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The Incidence of *Eimeria* Species in Naturally Infected Calves

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ABSTRACT

The present study reports the incidence of oocysts in faeces of calves in North Wales, naturally infected with *Eimeria species.* The *Eimeria* species encountered were *E. bovis, E. auburnesis, E. canadensis, E. ellipsoidalis/alabamensis, E. brasiliensis, E. cylindrica, E. zuernii, E. wyomingensis, E. subspherica* and *E. pellita.* Multiple-infection was commonly seen due to two to seven species often represented in a single faecal sample. These species in the faecal samples of 3 - 4 months old calves were examined at monthly intervals and the seasonal incidence was determined over the period of 12 months. A significant reduction in the incidence was seen with time and this was most marked in the case of pathogenic species. No association between weight gain and number of oocysts shed was evident even when large number of oocysts of known pathogenic species was present.

Key Words: *Eimeria* spp.; Coccidiosis; Calves; North Wales

INTRODUCTION

Coccidiosis is considered to be the most common disease of cattle in general, in calves in particular, throughout the world. It is generally a disease of the intestinal tract of young calves at less than six months of age and is particularly severe in early-weaned animals. Infection of this age group ranges from zero, immediately after birth, to 100% in animals up to two months of age (Fitzgerald, 1962; Joyner *et al*., 1966).

Bovine coccidiosis causes heavy losses to the cattle industry; therefore, a great deal of work regarding the prevalence and some other related aspects (life cycle & pathogenicity) to *Eimeria* species has been carried out throughout the world (Kasim & Al-Shawa, 1985; Parker & Jones, 1987; Weinandy, 1989; Kang, *et al*., 1989; Parker, 1991; Abrahamsen *et al*., 1994) including cattle. Most of the studies in previous years regarding the epidemiology of bovine coccidia have been conducted and recorded in different parts of England (Joyner *et al*., 1966). However, with the exception of two short studies (Wade, 1990; Oakley, 1990), little work has been carried out on the distribution of bovine coccidiosis in North Wales. The present study was carried-out to record some epidemiological aspects of *Eimeria* species in naturally infected calves.

MATERIALS AND METHODS

Animals and sampling. The calves, 3 - 4 months of age at the start of the experiment, were sampled for one year from June 1998 to May 1999. All the animals were kept during winter (December-May) in sheds at Abar Cattle Farm, North Wales, Bangor UK. The calves were normally kept in a group of 15 animals on straw in metal pens with solid partitions and barred fronts but with access to food and water. The animals were kept on normal commercial diets of hay, silage and concentrate feed during winter and pasture only in summer. Calves were weighed at monthly intervals for 12 months and the general condition of each animal recorded. Individual faecal samples were obtained per rectum from each animal. The samples were stored in plastic screw-top jars filled to the top to avoid air and hence to slow down the rate of oocyst sporulation. Samples were stored at 4°C and examined as soon as possible, but not later than 7 days.

Separation of *Eimeria* oocysts. Oocysts were separated by the modified McMaster technique (Anonymous, 1986) in saturated NaCl solution. Three grams of faeces were weighed and mixed with 42 mL of tap water and ground in a Pestle and Mortar. The suspension was then filtered through a mesh sieve with an aperture of 0.15 mm and the liquid collected below in a clean dry beaker. The filtered suspension was mixed and poured into a Clayton Lane centrifuge tube to within 1 cm of the top. The samples were then centrifuged at 1500 rpm for 2 min after which the supernatant was discarded. The packed, sediment mass at the bottom of the tube was emulsified with a saturated solution of NaCl until the volume equaled that of the initial aliquot filtrate. The tube was inverted several times with the help of the thumb until the sediment was evenly suspended and the two chambers of a McMaster slide were filled using a Pasteur pipette, care being taken not to introduce air into the chambers. All the oocysts within the ruled area, 1 cm^2 of each chamber, were counted using a microscope (10x eyepiece & objective). In calculating the numbers of oocysts

per gram of faeces, the mean number of oocysts from the two chambers of the McMaster slide was multiplied by 100. This factor was derived from the formula: X/ 0.15 x 45 x 1/3; where X = mean number of oocysts from two chambers, 0.15 = volume of sample in cm^2, 45 = total volume of sample i.e., 3 gram faeces + 42 mL water and 1/3 = correction factor for 1 g of faeces.

Statistical methods. Data were examined by analysis of variance (one way ANOVA) using a Minitab package 1993/94. Mean values and standard error of the difference between means (SED) are given in the tables.

RESULTS

Generally, all 15 animals were in good condition. Apparently they were alert, healthy and no signs of clinical coccidiosis were evident; even calves shedding had low number of oocysts of genus *Eimeria*. As evident form Fig. 1, a highly significant weight gain was recorded in these calves over the study period. The calves were seen to shed oocysts in their faeces yet no effect on weight gain was observed due to this intestinal parasite. During the course of the study a steady increase in weight gain was seen together with a gradual reduction in the incidence of total oocysts (Fig. 2). However, there appeared to be no correlation between these two phenomena (a 79.04% weight gain & a -78.46% reduction in oocyst numbers during 12 months period). Thus, the largest weight gains did not take place in the same months as those in which the greatest reduction in oocyst numbers. This suggested that the *Eimeria* spp at these levels of infection have no effect on the weight gain in naturally infected calves. This does not mean, however that larger infection rates with a different mix of species of *Eimeria* would not affect weight gain.

While all samples were infected at the start of sampling in June, considerable fluctuation in prevalence occurred from month to month (Fig. 3). Although the incidence fell during the winter months (January, February & March), the incidence rose only to fall in April and to rise again in May. It was difficult to see any pattern of change except to say that generally as the infection proceeds there was an overall reduction in prevalence percentage. It is difficult to say whether this is an age, immunity, climate or husbandry effect.

The mean percentage prevalence of each *Eimeria* species at each month of the year and their incidence over this period show that all ten species of *Eimeria* showed different prevalence rates each month (Table I). Thus in June *E. bovis* occurred in 33.33% of samples taken, while *E. auburnensis, E. canadensis, E. ellipsoidalis/alabamensis, E. brasiliensis, E. cylindrica, E. zuernii, E. wyomingensis* and *E. subspherica* occurred in 6.66, 26.66, 93.33, 6.66, 80.0, 93.33, 6.66 and 73.33%, respectively of the samples for this month. Generally from June to December, most species showed high prevalence in the samples. However, by January (6 months later) the prevalence rates had fallen to 20.0% for *E. bovis*, 26.0% for *E. canadensis*, 13.33% for *E. ellipsoidalis/alabamensis*, *E. cylindrica* and *E. wyomingensis*, 6.66% for *E. zuernii* and 0% for *E. auburnensis, E. brasiliensis, E. subspherica* and *E. pellita*, respectively. Finally, twelve months from the start of sampling (May) the data showed that while oocysts were still recorded from 46.7% of the samples, only four species of *Eimeria*, namely *E. bovis* (26.66%), *E. auburnensis* (13.33%), *E. canadensis* (6.66%) and *E. cylindrica* (6.66%) were still present. This showed that the range of species encountered reduced over the year. Furthermore, there was no indication that the most pathogenic species were the ones, which disappeared since *E. bovis*, considered to be the most pathogenic species, was still present.

A total of 178 faecal samples from calves aged 3 - 4 months were collected for one year at monthly intervals (Fig. 4). The mean number of oocysts (g^{-1} faeces) during June was 1073.30, while in subsequent months a decline in oocyst output g^{-1} faeces was seen such that by May, a reduction of -91.92% was seen with only 86 oocyst recorded. Thus a significant decrease in mean oocysts was observed over the twelve months period. The lowest mean figures were recorded for February and April (21.4, -98.0% & 6.7, -99.4%, respectively). Generally, it can be concluded that not only the number of species declined but those species, which survived were producing many fewer oocysts. This indicated that there was a direct relationship between the diversity and number of parasites and the age of the host animals. Furthermore, a continuous infection with coccidian parasites can result in the development of protective immunity, although complete immunity was seldom developed. This has implications for the management of this disease in the field since animals of this age (one year) can continue to infect younger stock.

All 10 species of the genus *Eimeria* showed different trends in incidence over 12 months of investigation (Table I). At the start of June, *E. zuernii*, *E. ellipsoidalis/alabamensis*, *E. subspherica*, *E. bovis*, *E. cylindrica* and *E. canadensis* showed the highest number of oocysts g^{-1} faeces (310, 300, 183, 66, 153 & 40, respectively). However, *E. auburnensis*, *E. brasiliensis* and *E. wyomingensis* produced only 6.0, 6.0 and 3.0, respectively while *E. pellita* was not recorded at all. During the subsequent months a general reduction in oocyst output by all the species was seen. A statistical examination shows that there was a significant reduction in *E. bovis*, *E. ellipsoidalis/alabamensis*, *E. auburnensis*, *E. cylindrica*, *E. zuernii* and *E. subspherica*, but the figures are inconclusive for *E. canadensis*, *E. brasiliensis*, *E. wyomingensis* and *E. pellita*. Furthermore, an examination of Table II showed that certain species, for example *E. ellipsoidalis/alabamensis*, *E. subspherica* and *E. pellita* were absent by January and February while others (*E. bovis*, *E. canadensis*, *E. auburnensis* & *E. cylindrical*) although present in small numbers, were still evident at the end of the sampling period.

Table I. The incidence (intensity of oocysts g^{-1}) of each species of *Eimeria* in faecal samples

	Months	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Species	*E. bovis*	33.33	73.33	46.66	64.29	26.66	60.00	86.66	20.00	0.00	20.00	6.66	26.66
	E. auburnensis	6.66	40.00	33.33	28.57	26.66	13.33	33.33	0.00	7.14	13.33	0.00	13.33
	E. Canadensis	26.66	66.66	46.66	57.14	53.33	40.00	40.00	26.66	21.42	33.33	0.00	6.66
	E. ellipsoidalis /alabamensis	93.33	26.66	60.00	35.71	26.66	13.33	46.66	13.33	0.00	0.00	0.00	0.00
	E. brasiliensis	6.66	46.66	13.33	21.42	6.66	13.33	13.33	0.00	7.00	6.66	0.00	0.00
	E. cylindrical	80.00	0.00	33.33	21.42	20.00	0.00	6.66	13.33	0.00	6.66	0.00	6.66
	E. zuernii	93.33	26.66	13.33	14.29	20.00	6.66	13.33	6.66	0.00	6.66	6.66	0.00
	E.wyomingensis	6.66	66.66	33.33	50.00	33.33	26.66	53.33	13.33	0.00	26.66	0.00	0.00
	E. subspherica	73.33	6.66	20.00	14.28	13.33	6.66	40.00	0.00	0.00	0.00	0.00	0.00
	E. pellita	0.00	0.00	6.70	0.00	6.00	0.00	13.33	0.00	0.00	0.00	0.00	0.00

Table II. The incidence (intensity of the oocysts g^{-1}) of the mean number of oocysts over twelve months (n=15)

	Months	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	SED	P
Species	*E. bovis**	66	73	43	110	50	80	203	40	00	23	3	56	38.93	0.000
	E. auburnensis	6	33	20	25	13	16	30	0	3	6	0	6	11.79	0.042
	E. Canadensis	40	70	56	53	56	56	50	110	10	33	0	20	47.56	0.689
	E. ellipsoidalis /alabamensis*	300	23	66	171	26	6	76	73	0	0	0	0	61.22	0.000
	E. brasiliensis	6	30	23	25	6	13	13	0	7	3	0	0	13.87	0.344
	*E. cylindrical**	153	0	20	25	10	0	6	0	0	6	0	3	12.81	0.000
	E. zuernii	310	23	6	10	10	6	6	3	0	6	6	0	15.90	0.000
	E. wyomingensis	3	66	26	42	23	16	73	66	0	16	0	0	29.66	0.064
	E. subspherica	183	6	10	7	6	3	30	0	0	0	0	0	16.08	0.000
	E. pellita	0	0	3	0	3	3	10	0	0	0	0	0	3.81	0.256

***** = Considerded to be most pathogenic species (according to the literature).

Fig. 1. The mean live weight gain (kg) of the calves over twelve months (n = 15)

*****By comparison with the starting weight

******By comparison with previous month

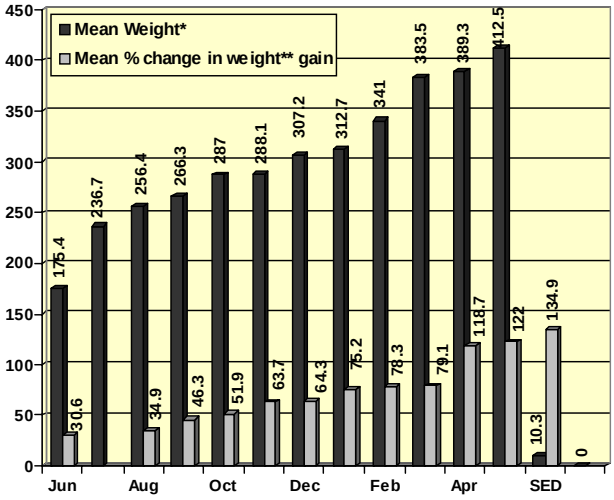
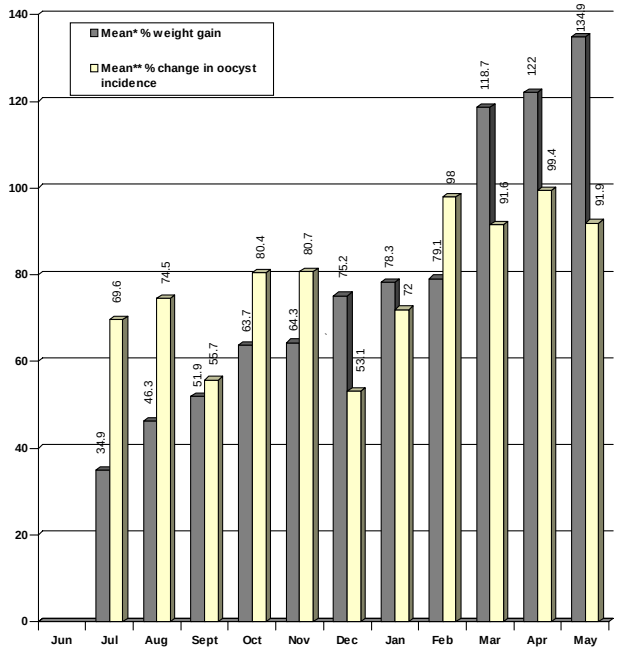


Fig. 2. The mean weight gain (kg) and percentage change (reduction) in mean oocysts during the experimental period

*Figures represent % change in mean weight gain of calves during twelve months period.

**Figures represent % change in oocyst incidence (decrease) during twelve months period.



DISCUSSION

During the present survey on the incidence of *Eimeria* infections in different age groups of cattle, no case of clinical coccidiosis were found and all the calves were healthy and alert, although calves shed oocysts in their faeces. When a comparison was made between oocyst outputs and mean weight gains of calves during twelve months, significant increase in weight gain (Fig. 1) together with a significant decrease in mean oocyst outputs (Fig. 2) was observed. These findings showed is no relationship between oocyst numbers and weight gain in naturally infected calves at least at these levels of infection. Similar findings have also been recorded by other workers (Wade, 1990; Oakley, 1990), who could not find any correlation

Fig. 3. The incidence (intensity of the oocysts g^{-1}) of the combined *Eimeria* sp. in faecal samples of calves during the study period (n = 15)

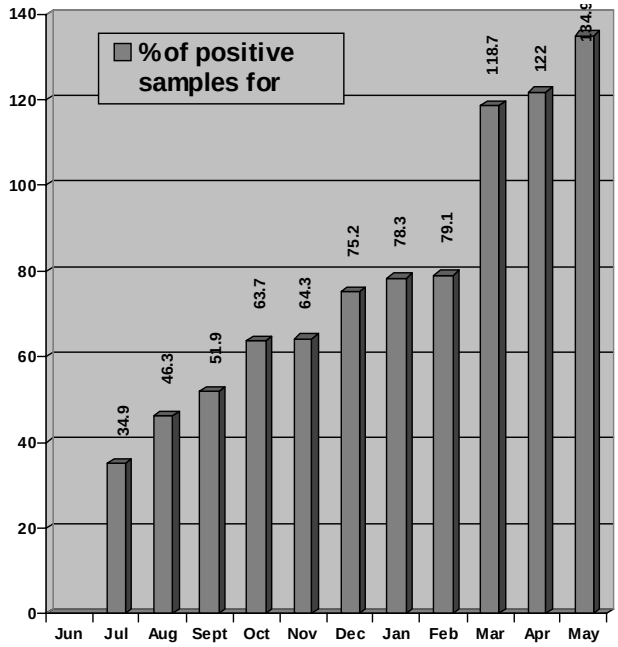
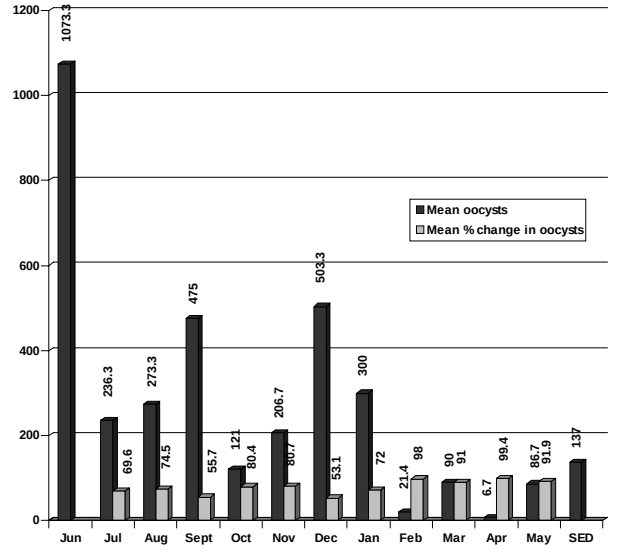


Fig. 4. The mean incidence of oocysts (g^{-1}) of combined and the percentage change (reduction) in oocysts during the experimental period



between the oocyst output and weight gain in naturally infected calves under 3 months of age. On the other hand, Svensson *et al*. (1994) demonstrated that heavy infections (860,000 oocysts g^{-1} faeces of *E. alabamensis*) with any pathogenic species either in naturally or experimentally infected animals could decrease weight gain in young calves (8 days old). Hooshmand *et al.* (1994) found a significant decrease in growth of calves experimentally infected (10 - 400 million sporulated oocysts) with *E. alabamensis* and observed clinical signs of coccidiosis in calves under two months of age. It is clear that the infection rate was probably too low in our animals since no clinical cases were encountered. This conclusion was confirmed by Ernst *et al.* (1984), who also failed to find clinical cases of naturally infected calves. Naturally infected calves, even at a young age can show appreciable weight gains in the presence of a range of *Eimeria* species provided the infection is low and no clinical symptoms are evident.

Species of bovine *Eimeria* can infect all age groups. During the present study ten species of *Eimeria* were identified from calves, which were reported previously by Wade (1990) and Oakley (1990) in their investigations in UK. However, with the exception of *E. bukidnonensis*, the species of *Eimeria* found in our survey, were also described from cattle in South-west England previously by Joyner *et al.* (1966) and USA (Ernst *et al.*, 1984).

The highest incidence of oocysts was recorded in June 100% (calves at pasture) and lowest in April (6.7%). Thus we found the highest incidence in early summer and the lowest in mid winter in Britain, while Sanyal *et al.* (1985) found the highest incidence in the autumn following the end of the monsoon, when temperatures was close to that in Britain. February in India is although warmer than that in Britain, it is the coldest period of the year in Britain. Munyua and Ngotho (1990) working on Kenyan cattle found a significant ($p < 0.05$) seasonal variation in the number of oocysts g^{-1} faeces. The higher output occurred in rainy seasons (March-May & October-November) compared with the dry months (December-February). The incidence of infection is thus determined by those times of the year when conditions are optimal for the survival and sporulation of oocysts. Warm moist conditions favour fast development of oocysts, which means that irrespective of season in different parts of the year maximum transmission will occur under these conditions. It is thus important to consider the effects of housing, since seasonal effects may be minimised. It is well known that young animals are likely to be infected with *Eimeria* species since they will not have had the opportunity to develop protective immunity. Thus, it is not surprising to find that the highest infection rates occurred in the young calves (Fitzgerald, 1962).

The range of species recorded was similar to that reported by other workers, which tended to be most frequent (Lee & Park, 1985; Ernst *et al*., 1987; Munyua & Ngotho, 1990). Svensson *et al.* (1994) recorded the mean monthly oocyst counts in calves (aged 8 - 12 weeks) kept indoors from March to October, as determined in this study. Furthermore, the number of oocysts per gram of faeces we recorded is within the same range as other workers have recorded in natural infestations of calves not showing signs of clinical coccidiosis (Fitzgerald, 1962; Oda & Nishida, 1990; Wade, 1990; Oakley, 1990; Svensson *et al*., 1994). A gradual decrease in oocysts was observed each month such that by month twelve a 78.9% reduction in mean oocyst

output was evident. Plotnikove *et al.* (1981) demonstrated that calves aged 20 days were already infected with coccidia and that maximum intensity and prevalence of infection were reached in 50 - 90 days. Subsequently, the intensity of infection decreased to 0.16 - 0.3 and 0.3 - 3.0 oocysts (per 20 x microscope fields), respectively over a two years period. A significant decrease in the mean number of oocysts of the pathogenic species was recorded during the experimental period compared with the less pathogenic species. This indicates that the host was able to affect oocyst output in the case of these species and also that a protective immunological response is at work. However, given that we are dealing with a natural infection, it is difficult to be certain which mechanisms involves this relationship.

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