

# Efficacy of toltrazuril as a metaphylactic and therapeutic treatment of coccidiosis in first-year grazing calves



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## Abstract

A multicentric, placebo-controlled, randomised, blinded and blocked field study was conducted to evaluate the efficacy and safety of toltrazuril (Baycox®, Bayer AG, Leverkusen, Germany) in the treatment of coccidiosis in first-year grazing calves naturally infected with *Eimeria* spp. Three-hundred and thirty-one calves were enrolled in the study and allocated to one of two treatments at a ratio of 1:1. One hundred and sixty-seven animals were treated once orally with 15 mg/kg toltrazuril, and 164 animals served as placebo-treated controls. Two treatment regimes were compared, a metaphylactic (treatment on the day, or 1 day after, turn out) and a therapeutic treatment (4 or 7 days after turn out). During an observation period of 14 days after treatment the animals were clinically examined for diarrhoea and faecal samples were regularly assessed for *Eimeria* oocysts. Other possible causes of diarrhoea were excluded on the basis of microbiological and virological examination. Animals were predominantly infected with *Eimeria alabamensis*. Number of days with diarrhoea in animals treated with toltrazuril was significantly lower compared to the placebo-treated group (therapeutic treatment:  $P=0.0024$ ; metaphylactic treatment:  $P<0.0001$ ). Furthermore, the number of animals with diarrhoea during the observation period for a minimum of at least 3 days, the number of animals positive for *Eimeria* oocysts, and the number of animals with both diarrhoea for a period of at least 3 days and positive for *Eimeria* oocysts, were significantly lower ( $P<0.01$ ) in

the toltrazuril- compared to the placebo-treated animals. Body weight in the toltrazuril-treated animals significantly exceeded that of the placebo-treated animals at the end of the observation period. Mean difference in body weight was higher in the metaphylactic (+7.3 kg) compared to the therapeutic treatment group (+3.4 kg). No adverse reactions were observed. The results indicate that toltrazuril is highly efficacious and safe in the metaphylactic and therapeutic treatment of coccidiosis caused by *E. alabamensis* in first-year grazing calves.

## Introduction

Coccidia of the genus *Eimeria* may cause watery to bloody diarrhoea in many vertebrates. In cattle, several *Eimeria* species have been shown to cause clinical disease associated with diarrhoea, high morbidity and even mortality. In the field, usually infections with more than one *Eimeria* species are found, but also monoinfections may occur (Gräfner et al. 1982; Svensson et al. 1993). In various European investigations *E. alabamensis* was identified as the dominating species in grazing cattle with clinical coccidiosis. Other species frequently found are *E. bovis*, *E. auburnensis*, *E. zuernii*, *E. ellipsoidalis* and *E. bukidnonensis* (Svensson et al. 1993, 1994; Svensson 1997). During clinical outbreaks of eimeriosis in grazing cattle in the northern part of the former German Democratic Republic (GDR) (Gräfner et al. 1982), *E. alabamensis* was the dominant-





ing species in ten out of 18 herds examined, and in three herds a monoinfection with *E. alabamensis* was diagnosed. Infections with *E. bovis* dominated in four herds, while in one herd a mixed infection with the three pathogenic species *E. alabamensis*, *E. bovis* and *E. zuernii* was found. This reflects the typical situation in northern Germany and, as far as is known, in central and northern Europe (Svensson et al. 1994; Samson-Himmelstjerna et al., in review). Typically *E. alabamensis* infections show a prepatent period ranging between 6 and 10 days. Clinical disease usually starts at 3–7 days post-infection with symptoms including watery diarrhoea, poor appetite, depression, abdominal pain and reduced growth rate leading to a significant reduction in weight gain or, at a low rate, even mortality (Hooshmand-Rad et al. 1994; Svensson et al. 1994).

The objective of this study was to evaluate the clinical and parasitological efficacy as well as the safety of toltrazuril in the metaphylactic and therapeutic treatment of first-year grazing calves under field conditions, using a single oral dose of 15 mg toltrazuril/kg bodyweight. Efficacy and safety parameters were compared between animals receiving toltrazuril and placebo-treated animals.

## Materials and methods

### Design of the study

The study was designed as a multicentric, placebo-controlled, randomised, blinded and blocked field study conducted on 14 dairy farms located in north-west Germany which had a history of clinical coccidiosis in calves after first turn out to pasture. It was performed in accordance with the European guidelines for the conduct of clinical trials as described in the directives 2001/82/EC, EC 92/18, and VICH GL9 (GCP) 15 June 2000, Good clinical practice, and the Guideline on statistical principles for veterinary clinical trials (EMA/CVMP/816/00-Final). Randomisation was based on the weight of the animals on the day of turn out to pasture.

### Two different treatment regimes were used:

1. Metaphylactic treatment: treatment of calves at the time of supposed infection (day of turn out or 1 day after turn out to pasture).
2. Therapeutic treatment: treatment of calves after the expected onset of clinical symptoms of coccidiosis 4–7 days after turn out to pasture.

For each treatment regime animals were allocated to either a toltrazuril group or a placebo group (water/milk application) at a 1:1 ratio, according to pre-established per centre (farm) randomisation lists that were available to the dispenser only (Table 1).

The primary evaluation criterion for the efficacy of the treatment was the number of days animals showed clinical signs of coccidiosis (diarrhoea) as measured by faecal scores. The tested hypothesis was that both metaphylactic and therapeutic treatment with toltrazuril lead to a significant reduction in clinical signs of coccidiosis as compared to the respective control group.

Secondary evaluation criteria were the number of *Eimeria* oocysts shed, the percentage of calves with diarrhoea for at least a 3-day period within the observation period and being positive for *Eimeria* oocysts, and weight gain during the observation period of 14 days.

### Study animals

Three-hundred and thirty-one calves – all but one (Spotted) Holstein Friesians – were enrolled in the study and allocated to one of two treatment regimes. In total, 53 male (25 in treatment, 28 in placebo groups) and 278 female (139 in treatment and 139 in placebo groups) calves with an age range of 2–15 months participated in the study. Body weight at the start of the study ranged from 61 kg to 357.5 kg with no significant difference between treatment and placebo group means. None of the animals was kept on pasture before the beginning of the trial. The number of animals enrolled per farm ranged between eight and 40. All herds were kept on pastures close to the farms which



**Table 1.** Treatment regime for toltrazuril- and placebo-treated calves

Day of treatment with regard to turn out <sup>a</sup>		No. of calves			
		Toltrazuril		Placebo	
Day 0	Metaphylactic treatment regime	40	69	40	69
Day 1		29		29	
Day 4	Therapeutic treatment regime	38	98	60	95
Day 7		60		35	
Total		166		164	

<sup>a</sup>Calves turned out to pasture on day 0

had been routinely used in the years before but had not been grazed in the current year.

### Clinical examination

A physical examination was performed by the examining veterinarian on the treatment day, followed by daily clinical observation for a 14-day observation period with an obligatory evaluation of faecal consistency.

### Body weight

All animals were weighed on the treatment day and at the end of the observation period of 14 days.

### Faecal samples

Faecal samples were collected from each animal before turn out, on the day of treatment and at regular intervals after treatment and examined for pathogenic *Eimeria* oocysts (*Eimeria alabamensis*, *E. bovis*, *E. zuernii*), using a modified McMaster technique. Oocysts were identified to species level and counted, i.e. up to 2,000 oocysts per species and sample [100,000 oocytes per gram of faeces (opg) calculated]; the surplus was disregarded and for all counts exceeding this

upper limit the number of 100,000 opg was used for calculation. Another faecal sample was collected before treatment for bacteriological and virological examination to confirm the absence of *Salmonella* spp. and/or coronavirus. This was repeated for each animal when it had diarrhoea for the first time. The microbiological examinations were done according to the standard methods of the Institute of Microbiology (Veterinary School Hannover) for the isolation and identification of

*Salmonella* spp. and, if applicable, *Clostridium perfringens*. Virological examinations were performed according to the test description of the coronavirus Elisa test kit (Bio-X Coronavirus Elisa kit; Bio-X diagnostics, Marche-en-Famenne, Belgium).

### Treatment allocation and treatment

Half of the animals on each pasture were treated with toltrazuril and the remaining half were allocated to the placebo-treated control group according to the randomisation procedure. Where possible, the pasture was split into two equal parts. Immediately following application animals were separated with regard to treatment. Toltrazuril was applied as a commercial oral suspension containing 50 mg active per ml (toltrazuril; BayerHealthCare, Leverkusen, Germany) at a dose rate of 15 mg/kg body weight. The toltrazuril dose was calculated based on the individual animal's body weight recorded on the same day. Animals allocated to the control group received the same dose volume, of an oral placebo, as the toltrazuril-treated animals.

### Animal selection and identification

All animals were reared on commercial farms and identified by individual ear tags. Only healthy animals that had not previously been treated with anticoccidials or corticosteroids were enrolled.





## Animal management and housing

Animals were kept on pastures providing between 18 m<sup>2</sup> and 105 m<sup>2</sup>/individual animal. The use of additional medication and/or therapies, which could directly affect the performance of the treatment product or the control group, i.e. sulphonamides, was not permitted. All animals had permanent access to water and on 12 of the 14 farms they received feed concentrates and on two farms maize silage or milk was fed additionally.

## Statistical analysis

All variables measured for animals during the study were stratified by treatment group and compared descriptively. For continuous variables, the following descriptive statistics were calculated where applicable: sample size, arithmetic and geometric means, SD, median, minimum, maximum, 25% percentile, and 75% percentile. For animal categorical or binary variables, absolute and relative frequencies were displayed. To assess treatment group comparability before treatment various animal characteristics were compared on the treatment day (baseline). For the comparison of the number of days where the calves in the different groups had clinical signs of coccidiosis, i.e. diarrhoea, an analysis of covariance (ANCOVA) was carried out using age, sex, and body weight as covariates. All other comparisons were carried out by employing a logistic regression model, using covariates age, sex and baseline body weight. The comparison of oocytes per gram of faeces between treatments was carried out using a repeated measures model for binary variables. Weight gain was analysed using an ANCOVA, using covariates age, sex and baseline body weight. For all statistical tests a nominal significance level of 5% ( $\alpha=0.05$ ) was used. Although multiple outcome variables were used to evaluate efficacy, no explicit multiple comparison correction of the *P*-values was used.

The occurrence of adverse events was reported descriptively.

## Results

Calves treated with toltrazuril or the placebo were comparable with regard to age, gender, weight and physical examination results prior to the onset of the study (data not shown).

Diarrhoea was first observed 4 days after turn out. The highest percentage of animals showing diarrhoea during the observation period was 90%, recorded for the placebo-treated animals of the metaphylactic treatment group. The toltrazuril-treated animals had significantly fewer days with diarrhoea than the placebo-treated calves, for both the therapeutic ( $P=0.0024$ ) and metaphylactic ( $P<0.0001$ ) treatment regimes (Fig. 1). The percentage of animals with diarrhoea was consistently lower throughout the observation period in toltrazuril compared to placebo-treated animals for both treatment regimes (Figs. 2, 3). During the observation period only *E. alabamensis* and *E. bovis* oocysts were recorded. A marked increase in oocyst numbers was observed in both placebo-treated groups and in the group receiving toltrazuril as a therapeutic treatment, with a maximum opg count at day 11 after turn out in both placebo-treated groups and on days 9–11 in the group treated therapeutically with toltrazuril. In contrast, oocyst numbers remained low in the group receiving toltrazuril as a metaphylactic treatment (Figs. 2, 3). The vast majority of oocysts belonged to *E. alabamensis*. The daily opg counts for *E. bovis* did not exceed 482 in the placebo-treated animals and 374 in the toltrazuril-treated animals.

The percentage of animals with diarrhoea for a period of at least 3 days and opg-positive for *Eimeria* spp. during the observation period was 54.7% and 58.0% for the therapeutic and metaphylactic treatment, respectively, in animals receiving placebo, and 31.3% and 11.6%, respectively, for animals receiving toltrazuril. In both the metaphylactic and the therapeutic treatment regimes the number of animals with diarrhoea, the number of animals with diarrhoea and positive for opg counts, and the number of animals with diarrhoea for at least a 3-day period, was significantly lower in the toltrazuril treatment group compared to the placebo-treated group (all  $P<0.01$ ).

Body weight gain in toltrazuril-treated animals significantly ( $P<0.01$ ) exceeded that in placebo-treated ani-

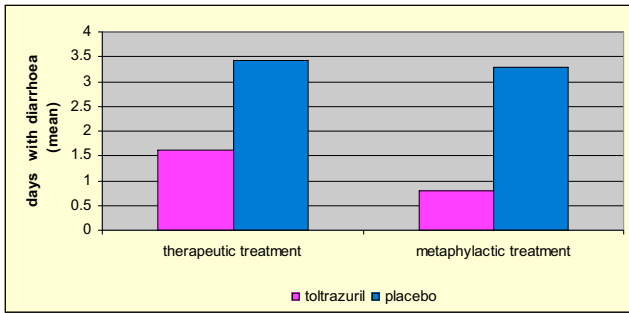


Fig. 1. Mean duration (days) of diarrhoea per group

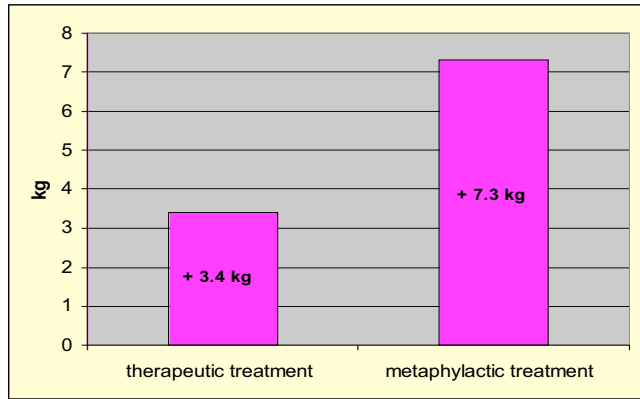


Fig. 4. Weight gain during observation period of toltrazuril- vs. placebo-treated calves after therapeutic and metaphylactic treatment

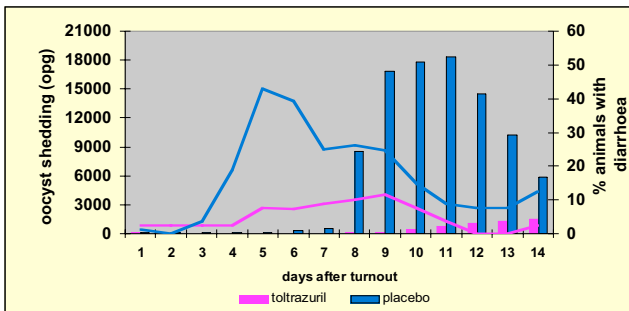


Fig. 2. Shedding of *Eimeria* oocysts (columns) and percentage of calves with diarrhoea (lines) after metaphylactic treatment with toltrazuril vs. placebo

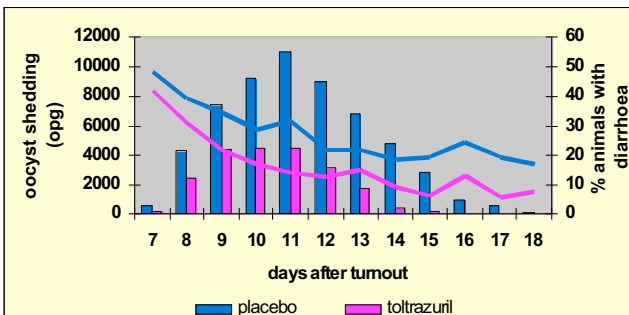


Fig. 3. Shedding of *Eimeria* oocysts (columns) and percentage of calves with diarrhoea (lines) after therapeutic treatment with toltrazuril vs. placebo

imals. Metaphylactic treatment with toltrazuril resulted in a higher body weight gain during the observation period (+7.3 kg) compared to placebo-treated animals than therapeutic treatment (+3.4 kg) (Fig. 4).

During the observation period no animal showed an infection with either *Salmonella* spp. or coronavirus and no animal was suspected to be infected with

*Clostridium perfringens* based on clinical signs.

No suspected adverse drug reaction, local or systemic, was reported for any animal treated with toltrazuril.

## Discussion

In central and northern Europe coccidiosis in grazing herds during the grazing season has previously been described (Gräfner 1989; Svensson et al. 1994; Snoep and Potters 2004). For northern Germany, reports exist which describe clinical outbreaks as well as the epidemiological situation in grazing cattle on a multi-herd basis (Gräfner 1989). The animals usually showed diarrhoea and weight loss during the first weeks following turnout and occasionally mortality was reported. The pattern of *E. alabamensis* infections in first-year grazing calves is characterized by significant increases in oocyst counts starting at approximately 7–9 days after turnout (Snoep and Potters 2004). Infections are considered to be mainly caused by oocysts which persisted on the pasture since the previous grazing season (Gräfner et al. 1982; Svensson et al. 1993, 1994). The findings of the present study are consistent with this view. In all herds and in nearly all of the calves *E. alabamensis* oocysts were found and in approximately 80% of the animals clinical coccidiosis was diagnosed. The pathogenic *Eimeria* species encountered in the present study were *E. alabamensis* and *E. bovis*, which were also





the most prevalent in an earlier study in the GDR (Gräfner et al. 1982). Although infection with this pathogen is usually considered to be acquired during the first few days of grazing (Gräfner et al. 1982; Svensson et al. 1993), it is also known that calves may be infected before turn out, usually leading to the excretion of low numbers of oocysts. It has been shown that *E. alabamensis* oocysts can be transmitted by contaminated hay (Svensson 1997). On several of the farms included in this study, anecdotal reports of calves which died with signs of clinical coccidiosis during the first 2 months following turn out were given. In contrast to *E. bovis* which can cause significant lesions in the large intestine with morbidity and mortality (Dauguschies et al. 1997; Taylor and Catchpole 1994), *E. alabamensis* is known to cause clinical cases with lower morbidity (Hooshmand-Rad et al. 1994). Nevertheless, such natural infections usually contribute to reduced weight gain during the first months of grazing and may occasionally require treatment to avoid a fatal outcome of the disease.

To date, in many countries the prophylactic and curative options for the treatment of calves infected with *Eimeria* spp. are unsatisfactory. For example, in Germany, currently only sulphonamides are registered for the treatment of bovine coccidiosis. However, repeated applications of these drugs for several days only result in the reduction of clinical symptoms but are unable to prevent these effectively. Experience with sulphonamide treatment against bovine coccidiosis has been described worldwide. A sulphonamide-bolus-based treatment using a baquiloprim/sulphadimidine bolus has been studied in Sweden and found to have significant effects on reduced weight loss and oocyst output in treated calves (Svensson 1998). Nevertheless, it is not marketed as a registered product and may be used in niche markets only due to the handling expenses. Other combinations of molecules tested for enhanced efficacy, such as sulphamonomethoxine/ormetoprim (Hasbullah et al. 1996), are reported to be effective as well. However, a multitude of reports concerning the unsatisfactory efficacy of these combinations in the field reflect the decreased compliance of users for this class of compounds.

Among other coccidiostatics used for prophylactic treatment, lasalocid has been known for many years to be effective in the prevention of clinical coccidiosis,

when fed to calves in milk starter. Although the application and efficacy of this compound is well described (Hoblet et al. 1989; Sinks et al. 1992; Waggoner et al. 1994; McMeniman and Elliott 1995; Quigley et al. 1997), a major constraint is the labour-intensive step of mixing the pure compound into the starter. Even though it is available as a commercial product (Bovatec, Hoffman-La-Roche), farmers have had to be persuaded to use it; however, once applied, the compliance rate reported was very good (unpublished observations). Further coccidiostatics such as decoquinate (Rhone-Poulenc) or Monensin, known to be effective against clinical coccidiosis in prophylactic use (Fitzgerald and Mansfield 1984; Fitzgerald and Mansfield 1989a, 1989b; Foreyt et al., 1986; Stromberg et al. 1986; Waggoner et al. 1994) are no longer registered for use in livestock animals in the European Union.

Immunisation has also been presented as a possible option for prevention in preliminary investigations. However, this has not yet become commercially available.

The present study showed that treatment with toltrazuril (Baycox), applied according to a therapeutic or metaphylactic treatment regime, was safe and highly efficacious against infections with *Eimeria* spp. in first-year grazing calves. Treatment with toltrazuril significantly decreased the number of days animals had diarrhoea. Furthermore, the number of animals with diarrhoea and/or the number of animals positive for *Eimeria* spp. was significantly lower in animals treated with toltrazuril compared to the placebo-treated animals, and treatment with toltrazuril resulted in a significantly higher body weight compared to placebo-treated animals.

The results obtained are in accordance with efficacy data for toltrazuril from an experimental *E. bovis* infection model with toltrazuril (Mundt et al. 2003) and from a natural coccidiosis outbreak in calves treated with toltrazuril (Bohrmann 1991). However, possible differences in formulation have to be considered since the off-label use of this compound in the 1990s did not employ the formulation used in the present study. Nevertheless, in both publications a significant reduction in oocyst shedding, as well as an improvement of clinical symptoms, were observed. Furthermore, in the experimental infection model against *E. bovis* the com-



pound proved to be fully effective in a prophylactic application (Mundt et al. 2003).

The results indicate that toltrazuril is highly efficacious when used to treat animals at, or shortly after, turn out to pastures contaminated with *E. alabamensis*. ●

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## References

- Bohrmann R (1991) Treatment with toltrazuril in a natural outbreak of coccidiosis in calves. *Dtsch Tierärztl Wochenschr* 98:343–345
- Dauguschies A, Buerger HJ, Akimaru M (1997) Effects of experimental infection with *Eimeria bovis* on the balance of sodium, potassium and water in calves. *Parasitol Int* 46:159–169
- Fitzgerald PR, Mansfield ME (1984) Control of bovine coccidiosis with monensin in nonresistant newborn calves. *Am J Vet Res* 45:1984–1988
- Fitzgerald PR, Mansfield ME (1989a) Effects of inoculations with *Eimeria zuernii* on young calves treated with decoquinate or narasin with or without dexamethasone. *Am J Vet Res* 50:1056–1059
- Fitzgerald PR, Mansfield ME (1989b) Effects of intermittent and continuous administration of decoquinate on bovine coccidiosis in male calves. *Am J Vet Res* 50:961–964
- Foreyt WJ, Rice DH, Wescott RB (1986) Evaluation of lasalocid as a coccidiostat in calves: titration, efficacy, and comparison with monensin and decoquinate. *Am J Vet Res* 47:2031–2035
- Gräfner G (1989) Zur derzeitigen Verbreitung, Bedeutung und Bekämpfung der Weideparasitosen des Rindes in der DDR. *Monatsh Veterinaermed* 44:435–437
- Gräfner G, Graubmann H-D, Kron A, Müller H, Daetz H-H, Plötner J, Benda A (1982) Zum Auftreten der Weidekokzidiose in Junggrinderbeständen. *Monatsh Veterinaermed* 37:776–779
- Hasbullah IH, Uchida T, Inamoto T, Nakai Y, Ogimoto K (1996) Medication of feedlot calves infected with *Eimeria* spp. by a combination of sulfamethoxine and ormetoprim. *J Vet Med Sci* 58:169–170
- Hoblet KH, Charles TP, Howard RR (1989) Evaluation of lasalocid and decoquinate against coccidiosis resulting from natural exposure in weaned dairy calves. *Am J Vet Res* 50:1060–1063
- Hooshmand-Rad P, Svensson C, Uggla A (1994) Experimental *Eimeria alabamensis* infection in calves. *Vet Parasitol* 53:23–32
- McMeniman NP, Elliott R (1995) Control of coccidia in young calves using lasalocid. *Aust Vet J* 72:7–9
- Mundt H-C, Daugschies A, Uebe F, Rinke M (2003) Efficacy of toltrazuril against artificial infections with *Eimeria bovis* in calves. *Parasitol Res* 90:S166–S167
- Quigley FD, Drewry JJ, Murray LM, Ivey SJ (1997) Effects of lasalocid in milk replacer of calf starter on health and performance of calves challenged with *Eimeria* species. *J Dairy Sci* 80:2972–2976
- Sinks GD, Quigley JD, Reinmeyer CR (1992) Effects of lasalocid on coccidial infection and growth in young dairy calves. *J Am Vet Med Assoc* 200:1947–1951
- Snoep JJ, Potters JB (2004) Coccidiosis causes diarrhea in calves in the pasture. Pasture coccidiosis caused by *Eimeria alabamensis*. *Tijdschr Diergeneeskde* 129:158–160
- Stromberg BE, Schlotthauer JC, Hamann KJ, Saatara Oz H, Bemrick WJ (1986) Experimental bovine coccidiosis: control with monensin. *Vet Parasitol* 22:135–140
- Svensson C (1997) The survival and transmission of oocysts of *Eimeria alabamensis* in hay. *Vet Parasitol* 69:211–218
- Svensson C (1998) Prevention of *Eimeria alabamensis* coccidiosis by a long-acting baquiloprim/sulphadimidine bolus. *Vet Parasitol* 74:143–152
- Svensson C, Hooshmand-Rad P, Pehrson B, Tornquist M, Uggla A (1993) Excretion of *Eimeria* oocysts in calves during their first three weeks after turn-out to pasture. *Acta Vet Scand* 34:175–182
- Svensson C, Uggla A, Pehrson B (1994) *Eimeria alabamensis* infection as a cause of diarrhoea in calves at pasture. *Vet Parasitol* 53:33–43
- Taylor MA, Catchpole J (1994) Coccidiosis of domestic ruminants. *Appl Parasitol* 35:73–86
- Waggoner JK, Cecava MJ, Kazacos KR (1994) Efficacy of lasalocid and decoquinate against coccidiosis in naturally infected dairy calves. *J Dairy Sci* 77:349–53