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Clinical and epidemiological characteristics of *Eimeria* infections in first-year grazing cattle

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Abstract

Infections with *Eimeria* parasites can lead to severe diarrhoea with considerable clinical and economic consequences in first-year grazing stock. To identify and characterise the cause of diarrhoea observed during previous years, 164 animals on 14 dairy farms in northwestern Germany were included in this study. The calves were physically and parasitologically examined prior to turnout and until 21 days post turnout (d.p.t.). Mean animal weights decreased from 194.9 kg at the start to 189.3 kg bodyweight at the end of the study. In all herds, oocyst counts were very low prior to turnout and increased after the calves had been kept on pasture for at least 7 days. On Day 9 post turnout, 90% and at the end of the study (21 d.p.t.) 70% of all animals showed *Eimeria*-positive faecal samples. During the course of the study, 79 (48.2%) animals passed faecal samples with more than 100,000 oocysts per gram. The predominant species identified was *Eimeria alabamensis*, which accounted for more than 83% of the oocysts counted. These parasitological findings matched the clinical observations. Diarrhoea was found in 130 (79.3%) of the study animals. At 5 d.p.t. and thus prior to the rise of faecal oocyst counts, a significant increase in diarrhoea was recorded. Calves showing diarrhoea excreted statistically significantly more often over 100,000 *E. alabamensis* oocysts per gram faeces (0.28; $p = 0.0002$) than calves without diarrhoea. Diarrhoea was also found during significantly more study days in animals with high oocyst counts (0.39; $p = 0.0001$). These data indicate that in endemic areas first-year grazing calves must be considered at risk to develop clinical coccidiosis due to *E. alabamensis* infection during the first 2–3 weeks post turnout.

Keywords: *Eimeria alabamensis*; Cattle; Diarrhoea; Epidemiology; Coccidiosis

1. Introduction

In grazing ruminants, diarrhoea may be caused by protozoan pathogens belonging to the genus *Eimeria*

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of the subclass Coccidia. In cattle, several *Eimeria* species have been shown to be capable of establishing clinical disease associated with watery to bloody diarrhoea, high morbidity, and, depending on which *Eimeria* species is dominant, high mortality rates as well. In the field, infections with more than one species were commonly found, but monoinfections were observed in some cases (Gräfner et al., 1982; Svensson et al., 1993). Investigations performed in Sweden and Germany have unambiguously identified *Eimeria alabamensis* as the predominant species in grazing cattle with clinical coccidiosis. Other species frequently found were *Eimeria bovis*, *Eimeria auburnensis*, *Eimeria zuernii*, *Eimeria ellipsoidalis* and *Eimeria bukidnonensis* (Svensson et al., 1993, 1994; Svensson, 1997). In Germany, clinical outbreaks of coccidiosis have occurred in grazing cattle in 17 herds from eight large cattle enterprises in the northern part of the former GDR (Gräfner et al., 1982). In three herds, *E. alabamensis* monoinfections were recorded, and this species was predominant in ten of the remaining herds with polyinfections. Infections with *E. bovis* dominated in four herds, while in one herd the three species *E. alabamensis*, *E. bovis*, and *E. zuernii* were present. Experimental infections and data obtained during field studies have demonstrated that the prepatent period of *E. alabamensis* ranges between 6 and 10 days (Hooshmand-Rad et al., 1994; Svensson et al., 1994). Clinical symptoms started 3–7 days post experimental infection (Hooshmand-Rad et al., 1994) and were observed to become prevalent at 5 days following turnout on contaminated pasture (Svensson et al., 1994). Apart from watery diarrhoea, other symptoms shown by experimentally infected calves were poor appetite, depression, abdominal pain, and reduced growth rate (Hooshmand-Rad et al., 1994). Significant reduction in weight gain was also found in natural infections. Compared to calves kept on clean pastures, the difference amounted to more than 24 kg during a period of 24 days and actually resulted in weight loss of 18 kg during that time (Svensson et al., 1994). Mortality of *E. alabamensis*-infected cattle appears to be low and remains below 1% even when polyinfections are present (Gräfner et al., 1982).

Our goals in the present study were to determine which *Eimeria* species were involved in natural infections and to describe their epidemiology in first-year grazing calves in typical dairy herds in northwestern Germany. Furthermore, we wanted to investigate possible clinical consequences of these infections.

2. Materials and methods

The investigations were performed in spring and early summer 2003 on 14 dairy farms in Ostfriesland and Weser-Ems counties (Lower Saxony, Germany). All farms had a history of suspected clinical coccidiosis during the last 6–8 years. On 10–15% of the farms, faecal samples were examined previously and coccidia were found. Groups of animals on all farms were repeatedly showing watery diarrhoea. Usually less than 5% of the animals died within the first 3 weeks post turnout, and more than 50% showed severe weight loss post turnout.

2.1. Animals, management, and feeding

A total of 164 first-year grazing Holstein Friesian calves (137 female, 27 male) were included. The animals were between 5 and 15 months old. None of the animals were kept on pasture before the beginning of the trial. The number of animals enrolled per farm ranged between 8 and 40. All herds were kept near the farms on pasture routinely used for young stock but which had not been grazed in the current year.

2.2. Clinical examination

All animals were physically examined prior to turnout concerning their attitude and appetite; the locomotive, respiratory, and cardiac system; their coat; and the consistency of the faeces. Animals were weighed on the day before or on the day of turnout and 7 and 14 days following turnout.

2.3. Faecal examination

Faecal samples were taken from the rectum once within 7 days before turnout, always on the day of turnout, and generally twice weekly until the end of the study 3 weeks post turnout. Thus, although not all animals were examined daily, faecal and clinical data were generated on each study day by visiting at least two farms per day. During the course of the study, the absence (faeces firm, thick mushy) or presence (faeces

thin mushy to watery, profuse) of diarrhoea was recorded daily.

Faecal samples of all animals were examined microbiologically according to standard operating procedures by the Institute of Microbiology (University of Veterinary Medicine, Hannover) for the isolation and identification of *Salmonella* spp. and *Clostridium perfringens*. Furthermore, virological examination of the samples was done using a Coronavirus ELISA test kit provided by Bio-X diagnostics (Marche-en Famenne, Belgium).

2.4. Parasitological examination

Four grams of faeces were examined using a modified McMaster procedure. Samples were mixed thoroughly in 60 ml of flotation solution (saturated NaCl). Two McMaster chambers were filled per sample, and parasitological specimens were identified and counted microscopically. The number of oocysts and helminth eggs per gram of faeces were determined according to Rommel (2000a).

Samples with more than 100,000 oocysts per gram (opg) were recorded as >100,000. The oocysts were identified according to Rommel (2000b) and Eckert et al. (1995).

2.5. Statistical methods

All data were analysed descriptively using appropriate descriptive statistics including mean, standard deviation, minimum, maximum, and 95% confidence intervals for continuous data, while frequencies and relative percentages along with respective 95% confidence intervals were displayed for categorical data. Associations between variables were analysed using the Spearman correlation coefficient. A nominal significance level of 5% ($\alpha = 0.05$) was used for all statistical tests. No multiple testing adjustments were carried out. All analyses were performed using the SAS statistical analysis software Version 8.2, SAS Institute Inc. Cary, NC, USA.

3. Results

At the beginning of the study all animals were healthy according to the results of the clinical examination. The mean weight was 194.9 kg (S.D. 62.6) and 189.3 kg (S.D. 59.2) at the start and end of the investigation, respectively. Apart from the occasion where the faeces of one animal tested positive for *Salmonella* spp., samples tested negative for both *Salomenlla* or Coronavirus. According to the clinical examinations, no indication of *C. perfringens* was apparent. The clinical examinations indicated that the calves were not suffering severely from the infections, with the exception of one calf which was given Stullmisan S® (Essex) to treat the observed excessive diarrhoea.

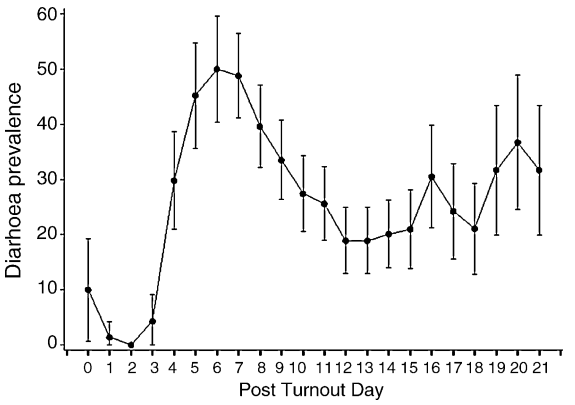

Fig. 1. Mean prevalence of diarrhoea on all farms over the course of the study with 95% confidence intervals.

Diarrhoea was found in 130 (79.3%) of the 164 study animals. Diarrhoea was already present in a few animals at, or around, turnout. The percentage of animals showing diarrhoea increased from Day 3 and peaked at Day 6 post turnout (Fig. 1). For a period of

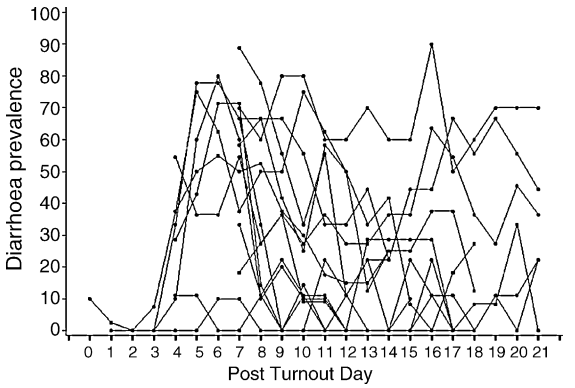

Fig. 2. Prevalence of diarrhoea on each of the 14 farms over the course of the study.

Table 1
Parasitological stages found in a total of 1124 faecal samples (percentage in parenthesis)

Identification	No. of samples
Eimeria alabamensis	936 (83.3)
Eimeria bovis	662 (58.9)
Eimeria zuernii	35 (3.1)
Eimeria negative	198 (17.6)
Strongyloides	16 (1.4)
Gastrointestinal strongyles	70 (6.2)
Not determined	106 (9.4)

another 4 days, between 40 and 50% of the study animals showed diarrhoea. The percentage of calves with diarrhoea then decreased, but remained at levels between 20 and 40% until the end of the study. The pattern of development of diarrhoea at the herd level is shown in Fig. 2. This graph illustrates that more than three quarters of the animals in several herds were affected.

Oocysts belonging to the species of the genus *Eimeria* were found in all 164 calves. At the beginning of the study the mean *Eimeria* herd prevalence was already above 70% followed by the peak prevalence of 100% at 9 days post turnout (Fig. 3). As the most prominent species, *E. alabamensis* was identified in 936 (83.3%) of a total of 1124 samples (Table 1). At turnout, a mean of 55.6% of the animals on all farms showed *E. alabamensis*-positive faeces. This increased to 99.0% on Day 9 post turnout, then decreased to 60.0% at the study's end (Table 2).

Table 2
Relative prevalence of *E. alabamensis* during the course of the study ($n = 164$)

Post turnout day	Percentage of *E. alabamensis* positive samples (number of samples examined)	95% confidence interval of %
0	55.6 (164)	(45.3–65.8)
2	60.0 (75)	(48.9–71.1)
3	48.3 (29)	(30.1–66.5)
4	57.3 (75)	(46.1–68.5)
5	45.6 (68)	(33.8–57.4)
6	60.0 (35)	(43.8–76.2)
7	61.0 (100)	(51.4–70.6)
8	84.4 (64)	(75.5–93.3)
9	99.0 (100)	(97.0–100.0)
10	93.1 (29)	(83.9–100.0)
11	94.8 (134)	(91.0–98.5)
12	93.1 (29)	(83.9–100.0)
13	88.9 (45)	(79.7–98.1)
14	98.9 (89)	(96.7–100.0)
15	82.4 (74)	(73.8–91.1)
16	88.0 (50)	(79.0–97.0)
18	71.6 (95)	(62.5–80.6)
21	60.0 (60)	(47.6–72.4)

During the course of the study, 79 (48.2%) calves showed opg counts >100,000 for *Eimeria* spp. Among these, all animals had *E. alabamensis*, and only one calf showed *E. bovis* opg counts of more than 100,000 (at Day 11 post turnout). For the complete study period, an average of 43.6% (S.D. 23.3) of the calves on the 14 sites had more than 100,000 *Eimeria* opg at

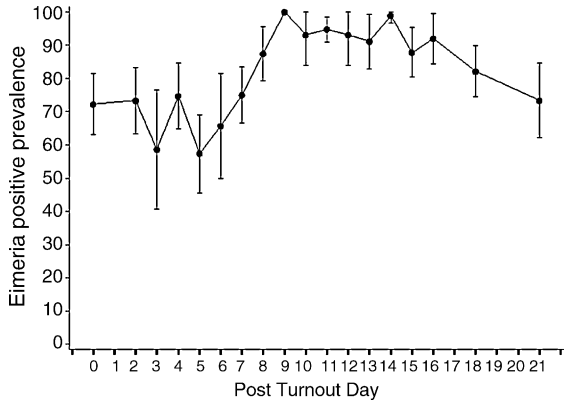

Fig. 3. Mean prevalence of animals showing *Eimeria* spp. positive faecal samples on all farms over the course of the study with 95% confidence intervals.

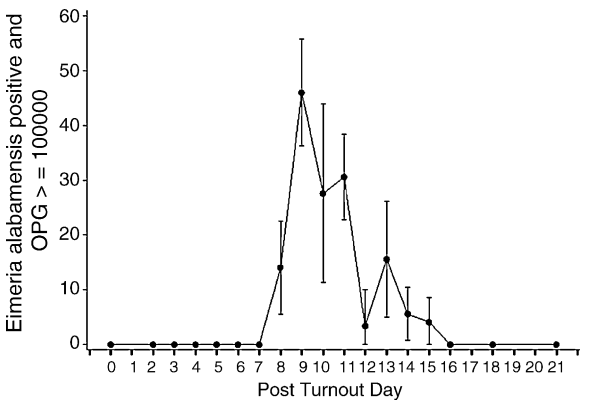

Fig. 4. Mean prevalence of animals on all farms showing faecal samples with ≥100,000 *E. alabamensis* opg over the course of the study (⊥ 95% confidence intervals).

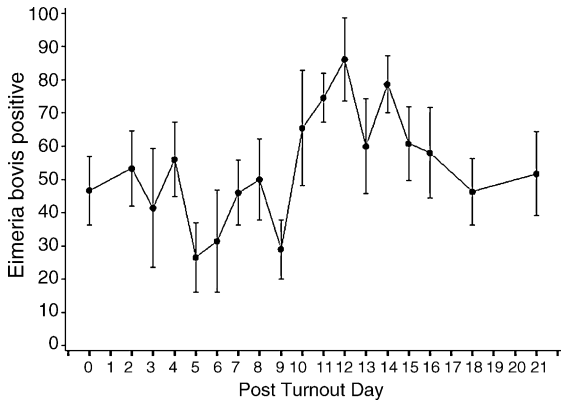

Fig. 5. Mean prevalence of animals on all farms showing *E. bovis* positive faecal samples (⊥ 95% confidence intervals).

least once. The relative peak of samples with such high counts for *E. alabamensis* was reached at Day 9 post turnout (Fig. 4). One week later the number of calves shedding over 100,000 *E. alabamensis* oozysts per gram fell to zero (Fig. 4). The number of *E. bovis* and *E. zuernii* positive calves peaked at Days 12 and 9 post turnout, respectively (Figs. 5 and 6). Both the mean *E. bovis* and the mean *E. zuernii* opg counts remained below 500 during the course of the study (data not shown).

The statistical examination of the data revealed that calves which showed signs of diarrhoea were significantly more often excreting over 100,000 *E. alabamensis* oocysts per gram faeces (0.28; $p = 0.0002$) than those without diarrhoea. Of the 130 animals for which diarrhoea was recorded, 72

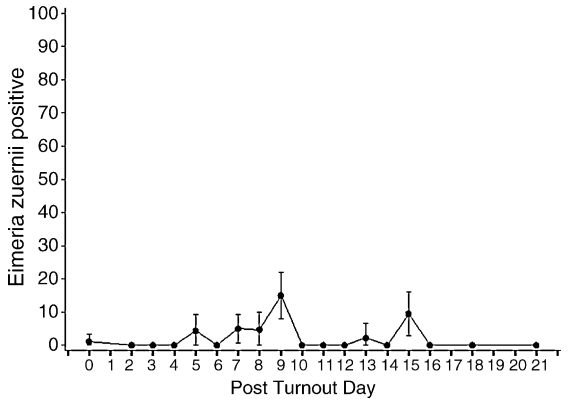

Fig. 6. Mean prevalence of animals on all farms showing *E. zuernii* positive faecal samples (⊥ 95% confidence intervals).

were found to shed more than 100,000 *E. alabamensis* oocysts per gram. Also, the number of days on which animals showed diarrhoea was correlated with high oocyst shedding (0.39; $p = 0.0001$). Among the group of animals with high oocyst counts, this was particularly true for 24 animals where persistence of diarrhoea for more than 1 week up to the end of the study was observed, compared to a total of nine with lower counts. Within the latter group, only two animals showed diarrhoea later than 8 days post turnout. Accordingly, the number of days where animals showed high *E. alabamensis* oocyst counts correlated with the number of days when diarrhoea was present (0.28; $p = 0.0003$). Out of a total of 79 calves showing faeces with more than 100,000 oocysts per gram for at least 1 day, 72 had diarrhoea.

4. Discussion

Only a few reports document the occurrence and consequences of coccidiosis in German grazing cattle based on multi-herd data (Gräfner et al., 1982; Gräfner, 1989). The district in which the current investigation was performed represents one of the most intensive dairy areas in Germany. Following repeated reports about cases of first-year grazing calves showing diarrhoea and weight loss during the first weeks following turnout, the present study was initiated to investigate the possible involvement of coccidia. The pattern of *E. alabamensis* infections in first-year grazing calves is characterised by significant increases in oocsyt counts starting at approximately 7–9 days post turnout (Svensson, 2000). Infections are considered to be mainly caused by oocysts which persist on the pasture from the previous grazing season (Gräfner et al., 1982; Svensson et al., 1993, 1994). A similar scenario was encountered in the present study. However, the high degree of reproducibility with which the parasitological and clinical parameters developed throughout the conduct of the study at the different sites was unexpected. In all herds and in nearly all calves, *E. alabamensis* oocsyts were found, and in approximately 80% clinical Eimeriosis was diagnosed. The spectrum of *Eimeria* species encountered in the present study includes those found most often in an earlier study in northeastern parts of Germany (Gräfner et al., 1982). Similarly,

E. alabamensis and *E. bovis* were the most prevalent species. At turnout, a significant number of animals showed *E. alabamensis*-positive faecal samples in spite of the fact that this pathogen is considered to be an infection acquired during the first few days of grazing (Gräfner et al., 1982; Svensson et al., 1993). However, it is also known that calves may be infected before turnout, which usually leads to the excretion of low oocyst numbers. *E. alabamensis* oocysts can be transmitted by feeding contaminated hay even when it has been stored for 8 months (Faber et al., 2002; Svensson, 1997). In-house infection and latent infections could relapse if animals are stressed during turnout (Levine, 1985). Reports have discussed a possible reactivation of arrested endogenous stages as a response to stress, such as unfavourable weather (Marquardt, 1976; Parker et al., 1986; Step et al., 2002).

Only a few reports are available on the clinical significance of naturally acquired bovine coccidiosis. Here, the calves on all farms started to show clinical symptoms of watery diarrhoea approximately 2 days before the increase in oocyst output began. Similar findings were reported in Swedish first-year grazing calves kept on permanent pastures which were proven to be contaminated in the previous year (Svensson, 2000). No animal died during the course of the present study, but one calf had to be treated palliatively. In the years preceeding and following this study, calves from the same farms have died, with signs of clinical coccidiosis during the first 2 months following turnout (data not shown). In contrast to *E. bovis*-dominated infections, coccidiosis predominantly caused by *E. alabamensis* is known to show a low morbidity. Nevertheless, such natural infections may contribute to reduced weight gain during the first months of grazing and may occasionally require treatment to avoid a fatal outcome of the disease. In the present study, infections with *E. alabamensis* were the most prevalent, showing the highest intensity in the faecal samples. Since *E. bovis* is more virulent than *E. alabamensis*, animals with *E. bovis* opg counts lower than 100,000 could also have been clinically affected, and these infections must also be considered as a cause of diarrhoea. Data from experimental (Hooshmand-Rad et al., 1994) but also natural (Svensson et al., 1994) *E. alabamensis* infections have shown that clinical signs of diarrhoea develop and peak within the first week post turnout. Essentially, the same was observed in the present study. In contrast, following infection with *E. bovis* and *E. zuernii*, the first signs of clinical disease are seen approximately 2–3 weeks post infection (Daugschies et al., 1986; Mundt et al., 2005). Therefore, the fact that diarrhoea began to develop after only 4 days post turnout in the herds investigated here, with the highest incidence at 6 days post turnout, suggests that the main cause of disease during this phase of the study can be attributed to infections with *E. alabamensis*. Furthermore, the low mean *E. bovis* and *E. zuernii* opg counts suggest that these pathogens did not unduly contribute to the clinical effects seen in this study.

Only sulphonamides are currently registered for treatment of bovine coccidiosis in Germany. However, even by repeated application of these drugs for several days, the clinical symptoms can only be reduced but not effectively eliminated. A sulphonamide bolus-based treatment, which has been studied in Sweden, was shown to be significantly effective in reducing weight loss and oocyst output in treated calves (Svensson, 1998). Immunisation has also been discussed as a possible option for prevention in preliminary investigations (Svensson et al., 1996). However, to date, the prophylactic and curative options are unsatisfactory in many countries. The known anti-coccidial compound toltrazuril has been shown to clinically cure *Eimeria*-infected calves and reduce oocyst output (Emanuel et al., 1988; Pilarczyk, 2001) but is not yet registered for use in cattle. From the data shown here, we conclude that clinical symptoms due to *Eimeria* spp. infections in endemic areas regularly occur during the first 1 or 2 weeks following turnout.

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