PREVALENCE OF PATHOGENIC SPECIES OF *EIMERIA* AT DUTCH DAIRY FARMS WITH CORRESPONDING RISK FACTORS

RESEARCH PROJECT VETERINARY MEDICINE UNIVERSITY UTRECHT
Elske van Balen
3381722

Project tutors:
Dr. T. van Werven
Dr. R. Jorritsma

Abstract

With this research the prevalence of *Eimeria* oocysts excretions of calves at Dutch dairy farms was examined, by performing two McMasters on rectally obtained feces samples. This way the OPG count as well as the oocysts determination was determined. All farms which participated in this research were client at the University Veterinary practice for farm animals. Samples were collected in October-December 2012.

In total 488 individual samples were collected of 66 farms. On every farm, calves were divided by pen and age into pens of age group I and II, which resulted in 143 pens in total. Age group I contained 42 completely analyzed pens with 144 individual samples, and age group II contained 49 completely analyzed pens with 166 individual samples. Of the 406 individual analyzed samples, 69.5% were positive on *Eimeria* pathogenic or apathogenic oocysts. The prevalence found of farms positive on at least pathogenic *Eimeria* oocysts was 87.9%, prevalence in age group I was 48.5% and in age group II 65.2%. Of the pathogenic *Eimeria* species, *E.alabamensis* was most common. Farm prevalence of *E.alabamensis* oocysts was 69.7%, prevalence in age group I was 34.9% and in age group II 45.5%. *E.bovis* was second most common found pathogenic *Eimeria* species, with a farm prevalence of 53%. Prevalence in age group I was 25.8% and in age group II 34.9%. Least found pathogenic *Eimeria* species was *E.zuernii* with a farm prevalence of 25.8%, prevalence in age group I was 10.6% and in age group II 18.2%. Prevalence's of all pathogenic *Eimeria* species were higher in older calves (age group II), compared to younger calves (age group I).

Although the OPG's of all individual samples of calves in age group I didn't differ significantly from calves in age group II, the OPG of individual samples of pens of age group I positive on oocysts of pathogenic *Eimeria* species were significantly higher compared to samples of age group II positive on oocysts of pathogenic *Eimeria* species. Also the OPG of individual samples of pens of age group I positive on oocysts of pathogenic *Eimeria* species were higher compared to pens of age group I positive on oocysts of apathogenic *Eimeria* oocysts. The same results were found for the mean OPG pen per and the highest OPG per pen.

No association was found between the OPG count and the occurring problems per pen, nor between the oocysts determination and the occurring problems per pen.

Because the OPG count of calves after weaning infected with pathogenic *Eimeria* oocysts is not higher compared to apathogenic oocysts, and no association is made between pathogenic *Eimeria* species and occurring problems nor for the OPG count and occurring problems, it is concluded that oocyst determination is necessary to find out if calves are infected with pathogenic or apathogenic *Eimeria* species. It is best to perform oocysts determination on pooled samples, because there was found a large range in OPG count between individual samples within pens.

It was tried to identify risk factors for *Eimeria* infections, but no clear risk factors were found.

Introduction

Rearing young stock is a very important part of managing a dairy farm.(1) During this first phase of a cow's life, the animal is very susceptible for all kinds of infections that can cause disease or even dead. Intestinal and respiratory problems are common problems which cause reduction of the health status of calves.(2, 3) Intestinal problems often cause diarrhea, which leads to weight loss, reduced growth and a higher first calving age, all resulting in economic losses.(1) Diarrhea is a very general symptom that occurs due to a lot of causes. Diarrhea can be caused by viruses, bacteria, protozoa and is favored by factors associated with housing and hygienic conditions.(1, 4)

Coccidiosis is a protozoic disease in calves caused by *Eimeria spp*.(5)(6-9) Clinical coccidiosis is characterized by diarrhea and leads to huge economic losses by reduced feed consumption and impaired growth that can be attributed to a higher feed conversion.(5, 9) Also when coccidiosis does not result in clinical problems it can cause economic losses by destroying the endothial cells of the intestine and therefore affecting the digestive process.(10, 11) This can result in impaired growth, and it possibly results in a higher age at calving age for heifers.(8)

For a better understanding of the clinical signs of coccidiosis, knowledge of the lifecycle of species of *Eimeria* is necessary. The lifecycle of all *Eimeria* species is very similar and consists of endogenous phase inside cells of the intestine of the host and an exogenous part. During the endogenous part merogony as well as gametogony takes place. The endogenous part starts with the uptake of sporulated oocysts by the host. The sporozoites invade the endothelial cells of the ilial vili after crossing the mucosal cells. In the endothelial cells merogony takes place and the sporozoits first develop to trophozoits and then develop to meronts. By asexual replication a lot of merozoits are formed within the meront. When the endothelial cell of the host ruptures, all the merozoits invade surrounding endothelial cells. Then merogony takes place again, but now smaller merozoits are formed. These smaller merozoits develop into male microgamonts and female macrogamonts after these endothial cell ruptures. Next the macrogamont gets fertilized by the microgamonts and a zygote is formed. A wall is built around the zygote which results in an oocyst. These oocysts are excreted by the feces of the host. Now the exogenous part of the lifecycle starts. Once the oocyst is excreted and the circumstances are suitable, sporulation starts and sporozoits are formed.(10)

Coccidiosis is a common problem in calves in general.(5, 10) Studies have shown high prevalence's of *Eimeria* oocyst excretion of 8% up till 100% at farms in general.(5) For example Kounty et al. showed a prevalence of *Eimeria* oocysts in manure of cattle in Austria of 97.97%.(9) However, recent studies which investigate the prevalence of *Eimeria* oocyst excretion in cattle in The Netherlands are missing. The most recent research that describes the prevalences of *Eimeria* oocyst excretion in The Netherlands was performed by Cornelissen et al. in 1995.(6) In this research all 38 farms were tested positive for a certain oocyst species. In total twelve species of *Eimeria* have been identified.(6) *E. bovis* was the most common infection of *Eimeria* in calves, with a prevalence of 27.8%.(6) Even though recent prevalence's of *Eimeria* oocyst excretion in cattle in The Netherlands is missing, assuming these facts it can be expected that the prevalence of *Eimeria* oocyst excretion in The Netherlands will be high, and therefore coccidiosis will be a common problem in The Netherlands.

Research has been performed by others to identify risk factors which contribute to the *Eimeria* infection, and a lot of factors have been mentioned in literature. Cornelissen et al. mentioned the *Eimeria spp.* that are involved, the age of the calves, the number of oocysts ingested and the presence of other infections at the same time are important factors that contribute to a coccidiosis infection becoming clinical.(6) Risk factors like larger herd size (5), having a non-slatted floor (5, 10), poor hygienic conditions (9, 10), poor climatic conditions in the stable(9, 10), high stress levels of the calves(9) and high animal density (10) are mentioned to contribute to a higher oocyst excretion of *Eimeria*. The mix of risk factors and their importance may differ between countries, while e,g,

housing conditions have changed over time. Thus, it would be interesting to update the knowledge on risk factors in The Netherlands.

By knowing the degree of coccidiosis infection in calves and the corresponding risk factors specifically on Dutch dairy farms, there will be better understanding in the coccidiosis problems in The Netherlands. Hopefully this will lead to improvement of young stock, a younger calving age in heifers and to smaller economic losses due to coccidiosis on Dutch dairy farms.

The aim of this cohort-study was therefore to investigate the prevalence of pathogenic *Eimeria* oocyst excretion at Dutch dairy farms, in order to determine a species specific prevalence of coccidiosis in The Netherlands. In this research an attempt is made to identify a correlation between the amount of *Eimeria* oocystes found in the feces samples and the species responsible for the excreted oocystes in the feces in order to improve the detection of the infection. Risk factors which contribute to the *Eimeria* infections at Dutch dairy farms in the Netherlands are examined by administered questionnaires.

Materials and methods

In order to investigate the prevalence of the excretion of *Eimeria* oocysts in calves, feces samples are taken rectally from calves at Dutch dairy farms. The samples were collected from October-December.

Selection of farms

Farmers, who are client at the University Veterinary practice for farm animals, were approached if they wanted to corporate with this research irrespective of the health status of their calves. This resulted in the inclusion of a group of 66 dairy farms. At each farm three to five samples were collected per age group. Age group I contains samples of calves older than 21 days until weaning, age group II contained samples of weaned calves till 164 days old. Samples were collected according to the flowchart shown in figure 1. The samples were temporarily stored individually in a rectal examination glove.

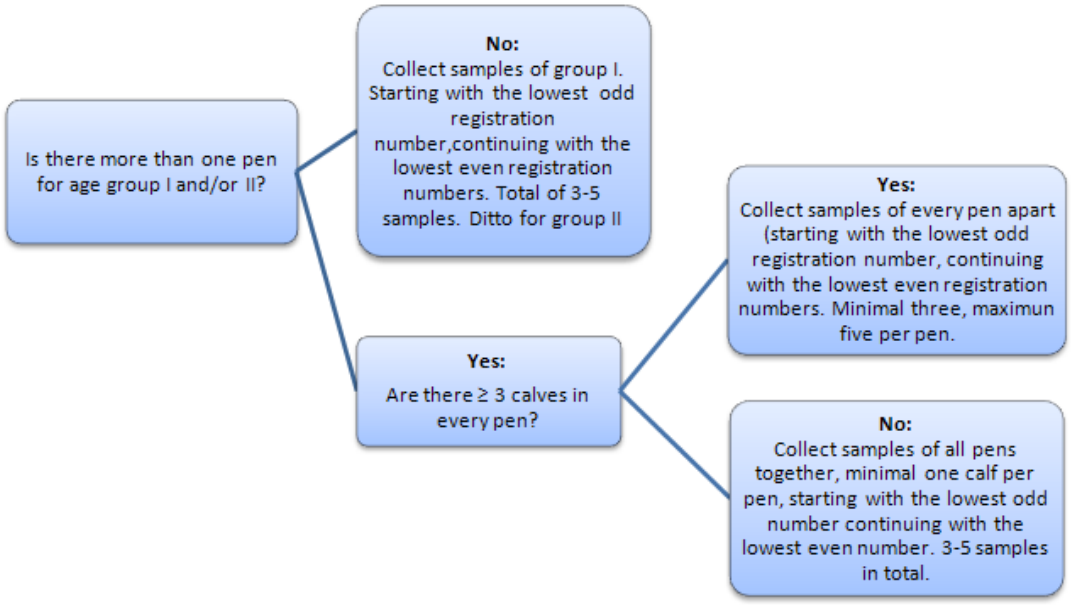

Fig. 1: Flowchart of sampling.

Individual McMaster

A McMaster of a subsample of three grams of the individual feces samples was performed at the University Veterinary practice for farms animals. Three grams of the fecal samples were mixed with 42 ml of saturated salt solution (1,2 gram/cm^{-3}). After having the mixture of feces and salt solution sieved, the mixture was put into a McMaster slide. The slide was examined with a light microscope, using the 200x magnification. The minimum detection level using this McMaster test was 50 OPG; no differentiation of the oocysts was made.

McMaster of pooled samples

The remainder of all fecal samples was sent to the lab of the Animal Health Centre in Deventer. Here the samples were pooled per pen or per age group according to the flowchart in figure 1. Then, another McMaster was performed to identify the different types of *Eimeria*. For this Mc Master, five grams of feces was used. The differentiation was based on morphological criteria. Difference was made between the total of non-pathogenic *Eimeria* species, *E. zuernii, E. bovis* and *E. alabamensis.* Figure 2 shows microphotographs of *E.alabamensis, E.bovis* and *E.zuernii*. The outcome 'negative on oocysts of pathogenic *Eimeria* species' was made when they found at least 100 oocysts of apathogenic *Eimeria* species, or no oocysts at all. The outcome of this test gave qualitative results about the presence of *Eimeria* oocystes in the pooled feces samples.

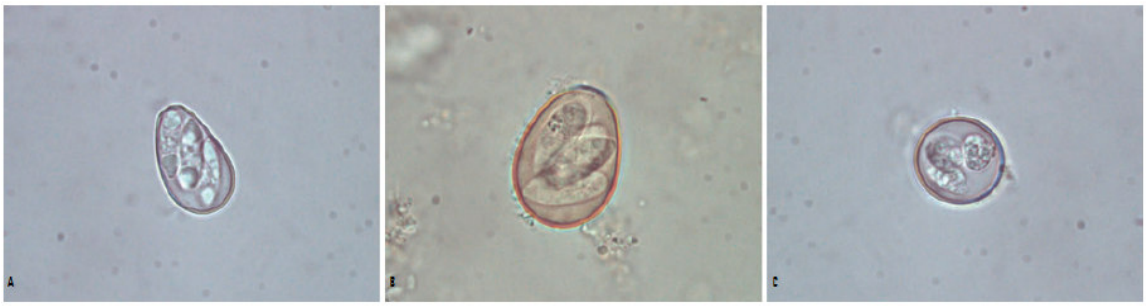

Fig. 2.: Microphotographs of A: E.alabamensis, B: E.bovis and C: E.zuernii (Magnification 1000x) (10)

Questionnaire

To identify risk factors for the excretion of *Eimeria* oocystes in the feces of calves, a questionnaire was filled in by the veterinarian, who also took the feces samples, along with the farmer. This questionnaire contained questions about the housing, feeding and care of the calves.

Statistics

Analyses were performed using the analytical software SPSS version 20. Associations between OPG values, results of the Animal Health Center and results of the questionnaire were analyzed using a Kruskal Wallis test or a Man Whitney U test. A P-value <0.05 was considered significant.

Results

McMaster results of individual samples

In total, 488 individual samples were taken from 66 farms. The total number of pens was 143. The average number of individual samples per farm was 7,4 samples and the average number of samples per pen was 3,4. The average number of individual samples in age group I was 3.8, with an average of 3,4 samples per pen. The average number of samples in age group II was 3,6, with an average of 3,4 samples per pen.

From 91 pens, all samples were analyzed, leaving 52 pens in which not all individual samples were analyzed. In the completely sampled 91 pens, 3.4 samples were collected on average, resulting in a total of 310 individual samples. Divided into age groups, 144 samples were collected from 42 pens in group I. In age group I, three groups contained two pens, while all other age groups in this category consisted of one pen only. Another 166 samples were collected from 49 pens in age group II. In age group II only two groups contained two pens. Of these samples, the minimum OPG in age group I is 50 and the maximum OPG is 123 000. The median OPG of these samples in age group I is 350. In age group II the minimum OPG of these samples is 50 and the maximum OPG 9100. The median OPG of these samples in age group II is 200. In both age groups, a dig difference in OPG count of individual samples within one pen is seen. The biggest range in OPG count within one pen was a minimum OPG of 250, and a maximum OPG of 123 000.

OPG distribution

Of the total 488 individual samples, 406 samples were analyzed by the McMaster method to determine the oocysts per gram feces. 284 samples contained oocysts and 122 samples contained no oocysts of *Eimeria* species. The frequency distribution of the positive individual samples is shown in figure 3. OPG's over a value of 5 000, 14 samples were excluded because of illustrational reasons.

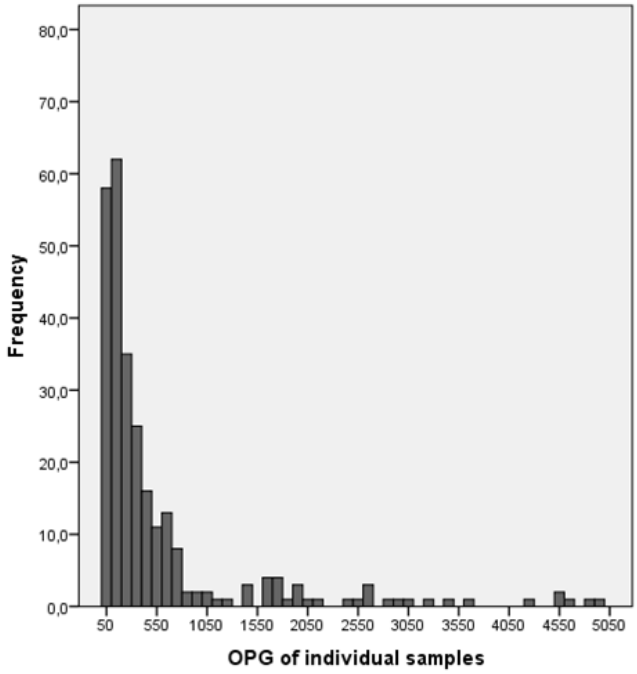

Fig.3: OPG distribution of the McMaster of individual samples positive on oocysts of Eimeria species. Number of samples: 284, with a minimum OPG of 50 and a maximum OPG of 123 000. The median OPG is 150.

The results of the McMaster of individual samples positive on *Eimeria* oocysts are divided into age groups and shown in figure 4, which illustrates the distribution of the OPG per age group.

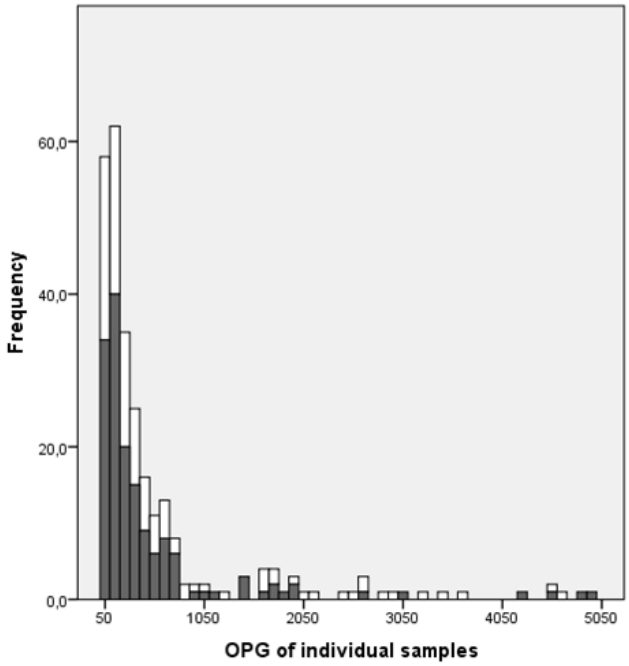

Fig.4: OPG distribution of the McMaster of individual samples positive on oocysts of Eimeria species of age group I and II. The blank bars represent age group I, and the grey bars represent age group II. Age group I contains 124 results, with a minimum OPG of 50 and a maximum OPG of 123 000. The median OPG in age group I is 300. Age group II contains 160 samples, with a minimum of 50 and a maximum of 42 000. The median OPG in age group II is 200.

Statistics showed no significant difference in OPG of age group I and age group II (p-value 0.051).

Prevalences of positive results on oocysts of pathogenic *Eimeria* species

Based up on the results of the McMaster of individual samples, the prevalence of samples positive on oocysts of Eimeria species, pathogenic as well as apathogenic is 69.95%. This prevalence is based on 406 samples.

Prevalence of oocysts of apathogenic and pathogenic *Eimeria* species found in feces samples per farm, which contains both age groups, and apart per age group is given in table 1. A farm or age group is ´positive´ if any oocyst of that category is found at that farm or age group, regardless the amount of oocysts found. For the result per farm, results per pen were merged. Also if an age group contained more than one pen, the results of the pens were merged.

	No oocysts	Only non-apathogenic oocysts	Only pathogenic oocysts	Both pathogenic and non-pathogenic ooysts
Farm in total	5 (7.6%)	3 (4.5%)	7 (10.6%)	51 (77.3%)
Group I	21 (31.2%)	13 (19.7%)	3 (4.5%)	29 (43.9%)
Group II	12 (18.2%)	11 (16.7%)	11 (16.7%)	32 (48.5%)

Table 1. Prevalence of pathogenic oocysts, non-pathogenic oocysts and their combination per farm (N=66), per age group within the farm (N=66) (group I: between 21 days and weaning; group II: between weaning and 164 days of age) and for all pens together (143).

Prevalences of pathogenic species of farms and for the age groups apart were specified for specific *Eimeria* species in table 2.

	Any of the pathogenic *Eimeria* species	**At least *E. alabamensis***	**At least *E.bovis***	**At least *E.zuernii***
Farm in total	58 (87.9%)	46 (69.7%)	35 (53.0%)	17 (25.8%)
Age group I	32 (48.5%)	23 (34.9%)	17 (25.8%)	7 (10.6%)
Age group II	43 (65.2%)	30 (45.5%)	23 (34.9%)	12 (18.2%)

Table 2. Prevalence of pathogenic oocysts, and specific for at least oocysts of E. alabamensis, E.bovis and E.zuernii for 66 farms and divided into the two age groups (group I: between 21 days and weaning; group II: between weaning and 164 days of age) within farms.

Prevalences of table 1 can be disaggregated for the each pathogenic *Eimeria* species with or without the presence of oocysts of apathogenic *Eimeria* species; this is shown in table 3.

	No oocysts	**Oocysts of apathogenic ES**	**Oocysts of E.alabamensis**	**Oocysts of E.alabamensis and apathogenic ES**	**Oocysts of E.bovis**	**Oocysts of E.bovis and apathogenic ES**	**Oocysts of E.zuernii**	**Oocysts of E.zuernii and apathogenic ES**	**combination of oocysts of pathogenic ES**	**Combination of oocysts of apathogenic and pathogenic ES**
Farm in total	5 (7,6 %)	3 (4.5%)	2 (3.0%)	12 (18.2%)	2 (3.0%)	8 (12.1%)	0 (0.0%)	1 (1.5%)	4 (6.1%)	29 (43.9%)
Age Group I	21 (31.8%)	13 (19.7%)	2 (3.0%)	8 (12.1%)	1 (1.5%)	5 (7.6%)	0 (0.0%)	3 (4.5%)	1 (1.5%)	12 (18.2%)
Age Group II	12 (18.2)	11 (16.7%)	4 (6.1%)	9 (13.6%)	3 (4.5%)	7 (10.6%)	0 (0.0%)	2 (3.0%)	4 (6.1%)	14 (21.2%)

Table 3. Prevalence of positive results of specific pathogenic oocysts, with or without presence of oocysts of apathogenic Eimeria species (ES) for 66 farms and divided into the two age groups (group I: between 21 days and weaning; group II: between weaning and 164 days of age) within farms.

Comparison between the OPG count and oocysts determination

Comparison is made between the OPG count of the McMaster of individual samples of pens which were completely analyzed and the oocyst determination of the McMaster performed by the Animal Health Center. Comparison between the OPG count of pens of the complete farm, so age group I as well as age group II, positive on oocysts of only apathogenic or at least pathogenic *Eimeria* species is made. In total, there were 30 samples of pens positive on oocysts of only apathogenic *Eimeria* species and 153 samples of pens positive on at least pathogenic *Eimeria* species. Comparison of these OPG counts of individual samples showed that samples with oocysts of at least one pathogenic *Eimeria* species have a significant higher OPG compared to samples positive only on oocysts of apathogenic *Eimeria* species (P-value 0.023).

The same comparison is made for the mean OPG count per pen, of completely analyzes pens also containing individual McMaster results of 0 oocysts per gram, and for the highest OPG per pen, of completely analyzed pens. For both comparisons, the category of samples positive on oocysts of only apathogenic *Eimeria* species contained 13 mean McMaster results and the category of samples

positive on oocysts of at least pathogenic *Eimeria* species contained 56 McMaster results. For both comparisons a significant difference was shown. The mean oocyste count in samples with at least one pathogenic *Eimeria* species was higher compared to samples with only apathogenic *Eimeria* species only (P-value: 0.015), and the highest OPG per pen in samples with at least one pathogenic *Eimeria* species was higher compared to samples with apathogenic *Eimeria* species only (P-value 0.011).

Figure 5 shows the distribution of the individual OPG count >0, of completely analyzed pens, apart for pens of age group I or II positive in on oocysts of only apathogenic *Eimeria* species and pens positive on oocysts of at least pathogenic *Eimeria s*pecies. OPG's over a value of 5000 are excluded because of illustrational reasons. Statistics showed the OPG of individual samples in these categories differ significantly (P-value 0.008). The total amount of McMaster results used for this test was 183. All combination between the four categories were tested, and the OPG of individual samples of age group I with at least one pathogenic oocyst was significantly higher than in age group II with at least one pathogenic oocysts (P-value 0.009). Also, in the OPG of individual of samples in age group I with at least one pathogenic oocyst was significantly higher than the OPG of samples in age group I without pathogenic oocysts (P-value 0.049). Also the OPG of individual samples was significant higher for age group I positive on oocysts of at least pathogenic *Eimeria* species compared to age group II with only apathogenic *Eimeria* species (P-value 0.025).

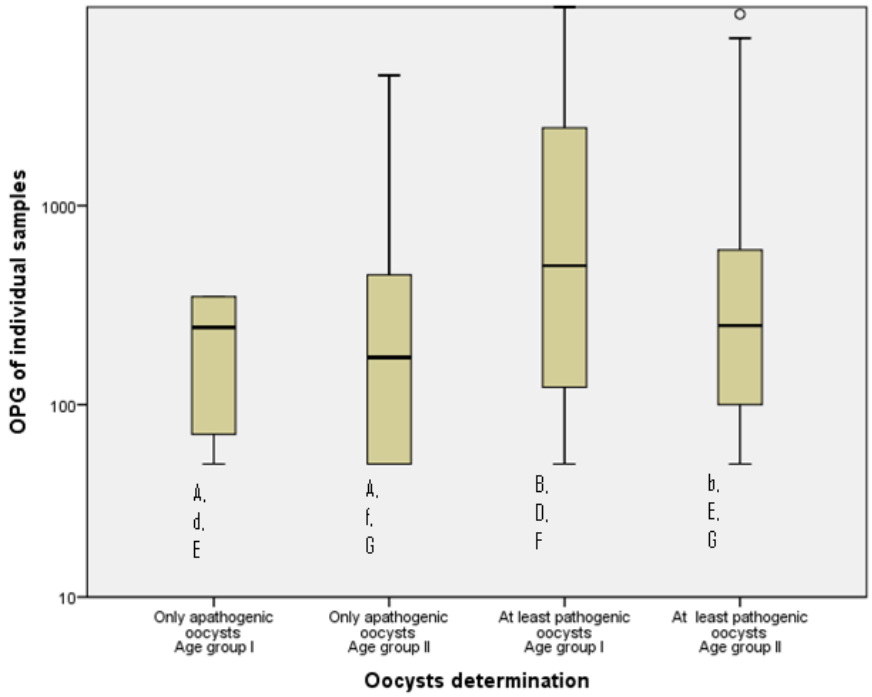

Fig. 5. OPG counts >0 of individual samples of completely analyzed pens apart for age group I and group II positive on oocysts of apathogenic or at least pathogenic Eimeria species. With a maximum OPG of 5000.

Distribution of the mean OPG per pen, which contain also values of 0 OPG, of completely analyzed pens, for pens of age group I or II positive in on oocysts of only apathogenic *Eimeria* species and pens positive on oocysts of at least pathogenic *Eimeria s*pecies, is shown in figure 6. Mean OPG's per pen over 4000 are excluded for illustrational reasons. All categories together contained 69 mean McMaster results. Statistics were performed and showed a significant difference between the categories (P-value 0.010). Again, all combinations between the four categories were tested. And a significant difference was shown between age group I and II positive on oocysts of pathogenic

Eimeria species (P-value 0.015). Age group I had a significant higher mean OPG per pen compared to age group II. Age group I positive on oocysts of at least pathogenic Eimeria species was significant higher than age group I positive on only apathogenic oocysts. (P-value 0.024). Between age group II positive on apathogenic *Eimeria* oocysts and age group I positive on oocysts of pathogenic *Eimeria* species, age group I positive on oocysts of pathogenic *Eimeria* species had a significant higher mean OPG per pen (P-value 0.029).

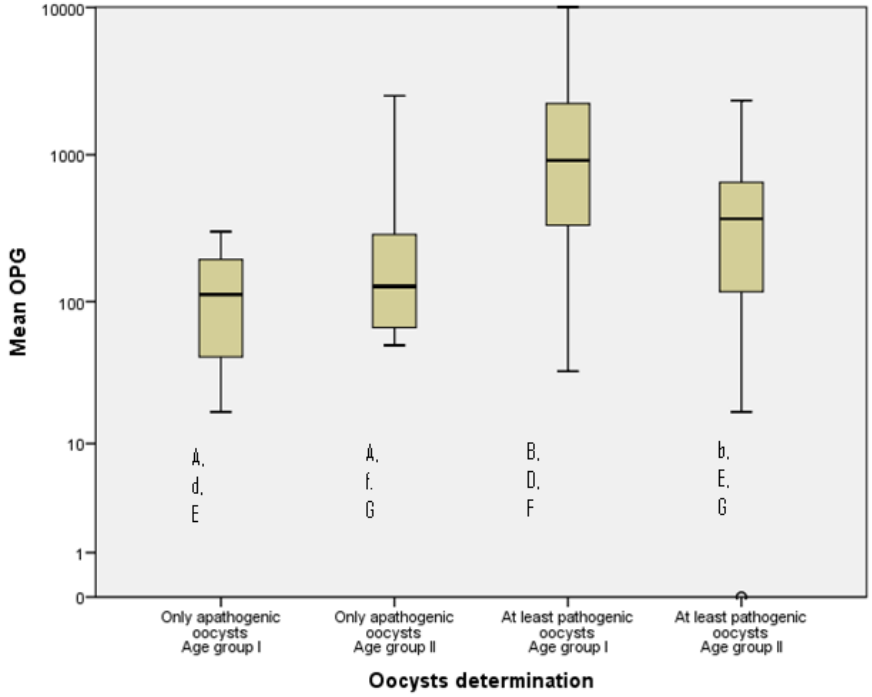

Fig.6. Mean OPG per pen (which also contains individual OPG's of 0) of completely analyzed pens apart for age group I and group II positive on oocysts of apathogenic or at least pathogenic Eimeria species. With a maximum OPG of 4000.

The same statistics were performed for the highest OPG in a pen, only using OPG's >0, for pens of age group I or II positive in on oocysts of only apathogenic *Eimeria* species and pens positive on oocysts of at least pathogenic *Eimeria s*pecies. The distribution of highest OPG per pen for these categories is shown in figure 9. Highest OPG's per pen over 5000 are excluded for illustrational reasons. Again, a significant difference was shown between these categories (P-value 0.009), and all combinations between the four categories were tested. Like comparison with the OPG of individual samples and the mean OPG per pen, the highest OPG per pen significantly differs between age group I and II positive on oocysts of at least pathogenic *Eimeria* species (P-value 0.009). Highest OPG per pen of age group I with oocysts of pathogenic *Eimeria* species is significantly higher compared to age group II. Highest OPG per pen of age group I positive on oocysts of at least pathogenic *Eimeria* species is significantly higher compared to age group I positive on oocysts of only apathogenic oocysts (P-value 0.020). Also there is a significant difference in highest OPG pen pen between age group II positive on oocysts of only apathogenic *Eimeria* species and age group I positive on oocysts of pathogenic *Eimeria* species (P-value 0.028). Highest OPG per pen of age group I positive on oocysts of pathogenic *Eimeria* species is significantly higher.

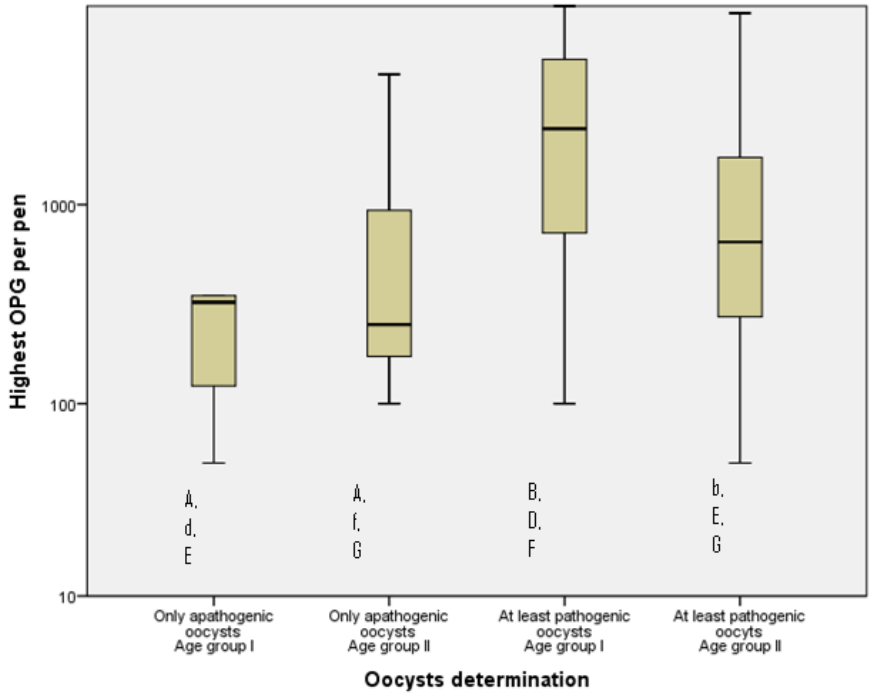

Fig.9. Highest OPG per pen, only OPG's>0, of completely analyzed pens apart for age group I and group II positive on oocysts of apathogenic or at least pathogenic Eimeria species. With a maximum OPG of 5000.

Comparison between OPG count and occurring problems

A comparison was made between the OPG count of completely analyzed pens and the problems which occur per pen according to the farmer. Occurring problems were divided into six categories: No occurring problems, diarrhea, poor calves, respiratory problems, other problems and a combination of problems. Now an OPG value of 0 oocysts is taken into account because the occurring problems didn't had to be caused by *Eimeria* species. Statistics showed no significant difference in OPG of individual samples for these categories for both age groups (N 310, P-value 0.201). Same test did not show a significant difference in mean OPG per pen for the occurring problems (N 91, P-value 0.510), nor in the highest OPG per pen for the occurring problems (N 91, P-value 0.421).

When the occurring problems are classified into age groups, no significant difference in OPG of individual samples is found for the occurring problems (P-value 0.073), nor for mean OPG per pen (P-value 0.552) or the highest OPG per pen (P-value 0.513).

Comparison between oocyst determination and occurring problems

Besides the comparison between the oocyst count and the occurring problems, also a comparison is made between the oocysts determination and the occurring problems. In total information of 141 pens is taken into account, 72 pens of age group I and 69 of age group II.

	No occurring problems N=73		**Diarrhea** N=24		**Poor calves /drop behinds,** N=4		**Respiratory problems,** N=12		**Other problems,** N=2		**Combination of problems,** N=26	
	I	II	I	II	I	II	I	II	I	II	I	II
No pathogenic Eimeria species, N= 65	23, (35%)	12, (18%)	7, (11%)	2, (3%)	0, (0%)	1, (2%)	1, (2%)	4, (6%)	1, (2%)	1, (2%)	8, (12%)	5, (8%)
E.alabamensis, N=24	2, (8%)	10, (42%)	3, (13%)	2, (8%)	0, (0%)	0, (0%)	2, (8%)	1, (4%)	0, (0%)	0, (0%)	3, (13%)	1, (4%)
E.bovis, N=16	3, (19%)	7, (44%)	1, (6%)	0, (0%)	1, (6%)	1, (6%)	1, (6%)	1, (6%)	0, (0%)	0, (0%)	0, (0%)	1, (6%)
E.zuernii, N=5	0, (0%)	2, (40%)	1, (20%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	2, (40%)	0, (0%)
A combination of pathogenic species, N=31	7, (23%)	7, (23%)	4, (13%)	4, (13%)	0, (0%)	1, (3%)	1, (3%)	1, (3%)	0, (0%)	0, (0%)	1, (3%)	5, (16%)

Table 4. *Presence or absence of oocysts of pathogenic Eimeria species, regardless the presence of oocysts of apathogenic Eimeria species, in combination with occurring problems per pen for both age groups. In total N=141.*

Because of the many categories, there is a small amount of results per category. Therefore, table 4 is reduced to table 5. Table 5 shows the absence or presence of problems per pen and the absence or presence of oocysts of pathogenic *Eimeria* species. Statistics showed no association between the presence or absence of oocysts of specific pathogenic *Eimeria* species and occurring problems for all pens (P-value 0.649), also no significant difference is shown for age group I (P-value 0.092) nor for age group II (P-value 0.373).

	Problems absent N=73		**Problems present** N=68	
	I	II	I	II
Pathogenic Eimeria species absent N=65	23	12	17	13
Pathogenic Eimeria species present N=76	12	26	20	18

Table 5. Presence or absence of problems per pen, and the presence or absence of oocysts of pathogenic Eimeria species per pen for both age groups. In total N= 141.

The table 6 shows the presence or absence of diarrhea per pen and the absence or presence of oocysts of pathogenic *Eimeria* species per pen. Again, statistics showed no association between the presence or absence of oocysts of specific pathogenic *Eimeria* species and occurring diarrhea for all pens (P-value 0.354), also no significant difference is shown for age group I (P-value 0.281) nor for age group II (P-value 0.482).

	Diarrhea absent N=81		**Diarrhea present** N=60	
	I	II	I	II
Pathogenic Eimeria species absent N=101	33	23	7	38
Pathogenic Eimeria species present N=40	23	2	9	6

Table 6. Presence or absence of diarrhea per pen, and the presence or absence of oocysts of pathogenic Eimeria species per pen for both age groups. In total N= 141.

Questionnaire

A questionnaire is filled in by the veterinarian in association with the farmer. The questionnaire contained seventeen questions about housing types of the calves during the period of individual housing, before weaning and after weaning, it also contained questions about cleaning, disinfection, way of offering food to the animals and other management aspects. The appendix shows the result of the total questionnaire combined with the presence or absence of oocysts of pathogenic *Eimeria* species per farm and per age group, according to the results of the oocysts determination.
Because of the few farms negative on oocysts of pathogenic *Eimeria* species, no statistics have been performed on this data.

Discussion

The aim of this study was to investigate the prevalence of pathogenic *Eimeria* oocysts excretion at Dutch dairy farms. The aim of this study was to investigate the prevalence of pathogenic *Eimeria* oocysts excretion at Dutch dairy farms. According to the results of the McMaster of pooled samples performed at the Animal Health Center, we found at least one calf excreting pathogenic oocysts on 87.9% of the farms, while at least one calf was excreting only non-pathogenic cysts was found on 4.5% of the farms. Therefor the prevalence of oocysts of *Eimeria* species in general found in this research is 92.4% according to the outcome of the McMasters performed at pooled samples at the Animal Health Center. According to the results of the McMaster performed on 406 individual samples, 284 samples were positive on *Eimeria* oocysts. This is a prevalence of 70.0%.

The animal prevalence at Dutch dairy farms reported by Cornelissen et al. is lower, 46% for calves.
This prevalence is lower compared to the prevalence found in this study. The difference between the study of Cornelissen et al. and our study is that they used a sucrose-flotation technic with a sensitivity of 10 oocysts per gram of feces and not the McMaster technic with a saturated salt solution with a minimal detection level of 50 oocysts per gram.(6) Because the test used by Cornelissen et al. is more sensitive compared to our test, the prevalence found in their study may appear lower. Also the degree of infection with *Eimeria* species at Dutch dairy farms in the Netherlands may differ in 17 years.

Researches performed in other countries also found other prevalences of *Eimeria* species, for example Rehman et al. found a prevalence of 47.09% in cattle in Pakistan. However, in this research cattle of all ages were used, and the prevalence is based on flotation technic of individual samples.(7) Bangoura et al. also found a high prevalence of *Eimeria* spp. oocysts excretion at farms of calves of 4 weeks old till 9 months old, namely a prevalence of 95.4% farms positive on *Eimeria* oocyst excretion.(5) Dong et al however, found a much lower prevalence of *Eimeria* oocyst excretion of individual samples at dairy farms in China. A prevalence of 51.8% was found in calves between 1 day old and 4 months, and a prevalence of 46.4% was found in weaners between 4

months old and 12 months old.(12) Koutney et al. found oocysts of *Eimeria* spp. on 97.97% of the investigated farms in Austria, and an individual calf prevalence of 83.7%. However, the method used in this research differs with our study, Koutny et al. used a sucrose-flotation technic.(9)

Apparently there is a big difference in prevalence of oocysts of *Eimeria* spp. over the world. Compared to other prevalences found by other researches, the prevalence of 92,4 based on positive farms seems to be 'normal' and the prevalence of 70.0 based on individual calves seems high. There has to be taken into account that this prevalence on farm level is based on results of pooled samples. A farm is called 'positive' if there is at least one calf excreting oocysts of *Eimeria* spp., so there may be a huge difference between the prevalence of individual calves positive on Eimeria oocysts between positive farms. What does match other researches is the higher prevalence with higher age group.(9)(13)(14)(15). Although Dong et al reported no significant difference in sample prevalence between calves and weaners.(12)

In both age groups, *E.alabamensis* was most prevalent, followed by *E.bovis* and *E.zuernii*. This is in contradiction with the results of Cornelissen et al. who found *E.bovis* as most common species at Dutch dairy farms in 1995.(6) Also other researchers found other *Eimeria* species as most common species. Like Dong et al., Rehman et al., Koutny et al., Waruiru et al., Lassen et al., and Cicek at al. also found *E.bovis* as most common pathogenic Eimeria species in Pakistan, China, Austria, central Kenya, Estonia and Turkey.(12)(7)(9)(13)(14)(15) Bangoura et al. found *E.zuernii* as most common *Eimeria* species in Germany.(5) What is also noteworthy besides the results of other researches, *E.alabamensis* is known as common species in calves on pasture (9, 10) (14), while the calves in this research haven't been on pasture at all. Apparently *E.alabamensis* can also be transmitted by contaminated hay (16), probably contaminated hay may cause the *E.alabamensis* infection in calves who aren't kept on pasture.

Further on, pathogenic *Eimeria* species are most commonly found in combination with apathogenic *Eimeria* species. In this research *E.zuernii* isn't found without apathogenic *Eimeria* species at all. However, *E.zuernii* without other pathogenic *Eimeria* species only occurred three times in age group I and 2 times in age group II. This number of occurrence is too small to conclude that *E.zuernii* only comes in combination with another pathogenic or apathogenic *Eimeria* species.

The OPG count in this study shows a large variation. The number of oocyst found in feces varies between 0 and 123 000. The OPG count per pen also shows a large variation, for example within one pen the range was 250 up to 123 000. Kounty et al. claimed the OPG value can differ during differ stages of infection. Therefore no conclusions should be made out of a single individual sample.(9) Unlike other studies, we found that the OPG of samples of calves excreting *Eimeria* oocysts was not different between age group I and II. Other studies reported a decreasing OPG with increasing age. (5, 6, 9, 12-14)

When the comparison was made between the OPG count and OPG determination, it was shown that individual OPG >0 of samples of completely analyzed pens positive on oocysts of at least pathogenic *Eimeria* species was higher compared to the OPG of pens positive on oocysts of only apathogenic *Eimeria* species. This was shown for the complete farm, so age group I as well as age group II, and for pens of age group I apart. However, this difference in OPG was not shown for pens of age group II. The OPG count > 0 of individual samples of completely analyzed pens of age group I and II did not differ significantly. So apparently, the difference in OPG for pathogenic and apathogenic *Eimeria* species is only seen in the younger calves, is not seen in older calves. In older calves, the OPG count is not useful for predicting the *Eimeria* species involved. The OPG of individual samples of completely analyzed pens were found higher for age group I positive on oocysts of pathogenic *Eimeria* species compared to age group II positive on pathogenic *Eimeria* species. This does corresponds to the

findings of Koutny et al, Bangoura et al, Dong et al, Waruiru et al., Lassen et al, and Cornelissen et al, unlike the previous finding that the OPG of excreting calves doesn't differ between age group I and II. (5, 6, 9, 12-14) All these results are the same for the mean OPG of completely analyzed pens, with OPG with a value of 0 also taken into account, and the highest OPG per pen of completely analyzed pens, with a minimum OPG of 50.

Taking this into account, we can conclude that it is difficult to predict the Eimeria species involved only based on the OPG count of feces samples of calves. With the OPG count of young calves, a prediction can be made between at least pathogenic or only apathogenic *Eimeria* species. But this doesn't apply for older calves. Therefore, only for calves in age group I the OPG count is useful for prediction if calves are infected with at least pathogenic or only apathogenic *Eimeria* species. It doesn't differ for the OPG count of individual samples, the mean OPG per pen or the highest OPG per pen. But because the big difference in OPG count of indivual samples within one pen, it is best to use the mean OPG per pen if men want to predict the *Eimeria* species involved in calves of age group I based on the OPG count. For calves of age group II, or when calves of both age groups are mixed within one pen, oocysts determination is necessary for finding out whether at least pathogenic or only apathogenic *Eimeria* species are involved.

We found no association between the OPG count and presence of problems per pen. Although Koutny et al have shown a correlation between the OPG and diarrhea, they also state there is no correlation between the oocyst excretion and the severity of clinical problems due to pathogenic *Eimeria* species.(9)

Also no correlation was found between the presence of pathogenic *Eimeria* spp and the presence of any problem per. This is a rather remarkable finding, since pathogenic *Eimeria* species are called pathogenic because they are thought to cause clinical symptoms.(6, 7, 9, 10, 17) Possible explanations for this finding is that the occurring problems per pen are not well observed by the farmer, or the veterinarian who filled in the questionnaire didn't ask clear enough or other causes besides *Eimeria* species are responsible for clinical symptoms. Another possibility is that the diarrhea was already gone or reduced, while *Eimeria* oocysts were still excreted. However, we found that the OPG of excreting calves is higher for calves infected with at least pathogenic *Eimeria* species in age group I compared to age group II. So a possible explanation is that the excretion of at least pathogenic *Eimeria* oocysts may reduce, but pathogenic oocysts may still be excreted with a lower OPG while the diarrhea was already gone or reduced.

The questionnaire filled in by the veterinarian in association with the farmer implies that the housing type and cleaning of the housing after every calf at the period of individual housing does not lower the risk of *Eimeria* infection at the farm or per age group. Of the 57 farms positive on pathogenic Eimeria species, 51 farms didn't disinfect the individual housing after every calf. This implies that if a farmer does not disinfect the individual housing, it brings a higher risk of the presence of pathogenic *Eimeria* species at the farm. However, this clear distribution does not apply to age group I. Of the total age groups who didn't disinfect the individual pens, 27 are negative on pathogenic *Eimeria* species and 19 are positive on pathogenic *Eimeria* species. Bangoura et al mentions the importance of cleaning and disinfection, but didn't found a lower prevalence of pathogenic Eimeria species if there hygienic measurements were taken like cleaning.(5) Therefor, there can be concluded cleaning and disinfection is important to prevent problems with *Eimeria* species, but proper cleaning and disinfection is difficult to perform. Most farms positive on pathogenic *Eimeria* species kept their calves on straw. This implies a risk of infection if calves are kept at straw. However, in age group I in total 40 pens with calves are kept on straw of which 21 pens are negative on pathogenic *Eimeria* species and 19 pens positive. Bangoura et al. and Daugschies et al. found having a slatted floor reduced the risk of presence of oocysts of pathogenic *Eimeria* species on the farm or age group.(5,

10) Having the calves classified by multiple groups within age groups does not seem to make any difference in prevalence of positive farms or age groups. The same as for cleaning and disinfection of the housing of calves during individual housing, applies for cleaning and disinfection of the housing of calves before and after weaning. It does not seem to make any difference in the amount of positive or negative farms or age groups. Of 58 farms positive on oocysts of pathogenic *Eimeria* species, 48 farms don't own an automatic calf feeder. This seems to imply having an automatic calf feeder reduced the risk on the presence of pathogenic *Eimeria* oocysts at a farm. But in age group I, 12 groups on farms use an automatic calf feeder of which 7 groups are negative and 5 groups are positive on oocysts of pathogenic *Eimeria* species. Factors such as moving calves to a different pens while weaning, housing type after weaning, all-in all-out principle when calves move to a different pen after weaning, numbers of liter milk when weaning and the weaning age does not seem to make a difference in the amount of farms or age groups positive or negative on oocysts of pathogenic *Eimeria* species. The way of feed supply before and after weaning also does not imply a clear difference in amount of farms or age groups positive or negative on oocysts of pathogenic *Eimeria* species. Although Rehman et al. found a higher risk of infection in the calves are fed on the ground instead of mangers.(7)

Conclusion

This study shows the prevalence of pathogenic *Eimeria* species in total, and specified by pathogenic species in calves at Dutch dairy farms. It shows *E.alabamensis* is the most common pathogenic *Eimeria* species, followed by *E.bovis* and *E.zuernii*. The prevalence of apathogenic and pathogenic *Eimeria* species in total (70%) is higher compared to the last known prevalence of *Eimeria* species in calves at Dutch dairy farms reported by Cornelissen et al (46%) in 1995. (6) This demonstrated that infection with *Eimeria* species is a common problem in calves at Dutch dairy farms. Although this study doesn't show any association between the pathogenic *Eimeria* species and occurring problems, based on literature it may be assumed that infections with pathogenic *Eimeria* species cause big economic losses at a dairy farm. The high prevalence of *Eimeria* infections in combination with the economic losses makes *Eimeria* infections of big importance at Dutch dairy farms. Because the OPG count of calves after weaning infected with pathogenic *Eimeria* oocysts is not higher compared to apathogenic oocysts, and no association is made between pathogenic *Eimeria* species and occurring problems nor for the OPG count and the occurring problems, it can be concluded that only a OPG count fails to find out whether calves are infected with apathogenic or pathogenic *Eimeria* species. Therefore, oocysts determination is necessary. Because of the big range in OPG found in the individual McMasters within one pen, no conclusion can be made out of one individual sample. It is better to make a pooled sample of several calves. An attempt was made to identify risk factors, but no clear risk was found.

Acknowledgements

The author would like to thank all the veterinarians at the University Veterinary practice for farm animals for collecting the samples and filling in the questionnaire. I would also like to thank the veterinary assistant's at the University Veterinary practice for farm animals, who helped with the logistics of sampling and the microscopic work. I would like to thank the farmers, who agreed participating in this research. And at last I would like to thank both project tutors, who helped writing this research report.

References

1. Lorenz I, Fagan J, More SJ. Calf health from birth to weaning. II. management of diarrhoea in pre-weaned calves. Ir Vet J. 2011 Sep 14;64(1):9,0481-64-9.

2. Svensson C, Linder A, Olsson SO. Mortality in swedish dairy calves and replacement heifers. J Dairy Sci. 2006 Dec;89(12):4769-77.

3. Gulliksen SM, Lie KI, Loken T, Osteras O. Calf mortality in norwegian dairy herds. J Dairy Sci. 2009 Jun;92(6):2782-95.

4. de Graaf DC, Vanopdenbosch E, Ortega-Mora LM, Abbassi H, Peeters JE. A review of the importance of cryptosporidiosis in farm animals. Int J Parasitol. 1999 Aug;29(8):1269-87.

5. Bangoura B, Mundt HC, Schmaschke R, Westphal B, Daugschies A. Prevalence of eimeria bovis and eimeria zuernii in german cattle herds and factors influencing oocyst excretion. Parasitol Res. 2012 Feb;110(2):875-81.

6. Cornelissen AW, Verstegen R, van den Brand H, Perie NM, Eysker M, Lam TJ, et al. An observational study of eimeria species in housed cattle on dutch dairy farms. Vet Parasitol. 1995 Jan;56(1-3):7-16.

7. Rehman TU, Khan MN, Sajid MS, Abbas RZ, Arshad M, Iqbal Z, et al. Epidemiology of eimeria and associated risk factors in cattle of district toba tek singh, pakistan. Parasitol Res. 2011 May;108(5):1171-7.

8. Lassen B, Ostergaard S. Estimation of the economical effects of eimeria infections in estonian dairy herds using a stochastic model. Prev Vet Med. 2012 Oct 1;106(3-4):258-65.

9. Koutny H, Joachim A, Tichy A, Baumgartner W. Bovine eimeria species in austria. Parasitol Res. 2012 May;110(5):1893-901.

10. Daugschies A, Najdrowski M. Eimeriosis in cattle: Current understanding. J Vet Med B Infect Dis Vet Public Health. 2005 Dec;52(10):417-27.

11. Mitchell ES, Smith RP, Ellis-Iversen J. Husbandry risk factors associated with subclinical coccidiosis in young cattle. Vet J. 2012 Jul;193(1):119-23.

12. Dong H, Zhao Q, Han H, Jiang L, Zhu S, Li T, et al. Prevalence of coccidial infection in dairy cattle in shanghai, china. J Parasitol. 2012 Oct;98(5):963-6.

13. Waruiru RM, Kyvsgaard NC, Thamsborg SM, Nansen P, Bogh HO, Munyua WK, et al. The prevalence and intensity of helminth and coccidial infections in dairy cattle in central kenya. Vet Res Commun. 2000 Feb;24(1):39-53.

14. Lassen B, Viltrop A, Raaperi K, Jarvis T. Eimeria and cryptosporidium in estonian dairy farms in regard to age, species, and diarrhoea. Vet Parasitol. 2009 Dec 23;166(3-4):212-9.

15. Cicek H, Sevimli F, Kozan E, Kose M, Eser M, Dogan N. Prevalence of coccidia in beef cattle in western turkey. Parasitol Res. 2007 Oct;101(5):1239-43.

16. Svensson C. The survival and transmission of oocysts of eimeria alabamensis in hay. Vet Parasitol. 1997 May;69(3-4):211-8.

17. Mundt HC, Bangoura B, Rinke M, Rosenbruch M, Daugschies A. Pathology and treatment of eimeria zuernii coccidiosis in calves: Investigations in an infection model. Parasitol Int. 2005 Dec;54(4):223-30.

Appendix

Result of the questionnaire in combination with positive (oocysts of pathogenic Eimeria species present) or negative (oocysts of pathogenic Eimeria species absent) outcome per farm and age group.

	Farm in total N=66		**Age group I N=66**		**Age group II N=66**	
	Negative N= 8	**Positive N= 58**	**Negative N=34**	**Positive N=32**	**Negative N= 23**	**Positive N=43**
1A) Period of individual housing:						
1: Individual iglo hutch	2, (25%)	16, (27,59%)	10, (29,41%)	8, (25%)	6, (26,09%)	12, (27,91%)
2: non-iglo individual hutch	5, (62,5%)	37, (63,79%)	22, (64,71%)	20, (62,5%)	13, (56,52%)	29, (67,44%)
3: other housing type	0, (0%)	3, (5,17%)	0, (0%)	3, (9,38%)	2, (8,70%)	1, (2,33%)
4: combination of housing types present of farm	0, (0%)	2, (3,45%)	1, (2,94%)	1, (3,13%)	1, (4,35%)	1, (2,33%)
5: unknown	1, (12,5%)	0, (0%)	1, (2,94%)	0, (0%)	1, (4,35%)	0, (0%)
1B) cleaning of housing after every calf:						
1: yes	6, (75%)	30, (51,72%)	20, (58,82%)	16, (50%)	12, (52,17%)	24, (55,81%)
2: no	2, (25%)	28, (48,28%)	14, (41,18%)	16, (50%)	11, (47,83%)	19, (44,19%)
1C) Disinfection housing after every calf:						
1: yes	2, (25%)	6, (10,34%)	5, (14,71%)	3, (9,38%)	3, (13,04%)	5, (11,63%)
2: no	5, (62,5%)	51, (87,93%)	27, (79,41%)	29, (90,63%)	19, (82,61%)	37, (86,05%)
3: onbekend	1, (12,5%)	1, (1,72%)	2, (5,88%)	0, (0%)	1, (4,35%)	1, (2,33%)
2A) Housing type period till weaning:						
1: straw	6, (75%)	34, (58,62%)	21, (61,76%)	19, (59,38%)	16, (69,57%)	24, (55,81%)
2: straw+ concrete or slatted floor next to the feedgate	0, (0%)	6, (10,34%)	1, (2,94%)	5, (15,63%)	1, (4,35%)	5, (11,63%)
3: group iglo hutch	1, (12,5%)	3, (5,17%)	4, (11,76%)	0, (0%)	1, (4,35%)	3, (6,98%)
4: other housing type	1, (12,5%)	11, (18,97%)	6, (17,65%)	6, (18,75%)	4, (17,39%)	8, (18,60%)
5: combination of housing types present on farm	0, (0%)	0, (0%)	2, (5,88%)	2, (6,25%)	1, (4,35%)	0, (0%)
2B) Multiple age groups:						
1i: Yes, difference in age < 3 weeks	2, (25%)	20, (34,48%)	9, (26,47%)	13, (40,63%)	10, (43,48%)	12, (27,91%)
1ii: yes, difference in age > 3 weeks	0, (0%)	13, (22,41%)	7, (20,59%)	6, (18,75%)	3, (13,04%)	10, (23,26%)
2: no	6, (75%)	22, (37,93)	16, (47,06%)	12, (37,5%)	9, (39,13%)	19, (44,19%)
3: combination of answers	0, (0%)	3, (5,17%)	2, (5,88%)	0, (0%)	1, (4,35%)	2, (4,65%)
2C) Cleaning housing before weaning:						
1: yes	1, (12,5%)	30, (51,72%)	14, (41,18%)	17, (53,13%)	9, (39,13%)	22, (51,16%)

2: no	6, (75%)	26, (44,83%)	17, (50%)	15, (46,88%)	13, (56,52%)	19, (44,19%)
3: combination of answers	0, (0%)	3, (5,17%)	1, (2,94%)	0, (0%)	0, (0%)	1, (2,33%)
4: unknown	1, (12,5%)	1, (1,72%)	2, (5,88%)	0, (0%)	1, (4,35%)	1, (2,33%)
2D) Disinfection housing before weaning:						
1: yes	1, (12,5%)	6, (10,34%)	4, (11,76%)	3, (9,38%)	1, (4,35%)	6, (13,95%)
2: no	6, (75%)	51, (87,93%)	29, (85,29%)	28, (87,5%)	21, (91,30%)	36, (83,72%)
3: unknown	1, (12,5%)	1, (1,72%)	1, (2,94%)	0, (0%)	1, (4,35%)	1, (2,33%)
2E) Automatic calf feeder						
1: yes	2, (25%)	10, (17,24%)	7, (20,59%)	5, (15,63%)	4, (17,39%)	8, (18,60%)
2: no	6, (75%)	48, (82,76%)	N=27, 79,41%	27, (84,38%)	19, (82,61%)	35, (81,40%)
2F) Moving calves to a different pen at weaning:						
1: yes	4, (50%)	32, (55,17%)	22, (64,71%)	14, (43,75%)	8, (34,78%)	28, (65,12%)
2: no	4, (50%)	26, (44,83%)	12, (35,29%)	18, (56,25%)	15, (65,22%)	15, (34,88%)
3A) Housing type after weaning:						
1: straw	5, (62,5%)	15, (25,86%)	12, (35,29%)	8, (25%)	9, (39,13%)	11, (25,58%)
2: straw + concrete or slatted floor next to feed gate	0, (0%)	4, (6,90%)	3, (8,82%)	1, (3,13%)	1, (4,35%)	3, (6,98%)
3: lying box + slatted floor	2, (25%)	29, (50%)	14, (41,18%)	17, (53,13%)	10, (43,48%)	21, (48,84%)
4: different housing type	0, (0%)	3, (5,17%)	2, (5,88%)	1, (3,13%)	0, (0%)	3, (6,98%)
5: combination of answers	1, (12,5%)	7, (12,07%)	2, (5,88%)	5, (15,63%)	2, (8,70%)	5, (11,63%)
6: unknown	0, (0%)	0, (0%)	1, (2,94%)	0, (0%)	1, (4,35%)	0, (0%)
3B) All in-all out grouphousing						
1: yes	3, (37,5%)	23, (39,66%)	12, (35,29%)	14, (43,75%)	10, (43,48%)	16, (37,21%)
2: no	5, (62,5%)	35, (60,34%)	22, (64,71%)	18, (56,25%)	13, (56,52%)	27, (62,79%)
3C) Cleaning housing after weaning:						
1: yes	1, (12,5%)	34, (58,62%)	18, (52,94%)	17, (53,13%)	9, (39,13%)	26, (60,47%)
2: no	7, (87,5%)	23, (39,66%)	15, (44,12%)	15, (46,88%)	14, (60,87%)	16, (37,21%)
3: unknown	0, (0%)	1, (1,72%)	1, (2,94%)	0, (0%)	0, (0%)	1, (2,33%)
3D) Disinfection housing after weaning:						
1: yes	2, (25%)	5, (8,62%)	4, (11,76%)	3, (9,38%)	2, (8,70%)	5, (11,63%)
2: no	5, (62,5%)	52, (89,66%)	28, (82,35%)	29, (90,63%)	20, (86,96%)	37, (86,05%)
3: unknown	1, (12,5%)	1, (1,72%)	2, (5,88%)	0, (0%)	1, (4,35%)	1, (2,33%)
4A) Feed supply till weaning:						
1: manger / rack	5, (62,5%)	41, (70,69%)	27, (79,41%)	19, (59,38%)	14, (60,87%)	32, (74,42%)

2: on the floor/feeding passage	3, (37,5%)	13, (22,41%)	6, (17,65%)	10, (31,25%)	6, (26,09%)	10, (23,26%)
3: combination of answers	0, (0%)	4, (6,90%)	1, (2,94%)	3, (9,38%)	3, (13,04%)	1, (2,33%)
4B) Number of liters milk at weaning						
1: 0-2 liter	6, (75%)	45, (77,59%)	26, (76,47%)	25, (78,13%)	18, (78,26%)	33, (76,74%)
2: >2 liter	2, (25%)	11, (18,97%)	7, (20,59%)	6, (18,75%)	4, (17,39%)	9, (20,93%)
2: unknown	0, (0%)	2, (3,45%)	1, (2,94%)	1, (3,13%)	1, (4,35%)	1, (2,33%)
4C) Weaning age:						
1: <10 weeks	0, (0%)	19, (32,76%)	12, (35,29%)	7, (21,88%)	3, (13,04%)	16, (37,21%)
2: 10-12 weeks	2, (25%)	30, (51,72%)	13, (38,24%)	19, (59,38%)	10, (43,48%)	22, (51,16%)
3: > 12 weeks	3, (37,5%)	8, (13,79%)	5, (14,71%)	6, (18,75%)	7, (30,43%)	4, (9,30%)
4: unknown	3, (37,5%)	1, (1,72%)	4, (11,76%)	0, (0%)	3, (13,04%)	1, (2,33%)
5A) Feed supply after weaning:						
1: manger /rack	5, (62,5%)	17, (29,31%)	16, (47,06%)	6, (18,75%)	8, (34,78%)	14, (32,56%)
2: on the ground/feed passage	3, (37,5%)	30, (51,72%)	14, (41,18%)	19, (59,38%)	13, (56,52%)	20, (46,51%)
3: combination of answers	0, (0%)	11, (18,97%)	4, (11,76%)	7, (21,88%)	2, (8,70%)	9, (20,93%)