as well (e.g. at centre II). On the basis of oocyst excretion (extent and intensity), the occurrence and intensity of diarrhoea and other causes of diarrhoea, the coccidial infection observed in this study can be regarded to be sub-clinical.

There were no distinct differences between the groups in terms of body weight development. Differences could not be expected in view of the tendentially sub-clinical coccidiosis and the fact that other factors had a notable influence on the development of the calves (e.g. centre II: diarrhoea; centre III: respiratory diseases). In view of the high variability and the small size of the groups, it is thus not possible to infer any conclusions about the effect of sub-clinical coccidiosis on body weight development from this study.

However, it is known that sub-clinical coccidiosis can also lead to financial losses in calves, mainly due to lower weight gain (Fitzgerald 1980, Daugschies *et al.* 2005). The success of a treatment can be determined by oocyst excretion, i.e. the presence

or absence of *E. bovis* and *E. zuernii* oocysts. Oocyst excretion is the most sensitive parameter for evaluating coccidial infection. The goal of a treatment is thus the suppression of oocyst excretion as great as possible. In this context, if oocysts can be detected in the faeces, the extent of excretion is less important in terms of evaluating the suppression of coccidiosis by drug products because complete elimination of oocyst excretion, in particular, minimises the infectious pressure on calves subsequently housed in the same pens und thus controls coccidiosis in the longer term (Mundt *et al.* 2005a).

It was demonstrated clearly in all centres that treatment with toltrazuril was more effective in controlling oocyst excretion than treatment with diclazuril. In all the centres individually, and in the evaluation of all centres, it emerged that the level of oocyst excretion was lowest in the group treated with toltrazuril, i.e. fewer samples of faeces positive for *E. bovis* and/or *E. zuernii* were found.

In addition to the evaluation carried out for the full period following treatment, the efficacy of treatment with toltrazuril or diclazuril was also evaluated for two three-week periods following treatment. Assuming a prepatent period of 22 to 23 days for *E. bovis* (Daugschies *et al.* 1986) and 16 to 17 days for *E. zuernii* (Ernst and Benz 1986, Stockdale and Niilo 1976), each animal in each pen could theoretically have gone through two complete cycles of infection during the time between stabling and the last day of the study (56 days after stabling). To establish the durability of the treatment, the animals were, therefore, also evaluated separately for the first three weeks following treatment (up to day 35 after stabling), which roughly corresponds to the first cycle of infection, and the following three-week period up to the end of the study, which corresponds to the second cycle.

It was shown that coccidiosis was prevented more durably with toltrazuril than with diclazuril; toltrazuril also had a superior initial action. The number of days on which excretion was observed to take place was significantly lower in the toltrazuril group than in either the diclazuril group or the sham-treated group during both assumed cycles of infection. This enables longer-lasting protection in the form of treatment with toltrazuril to be given to animals in herds affected by coccidiosis. Since exposure of the calves to pathogenic species of *Eimeria* evidently persists for several weeks after they have been housed, as the course of the infection in the untreated control groups shows, this protection is an important factor in the long-term control of coccidiosis in calves. The multiplication of pathogenic *Eimeria* spp. in a stable should be limited as far as possible, even if clinical coccidiosis is not present. This can be achieved by an effective and long-lasting control with toltrazuril. Furthermore, good herd management as well as hygiene contributes to this.

The present study showed that a single preventive treatment of 15 mg toltrazuril per kg body weight achieved effective control of infection caused by *E. bovis* and *E. zuernii*. This treatment considerably reduced oocyst excretion. The suppression of oocyst excretion achieved by toltrazuril was significantly more effective and durable than that achieved by diclazuril. In two centres in which animals that were already infected were housed, the treatment was also therapeutic (centres I and II). In this context, too, oocyst excretion was suppressed to a very large extent. This is in line with the experience reported by Staschen (2004), who demonstrated a reduction in coccidiosis following therapeutic treatment. Even if a therapy in a strict sense does not provide protection for an individual, it is beneficial for the rest of the animals in the same group who may still be in the phase of late prepatency. However, preventive treatment produced superior results in a direct comparison and should therefore be preferred.

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