

## Patterns of *Eimeria* excretion in young Icelandic calves

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### ABSTRACT

Faecal samples were collected from a total of 11 calves on three dairy farms (four from two farms and three from one) where calves generally thrive well and no anti-coccidial treatment is habitually carried out. One of the farms keeps calves in groups on hay/straw bedding, one keeps calves in pairs on plastic slats and then in groups on concrete slats, and the third one keeps calves in groups on concrete slats. Faecal consistency and the total number of *Eimeria* spp. oocysts per gram faeces (OPG) were determined and species identification was carried out by morphology. *Eimeria* oocysts were detected in all calves at some point of the study period, and diarrhoea was seen in 55% of the calves. The highest peak in oocyst excretion was 69,300 OPG. The first peak in oocyst excretion was seen 2-3 weeks after calves had been moved to group pens, and a second peak was seen 2-3 weeks later. Nine *Eimeria* species were found, including *E. bovis* and *E. zuernii*. The results are in accordance with previous studies showing that one of the risk factors for *Eimeria* infection in calves is entering a group pen where older calves are already being kept.

**Keywords:** Bovine coccidiosis, Iceland, *Eimeria* species, diarrhoea

### YFIRLIT

*Þróun smits og tegundasamsetningar hnísla í íslenskum ungkálfum*

Saursýnum var safnað úr alls 11 kálfum á þremur mjólkubúum (fjórum á tveimur búum og þremur á einu búi) þar sem kálfar þrífast almennt vel og eru ekki meðhöndlaðir með hníslasóttarlyfjum að staðaldri. Á einu búanna voru kálfar í hópstíu á heyi/hálmi, á öðru voru kálfar í parastíu á plastrimlum þar til þeir voru færðir í hópstíu á steiptum bitum, og á þriðja búinu voru kálfar í hópstíu á steiptum bitum. Þéttleiki saurs og fjöldi *Eimeria* þolhjúpa á hvert gramm saurs (OPG) voru metin. Tegundagreining var byggð á útliti þolhjúpa. Hnísar fundust í öllum kálfunum á rannsóknartímanum og 55% kálfanna fengu niðurgang á rannsóknartímanum. Mesti fjöldi hnísla var 69,300 OPG. Fyrsti toppur í útskilnaði hnísla sást 2-3 vikum eftir að kálfarnir höfðu verið settir í hópstíu með eldri kálfum. Annar toppur sást 2-3 vikum eftir það. Níu tegundir hnísla fundust, þar með taldir mestu skaðvaldarnir, *E. bovis* og *E. zuernii*. Niðurstöðurnar eru í samræmi við fyrri rannsóknir um að stór áhættuþáttur í hníslasmiti í kálfum sé að koma í hópstíu þar sem eldri kálfar eru fyrir.

### INTRODUCTION

*Eimeria* is a genus of apicomplexan parasites that parasitize the intestinal tract in several species of livestock, particularly young animals of poultry, rabbits and ruminants. Some *Eimeria* species cause coccidiosis, one of the most

common and economically important diseases in livestock worldwide (Lassen & Østergaard 2012). Symptoms of coccidiosis are diarrhoea (watery to bloody or mucous), reduced appetite, depression, fever, abdominal pain, tenesmus,

dehydration and weight loss, even persistent ill-thrift (Dauguschies & Najdrowski 2005). *Eimeria* spp. are strictly host-specific with a complex life cycle where they undergo rounds of asexual and sexual reproduction intracellularly (Hooshmand-Rad et al. 1994). During this life cycle they damage the intestinal wall of the host, and the intestinal mucosa, and impair the absorptive capacity of the digestive tract (Friend & Stockdale 1980). In Europe at least 13 species have been described in cattle by morphological methods (Dauguschies & Najdrowski 2005), whereof nine have been found in Icelandic dairy cattle (Einarsdóttir 1995). Two of them, particularly (*Eimeria bovis* and *E. zuernii*), are known to be highly pathogenic, causing clinical outbreak of coccidiosis (Bangoura & Dauguschies 2007, Friend & Stockdale 1980). To a lesser extent, *E. alabamensis* has been reported to induce clinical coccidiosis in pastured cattle (Hooshmand-Rad et al. 1994). Infection occurs orally with calves ingesting infective oocysts in the environment. Oocysts become infectious in the environment by sporulation, a process dependent on environmental factors such as temperature and relative humidity (Marquardt et al 1960, Pyziel and Demiaszkiewicz 2015). They can remain in the environment for months (Lassen et al. 2013). Herd prevalence of *Eimeria* spp. in calves can reach 100%, mixed infections being most common, with increased risk for clinical disease in the age group 3 weeks to 6 months, due to lack of immunity (Dauguschies & Najdrowski 2005). Research into the pathogenesis of *E. bovis* and *E. zuernii* has shown that 18-21 days after a high-dose infection, extensive damage can occur in the intestinal mucosa, mostly appearing at the end of the parasite lifecycle, just before oocysts appear in faeces (Friend & Stockdale 1980, Stockdale 1977). Metaphylactic treatment with anticoccidial products such as diclazuril or toltrazuril around 15 days after presumed infection is therefore recommended (Philippe et al. 2014). Important risk factors influencing the severity of *Eimeria*-related disease in calves are population density, stress (due to density, transfer between pens or herds, weaning,

changes in feed), lacking hygiene etc. (Sánchez et al. 2008).

The objectives of this study were to investigate the excretion pattern and species combination of *Eimeria* spp. in Icelandic housed dairy calves during their first three months of life. To this end, faecal samples from individual calves were taken repeatedly throughout the study period, a minimum of 10 times for each calf.

## MATERIALS AND METHODS

### *Farm management systems*

Three Icelandic dairy farms were chosen for sampling by convenience of accessibility, two in Wiceland and one in NEIceland. On farm A, calves were kept in groups of up to 14 on straw/hay bedding and fed soured milk/milk replacer until 9-11 weeks old. Four heifer calves were sampled consecutively. On farm B, calves were kept in pairs on plastic slats and moved to a group of up to 5 calves on concrete slats at around 5 weeks old and fed milk/milk replacer; four calves (three heifers, one bull) were sampled. On farm C, calves were kept in the calving pen on straw bedding for approximately two weeks, then moved to a group of up to 7 calves on rubber-covered concrete slats and fed fresh milk; three heifer calves were sampled. On all farms, calves received colostrum from their mothers in two to three feedings during the first 24 hours before being moved to their pen and had access to good hay from the start. General health and thrift of calves was good on all farms, and no preventive treatment of calves against endoparasites was habitually carried out on the farms. Information on size and milk yield of the farms, calf density in group pens and duration of stay in each pen can be found in Table 1.

### *Faecal samples*

Faecal samples (20-50 ml) were collected weekly rectally by the farmers using a single-use glove and put into a sterile container from each calf. Samples were refrigerated and sent within 24 hours to the Institute for Experimental Pathology. Faecal consistency was assessed on a scale of one to five (1: pelleted, dry; 2: soft

**Table 1.** Pen size for each calf (m<sup>2</sup>/calf), type of flooring and length of stay (in days) in each pen on farms A, B and C.

Farm A, W Iceland: 72 cows/year, total milk yield 540,000 L/year							
Calving pen		Group pen			Weaning pen		
Flooring type	No. days in pen	m <sup>2</sup> /calf	Flooring type	No. days in pen	m <sup>2</sup> /calf	Flooring type	No. days in pen
Straw/hay	1-3	3,9-6,1	Straw/hay	60-71	3,75-5	Straw/hay	21

Farm B, W Iceland: 32 cows/year, total milk yield 205,000 L/year					
Individual/pair pen			Group pen		
m <sup>2</sup> /calf	Flooring Type	No. days in pen	m <sup>2</sup> /calf	Flooring type	No. days in pen
0,8-1,6	Plastic slats	39-72	1,02	Concrete slats	0-55*

Farm C, NE Iceland: 50 cows/year, total milk yield 350,000 L/year					
Calving pen			Group pen		
m <sup>2</sup> /calf	Flooring type	No. days in pen	m <sup>2</sup> /calf	Flooring type	No. days in pen
13-27	Straw/hay	12-14	1,5-2,25	Concrete slats w/rubber covering	68-110

\* The youngest calf stayed in an individual pen throughout the study period.

pelleted; 3: not pelleted, paste; 4: semiliquid/liquid; 5: haemorrhagic and/or mucous). Faecal consistency of 4-5 was classified as diarrhoea.

#### *Parasitological methods and oocyst count*

Faecal oocyst counts were performed using a modified McMaster technique (a slight modification of a technique originally described by Roepstorff & Nansen [1998]) where 4 grams of faeces were suspended in 56 ml of tap water, mixed thoroughly and then sieved through a sieve with an aperture of 0.8 mm. The strained fluid was centrifuged for five minutes at 2,000 rpm (500 G), the supernatant was discarded, and the pellet mixed in saturated magnesium sulphate solution (FASOL® reagent, Kruuse, Denmark), with specific density of 1.225 g/ml. After thorough mixing, two McMaster slides were filled per faecal sample giving a detection limit of 25 OPG (oocysts per gram faeces).

#### *Sporulation and species identification*

Prominent peaks (highest number of OPG) were chosen for each calf for further species identification and the samples were mixed thoroughly with 3% potassium dichromate

and left to sporulate at room temperature for approximately 2 weeks, and then stored in a refrigerator until analysis was performed. Two peak points were chosen for each calf, when possible, and 100 oocysts were identified directly from each peak according to the size and morphology, using published descriptions (Eckert et al. 1995, Dausgies & Najdrowski 2005).

A Leica DMLB microscope was used to count oocysts in the McMaster slides at 10x magnification, and 100x magnification was used to diagnose the oocysts to species level. Oocysts were diagnosed to species by the same person, but a colleague was consulted regarding any uncertainties.

#### *Data handling*

All data handling and graphs were done in Microsoft Excel.

## RESULTS

A total of 130 samples were taken regularly from eleven calves on three farms, eight to 14 samples from each calf, through a period of 16 weeks.

### Faecal consistency

Faecal consistency varied between farms and within farms, with most samples (90.8%) scoring 3 or lower, indicating a general state of soft to dry, formed faeces. Out of the 130 samples, 9.2% (12 samples) scored 4 or 5, indicating diarrhoea. Diarrhoea was diagnosed on all farms, in 55% of the 11 calves, with the highest flock prevalence on farm A. Nine diarrhoea samples were collected on farm A, with three calves first showing diarrhoea at 3-4 weeks of age (Figure 1), and two of them showing another bout of diarrhoea at 11 weeks of age. Two calves on farm B had diarrhoea at three and ten weeks of age, respectively (Figure 2), and one calf on farm C had diarrhoea at six weeks of age (Figure 3).

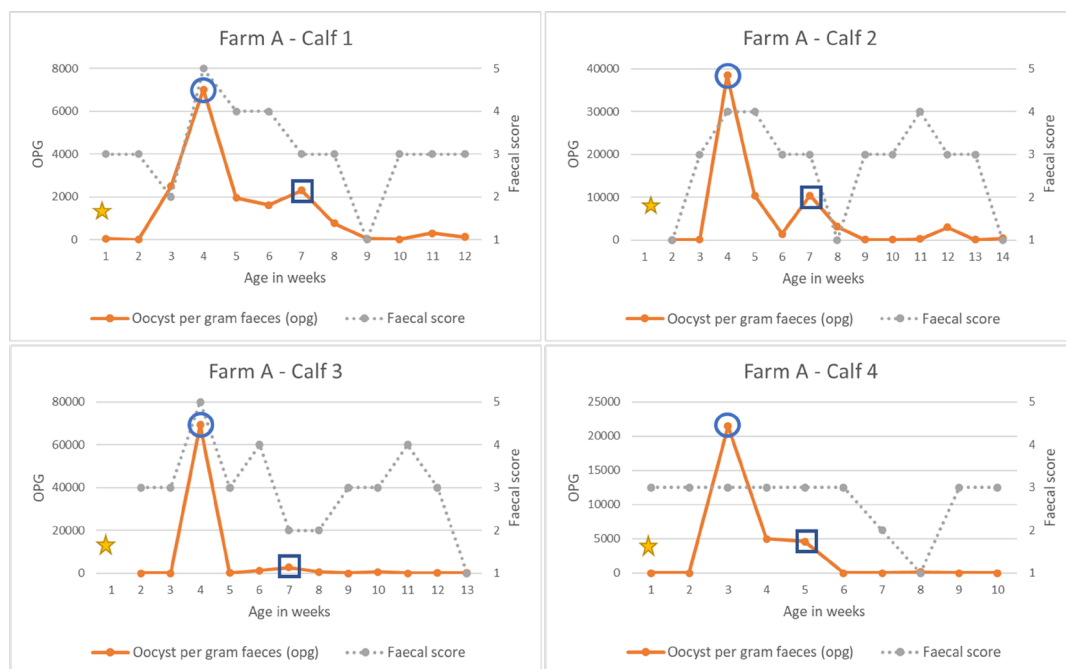
### Oocyst counts

Oocyst counts and coinciding faecal scores for each calf on all three farms are shown in Figures 1-3. Oocysts were found in samples from all calves at some point in the study, therefore yielding 100%

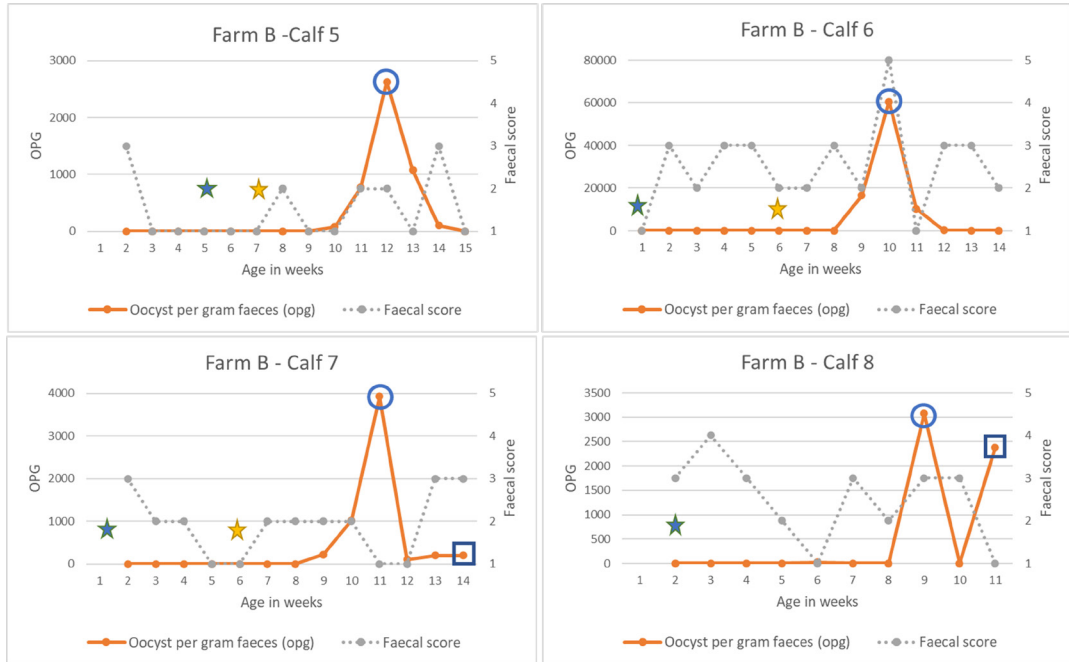
farm prevalence and 100% flock prevalence. However, all samples taken before the calves were moved to group pens yielded an oocyst count of zero. The highest number of oocysts reached 69,300 OPG, with 7% of calves excreting over 10,000 OPG, and considerable individual variation. Three calves on farm A with diarrhoea at 3-4 weeks of age also had peaks in oocyst numbers during that same period (21,450-69,300 OPG). Overall, diarrhoea was not prevalent, but prevalence did increase with increasing OPG (Table 2). On farm C, sampling was unstable, leading to missing data points during the 16-week period, making inference problematic.

### *Eimeria* species composition

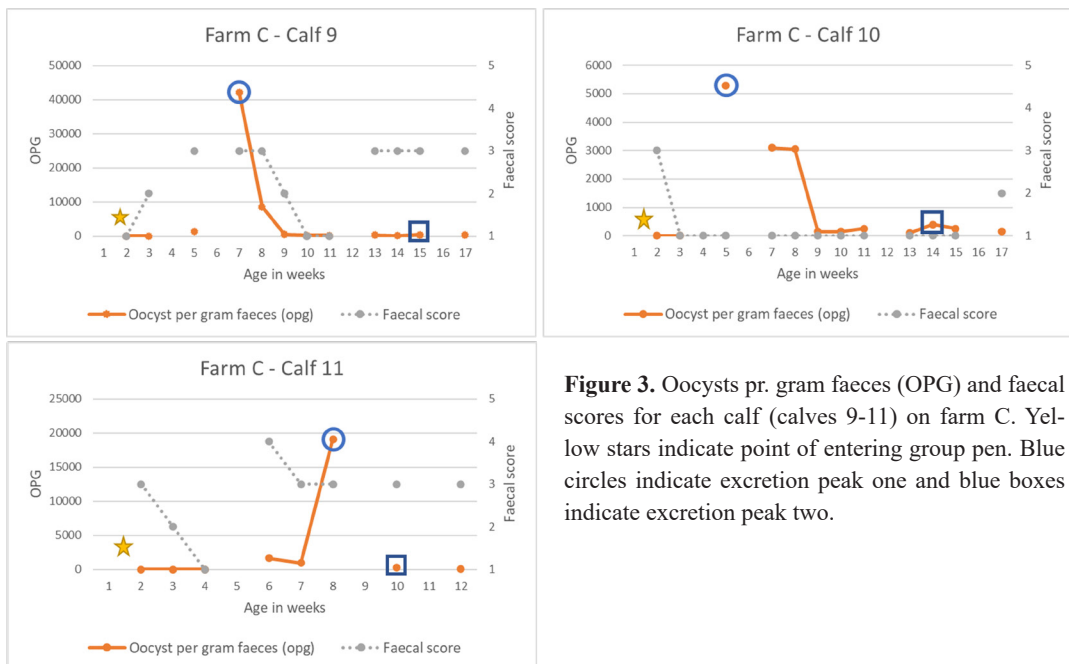
For most calves, one or two peaks were seen in oocyst numbers, as shown in Figures 1-3. On farm A, the first peak was seen 2-3 weeks after calves entered the group pen and a second, much lower peak was seen 2-3 weeks later. On farm B it took 4-5 weeks from entering a group pen for the first peak to appear and where discernible,



**Figure 1.** Oocysts pr. gram faeces (OPG) and faecal scores for each calf (calves 1-4) on farm A. Yellow stars indicate point of entering group pen. Blue circles indicate excretion peak one, and blue boxes indicate excretion peak two.



**Figure 2.** Oocysts pr. gram faeces (OPG) and faecal scores for each calf (calves 5-8) on farm B. Blue stars indicate point of entering pair pen. Yellow stars indicate point of entering group pen. Blue circles indicate excretion peak one and blue boxes indicate excretion peak two, no peak 2 was seen in calves 5 and 6. Calf 8 did not enter group pen during the study.

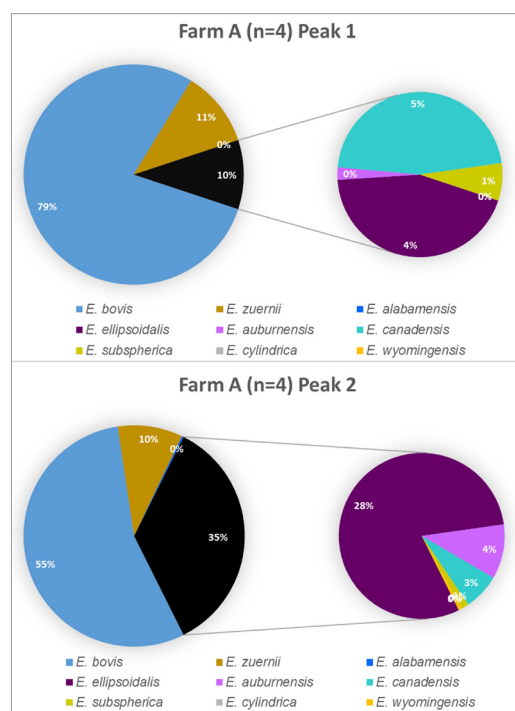


**Figure 3.** Oocysts pr. gram faeces (OPG) and faecal scores for each calf (calves 9-11) on farm C. Yellow stars indicate point of entering group pen. Blue circles indicate excretion peak one and blue boxes indicate excretion peak two.

**Table 2.** Percentage of faecal samples categorized as diarrhoea/no diarrhoea by range of oocysts pr gram faeces (OPG). Diarrhoea: Faecal score 4 and 5; No diarrhoea: Faecal score 1-3.

Oocysts per gram (OPG)	Diarrhoea (%)	No diarrhoea (%)
0	2.3	97.7
1-99	5.6	94.4
100-999	2.9	97.1
1.000-9.999	20.8	79.2
≥10.000	40.0	60.0

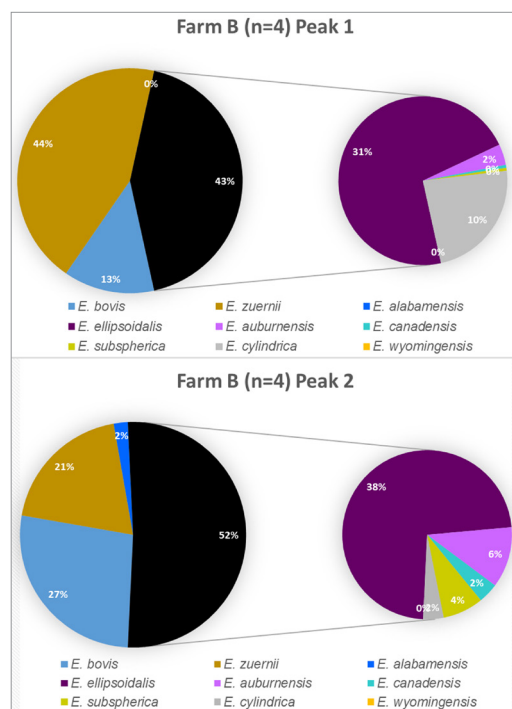
a second peak was seen 2-3 weeks later. On farm C the first peak appeared 5-6 weeks after entering the group pen and a second peak around 3 weeks later. The samples demonstrating



**Figure 4.** Relative *Eimeria* species composition in two oocyte peaks identified in all calves on farm A. The duration from entering group pens, until the two peaks in oocyst counts appeared, was as follows: Peak 1: 2-3 weeks, peak 2: 4-6 weeks. A total of nine *Eimeria* species were identified, with species numbers increasing from peak 1 to peak 2.

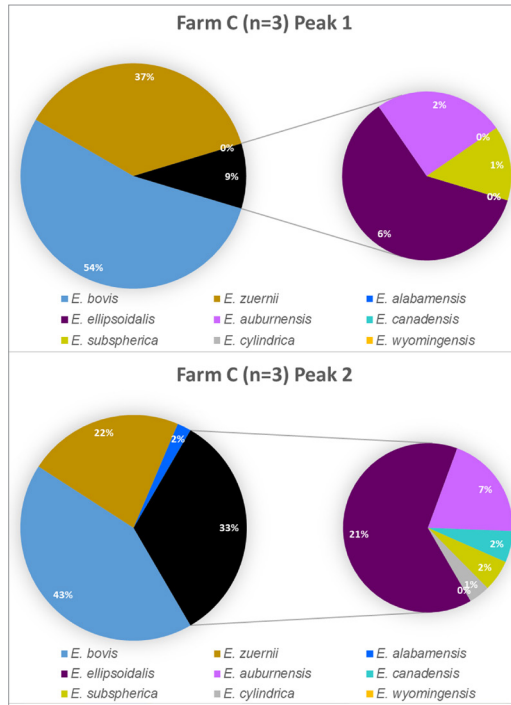
these peaks were chosen for *Eimeria* species identification and composition analysis. Calf 7 on farm B and calf 11 on farm C did not provide an obvious second peak. Therefore, samples timed according to the second peak in other calves were analysed, identifying 25 oocysts. In total, 20 samples were analysed and nine *Eimeria* species identified. The most pathogenic species, *E. bovis* and *E. zuernii*, were identified on all farms, and in both peaks for nine of eleven calves. Calves numbers 5 and 6 on farm B were introduced very late into the group pen, and the timing of an expected second peak was outside of the study period.

Figures 4-6 show the *Eimeria* species composition in two peaks for the combined



**Figure 5.** Relative *Eimeria* species composition in two oocyte peaks identified in all calves on farm B. The duration from entering group pens, until the two peaks in oocyst counts appeared, was as follows: Peak 1: 4-5 weeks, peak 2: 8 weeks. A total of nine *Eimeria* species were identified, with species numbers increasing from peak 1 to peak 2. On farm B only 2 calves exhibited peak 2, one of which did not enter the group pen during the study period.





**Figure 6.** Relative *Eimeria* species composition in two oocyst peaks identified in all calves on farm C. The duration from entering group pens, until the two peaks in oocyst counts appeared, was as follows: Peak 1: 5-6 weeks, peak 2: 8-9 weeks. A total of nine *Eimeria* species were identified, with species numbers increasing from peak 1 to peak 2.

number of calves on each farm. On all farms, in peak 1, *E. bovis* and *E. zuernii* were most noticeable. In general, the third most common species was *E. ellipsoidal*. When a second peak was discernible, *E. bovis* and *E. zuernii* were still the most common on farms A and C, whereas on farm B, *E. ellipsoidal* was more common in the second peak than *E. bovis* and *E. zuernii* in calves numbers 7 and 8. Calf number 8 on farm B was not transferred to the group pen during the study, and only presented four *Eimeria* species (*E. cylindrica*, *ellipsoidal*, *bovis* and *zuernii*), the last two not increasing until the second peak, 9 weeks after entering the pair pen.

## DISCUSSION

All calves were found to excrete *Eimeria* oocysts at some point during their first three months of life. This shows that an *Eimeria* prevalence of up to 100% can be expected in Icelandic calves even though serious clinical signs are not present. The total oocyst counts in this study (over 10,000 OPG in 7% of samples) correspond well to previous studies on natural *Eimeria* infections in this age group. In housed, young calves in Germany, maximum oocyst counts were around 84,000 OPG during the first nine weeks of the calves' life, and the prevalence reached 67% (Faber et al. 2002). In a Danish study the prevalence was around 61% and nearly 4% of the calves had OPG values over 10,000, although in that study the calves were sampled only once, 3-4 weeks following re-housing into common pens (Enemark et al. 2013).

It is evident that calves are infected soon after entering group pens, irrespective of their age and the density of calves in the pen. Sánchez et al. (2008) found calves excreting over 10,000 OPG at four weeks of age, with another peak of 2,000 OPG appearing three weeks later. Similar patterns were seen on all three farms in the present study; however the data from farm C were too sporadic to lend themselves to much comparison. Calf no. 8 on farm B was either penned on its own or with one older calf during the study period and showed a different species composition to the other calves on the farm. The general pattern in this study fits the results of Tomczuk et al. (2015), where a much higher ratio of pathogenic *Eimeria* species was found in calves kept in group pens than calves kept individually or reared by their mothers.

On farm A, where calves were kept in a group pen on straw or hay bedding, with a low density of calves, the peaks of oocyst numbers were generally higher than on farm B, where calves were kept in a group pen on concrete slats. The difference could be due to different floor-types, as previous studies have implied that straw or hay bedding does increase the risk of *Eimeria*-infection and coccidiosis, when compared to slatted and smooth floors

(Bangoura et al. 2011, Lopez-Osorio et al. 2020). Environmental factors play a big role when it comes to sporulation rate and success of the oocysts (Kheysin 1972, Marquardt et al. 1960, Pyziel and Demiaszkiewicz 2015). Factors like relative humidity and temperature might be higher in straw or hay bedding and thereby increase the sporulation rate of the oocysts in the surroundings. Meanwhile a drier environment or lower temperatures on slatted or smooth floors could cause the sporulation to take longer. The development of *Eimeria* infection in housed calves is highly reliant on factors such as calf density (Sánchez et al. 2008) and faecal contamination of the surroundings (Lopez-Osorio et al. 2020) and it is important to prevent the build-up of sporulated oocysts in the calves' surroundings. Studies have shown that disinfectants containing cresol or acetic acid, among others, are effective against oocysts if left in contact with infected surfaces for a sufficiently long time (Bangoura et al. 2011, You 2014).

Another difference between farms A and B was the age of the calves when entering group pens, possibly making the calves on farm B better equipped immunologically to deal with the increased infection pressure put on them by their older pen-mates. It has been indicated that antibodies against *E. bovis* increase from three weeks of age (Faber et al. 2002), although the process is less known for calves where environmental infection pressure is low. According to research by Fiege et al. (1992) a weak natural infection with *E. bovis* early in life did not provide calves with protection when later experimentally infected with 100,000 *E. bovis* oocysts, although the concept of "weak infection" was not clearly defined. Other studies on *E. bovis* indicate that a single infection involving large numbers of oocysts, or repeated infection with moderate numbers of oocysts, will lead to an induction of immunological resistance (Cornelissen et al. 1995).

The calves in this study were able to manage *Eimeria* infection by themselves, as the second peak in oocyst numbers was much lower than the first. This was also demonstrated by Fiege

et al. (1992), who infected calves with 100,000 *E. bovis* oocysts twice, at 15 and 20 weeks of age, with reduced excretion of oocysts after the second infection. Bangoura et al. (2011) found that the total oocyst count, as well as the count of *E. bovis* and *E. zuernii* oocysts, decreased with increasing age.

In the present study, 55% of the calves had diarrhoea at some point during the study, and all but one calf were in a group pen when samples indicative of diarrhoea were collected. A study by Enemark et al. (2013) showed that 36% of calves had diarrhoea 3-4 weeks after being moved to a group pen. The grading system used in the present study might not have been sensitive enough to discover diarrhoea in some instances although it has been widely used (Enemark et al. 2013, Mundt et al. 2005, Svensson 2000), but a less subjective method of measuring the ratio of dry matter in faecal samples could have yielded more accurate results (Hooshmand-Rad et al. 1994). Calf 8 on farm B was put in a pair pen with a 4-week-old calf and had diarrhoea at 3 weeks of age, which might be related to the adjustment to milk replacer, or mild infection with microorganisms, such as protozoa, as the diarrhoea subsided over four days without requiring treatment, and the older calf had not had diarrhoea.

No definite reference value has been defined for oocyst numbers correlated with clinical coccidiosis, but for practical reasons researchers and clinicians use various guidelines. One of five samples in a herd with mild clinical signs, yielding 2,500 OPG, is proposed to be a strong indication of coccidiosis in the herd (Andrews 2000). However, for clinical relevance, it is important to know which *Eimeria* species are present. Sánchez et al. (2008) concluded that oocyst counts of *E. ellipsoidalis* over 12,000 OPG were not associated with diarrhoea, whereas *E. bovis* counts of 4,000 OPG were correlated to diarrhoea in three to five-week-old calves. Bangoura et al. (2011) demonstrated a significantly increased ratio of calves with diarrhoea among those excreting over 500 OPG of *E. bovis* or *E. zuernii*. In a study by Cornelissen et al. (1995), clinical coccidiosis was



not associated with counts of over 1,000 OPG, even for *E. bovis* or *E. zuernii*, demonstrating subclinical infections by these species in calves.

In the present study, samples were collected weekly and therefore it is difficult to know how long episodes of diarrhoea lasted; however on farm A, samples were repeatedly categorised as diarrhoea from the same calves. During an experimental *E. zuernii* infection, calves were noted to have diarrhoea for up to 11 days, with considerable variation in the total duration and association to oocyst numbers (Mundt et al. 2005). We cannot rule out that calves exhibiting the most severe diarrhoea (bloody and mucous) did suffer chronic consequences, but all calves developed well. It would have been preferable to weigh the participating calves regularly, to gather data on their development. However, as the study involved a small number of calves, and there was no control group or reliable data on the weight gain in general on the farms, it was decided not to weigh the calves.

The nine *Eimeria* species identified were the same as previously identified in single samples from Icelandic cattle by Einarsdóttir (1995). The number of species found agrees with research in other countries, although some variation in species can be expected. Seven to nine species have been found in German calves (Faber et al. 2002), and 12 species in Dutch (Cornelissen et al. 1995) and Polish calves (Klockiewicz et al. 2007). In all instances, the infection was a mixed one, with at least two, and in most cases four, *Eimeria* species. Species identification of *Eimeria* oocysts by morphological methods is time consuming and requires experience and good knowledge of the character of each species. Even though morphological identification did not pose any considerable problems in the present study, future studies could include PCR methods to guarantee more accurate identification (Lopez-Osorio et al. 2020, Ekawasti et al. 2019).

The species combination seen in this study is in accordance with previous observations (Faber et al. 2002). Research has shown that oocysts of *E. bovis*, *E. zuernii* and *E. auburnensis* are found in faecal samples from three-week-old

calves, indicating that infection can occur very soon after birth, as the prepatent period for these species can be as short as 15 days (Rommel 2000). The prepatent period for *E. ellipsoidalis* is between 8-13 days and therefore it would be theoretically possible for two-week-old calves to excrete oocysts of this species (Bangoura & Bardsley 2020). The third pathogenic species, *E. alabamensis*, was not common in the present study, although it was identified in the second oocyst peak on all three farms, a pattern evident in previous studies (Faber et al. 2002). It has been shown that it can be beneficial for calves to encounter and develop immunity against this species during housing to better withstand the increased infection pressure when put to grass (Svensson 2000).

The study demonstrates the importance of considering *Eimeria* as a cause of ill-thrift in young calves or diarrhoea in calves aged three to 12 weeks. The aim of this study was to monitor the development of *Eimeria* in young calves. Diarrhoea was not a persistent health problem in the studied herds, and therefore other pathogens, such as *Cryptosporidium* spp., *Giardia* spp., or bacteria and viruses were not investigated in this study.

## CONCLUSION

This study has shown that Icelandic dairy calves are at risk of being infected by highly pathogenic *Eimeria* species, and that there is reason to consider these pathogens when dealing with ill-thrift and diarrhoea in calves during the first months. To reduce the effect of *Eimeria* infection on the development and health of young calves it is important to realise that oocysts are invariably found in their surroundings, and therefore it is imperative to focus on the risk factors of clinical coccidiosis. It is also important to focus on the factors increasing the numbers of infective oocysts in the environment, such as floor type, hygiene measures and density of calves in the pen, so that the risk of coccidiosis is minimised. Calves will recover from mild *Eimeria* infection, but for that to occur the infection will need to be enough to encourage an immunological reaction.

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