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Original Article

# Oocyst shedding patterns of *Eimeria* species and their association with management and performance at ten rose veal starter farms in the Netherlands

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ABSTRACT

Coccidiosis at rose veal starter farms is often diagnosed however, this was the first study performed considering this topic on this type of rearing unit. The objective of this study was to identify *Eimeria* species faecal shedding patterns at ten Dutch rose veal starter farms during rearing. Further objectives were to investigate associations with (gut) health, production and management decisions. Faecal samples from twelve randomly selected calves per farm were collected weekly during 9 consecutive weeks. Thereafter samples were pooled in a predetermined composition of six. These calves were clinically evaluated every sample visit and weighed at the first and last visit. Laboratory tests included a flotation test of the samples. If this yielded *Eimeria* oocysts, both oocysts per gram feces (OPG) and species differentiation were established using a modified McMaster method. Management parameters and technical herd results were identified after finalizing the study period using a questionnaire. Studied patterns in oocyst shedding included the pool's cumulated OPG, maximum OPG level and the number of sample days (SD) that OPG exceeded a confirmed level. Statistical analysis included univariate and multivariate analysis. Associated ($p < 0.10$) OPG patterns considering rumen fill, faecal consistency and average daily weight gain (ADG) were tested using a model with herd included as random effect.

*Results:* on all ten farms *E. alabamensis, E.bovis* and *E. zuernii* besides non-pathogenic species were identified, often as mixed infections. Peak OPG occurred predominately at SD 21 and 28 (*E. alabamensis and E. bovis*), and at SD 42 (*E. zuernii*). In 16 pools, OPG levels ≥500 for *E. bovis* or *E. zuernii,* were found. Significant correlation ($p < 0.0001$) showed between the $\log_{10}$ OPG of pathogenic *Eimeria* and of all *Eimeria* species. Multivariate analysis showed significant correlation between cumulative faecal consistency scores and cumulative $\log_{10}$ OPG of pathogenic *Eimeria species* ($\beta = 0.16$; $p = 0.008$). Pools exceeding 750 OPG for *E. bovis* showed 93 g lower ADG and pools experiencing ≥2 SD with >1000 OPG for *E. alabamensis* 141 g lower ADG. From the questionnaire we identified lower cumulated OPG of all *Eimeria* species except for *E. bovis* at farms where the units were cleaned before arrival of the calves.

*Conclusion:* As a rule, on Dutch rose veal starter farms, mixed *Eimeria* infections occur, but shedding patterns differ between farms. Clinical and growth performance is related to OPG patterns found. Cleaning units before arrival of calves lowers oocyst shedding during the rearing period.

## 1. Introduction

The number of rose veal calves in the Netherlands was 373,000 calves in 2019 (CBS stat line rundveestapel, 2019). Calves reared under rose veal conditions originate from dairy farms and typically enter the farm at a minimum of 2 weeks of age. The calves are fed milk concentrates and roughage and are weaned between 5 and 7 weeks on-farm. The total fattening period is divided in a starter period of roughly ten weeks and a finishing period until the age of 8 to 12 months. After the starter period calves enter the finishing phase, either at the same

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location or in a different unit or even on a different farm. The main health problems at rose veal starter farms are caused by respiratory and digestive disorders. Digestive disorders can be caused by viruses such as rota and corona viruses, bacteria such as salmonella species or protozoal infections such as *Cryptosporidium parvum* or *Eimeria* species. Feeding management and antibiotic treatment can also lead to or worsen digestive disorders and cause small intestinal bacterial overgrowth (Constable, 2004). When it comes to protozoal infections due to *Eimeria* species, high prevalence has been found in dairy calves from 5 weeks up to 3 months of age (Cornelissen et al., 1995). The prepatent period of coccidiosis differs between *Eimeria* species. Following initial infection oocysts appear in the feces after 6–11 days (*E. alabamensis*), 16–21 days (*E. bovis*) and 15–17 days (*E. zuernii*) (Svensson, 2000; Ernst and Benz, 1986a; Ernst and Benz, 1986b). In cases of clinical coccidiosis calves show watery, mucous and sometime bloody feces in combination with other clinical disorders like anorexia, depression and in rare cases mortality (Ernst and Benz, 1986a; Ernst and Benz, 1986b). In housed animals clinical disease is often caused by infection with *E. zuernii* or *E. bovis*, generally linked with conditions of high infection pressure (Stockdale et al., 1981; Daugschies et al., 1986). Immune suppression due to stress can lead to high numbers of oocysts excreted per gram of feces (OPG) causing a more severe environmental contamination (Bürger, 1983; Svensson et al., 1994; Cornelissen et al., 1995; Lucas et al., 2007). To a lesser extent, *E. alabamensis* can also cause clinical disease. (Daugschies and Najdrowski, 2005). *E. alabamensis* was found to cause clinical disease in calves kept on pasture (Svensson et al., 1994). Oocyst shedding in feces is not necessarily accompanied by clinical disease since calves can also experience subclinical coccidiosis (Cornelissen et al., 1995). This can occur either with *E. bovis* and *E. zuernii* under low infection pressure conditions or when infection with large amounts of *Eimeria* species with low pathogenicity takes place (Cornelissen et al., 1995). Whereas clinical coccidiosis shows only in few individual calves, subclinical infections can occur in a larger number of calves in a group. Calves with subclinical coccidiosis experience lower impact on growth performance than clinically affected cases. However, since these are more numerous, they are often responsible for most of the economic losses (Gradwell et al., 2005). It is to be expected that coccidial infections occur in rose veal starter farms due to several circumstances. Calves in rose veal starter farms are at a susceptible age (between 2 and 12 weeks after birth) for coccidial infections (Daugschies and Najdrowski, 2005). Rose veal calves originate from dairy farms, where they possibly get infected with coccidia leading to introduction of coccidia at the rose veal starter farm (Daugschies and Najdrowski, 2005). Moreover, it is possible that calves will take up oocysts from the environment after arrival at the rose veal starter farm. Oocysts were proven to survive in the environment for several months, allowing contamination of pens from previous batches of calves (Daugschies and Najdrowski, 2005; Bürger, 1983; Svensson, 1997). Finally rose veal calves experience several stressful and immune suppressive events such as transportation, transitions in housing, feeding and management. These events combined with the occurrence of the immunity gap between passive immunity and acquired immunity could possibly lead to higher shedding of oocysts in the environment (Chase et al., 2008). We do know that clinical coccidiosis is commonly diagnosed and treated at rose veal starter farms and that coccidiosis was included in the list of the 34 most important calf diseases with their respective transmission routes regarding veal calves (Damiaans et al., 2019). Despite this, the knowledge about coccidiosis in this type of farm is mainly based on practical experience and extrapolated knowledge of other farm types. To our knowledge, no studies are available about *Eimeria* infection patterns and the impact on calf performance on this type of rearing unit. This study aimed to provide insights into infections with *Eimeria* species on this type of farm. The objective was to study faecal oocyst shedding patterns of pathogenic and non-pathogenic *Eimeria* species. Furthermore, the study aimed to establish how oocyst shedding patterns were associated with health parameters and growth performance of calves. Therefore, weekly faecal examination was performed on two pools of six calves for nine consecutive weeks on ten rose veal farms in the Netherlands. Species differentiation and differentiated oocysts per gram feces (OPG) were established. A management questionnaire was run on each of the ten farms to identify factors that possibly influence the excretion of oocysts. Better understanding of the dynamics of oocyst shedding, associations with clinical performance, growth and management factors is key for farmers and veterinarians. It helps them to prevent calves from becoming ill and minimize damage due to *Eimeria* infections. The knowledge gained in this study could therefore help to improve animal welfare and herd performance.

## 2. Material and methods

### 2.1. Farms

Ten rose veal farms, each with a minimum of 450 calves, were selected by one Veterinary Practice (Thewi veal calf veterinarians Tilburg, the Netherlands). Only farms that implemented an all-in all-out strategy and which reared calves originating from the Netherlands, Germany or Belgium were selected. On these farms no metaphylactic treatment with registered anticoccidials (diclazuril is the only registered product for coccidiosis treatment or metaphylactic treatment for veal calves in the Netherlands) was allowed during this study period. Farms were coded with a letter according to the order of enrollment (A to J).

### 2.2. Animals

In total 120 animals, 12 per farm, both male and female were included. Calves were of mixed commercial breeds, mainly (male) Holstein Friesian (HF) (85%) or (female) mixed HF / Belgian Blue (15%). The selected calves were randomly assigned to the study by the investigators at the first visit while they were housed in individual pens. Absence of clinical disorders and a bodyweight between 45 and 55 kg were inclusion criteria for enrolment. Individual animals were identified by using their ear tag numbers. To allow for quick and easy recognition, the animals identified for close follow-up were given an additional color mark in order to be able to identify them throughout the study.

### 2.3. Sample size

Animal prevalence of *Eimeria* oocysts of 46% and 70% was found at Dutch dairy farms (Cornelissen et al., 1995; Van Balen, 2013). It is to be expected that on rose veal farms a similar or higher prevalence will be present, since calves originate from many dairy farms. Pooled samples were chosen because this was proven to dampen the considerable variation in oocysts per gram feces (OPG) within individual calves (Van Balen, 2013). Therefore, a sample size of 12 calves (each in a separate pen), divided into two pools of six, was chosen to represent each herd. The first 6 samples taken were assigned to pool 1 and the others to pool 2. In order to evaluate the repeated pooled samples as a good representation of oocyst shedding patterns of the complete herd, the twelve samples were divided into two pools. In this way both pools serve as each other's internal control.

### 2.4. Sampling

On every farm faecal sampling during the rearing period was performed nine times at weekly intervals. The two pools of for the *Eimeria* differentiation and oocyst counting contained the same six animals every week. The first sampling day (SD0) was within eight days after arrival of the first calves on the farm (farms would bring in calves over three to four consecutive days). After SD0 the calves were sampled every seven days. Sample preservation was achieved through storage between 4 and 7 °C. To collect the feces, the investigator restrained the calf in the pen corner, palpated the calf rectally with a gloved finger and waited for

the calf to defecate. The feces was collected in a sampling vial (Cellstor 60 ml, Cellpath, Newtown, United Kingdom) and provided with a label containing the following information: Lab study code, farm code (A-J), pool number (1 or 2), sample day number (SD0-SD56), calf identification (ID number), and date of sampling.

### 2.5. Flotation and determining the oocysts per gram feces (OPG)

The samples were sent to the commercial laboratory DGZ-Vlaanderen (Torhout, Belgium). The DGZ laboratory applied a protocol adapted from Janssen Pharmaceutical (Thienpont et al., 1986). Faecal samples were pooled to a maximum of six, according to predetermined pool composition. In order to clean the pooled sample from coarse parts and to avoid a too dark color of the samples, the following adaptation was made on the flotation with centrifuging described by Thienpont et al., 1986: Of each pool, 10 g (g) was thoroughly mixed with ±100 ml (ml) of cold tap water, after which the pool sample was sieved at room temperature on a vibration-free surface. After at least 2 h, the supernatant was removed before 10 ml $ZnCl_2$ solution (2 kg (kg) $ZnCl_2$ (VWR, Haasrode, Belgium) + 1 kg NaCl (VWR, Haasrode, Belgium) in 2 l tap water) with a density of 1.56 g per cubic centimeter ($cm^3$) was added. Subsequently, the sample was mixed thoroughly, poured into V-bottomed plastic 15 ml-sized tubes. Thereafter centrifuged for 3 min at 1500 *g*. Tubes were filled with $ZnCl_2$ solution until a convex meniscus was formed. A coverslip was put on top of the convex meniscus for at least 2 min, after which the coverslip was examined microscopically. Flotation enables the laboratory personnel to enlarge, measure and identify oocysts better than the Mc Master technique allows. Detected *Eimeria* species were differentiated into *Eimeria zuernii, Eimeria alabamensis, Eimeria bovis* or into the remaining group of non-pathogenic *Eimeria* species. When flotation was positive, the count of oocysts per gram (OPG) was performed with the modified McMaster method (Thienpont et al., 1986). Of each feces pool, 1 g was thoroughly mixed with 30 ml salt sugar solution (500 g sucrose (VWR); 832.5 g NaCl (VWR); 25 ml 4% formol (VWR) in 2.5 l warm tap water) with a density of 1.20 g per $cm^3$. Subsequently 150 µl of sample is added onto a double McMaster counting chamber. The oocysts were counted in both chambers, after which the OPG was calculated. When no oocysts were counted in the McMaster following a positive flotation, a result <100 was reported which we defined in the dataset as 1.

### 2.6. Clinical observations and weighing of the calves

The selected calves were weighed at the first and last sampling day (SD0 and SD56) by the investigator. The investigator carried out a weekly clinical evaluation including scoring of rumen fill, alertness, temperature, respiratory disease, faecal consistency, cleanliness hindquarters and cleanliness of the pen, based on clinical score definitions. Detailed description of the scoring system is given in Table 1.

### 2.7. Herd technical results

Herd technical results were collected after the calves left the farm. The following parameters were available for all farms; mortality (% animals), average weight at arrival (kg), average weight at delivery (kg), average daily weight gain (gram), average growth per calf (kg), number of growth days (days), antibiotic use per calf (animal daily dosages; ADD). The total amount of concentrate, milk powder, corn, muesli, and straw (kg) per calf was established for each herd except for herd A. Despite repeated effort of the investigator to derive this information on herd A this could not be shared with us.

### 2.8. Questionnaire

On every farm a questionnaire was completed by the investigator at or after SD56. The registrations were based on interviews with the farmers/ farmers' representative. The questionnaire covered the following topics: Farm parameters (number of calves, type of farm), animal parameters (breed, gender, and country of origin), hygiene (cleaning and disinfecting units, water pipes, milk pipes), climate control, milk replacer supply, water supply, concentrate and roughage supply, supplements supply, management, and medication (Supplementary data table 8).

**Table 1**
Clinical scoring protocol.

| Clinical score | 0 | 1 | 2 |
|---|---|---|---|
| Rumen fill | Full, rumen bulges outside rib wall | Empty, rumen fill below rib wall | |
| Condition | Good muscle development and round body contours | Moderate, slightly sharper body contours and less muscle development | Skinny sharp bony appearance |
| Alertness | Normal = when entering the room/ approaching the calf, the calf is up and active | Depression moderate = calf does not get up upon approaching, does only get up after light back pressure<br>little or no interest in social interaction and/or food; calf stands with its head down and/or tends to lay down almost immediately after it got up. Animal has a rough coat | Depression severe = calf is moribund, does not get up anymore or gets up very difficult, does not respond to stimuli<br>no interest in social interaction, food and surroundings, appears anxious. |
| Temperature | No fever, rectal temperature below 39,5 °C | Fever rectal, temperature higher than 39,5 °C | |
| Respiratory | No signs of respiratory disease present | Signs of respiratory disease present; coughing and or panting and or mucous or purulent material at nostrils | |
| Faecal consistency | Normal solid, soft and malleable | Pasty, falls apart not malleable | Watery and/or mucous and/or bloody feces |
| Hindquarter cleanliness | Clean hindquarters | Hindquarters dirty with stuck dry feces | Hindquarters dirty with wet feces |
| Pen cleanliness | Clean floor | Several spots of pasty or wet feces on the slatted floor | Entirely wet and dirty floor |

Clinical scoring according to this protocol was used to evaluate the clinical performance of 12 randomly selected calves per farm at ten rose veal starter farms at each of nine weekly faecal sampling occasions.

### 2.9. Statistics and statistical analysis

All statistical analysis was performed using JMP computational software (version 14, SAS Institute Inc.). A significant difference between the levels of a classification variable was considered at $p \leq 0.05$, whereas differences between $p > 0.05$ and $p \leq 0.10$ were considered a statistical tendency.

#### 2.9.1. Descriptive and univariate analysis on Eimeria shedding patterns

Distribution analysis was used to establish the presence and the OPG level of the different *Eimeria* oocysts in the pooled samples per sample day. Thereafter, logistic regression was used to establish correlation between presence of pathogenic and non-pathogenic *Eimeria* per sample day and for the complete study period. A multivariate logistic regression (MLR) analysis was used to establish correlations between oocyst counts ($log_{10}$ OPG) of different *Eimeria* species per sample day.

*2.9.2. Descriptive and univariate analysis of clinical scoring*

Clinical scores were derived of all individual calves in the study. Therefore 120 datapoints are available. First distribution analysis was used to establish the clinical scoring patterns per sample day. MLR was used to establish which clinical signs show up simultaneously in individual calves. Per calf an average clinical score was calculated over the 9 sample moments to relate this to the average daily weight gain (ADG). MLR was used to establish correlation between average daily weight gain and the average clinical score per calf for every clinical parameter. Finally, these average clinical scores were tested for correlation using MLR again.

*2.9.3. Descriptive and univariate analysis of mean clinical scores per pool and the presence and number of oocysts of different Eimeria species per sample day*

For every pool, a mean clinical score was calculated per sample day. This was followed by a MLR to establish possible correlation between OPG of the different *Eimeria* species per sample day and the mean clinical scores of the pools at that same sample day.

Distribution was used to identify the sample day on which a clear peak in OPG of pathogenic *Eimeria* occurred (SD-$OPG_{max}$) and the sample day on which the peak in clinical score occurred (SD-$CS_{max}$) for faecal consistency, hindquarter cleanliness, pen cleanliness and respiratory disease. Interval (days) between SD $OPG_{max}$ and $SDCS_{max}$ was calculated. Distribution analysis was established for the outcomes of SD $OPG_{max}$ minus $SDCS_{max}$.

*2.9.4. Univariate and multivariate analysis OPG patterns and mean cumulative clinical scores per pool and mean ADG*

Studied patterns in oocyst shedding included cumulative ($log_{10}$) OPG levels, cumulative OPG, maximum OPG levels and number of sample days (SD) at a confirmed OPG level per pool. For the analysis of OPG patterns versus mean cumulative clinical scores per pool a $log_{10}$ cumulative OPG was determined to represent the total oocyst load of the calves in the pool. Therefore, the OPG's of SD0 up until SD56 were added up and the transcendental $log_{10}$ of the sum was calculated. For each pool, a mean cumulative clinical score was calculated to represent the total disease load of the calves in the pool for the parameter's rumen fill and faecal consistency. Therefore, all scores per clinical parameter of SD0 up until SD56 were added up of all calves in the pool whereafter the sum was divided with the number of calves in the pool. MLR was used to establish correlations. Differentiation was used to establish cumulative OPG and the maximum OPG level reached per *Eimeria* species per pool. The pools were categorized according to the median cumulative OPG and according to the maximum OPG in two groups. Secondly the number of days a pool reached an OPG level at or higher than the median maximum was established per pool. For this variable, the pools were categorized in 3 groups: 0, 1 and $\geq$ 2 days higher than the median maximum OPG. Univariate analysis using one-way ANOVA followed by comparing means by students *t*-tests were used to establish the effect of difference in shedding patterns category on the mean cumulative clinical scores per pool and on mean ADG. In a second step, to determine the OPG patterns affecting mean cumulative clinical scores and mean ADG, an explanatory standard least square model was fitted using farm as a random effect. Significant associated shedding pattern factors ($p \leq 0.05$) and tendency's ($p \leq 0.1$) were tested in the model. The variables were tested for inclusion using a manual backward selection procedure (*p*-value for retention $\leq$0.10). From the outcomes of interest from the questionnaire data and technical herd parameters the effect of differences in management approaches on cumulative pool $log_{10}$ OPG's for *E. alabamensis, E. bovis, E. zuernii,* pathogenic *Eimeria* and *Eimeria* species was addressed. As a first step, ANOVA were carried out for ordinal parameters and logistic regression for continuous parameters (Supplementary data tables 7 and 8). When results of the ANOVA analysis yielded a *p-value of* $\leq$ *0.10*, Students *t*-test were used for comparison of means across all groups to control experiment-wise error rate. In a second step, to determine the factors affecting cumulative pool OPG's for the different *Eimeria* species, an explanatory standard least square model was fitted. Significant associated factors ($p \leq 0.05$) and tendency's ($p \leq 0.1$) were tested in the model. The variables were tested for inclusion using a manual backward selection procedure (*p-value for retention* $\leq$ *0.20*).

## 3. Results

### 3.1. Similarity in shedding patterns between pools per farm

During the study period, one calf died due to complications during respiratory infection. This calf was excluded from the study. The corresponding pool in which this calf was included stayed in the study, because the pool still contained five calves. For *E. alabamensis* pool 1 and 2 showed similar patterns in prevalence and oocyst flare ups during the entire study on farms A, B, D, E, G, H, I, J. On farms C and F *E. alabamensis* was found in only one pool. For *E. bovis*, this similarity in both pools was the case on all farms. For *E. zuernii* similarity was present at farm B, D, E, G, H, I and J; At farm A, C and F *E. zuernii* was only found in one of the two pools.

### 3.2. Morphological differentiation of oocysts

On all ten farms *E. alabamensis, E. bovis* and *E. zuernii* were found as well as non-pathogenic species. In 90% of the pool groups *E. alabamensis and E. zuernii* were found and all pool groups showed *E. bovis* and non-pathogenic *Eimeria* species at some point during the study period. *E. alabamensis* was present in 34% of the pooled samples, *E. bovis* in 43% and *E. zuernii* in 20%. Of all pooled samples, 56% were positive for pathogenic *Eimeria* and 64% for non-pathogenic *Eimeria.*

Fig. 1 shows the part of the 20 samples positive for the three pathogenic *Eimeria* species per sample day over the entire study period. Fig. 2 shows the part of the 20 samples being positive on either pathogenic or non-pathogenic *Eimeria* species. Significant correlation existed on all sample days except for day 7, 14 and 42 in being positive for either pathogenic or non-pathogenic species. Overall a significant correlation of 0.60 existed between the presence of pathogenic and non- pathogenic *Eimeria* ($p < 0.001$).

### 3.3. Patterns of Eimeria oocyst positive samples per farm

All farms and all pools showed combinations of different *Eimeria* species throughout the study, but patterns in oocyst shedding differed between farms and between *Eimeria* species. *E. bovis* and *E. alabamensis* appeared at the same sample day at five farms; at two farms *E. alabamensis* appeared first; at three farms *E. bovis* appeared first. *E. zuernii* appeared at a later sample day than *E. alabamensis* at seven farms and a later sample day than *E. bovis* at eight farms. *E. alabamensis* was first seen at SD7 on one farm, at SD14 on five farms, at SD21 on three farms and at SD28 on one farm. *E. bovis* was first found at SD7 on three farms, at SD14 on three farms at SD21 on three farms, and at SD28 on one farm. *E. zuernii* was first found at SD14 on one farm, at SD21 on two farms, at SD28 on three farms, at SD35 on one farm, at SD42 on two farms, and at SD49 on one farm. Non-pathogenic *Eimeria* species appeared for the first time at SD7 on three farms, at SD14 on four farms, at SD21 on two farms and at SD28 on one farm.

### 3.4. Oocysts per gram feces

Oocyst shedding for *E. alabamensis, E. bovis and E. zuernii* differed per farm and per pool. For *E. alabamensis* seven out of the 20 pools reached a peak excretion between 100 oocysts per gram feces (OPG) and 1000 OPG; ten pools reached an excretion between 1000 OPG and 10,000 OPG and one pool peaked higher than 10,000 OPG (actual 30,500 OPG). Maximum OPG was reached at SD 14 in four pools, at SD 21 in six pools,

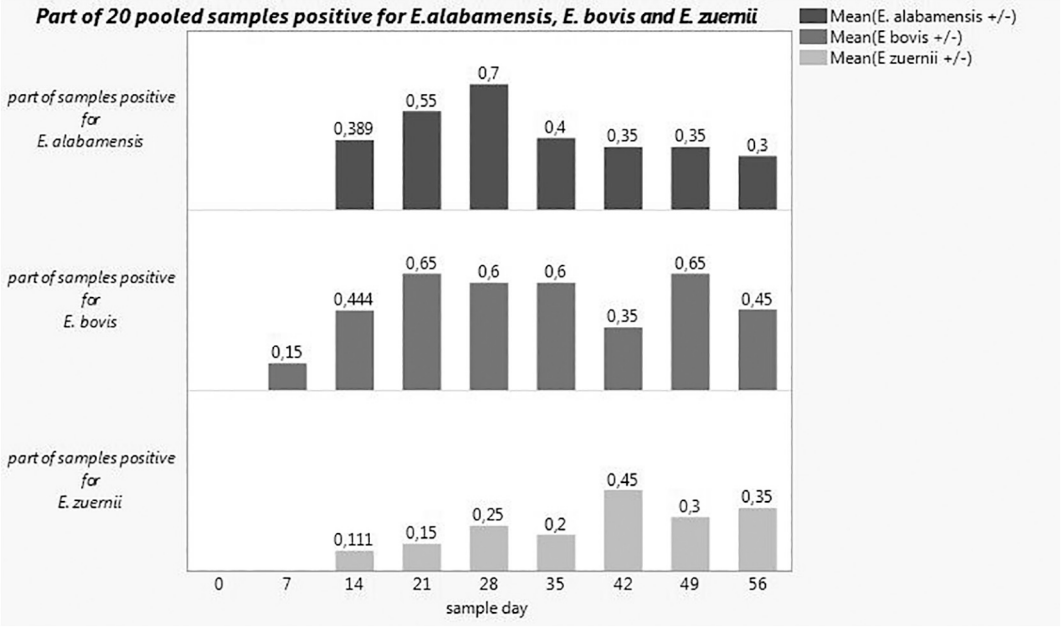


**Fig. 1.** Part of total number of pooled samples that test positive for respectively *E. alabamensis, E. bovis* and *E. zuernii* oocysts per sample day. X- axis representing the sample days 0, 7,14,21, 28, 35, 42, 49 and 56. Y- axis is representing the part of the 20 pooled samples that test positive: For example 0,7 represents 0,7 * 100% = 70% of the samples test positive for *E. alabamensis* on sample day 28.

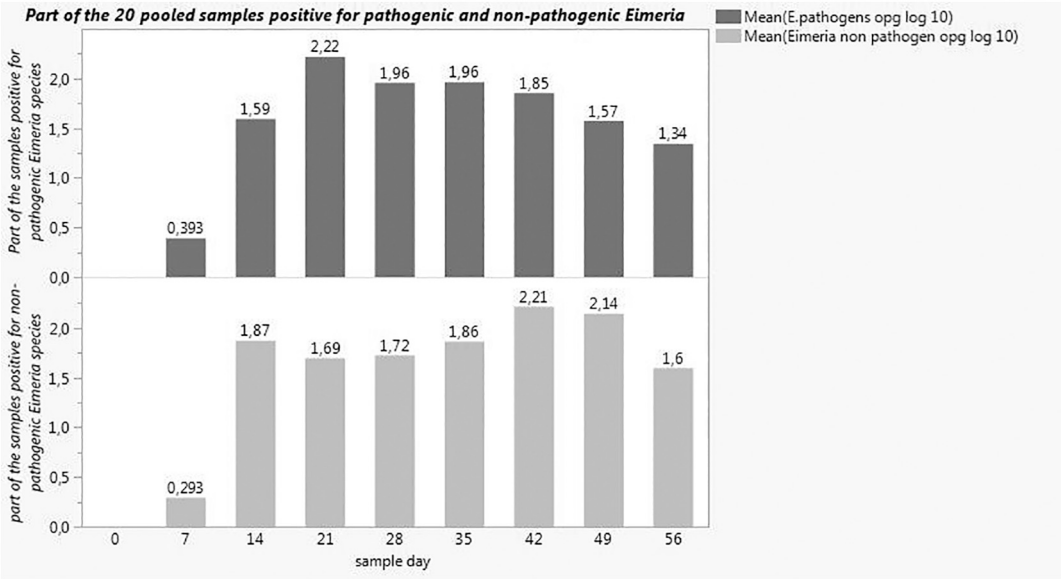


**Fig. 2.** Part of total number of pooled samples that test positive for respectively pathogenic and non-pathogenic *Eimeria* species oocysts per sample day. The X- axis is representing the sample days 0, 7,14,21, 28, 35, 42, 49 and 56. The Y- axis is representing the part of the 20 pooled samples that test positive: For example 0.85 represents 0.85 * 100% = 85% of the samples test positive for pathogenic *Eimeria* species on sample day 21.

at SD 28 in four pools and at SD 42 in four pools.

For *E. bovis* two pools reached a peak excretion between 100 OPG and 500 OPG, 11 pools reached an excretion between 500 OPG and 1000 OPG, six pools between 1000 OPG and 10,000 OPG and one pool peaked higher than 10,000 OPG (actual 10,200). Maximum OPG for *E. bovis* was reached at SD 14 in three pools, at SD 21 in six pools, at SD 28 also in six pools, at SD 35 in one pool, at SD 42 in three pools and at SD 56 in one pool.

For *E. zuernii* four pools showed no oocysts, eight pools reached an excretion between 100 OPG and 500 OPG, one pool between 500 OPG and 1000 OPG, four pools between 1000 OPG and 10,000 OPG and three pools excreted more than 10,000 OPG (actual 11,800; 37,600 and 86,000). Maximum OPG for *E. zuernii* was reached at SD 14 in two pools, at SD 21, SD 28 and at SD 35 in one pool, at SD 42 in six pools, at SD 49 in three pools and at SD 56 in three pools. Within the samples of the 20 pools a significant correlation was present between $\log_{10}$ OPG of different *Eimeria* species per sample day. The $\log_{10}$ OPG for pathogenic and non-pathogenic *Eimeria* species had a correlation coefficient of 0.60 ($p = 0.0001$). $\text{Log}_{10}$ OPG for pathogenic *Eimeria* and all *Eimeria* had a correlation coefficient of 0.83 ($p = 0.0001$). (Supplementary data Table 1).

### 3.5. Clinical scores

#### 3.5.1. Distribution analysis of clinical scores

Mean clinical scores per sample day for condition and alertness maintained at a low level through all farms with no clear peak (Supplementary data Table 2). Rumen fill scores though, showed higher at the first 3 sample days of the study period (indicating a lower rumen fill). Temperature and respiratory disease scores were higher towards sample days 21–28 and at the end of the study period; sample day 49 and 56. Table 2 shows the mean faecal clinical scores (faecal consistency, hindquarter cleanliness, pen cleanliness) per farm and per sample day. Faecal parameter scores differed between farms but showed a concentration of higher scores from SD 0 until SD 21 and at SD 42 and SD 49.

#### 3.5.2. Univariate analysis of clinical parameters and average daily weigh gain at calf level

Significant correlation was identified between all three faecal parameters. Respiratory disease scores correlated with the following parameters: alertness, temperature, and condition. Rumen fill scores showed significant correlation with condition and faecal consistency scores. Furthermore, daily weight gain per calf showed significant negative correlation with cumulated scores per calf of rumen fill, temperature, alertness, faecal consistency, cleanliness of hindquarters and cleanliness of the pen ($p < 0.05$). For cumulative respiratory disease score the negative correlation was not significant (Supplementary data Table 5). Between many clinical parameter scores per calf per sample day a significant correlation was established ($p < 0,05$) (Supplementary data Table 3).

#### 3.5.3. Univariate analysis at pool level

The average clinical scores over the entire study period of the following parameters were related ($p < 0.05$). Faecal consistency correlated with alertness (0.49), temperature (0.47) and hindquarter cleanliness (0.54). Temperature correlated with alertness (0.72) and respiratory disease (0.53) (Supplementary data Table 4).

### 3.6. Average clinical scores per sample day and the presence and number of oocysts of different Eimeria species

The data showed no correlation between the presence of oocysts in the 20 pooled faecal samples and the pool average of clinical scores at the same sample day. Nor was there a correlation found between the OPG levels of different *Eimeria* species and average clinical scores in that pool at the same sample day. The mean peak in faecal consistency score per farm preceded the peak in oocyst shedding of pathogenic *Eimeria* by 4.5 days ($n = 14$; SEM 1.7 CI95%: 8.3; 0.7) (Supplementary data table 6).

### 3.7. Results of univariate and multivariate analysis of 3 pool OPG patterns versus mean cumulative (MC) scores of 2 clinical parameters

#### 3.7.1. Cumulative $Log_{10}$ OPG (C.Log OPG)

The MC rumen fill score per pool showed significant correlation with the C. Log OPG of *E. zuernii* ($p < 0.05$) and a tendency to correlation for non-pathogenic *Eimeria* ($N = 20$; $p < 0.10$). (Table 3). For the MC faecal consistency score per pool a significant correlation with C.Log OPG of pathogenic *Eimeria* and *Eimeria* species ($p < 0.05$) was found and a tendency with C.Log OPG of *E. bovis, E. zuernii and* non-pathogenic *Eimeria* ($p < 0.10$) (Table 3).

#### 3.7.2. Maximum OPG (OPG max)

MC rumen fill scores tend to be higher when OPG max reached the median level $\geq 750$ (*E. bovis*) ($p < 0.10$). For MC faecal consistency significant higher scores were reached when OPG max was higher than

**Table 2**
Mean faecal parameter scores of 12 calves per farm per sample day and for the entire study period.

| | | Sample day | | | | | | | | | Study period |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Farm | Faecal parameter | SD0 | SD7 | SD14 | SD21 | SD28 | SD35 | SD42 | SD49 | SD56 | |
| A | Faecal consistency | 0 | 0.17 | 0.17 | 0.25 | 0.08 | 0.08 | 0.08 | 0 | 0 | 0.09 |
| | Hindquarter cleanliness | 0 | 0.17 | 0.25 | 0.08 | 0 | 0 | 0 | 0.08 | 0.08 | 0.07 |
| | Pen cleanliness | 0 | 0 | 0 | 0.08 | 0.17 | 0.25 | 0.17 | 0 | 0 | 0.07 |
| B | Faecal consistency | 0.17 | 0 | 0.17 | 0.25 | 0 | 0.08 | 0.08 | 0.08 | 0 | 0.09 |
| | Hindquarter cleanliness | 0 | 0 | 0.17 | 0.17 | 0 | 0 | 0 | 0.08 | 0 | 0.05 |
| | Pen cleanliness | 0 | 0 | 0 | 0 | 0 | 0.08 | 0 | 0 | 0 | 0.01 |
| C | Faecal consistency | 0.08 | 0.25 | 0.17 | 0.08 | 0.08 | 0 | 0 | 0 | 0 | 0.07 |
| | Hindquarter cleanliness | 0 | 0.25 | 0.17 | 0 | 0 | 0 | 0 | 0 | 0 | 0.05 |
| | Pen cleanliness | 0 | 0.17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.02 |
| D | Faecal consistency | 0.25 | 0.08 | 0.08 | 0.17 | 0.17 | 0.08 | 0.25 | 0 | 0.08 | 0.13 |
| | Hindquarter cleanliness | 0 | 0 | 0 | 0 | 0 | 0 | 0.08 | 0 | 0 | 0.01 |
| | Pen cleanliness | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 |
| E | Faecal consistency | 0.25 | 0.17 | 0.33 | 0.08 | 0.08 | 0.17 | 0.08 | 0 | 0.25 | 0.16 |
| | Hindquarter cleanliness | 0.08 | 0.08 | 0 | 0.08 | 0 | 0.08 | 0.08 | 0 | 0.08 | 0.05 |
| | Pen cleanliness | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 |
| F | Faecal consistency | 0 | 0.17 | 0.25 | 0.42 | 0.25 | 0.17 | 0.25 | 0.17 | 0.08 | 0.20 |
| | Hindquarter cleanliness | 0 | 0.08 | 0.25 | 0.17 | 0.17 | 0 | 0.17 | 0.08 | 0.08 | 0.11 |
| | Pen cleanliness | 0 | 0 | 0 | 0 | 0.08 | 0.08 | 0 | 0 | 0 | 0.02 |
| G | Faecal consistency | 0.08 | 0.42 | 0.08 | 0 | 0 | 0 | 0.25 | 0 | 0.08 | 0.10 |
| | Hindquarter cleanliness | 0.08 | 0.17 | 0.17 | 0 | 0 | 0 | 0.08 | 0.08 | 0.08 | 0.07 |
| | Pen cleanliness | 0.08 | 0.08 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.02 |
| H | Faecal consistency | 0.17 | 0.17 | 0.25 | 0.25 | 0.17 | 0.17 | 0.17 | 0.08 | 0 | 0.16 |
| | Hindquarter cleanliness | 0.25 | 0.08 | 0 | 0.08 | 0.08 | 0 | 0.25 | 0.08 | 0.09 | 0.10 |
| | Pen cleanliness | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.36 | 0.10 |
| I | Faecal consistency | 0.25 | 0.42 | 0.17 | 0.17 | 0 | 0 | 0 | 0.36 | 0 | 0.15 |
| | Hindquarter cleanliness | 0 | 0.25 | 0 | 0.17 | 0.09 | 0 | 0 | 0.27 | 0 | 0.09 |
| | Pen cleanliness | 0 | 0.08 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 |
| J | Faecal consistency | 0.25 | 0.33 | 0 | 0 | 0.17 | 0.08 | 0.25 | 0 | 0.09 | 0.13 |
| | Hindquarter cleanliness | 0 | 0 | 0 | 0 | 0.08 | 0 | 0.08 | 0 | 0 | 0.02 |
| | Pen cleanliness | 0 | 0.17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.02 |
| all farms | Faecal consistency | 0.15 | 0.22 | 0.17 | 0.17 | 0.10 | 0.08 | 0.14 | 0.07 | 0.06 | |
| | Hindquarter cleanliness | 0.04 | 0.11 | 0.1 | 0.08 | 0.04 | 0.01 | 0.08 | 0.07 | 0.04 | |
| | Pen cleanliness | 0.01 | 0.05 | 0 | 0.01 | 0.03 | 0.04 | 0.04 | 0.03 | 0.03 | |

**Table 3**
Most important significant and trending correlation found between $log_{10}$ cumulative OPG [a] and the mean cumulative clinical scores [b] per pool.

| *Eimeria* | Clinical parameter | Correlation | CI 95% | *p* |
|---|---|---|---|---|
| $log_{10}$ cum OPG *Eimeria* species/pool | Faecal consistency mean cumulative score/pool | 0.48 | 0.04; 0.76 | < 0.05 |
| $log_{10}$ cum OPG pathogenic *Eimeria*/pool | Faecal consistency mean cumulative score/pool | 0.47 | 0.04; 0.76 | < 0.05 |
| $log_{10}$ cum OPG *E. zuernii*/pool | Rumen fill mean cumulative score/pool | 0.46 | 0.02; 0,75 | < 0.05 |
| $log_{10}$ cum OPG *E. zuernii*/pool | Faecal consistency mean cumulative score/pool | 0.41 | −0.04; 0.72 | < 0.10 |
| $log_{10}$ cum OPG *E. bovis*/pool | Faecal consistency mean cumulative score/pool | 0.41 | −0.04; 0.72 | < 0.10 |
| $log_{10}$ cum OPG non-pathogenic *Eimeria*/pool | Rumen fill mean cumulative score/pool | 0.41 | −0.04; 0.79 | < 0.10 |
| $log_{10}$ cum OPG non-pathogenic *Eimeria*/pool | Faecal consistency mean cumulative score/pool | 0.40 | −0.05; 0.72 | < 0.10 |

Faecal samples were collected from 12 calves per farm on ten rose veal starter farms and analyzed in predetermined pools of six calves. First sample was taken within eight days after arrival of the calves where after weekly for nine consecutive weeks. [a] $Log_{10}$ cumulative OPG was determined per pool by adding up the OPG's of SD0 up until SD56 and calculate the transcendental $log_{10}$ of the sum. [b] Mean cumulative scores per clinical parameter were determined by adding up all scores of of SD0 up until SD56 of all calves per pool and divided with the number of calves in the pool.

the median OPG of 750 (*E. bovis*) and 200 (*E. zuernii)* ($p < 0.05$) (Table 4).

### 3.7.3. Number of sample days at OPG max

MC rumen fill was significantly higher (indicating a lower rumen fill) when the median level ≥ 750 (*E. bovis*) was reached at 2 or more sample days ($p < 0.05$). MC faecal consistency tended to be higher when the median level of 750 (*E. bovis*) and 200 (*E. zuernii)* were reached at 2 or more sample days ($p < 0.10$) (Table 4).

### 3.7.4. Multivariate analysis OPG pattern affecting MC rumen fill and MC faecal consistency scores

Multivariate analysis ($n = 20$; $R^2 = 0.81$; $p = 0.06$) showed eventually that cumulative $log_{10}$ OPG of *E. zuernii* had a positive correlation with MC rumen fill score per pool ($\beta = 0.05$; $p = 0.06$).

For the MC faecal consistency score per pool multivariate analysis showed eventually (n = 20; $R^2 = 0.88$; $p = 0.008$) a positive correlation with cumulative $log_{10}$ OPG of pathogenic *Eimeria species* ($\beta = 0.16$; $p = 0.008$).

### 3.8. Patterns of pathogenic Eimeria species versus the mean average daily weight gain (ADG) per pool

Univariate analysis showed significant lower ADG in case cumulative pool OPG ≥ 5450 (pathogenic *Eimeria*) ($p < 0.05$) and that ADG tended to be lower when the cumulative pool OPG was ≥2100 (*E. alabamensis*) or ≥ 10,000 (*Eimeria* species in general) ($p < 0.10$) (Table 5). Furthermore, the mean ADG of the pool was significantly lower when the OPG max reached the median level of ≥750 (*E. bovis*) and tended to be lower if OPG max reached ≥2800 (pathogenic *Eimeria*) ($p < 0.10$) (Table 5). Finally, the mean ADG per pool was significantly lower if OPG levels of ≥1150 (*E. alabamensis*) ($p < 0.05$) were reached at two or more sample days and tended to be lower if OPG max reached at two or more SD was ≥750 (*E. bovis*) or ≥ 2800 (pathogenic *Eimeria*) ($p < 0.10$) (Table 5). In the multivariate analysis ($n = 20$; $R^2 = 0.58$; $p = 0.0413$), least square mean values indicated a lower ADG in those pools that showed a peak OPG for *E. bovis* (difference 93 g) that was higher than the median of 750 OPG (LSM: 855 and 947 g / day for higher and lower than 750 OPG respectively, $p = 0.0271$) and in pools that showed more than 2 sample days with an OPG level of 1000 for *E. alabamensis* (difference 141 g) (LSM: 807; 958 and 948 g / day for ≥2; 1 and 0 SD > 1000 OPG respectively, $p = 0.0471$).

**Table 4**
Mean cumulative clinical score per pool for the group of pools with a higher or lower maximum OPG level than median and for the group of pools that showed 0, 1 or ≥ 2 sample days with an OPG level higher than the median for different *Eimeria* species.

| *Eimeria* species | maximum OPG level per pool | N | rumen fill (mean ± sd) | *p* | faecal consistency (mean ± sd) | *p* | number of sample days | N | rumen fill (mean ± sd) | p | faecal consistency (mean ± sd) | p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. alabamensis* | < 1150 | 10 | 0.80 ± 0.07 | | 0.52 ± 0.05 | | 0 | 9 | 0.80 ± 0.08 | | 0.48 ± 0.05 | |
| | ≥ 1150 | 10 | 0.79 ± 0.07 | 0.92 | 0.55 ± 0.05 | 0.70 | 1 | 8 | 0.84 ± 0.08 | | 0.59 ± 0.06 | |
| | | | | | | | ≥2 | 3 | 0.73 ± 0.13 | | 0.61 ± 0.09 | |
| *E. bovis* | < 750 | 10 | 0.72 ± 0.07 | | 0.46 ± 0.05 | | 0 | 10 | 0.72 ± 0.06 | | 0.46 ± 0.05 | |
| | ≥ 750 | 10 | 0.88 ± 0.07 [a] | < 0.10 | 0.61 ± 0.05 [b] | < 0.05 | 1 | 5 | 0.80 ± 0.09 | | 0.60 ± 0.07 | |
| | | | | | | | ≥2 | 5 | 0.97 ± 0.09[c] | < 0.05 | 0.62 ± 0.07[e] | < 0.10 |
| *E. zuernii* | ≤ 200 | 11 | 0.74 ± 0.07 | | 0.47 ± 0.04 | | 0 | 11 | 0.74 ± 0.07 | | 0.48 ± 0.05 | |
| | > 200 | 9 | 0.87 ± 0.07 | 0.22 | 0.62 ± 0.05 [b] | < 0.05 | 1 | 4 | 0.92 ± 0.11 | | 0.61 ± 0.08 | |
| | | | | | | | ≥2 | 5 | 0.82 ± 0.10 | | 0.65 ± 0.07[e] | < 0.10 |
| pathogenic *Eimeria* | < 2800 | 10 | 0.78 ± 0.07 | | 0.48 ± 0.05 | | 0 | 10 | 0.78 ± 0.07 | | 0.49 ± 0.05 | |
| | > 2800 | 10 | 0.82 ± 0.07 | 0.71 | 0.59 ± 0.05 | 0.15 | 1 | 7 | 0.74 ± 0.08 | | 0.59 ± 0.06 | |
| | | | | | | | ≥2 | 3 | 1,01 ± 0.12[d] | < 0.10 | 0.61 ± 0.09 | |
| *Eimeria* species | < 5550 | 10 | 0.79 ± 0.07 | | 0.50 ± 0.05 | | 0 | 10 | 0.80 ± 0.07 | | 0.50 ± 0.05 | |
| | ≥ 5550 | 10 | 0.81 ± 0.07 | 0.88 | 0.58 ± 0.05 | 0.26 | 1 | 7 | 0.72 ± 0.08 | | 0.57 ± 0.06 | |
| | | | | | | | ≥2 | 3 | 1.01 ± 0.12[d] | < 0.10 | 0.61 ± 0.10 | |

Maximum OPG and number of sample days with a OPG level higher than median wazs analyzed in pooled faecal samples of calves in rose veal starter farms. Pools contain six calves each. Per farm 12 calves were scored and sampled. First sample was taken within eight days after arrival of the calves where after weekly for nine consecutive weeks. [a] $p < 0.10$ for difference < median; [b] $p < 0.05$ for difference with < median; [c] $p < 0.05$ for difference with 0 sample days; d $p < 0.10$ for difference with 1 sample day; [e] $p < 0.10$ for difference with 0 sample days.

**Table 5**
Comparing means of average daily weight gain for the group of pools with a higher or lower maximum OPG level than median and for the group of pools that showed 0, 1 or ≥ 2 sample days with an OPG level higher than the median for different *Eimeria* species.

| *Eimeria* species | average cumulative OPG | N | average daily weight gain (gram/day) (mean ± sd) | *p* | maximum OPG level per pool | N | average daily weight gain (gram/day) (mean ± sd) | *p* | maximum OPG level per pool | number of sample days | N | average daily weight gain (gram/day) (mean ± sd) | *p* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. alabamensis* | < 2150 | 10 | 923 ± 27.7 | | < 1150 | 10 | 887 ± 30.6 | | ≥ 1150 | 0 | 9 | 908 ± 28.6 | |
| | ≥ 2150 | 10 | 845 ± 27.7[b] | < 0.10 | ≥ 1150 | 10 | 881 ± 30.6 | 0.88 | | 1 | 8 | 900 ± 30.3 | |
| | | | | | | | | | | ≥2 | 3 | 773 ± 49.5[e] | < 0.05 |
| *E. bovis* | < 1100 | 10 | 907 ± 29.7 | | < 750 | 10 | 924 ± 27.6 | | ≥ 750 | 0 | 10 | 924 ± 28.4 | |
| | ≥ 1100 | 10 | 861 ± 29.7 | 0.28 | ≥ 750 | 10 | 844 ± 27.6[c] | < 0.10 | | 1 | 5 | 852 ± 40.0 | |
| | | | | | | | | | | ≥2 | 5 | 837 ± 40.0[f] | < 0.10 |
| *E. zuernii* | < 292 | 10 | 886 ± 30.6 | | ≤ 200 | 11 | 896 ± 28.9 | | > 200 | 0 | 11 | 896 ± 29.7 | |
| | ≥ 292 | 10 | 882 ± 30.6 | 0.92 | > 200 | 9 | 870 ± 32.0 | 0.55 | | 1 | 4 | 862 ± 49.3 | |
| | | | | | | | | | | ≥2 | 5 | 877 ± 44.1 | |
| pathogenic *Eimeria* | < 5450 | 11 | 927 ± 25.2 | | ≤ 2800 | 10 | 922 ± 29.4 | | > 2800 | 0 | 10 | 922 ± 28.4 | |
| | > 5450 | 9 | 833 ± 27.8[a] | < 0.05 | > 2800 | 10 | 847 ± 29.4[d] | < 0.10 | | 1 | 7 | 860 ± 33.9 | |
| | | | | | | | | | | ≥2 | 3 | 816 ± 51.8[f] | < 0.10 |
| *Eimeria* species | < 10,000 | 10 | 922 ± 28.0 | | < 5550 | 10 | 910 ± 27.9 | | ≥ 5550 | 0 | 10 | 909 ± 29.5 | |
| | > 10,000 | 10 | 847 ± 28.0[b] | < 0.10 | ≥ 5550 | 10 | 859 ± 27.9 | 0.23 | | 1 | 7 | 877 ± 35.3 | |
| | | | | | | | | | | ≥2 | 3 | 816 ± 53.9 | |

Cumulative OPG, maximum OPG level and the number of sample days with an OPG level higher than median were established out of pooled faecal samples of calves in rose veal starter farms. Pools contain 6 calves each. Per farm 2 pools of calves were weighed and sampled. First sample was taken within 8 days after arrival of the calves where after weekly for 9 consecutive weeks. Calves were weighed at the first and the last visit.

[a] $p < 0.05$ for difference with < median.
[b] $p < 0.10$ for difference with < median.
[c] $p < 0.10$ for difference with <750.
[d] $p < 0.10$ for difference with <2800.
[e] $p < 0.05$ for difference with 0 and 1 sample day.
[f] $p < 0.10$ for difference with 0 sample days.

### 3.9. Technical parameters of the herds

Average mortality of the herds was 0.85% (1.02; 0.68 CI 95%), average weight gain 63.1 kg (65.8; 60.3 CI 95%); 873 g/day (902; 844 CI 95%). Average number of growing days 72.3 (74.6; 69.9 CI 95%). The antibiotic use of the farms was 24.1 animal daily dosages on average and showed large variation (26.7; 21.5 CI 95%). The amount of different feed components fed per calf per day showed also large variation, average amount of concentrate was 1470 g/ calf/ day (1575; 1364 CI 95%), average amount of corn silage 599 (834; 364 CI 95%) and average amount of straw 134,5 (178; 90.3 CI 95%) also showed large variation within the group of farms. (Supplementary data table 7).

### 3.10. Questionnaire results

Full details on the questionnaire and answers concerning management are displayed in supplementary Table 8a -g. In this section we will only mention the parameters that appeared relevant for *Eimeria* shedding patterns.

Different strategies were used to prepare the units (Supplementary data table 8a). From no cleaning and keeping the barn empty for less than one week to complete cleaning and an empty barn period of more than 2 weeks. None of the farms performed any disinfection of the units. Regarding water management it was noticed that most farms (except farm D and F) provided water from a source other than tap water. (Supplementary data table 8b).

A lot of different approaches were identified concerning management in the onset of group housing: All farms provided extra care management to ease this transition for the calves, although farms differed in their choices: Three farms delayed the start of group housing for extra care calves, four farmers lowered the milk supply at the start of group housing, six farms provided extra roughage, three farms extra water and six farms provided extra medication (sodium-salicylate). (Supplementary data table 8e).

None of the farms used a herd treatment with trimethoprim-sulfamide antibiotics. The number of herd treatments differed from three up to six in this study period (Supplementary data table 8g).

The following results (3.10.1–3.10-5) are based on multivariate analysis and uses pool as statistical unit. The Explanatory standard least square models concern management explaining mean cumulative $\log_{10}$ OPG's (MC.Log OPG) per pool of the following five categories of *Eimeria* species: *E. alabamensis*, *E. bovis*, *E. zuernii*, pathogenic *Eimeria* species and *Eimeria* species in general of the pools.

In the multivariate analysis regarding C.Log OPG of *E. alabamensis* ($n = 20$; $R^2 = 0.71$; $p = 0.0019$) least square mean values indicated a higher C.Log OPG for *E. alabamensis* in those farms that did not clean the units prior to the delivery of the calves (LSM: 1.02; 0.55 and 0.60 for no cleaning, half cleaning or complete cleaning of the units respectively, $p = 0.0106$) and in farms that provided extra roughage at the onset of group housing (LSM 0.99 and 0.46 for yes and no respectively, $p = 0.0119$). The delivery timeframe of the calves and providing no extra water at onset of group housing were not significant but these variables remained into the model because $p < 0.2$.

The analysis regarding C.Log OPG of *E. bovis* ($n = 20$; $R^2 = 0.75$; $p = 0.0024$) showed a negative correlation with amount of milk powder per calf per study day ($\beta = -0.01$; $p = 0.0185$) and a positive correlation with the interval between arrival and group housing ($\beta = 0.191$; $p = 0.020$). Least square mean values indicated a lower C.Log OPG for

*E. bovis* in those farms that did not delay group housing for extra care calves (LSM: 0.76 and 1.1 for no and yes respectively, $p = 0.0359$). Providing water from a source or not and the amount of concentrate fed per calve per day were not significant, but these variables remained in the model because $p < 0.2$.

Regarding C.Log OPG of *E. zuernii* in the multivariate analysis ($n = 20$; $R^2 = 0.75$; $p = 0.002$) a positive correlation was found with weaning length ($\beta = 0.05$; $p = 0.0031$). Least square mean values also indicated a higher C.Log OPG for *E. zuernii* in those farms that did not clean the units before arrival of the calves (LSM: 0.74; 0.28 and 0.30 for no cleaning, half cleaning or complete cleaning of the units respectively, $p = 0.0021$) and for farms that delayed the start of group housing for extra care calves (LSM: 0.58 and 0.31 for yes and no respectively; $p = 0.0326$)

Multivariate analysis of C.Log OPG of pathogenic *Eimeria* species ($n = 20$; $R^2 = 0.84$; $p = 0.0002$), showed a positive correlation with amount of milk powder per calf per study day ($\beta = 0.29$; $p = 0.0114$). There was a negative correlation with the animal daily dosages of antibiotics used ($\beta = -0.68$; $p = 0.0004$). Least square mean values indicated a higher C. Log OPG for pathogenic *Eimeria* in those farms with delayed start group housing for extra care calves (LSM: 1.69 and 1.32 for yes and no respectively, $p = 0.0037$) and a lower C.Log OPG for pathogenic *Eimeria* in those farms that cleaned the units prior to arrival of the calves (LSM: 0.90; 1.73 and 1.88 for cleaning, half cleaning and no cleaning respectively, $p = 0.003$).

Finally in the analysis of C.Log OPG of *Eimeria* species in general ($n = 20$; $R^2 = 0.79$; $p = 0.0002$) least square mean values indicated a higher C.Log OPG for *Eimeria* species in those farms that did not clean the units previous to the delivery of the calves (LSM: 2.3; 2.13 and 1.48 for no cleaning, half cleaning or complete cleaning of the units respectively, $p = 0.0002$). The use of calcium carbonate and amount of concentrate fed per calf per day were not significant, but these variables remain into the model because $p < 0.2$.

## 4. Discussion

All three pathogenic *Eimeria* species were found on every farm at least in one sample. High prevalence of bovine coccidia have been described in Europe. In Dutch dairy farms, a prevalence of 87.0% has been demonstrated in a study concerning 66 farms (Van Balen, 2013), whereas even a higher prevalence of 95% was reported in German herds in calves between the age of 4 weeks up until nine months (Bangoura et al., 2012) and of 97,7% in Austrian herds (Koutny et al., 2012). Sampling the herds in our study over nine consecutive weeks led to higher herd prevalence for each *Eimeria* species compared to that at any single sample point. In our study we found many samples yielding a mix of *Eimeria* species which corresponded to other studies (Cornelissen et al., 1995; Bangoura et al., 2012; Koutny et al., 2012). Including criteria did not allow the farmers to use metaphylactic treatment during the study. Therefore, one could argue that the chosen farms could experience historically less problems with coccidiosis than Dutch rose veal starter farms in general. However, a metaphylactic approach to prevent coccidiosis consequences, is rarely used in Dutch rose veal starter farms (information derived in many conversations between Dutch veal veterinarians and Elanco Benelux). The majority of rose veal starter farmers in the Netherlands are using a curative approach to manage coccidiosis if clinical symptoms occur. Therefore, we do not consider this inclusion criterium to be an indication of lower or different occurrence of problems with coccidiosis in the studied farms. In general, peak prevalence for pathogenic *Eimeria* species showed at sample day (SD) 21. Although it has been shown that the onset of excreting oocysts of *E. bovis* and *E. zuernii* can start from the age of 3 weeks (Marshall et al., 1998), the samples in our study taken before SD 14 (age 2–4 weeks) yielded no (SD0) or only very few oocysts (SD7). Therefore, sampling calves before they have been on the farm for at least 14 days seems to be too early to diagnose infection based on pooled faecal samples on this type of farm. This can be due to a later onset of shedding related to a later infection or due to dilution of the number of oocysts caused by pooling. The calves enrolled in our study on farm I happened to be housed in a newly built clean barn. However, samples from farm I already showed oocysts of all three pathogenic species in the pooled samples at SD 14 (in this case exactly 14 days on farm). Considering the prepatent periods of *E. bovis* and *E. zuernii* the infections of these calves took place at the dairy farm of origin and were introduced with the calves.

The patterns in oocysts shedding differed between the ten farms and between *Eimeria* species. This corresponded to other data in literature (Daugschies and Najdrowski, 2005; Staschen et al., 2003). In contrast with the differences found between farms, pooled samples from the same farm showed similar patterns over the study period on most of the farms, both in prevalence and in patterns of OPG shedding flare ups of *Eimeria* species. Therefore, we consider this approach an acceptable representation of the oocyst shedding patterns of the herd.

Although prepatent periods of *E. bovis* and *E. zuernii* are similar (16–21 days and 15–17 days) in our study the highest number of sample positives of *E. zuernii* oocysts showed at sample day 42 whereas *E. bovis* showed the highest number of sample positives 3 weeks earlier. In German herds similar results were reported: OPG of *E. zuernii* correlated positively with the age of calves and the OPG of *E. bovis* correlated with the number of days after rehousing (Bangoura et al., 2012). Differences in immunogenicity are considered to play a role in differences in oocysts shedding patterns between *Eimeria* species (Faber et al., 2002). In our study *E. alabamensis* was found in 100% of the herds and in 34% of the samples. This is higher than the 6.3% found in dairy calves up to nine weeks of age (Faber et al., 2002) and higher than the 12% herd prevalence found in Dutch dairy herds (Cornelissen et al., 1995). In a more recent publication in Italy *Eimeria* prevalence's between 16 and 21% were found (Grandi et al., 2016). Possible explanations could be either the fact that rose veal starter farms house calves originating from many different dairy farms, but *E. alabamensis* could be more prevalent over the last decades as well.

Oocyst counts per gram feces (OPG) were different per sample day and showed different patterns on each farm. A consistent cut off point for OPG levels per *Eimeria* species for impact on growth performance or occurrence of clinical disease is difficult to identify from the literature. However, oocyst excretion per calf was classified was associated with more clinical signs when at least 500 *E. bovis* or *E. zuernii* oocysts were counted (Mundt, 2005, Bangoura et al., 2012). Bangoura et al., 2012 found higher OPG values were associated with a greater frequency of diarrhea (median 1075 OPG) and diarrhea correlated to a larger number of animals excreting relevant numbers (>500 OPG) for *E. bovis* and *E. zuernii*. In Germany correlation was found between individual OPG levels of 100,000 or more for *E. alabamensis* with diarrheal feces in calves after turning out to pasture (Von Samson-Himmelstjerna et al., 2006). Furthermore, species considered non-pathogenic such as *Eimeria auburnensis* and *Eimeria ellipsoidalis* have also been occasionally observed to cause diarrhea (Mielke et al., 1993). Considering the data from literature all farms showed pooled samples exceeding OPG's of 500 for *E. bovis* and five farms for *E. zuernii*. None of the pooled samples exceeded the level of 100,000 OPG for *E. alabamensis*. At 5 out of ten farms OPG of the pooled samples exceeded 4000 for *E. alabamensis* and at 1 farm one pool reached an OPG of 30,500. However, the sample interval of seven days allows missing a high peak in OPG, since evidence was found that peak excretion of oocysts of *E. zuernii* and *E. alabamensis* can occur within a week (Bangoura and Daugschies, 2007; Svensson, 2000).

The OPG level of 1075, (the median OPG level above which Bangoura et al., 2012 found association with greater diarrhea prevalence in individual calves) was exceeded in 19 pools.

The highly correlating $\log_{10}$ OPG of pathogenic *Eimeria* and $\log_{10}$ OPG of *Eimeria* species all together (correlation coefficient 0.83; $p = 0.0001$) indicates that without differentiation of pathogenic *Eimeria* species, OPG counts are still useful in diagnosing the truly relevant

infections.

Clinical score patterns showed some similarities between farms. Rumen fill scores and faecal scores were higher at the first half of the study period (indicating a lower rumen fill). Calves being fed with more milk replacer and a relatively smaller amount of roughage in the first half of the study period probably affected the rumen fill scores. The rumen volume is after all still developing between the age of 2 and 12 weeks. Although calves experienced a period of rumen development and were fed milk in the first weeks of the study period, we considered the differences in mean rumen fill scores between calves from different pools as indicators for appetite. Faecal scores in the early sample days can be affected by pathogens such as rota and corona viruses, *Cryptosporidium parvum*, *Salmonella* species or bovine virus diarrhea. Also feeding, management and antibiotic treatment can lead to higher faecal consistency scores in this study segment. However, there were also differences in clinical score patterns and cumulative clinical scores between pools. The average score per calf for all clinical parameters was negatively correlated with average daily weight gain (except respiratory disease for which negative correlation was not significant). This finding confirms the generally negative impact of disease on growth performance. We found no correlation between the average clinical scores per pool and the presence or OPG's of the different *Eimeria* species at the same sample day. The pooling of faecal samples means that we do not know how many calves in the sample group were contributing to the OPG level. A clear correlation of OPG levels and clinical symptoms on the same sample day can therefore be diluted due to only a few or even just one calf responsible for a high OPG. However, we did find significant correlation between pool cumulative $\log_{10}$ OPG of pathogenic *Eimeria* species and mean cumulative faecal consistency score in the multivariate analysis. Correlation of higher OPG's with faecal consistency is also established in other studies (Bangoura et al., 2012; Von Samson-Himmelstjerna et al., 2006). However, no correlation could be established for OPG levels and diarrhea in the study on Dutch dairy farms (Cornelissen et al., 1995). Disease symptoms and OPG peaks occurring at different moments during the infection can be a reason for these incongruences in studies. Cornelissen et al., 1995 sampled animals once and performed a clinical evaluation at the same time. In our study animals were followed over time. On average we found the peak in faecal consistency score 4.5 days prior to the peak in OPG for pathogenic *Eimeria* species. A similar interval for diarrhea due to *E. alabamensis* infection was reported earlier in literature (Svensson, 2000). Inconsistent findings for OPG levels and clinical symptoms at the same sample day can also be related to the relatively small number of calves experiencing clinical coccidiosis, compared to the calves experiencing subclinical coccidiosis (Gradwell et al., 2005). Furthermore, the association between higher OPG shedding and high faecal consistency scores per pool is not necessarily causative. There are many on farm factors that can cause stress, impaired immune function and/or digestive disorders and therefore possibly cause high scores for rumen fill and faecal consistency as well as higher numbers of oocysts in faecal samples.

Within this study a correlation was found between several pool OPG patterns and the mean average daily weight gain of the calves in that pool (Table 5). In the multivariate model the mean average daily weight gain per pool was 92 g lower when the OPG of *E. bovis* exceeded the median of 750 and 141 g lower when OPG *E.alabamensis* exceeded a level of 1000 at 2 or more sample days. Since the model included farm as random effect, differences in growth rate seemed not severely influenced by different strategies between herds. These findings indicate that infections with *Eimeria* species leading to higher numbers of oocysts in the feces during the study period impacted the average daily weight gain of the calves. A relationship between infections with pathogenic *Eimeria* species and growth performance has also been found in several other studies. Daugschies and Najdrowski, 2005, showed a decreased body weight. Mild experimental infections with *E. bovis* resulted in reduced feed consumption and loss of body weight (Fitzgerald and Mansfield, 1972; Daugschies et al., 1986). Other research found that calves infected with *E. zuernii* showed markedly reduced weight gain compared to the uninfected control (Bangoura and Daugschies, 2007). Furthermore, considerable weight loss was found due to *E. alabamensis* and *E. bovis* infections in first year grazing cattle (Von Samson-Himmelstjerna et al., 2006). To our knowledge, no relation between OPG levels in faecal samples and growth performance of calves has been described. It has been found, however, that calves infected with *E. zuernii* had a dose-dependent lower weight gain, especially during patency (Bangoura and Daugschies, 2007). The lower growth performance of pools with a peak excretion $\geq$750 OPG for *E. bovis* and $\geq$ 2 SD $>$ 1000 OPG for *E. alabamensis* in the current study could therefore be a possible indication that the initial dose of infection with pathogenic oocysts was also higher.

Technical parameters of the herds showed a lot of similarities, but also huge differences for example in solid food strategy. One calf out of 120 studied animals died due to complications accompanying respiratory disease, which is in accordance with the mean mortality rate of 0.85% in the studied herds. Mortality rates can be considered low in the studied herds varying from 0.15 to 1.35% with a mean value of 0.85% since mortality rates between 0 and 12.9% are reported previously in veal calves during first 56 days on farm (Timmerman et al., 2005). This indicates that it is not likely that an outbreak of severe diseases (including diarrhea) is disturbing the results of our study.

The questionnaire was taken at or after the last farm visit by the investigator. The nine visits per farm, allowed the investigator to observe most of the practices and measures, which possibly limited the amount of interview bias due to the socially desired response rather than the true situation. Because all herd visits were performed by a single person, investigator variability was avoided. Farms differed in their approach of preparing the units for new calves. The empty pen time prior to the arrival of the calves differed from $<$1 week to $>$2 weeks. Only half of the farms cleaned the unit and no farm provided disinfection before arrival of the calves, although several studies in the last decades showed cleaning and disinfection to be important and more effective than a period of vacancy of the units (Luyckx et al., 2016; Damiaans et al., 2019). All farms took extra measures in the transition to group housing and weaning. The multivariate analysis regarding the different measurements in respect to the cumulative $\log_{10}$ OPG of the different *Eimeria* species showed several similarities; Farms that cleaned the units prior to the arrival of the calves had lower cumulative $\log_{10}$ OPG counts for *E. alabamensis, E. zuernii*, pathogenic *Eimeria* and *Eimeria* species in general. Therefore, contact with oocysts on the rose veal starter farm left by the previous group of animals could also be a considerable factor in the infection with *E. zuernii* and *E. alabamensis* next to the introduction of *Eimeria* species from the dairy farms of origin. A preventive measurement to delay group housing of extra care calves seems to relate to higher cumulative $\log_{10}$ OPG's in case of *E. zuernii* and pathogenic *Eimeria* species. It needs to be considered however, that in these farms more extra care calves could be identified and as a result the farmers decided to postpone the group housing of this category.

## 5. Conclusion

Farmers, raising rose veal starter calves, have many management factors to consider in order to keep their animals healthy and well performing. This study showed that *Eimeria* infections provide an extra challenge on animal performance; High pathogenic *Eimeria* oocysts counts throughout the study period correlated with higher faecal consistency scores and a lower average daily weight gain. Furthermore, this study confirmed that cleaning units prior to the arrival of calves is beneficial for lower oocyst counts. The insights that were gained through the nine week investigation of *Eimeria* oocyst shedding on each of these ten rose veal starter farms can help farmers and veterinarians take better evidence-based decisions on the diagnostic approach of coccidia infections, preventative measurements and whether and when metaphylactic treatment would benefit health and performance of the

herd.

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The author declares the following financial interests/personal relationships which may be considered as potential competing interests:

## Animal welfare statement

This protocol (study number EIAC 1055) has been reviewed and approved by the Animal Care and Use Committee of Elanco (IACUC). Approval date was 2/19/2019.

## Declaration of Competing Interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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