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Eimeriosis in dairy calves

Effect of milk feeding strategies on infection pressure and disease development

Master's thesis

Daniel Haraldsson

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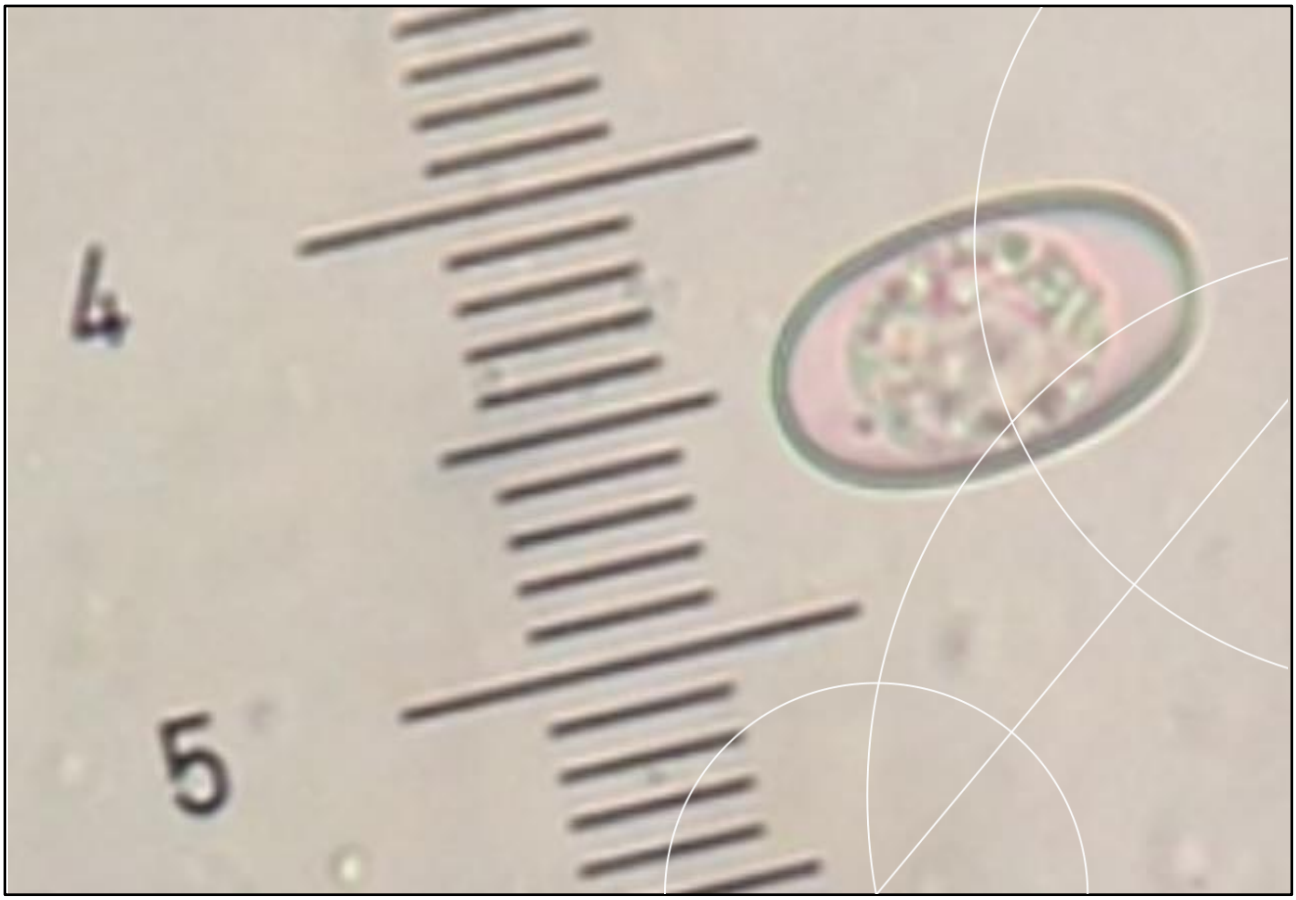

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Preface

This report is part of the authors Master's program in Veterinary Science at the University of Copenhagen, and is therefore primarily addressed to my supervisor and censor. It can also serve as an introductory text regarding the importance of Eimeria in the dairy industry and the pathological variations in calf populations. It is intended to shed a light on the importance of milk-feeding strategies on the prevalence of Eimeria spp. infections and symptoms.

All funds for equipment, transport, laboratory costs, etc. were provided by "Videncentret for Landbrug, Kvæg".

Acknowledgements

I would like to extend my thanks to the farmers and staff at both farms, as the enforcement of the feeding-strategy has meant added work-load for all involved.

I would also like to thank, Henrik Læssøe Martin, Henrik Martinussen and Rikke Engelbrecht from "Videncentret for Landbrug, Kvæg" for their involvement and help, during the data collection phase.

In the laboratory, my work would have been impossible, if not for the help of Leif Eiersted, Cynthia Dawn Juel, Aleksandra Tofteby and Boi-Tien Thi Pham. Thank you for accepting me into your already busy program and facilities.

My thanks to Senior Adviser Peter Lind, National Veterinary Institute, DTU, for his useful advice and patience during the statistics phase.

Thanks to Heidi Enemark, my Senior Adviser, for support and advice.

All the practical work was done in cooperation with Zascha Irving Pedersen, my fellow-student. Thanks you for your good humor, the 25.000km driven, the infusions of discipline and for making me want to get up in the cold winter mornings.

In conclusion, I would like to thank Heidi H. Petersen, Parasitologist, PhD for her unselfish help, support and morale-boosting presence at the office.

Abbreviations and Definitions

OPG	Oocysts per gram of faeces.
DTU-VET	National Veterinary Institute, Technical Univerisy of Denmark
Total OPG	The sum of all OPG-counts for a specific calf, throughout the study (sum of 12 values)
Faecal Score (FS)	0=firm, 1=normal in structure, 2=soft in structure, 3=thin without structure, 4=watery, 5=watery with blood in the stools and/or mucus (phlegm)
Diarrhea	For the purpose of this study, “diarrhea” was defined as a faecal score higher than 2.
Translated Diarrhea Score	For the purpose of this study, diarrhea scores were assigned values; 0=no diarrhea, 1=diarrhea, 2=watery diarrhea.

1 Abstract

1.1 Introduction

Eimeria spp. are protozoan, intestinal parasites of the family Eimeriidae, particularly seen in poultry, rabbits and ruminants. In cattle, clinical eimeriosis mainly affects calves and younger animals, causing diarrhea (scouring) and impaired production and growth. This, and the fact that Eimeria spp. are endemic on most farms in Denmark, accounts for substantial economic loss (Daugschies & Najdrowski, 2005; Enemark et al., 2013). Treatment of bovine Eimeriosis in calves is challenging, as intestinal lesions precede clinical signs, making timely treatment difficult (H.-C. Mundt et al., 2003).

Milk feeding intensity has been shown to correlate with weight gain and feed conversion (Bartlett et al., 2006; Diaz et al., 2001).

Intensively fed calves have been shown increased resistance to experimental infection with the Apicomplexan parasite *Cryptosporidium parvum (Ollivett et al., 2012).* It has yet to be discovered, whether a similar correlation is present in the case of Eimeria infections. Information on the correlation between Eimeria infections and general health are also sparse.

1.2 Objectives

The purpose of the study was to test the correlation between milk-ration (high vs. normal ration) and the shedding of Eimeria-oocysts, diarrhea and growth.

The following hypotheses were tested:

1) Intensive feeding with high amounts of energy and protein will:
   a. Reduce the excretion of oocysts.
   b. Increase growth (weight-gain).
   c. Improve the general health of the calves and thereby reduce the risk of clinical manifestation of eimeriosis (diarrhea).
2) There is a correlation between the excretion levels and diarrhea.
3) There is a correlation between the excretion levels and growth rate.

1.3 Methods

A cohort-study was conducted, where the subjects were assigned into two groups; a control-group, receiving a standard milk-ration, and a treatment-group, receiving increased milk-ration. Calves were followed from their 2^{nd} week of life, until their 13^{th} week of life. Parameters such as rectal temperature, respiratory symptoms, general appearance, oocyst shedding and diarrhea score were monitored weekly. Weight at birth and final weight at the termination of the study were recorded.

In the laboratory, the consistency of faecal samples was evaluated, and oocysts were counted and morphologically differentiated.

Statistical analysis was conducted in GraphPad Prism, through un-paired t-tests and correlation analysis.

The fieldwork was conducted in conjunction with another study, focusing on cryptosporidia/giardia infections in the same calves. This work concluded the veterinary master's thesis of Zascha Irving Pedersen (Pedersen, 2014).

1.4 Confinement

The study included 69 Holstein calves of mixed sex, from two dairy farms in Denmark. These were studied in the period 16th of January, until the 18th of June 2014. The farms were chosen with the criteria of being middle-/large-scale, having well-established and standardized calf-management, having a history of calf-diarrhea and being willing and able to effectively execute the feeding-regimes throughout the study period.

The calves were only monitored in the period between their 2nd and 13th week of life. No follow-up was conducted.

In terms of calf management, the two herds do not diverge significantly from the norm in the remainder of the Danish dairy-industry.

Relevant literature concerning parasite biology and characteristics, effect of various milk-feeding regimes and general literature on calf scouring was reviewed.

2 Background

2.1 Diarrhea overview

Together with respiratory disease, calf diarrhea (scouring) is the leading cause of calf mortality, in spite of both generally being described as low-mortality, high morbidity conditions (Johnson et al., 2011).

Scouring is usually a multi-factorial problem with pathogens being viral, bacterial and protozoal, which compounds the challenge. In the United States, 57% of weaning calf mortality is caused by scouring. Similar numbers have been reported from the Korean dairy-industry (53%). In Norway, the economic loss caused by calf death (various causes) is reported to be approx. USD 36 per calf produced (Cho & Yoon, 2014).

The detrimental effects of clinical and even subclinical eimeriosis, such as impaired performance, mortality and costs for treatment, result in severe economic losses, while studies have suggested that calf scouring is associated with lowered first-lactation milk production (Daugschies & Najdrowski, 2005; Svensson & Hultgren, 2008).

Rotavirus and *E. coli* are most frequently encountered as the cause of neonatal (<3 weeks) diarrhea in calves. Other pathogens of importance are *Coronavirus*, *Salmonella* and *Cryptosporidium* (Singh et al., 2009). *Rotavirus* and *Coronavirus* attack the intestinal cells of villi in the small intestine, reducing absorption potential. These infections are most prominent in the first 3 weeks of life. *E. coli* produces enterotoxin, resulting in excessive secretion and thus, fluid loss. The susceptibility to *E. coli* infections is greatest during the first 2 weeks of life (Singh et al., 2009). Salmonellosis is mostly caused by *S. Dublin spp*., with clinical signs including diarrhea and fever. Among the predisposing factors are high protein diets. *Cryptosporidia* are zoonotic. Infections from calves to humans are frequently reported. Goats, cats and mice have also been successfully, experimentally infected by *Cryptosporidia* (Current et al., 1983).

The most prominent causes of diarrhea in older calves (>3 weeks), are *Eimeria spp*. and *Giardia spp*.. *Giardia spp*. infection can result in subclinical or even asymptomatic infections, but can cause acute/chronic diarrhea, reduced weight gain and ill thrift. *Giardia spp*. are known to be zoonotic (Gillhuber et al., 2014).

2.2 Eimeria (Coccidia)

Eimeria spp. are protozoan, intestinal parasites of the family *Eimeriidae*, particularly seen in poultry, rabbits and ruminants. In cattle, clinical eimeriosis mainly affects calves and younger animals, causing diarrhea (scouring) and impaired production and growth. This, along with the fact that *Eimeria spp*. are endemic on most farms in Denmark, accounts for substantial economic loss (Daugschies & Najdrowski, 2005; Enemark et al., 2013). Treatment of bovine eimeriosis in calves is challenging, as intestinal lesions precede clinical signs, making timely treatment difficult (H.-C. Mundt et al., 2003).

2.2.1 Taxonomy

The family of *Eimeriidae* includes the genera of *Toxoplasma*, *Isospora*, *Eimeria*, *Cryptosporidium* and *Sarcocystis*. The genus *Eimeria* includes the bovine pathogens *E. bovis*, *E. zuernii* and *E. alabamensis*.

Clinical eimeriosis is often referred to as coccidiosis, although this is technically a misnomer, as this refers to the taxonomic subclass *Coccidia* (*Coccidiasina*), which includes all the genera mentioned above.

Eimeria (*Eimeriidae*) is a taxonomic family within the subclass of *Coccidia* (*Coccidiasina*), who in turn reside within the *Apicomplexa* phylum of parasitic protozoa. *Coccidiae* are obligate, intracellular parasites, including the families of *Toxoplasma*, *Isospora*, *Cryptosporidium*, *Sarcocystis*, and *Eimeria* (Schmidt & Roberts, 2009). Each subspecies of *Eimeria* is strictly host specific with no cross-infection or zoonotic potential (Gillhuber et al., 2014; Taylor et al., 2007).

DNA-based classification is currently unveiling new aspects of the taxonomy of the genus, increasing the understanding of common qualities, perhaps allowing for novel treatment options (H. David Chapman et al., 2013).

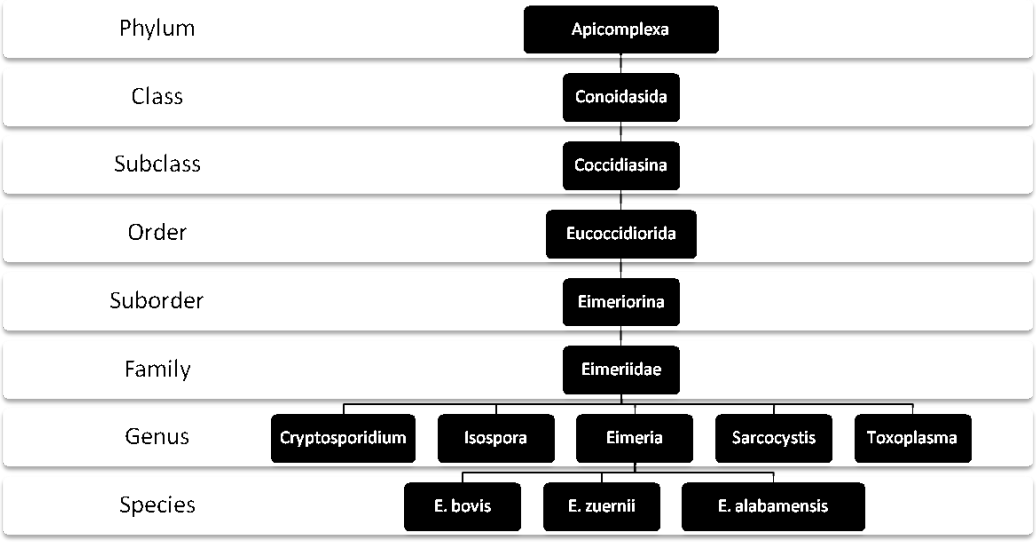


Figure 1: *Taxonomy of* Eimeria *spp.*

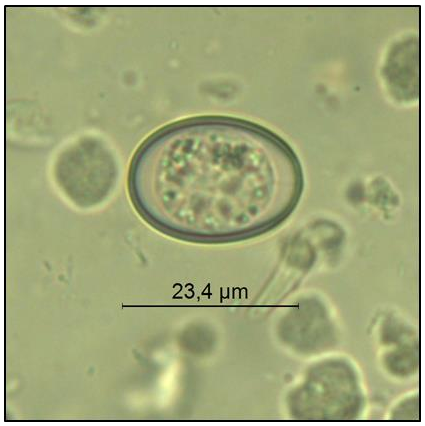
2.2.2 Morphology

Eimeria spp. are characterized by their small size, compared to other objects, usually detected by light microscopy of corprological samples. The oocysts are visible at 100x magnification, but observation of individual characteristics, allowing species differentiation, can usually only be carried out at 400x magnification. The pathogenic species *E. bovis* has an ovoid shape, is brownish-yellow in color, with one micropyle at the narrower end, measuring 23-34µm in length and 17-23µm in width. The smaller *E. zuernii* has a more subspherical shape, is mostly colorless, lacks a micropyle and is 15-22µm long and 13-18µm wide. Less relevant to this study, *E. alabamensis* has an ovoid shape, is mostly colorless, lacks a micropyle and is 13-24µm long and 11-16µm wide.

E. bovis

E. zuernii

E. ellipsoidalis



E. auburnensis

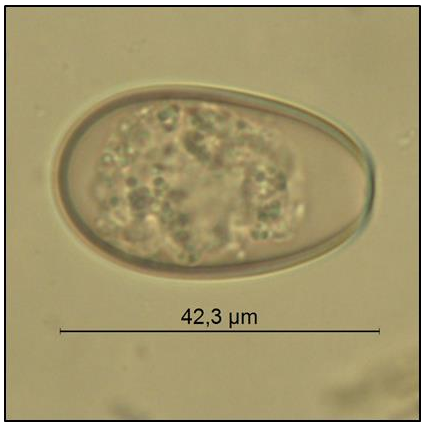


Figure 2: *Morphology of common Eimeria-species in 630x magnification, using standard light-microsopy. Photo of E. zuernii from Enemark et al. (2013) (magnification unknown).*

Table1: *Bovine* Eimeria-*species in alphabetical order* (Daugschies & Najdrowski, 2005).

Species	Length (µm)	Width (µm)
E. alabamensis	13-24	11-16
E. auburnensis	32-46	20-25
E. bovis	23-34	17-23
E. brasiliensis	34-43	24-30
E. bukidnonensis	47-50	33-38
E. canadensis	28-37	20-27
E. cylindrica	16-27	12-15
E. ellipsoidalis	20-26	13-17
E. illinoisensis	24-29	19-22
E. pellita	36-41	26-30
E. subspherica	9-14	8-13
E. wyomingensis	37-45	26-31
E. zuernii	15-22	13-18

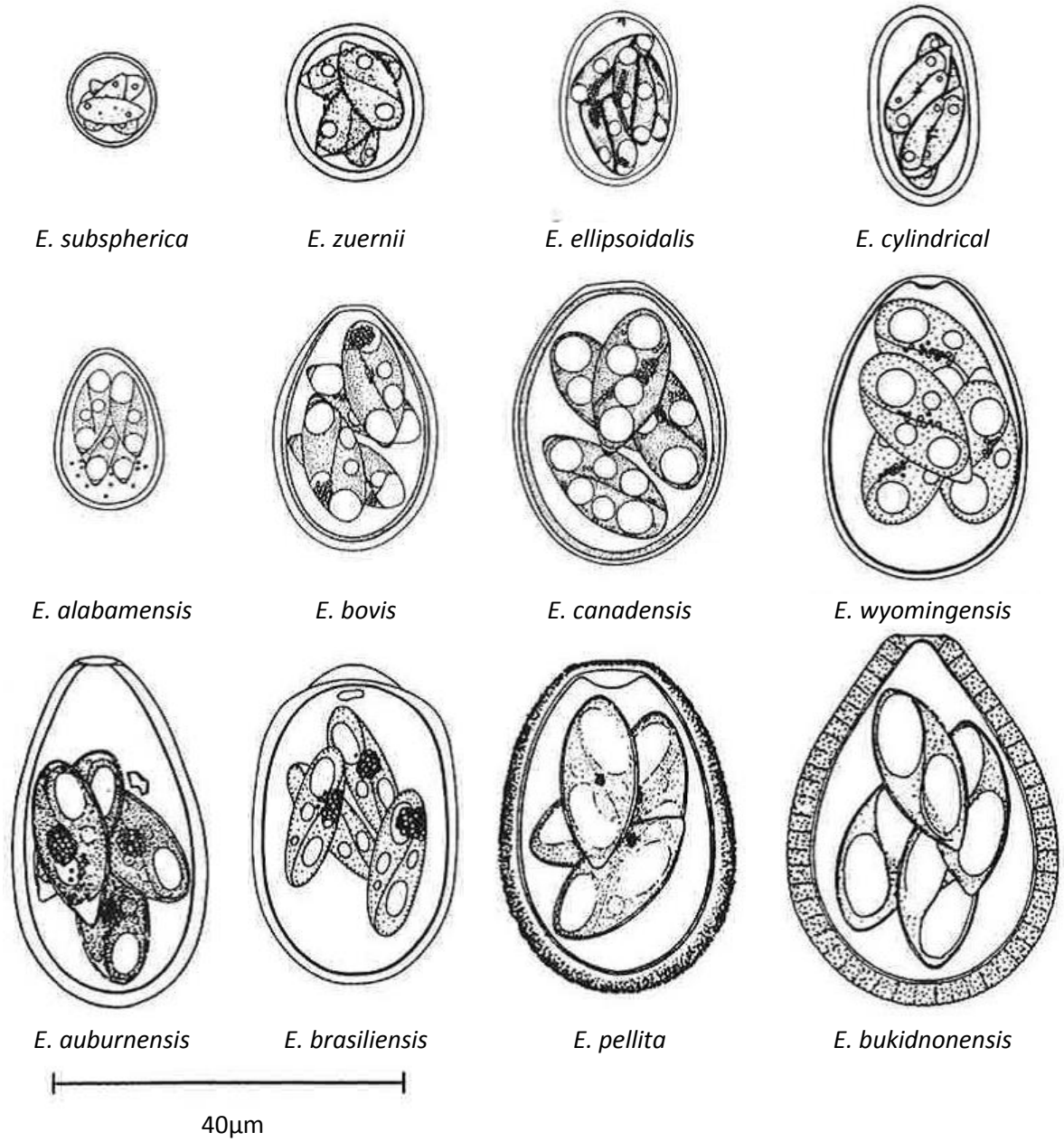


Figure 3: *Morphology of common bovine* Eimeria *spp.*

2.2.3 Life Cycle

The life cycle of Eimeria is completed within a single host (i.e. a monoxenous cycle) involving both asexual and sexual replication. All Eimeria spp. rely on a parasitic (endogenous) phase in the host and an environmental (exogenous) phase, in their development. The endogenous phase is strictly host- and tissue specific and is initiated when sporulated oocysts are ingested from contaminated environment. In the intestinal tract, under influence of stomach acid and bile salts, sporozoites excyst from the oocyst, invading endothelial cells of the central lymph capillaries of the ileal villi. Here, they transform into trophozoites and form first generation meronts (macromeronts) through repeated cycles of asexual multiplication (merogony). More than 10^5 merozoites are formed within these macromeronts, which finally rupture, releasing merozoites, which in turn invade neighbouring

mucosal cells, forming a second generation “micromeront”. From this micromeront, merozoites are formed, that then differentiate into microgamonts (male) and macrogamonts (female). From the microgamonts, a number of microgametes develop, who in turn fertilize the macrogamonts in a cycle of sexual multiplication (gamogony). The fertilized macrogamonts develop into zygotes. A wall forms around the zygote, which then is termed a non-sporulated oocyst, which is excreted to the environment. In the exogenous phase, these oocysts sporulate and become infective, resulting in the oocyst harbouring four sporocysts, containing two sporozoites each. The entire endogenous phase takes 16-22 days in case of the pathogenic species, also called the prepatent period. Upon excretion, the oocysts sporulate, becoming infective within a few days to a week. The oocysts remain infective for months and can even survive through a whole winter-season (reviewed by (Daugschies & Najdrowski, 2005)).

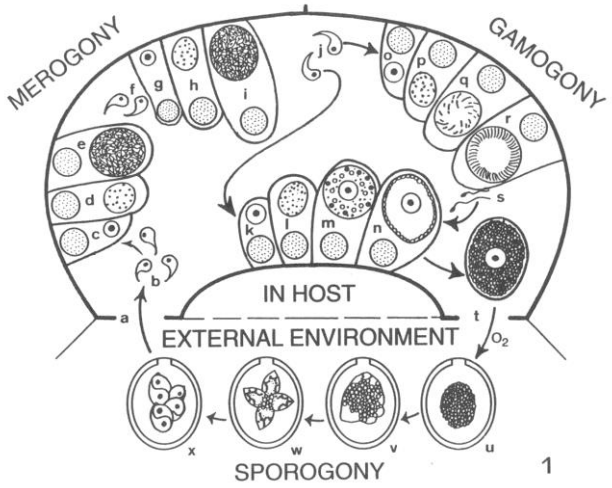


Figure 4: *Life Cycle of* Eimeria *spp. (Duszynski & Upton, 2008).*

2.2.4 Clinical manifestations and Pathology

Clinical disease is mainly attributed to the late stages of the life-cycle (second merogony and gamogony). Through their sheer numbers and destruction of host cells, the gamonts cause the majority of the histopathological changes. Of the bovine Eimeria spp., E. bovis and E. zuernii are considered to be most pathogenic, causing hemorrhagic diarrhea, containing fibrin and intestinal tissue, in calves. The most susceptible age-group is 3 weeks to 6 months (Bangoura et al., 2012; Daugschies & Najdrowski, 2005).

Other species, such as E. auburnensis and E. ellipsoidalis have occasionally been shown to cause diarrhea, while the last species of interest (E. alabamensis) is known to cause diarrhea in pastured cattle only (Daugschies and Najdrowski, 2005).

Calves, experimentally infected with E. zuernii, start losing weight on day 21, post-infection, which also is the day of maximal oocyst-output. Calves dying early-on (18-20 days post-infection), suffered from dehydration, while calves dying later-on (21-25 days post-infection) suffered from both dehydration and anaemia. Calves surviving the acute-phase (>day 25) would either improve rapidly or slowly decline until death (Stockdale et al., 1981). Active immunity develops rapidly after first antigen contact, its speed being dependent on the number of oocysts ingested (Daugschies &

Najdrowski, 2005). In general, clinical signs of eimeriosis may not become evident until 3-8 weeks post-infection (Fox, 1985).

2.2.5 Prevalence

The large numbers and long survivability of oocysts excreted by each infected animal, makes infection of calves and young cattle very difficult to avoid (Bürger, 1983). In Denmark, approx 96% of herds and 61% of individual calves are infected with Eimeria spp. For the two most pathogenic species, E. bovis and E. zuernii, the prevalence is approx 89% for herds and 42% for individual calves (Enemark et al., 2013). Similarly, high prevalences have been reported from Austria (Koutny et al., 2012), Hungary (Farkas et al., 2007), Germany (Bangoura et al., 2012) and Algeria (Ouchene et al., 2014), while numerous studies have reported prevalence as being "generally high", possibly as high as 100% (reviewed by (Daugschies & Najdrowski, 2005).

2.2.6 Pro-/metaphylaxis and treatment

Proper hygiene regime (reducing faecal-oral transmission) and ensuring unfavorable conditions for oocyst survival in the environment (temperature under 15°C and relative humidity less than 80%) are beneficial for reducing infection pressure on the herd. Medical treatment is most effective if applied against the late developmental stages, while administration should be conducted before the onset of clinical symptoms, during prepatency (Daugschies & Najdrowski, 2005).

Treatment after the onset of clinical signs is ineffective in the individual calf, due to the amount of intestinal damage already present, when oocyst-excreation commences (H.-C. Mundt et al., 2003). On the other hand, as illustrated by a study of E. zuernii infenctions, if treatment is initiated (toltrazuril), even as little as 2-3 days before onset of symptoms, symptoms can be drastically reduced (H. C. Mundt et al., 2005). Metaphylactic treatment of groups of calves, initiated after the first clinical signs in the group is therefore advised.

Profylaxis has mostly been practiced within the poultry industry, where resistance has been reported as little as one to four years post-introduction of the recent anticoccidials; salinomycin and diclazuril (H. D. Chapman, 1997).

2.2.7 Long term effects

Calves surviving serious clinical eimeriosis have been shown to have reduced potential for production and growth. In general, heifers suffering even from mild diarrhea early in life, have been shown to yield significantly less milk in their first lactation. It has also been shown that animals that survive severe eimeriosis show less growth and may not ever become profitable (Fox, 1985; Svensson & Hultgren, 2008).

2.2.8 Diagnostic procedures

Eimeria has traditionally been detected qualitatively by fecal smear or quantitatively by flotation (McMaster) and light microscopy. Single registrations of OPG are considered unreliable indicators of clinical status or parasitic load, as excretion varies within the infection cycle. Rather, faecal samples from several animals should be used (possibly pooled) to create a true estimate of the level of infection (Daugschies & Najdrowski, 2005). The possibility of using ELISA or Western Blot has been examined, but these methods are not practical (Faber et al., 2002; Fiege et al., 1992).

2.3 Feed strategies and scouring

Studies of calves, experimentally infected with the Apicomplexan parasite, *Cryptosporidium parvum*, have shown that calves fed intensively with 0.3 Mcal / kg (28% protein, 20% fat) had significantly less diarrhea, had a better hydration, showed more effective feed-conversion and had better growth than calves fed with 0.13 Mcal / kg (20% protein, 20% fat) (Ollivett et al., 2012).

It has been shown that by increasing the milk-intake of calves from the usual 4-6kg/day to 6-10kg/day, a significant increase in bodyweight (up to 23kg at 90 days of age) can be expected (Khan et al., 2007).

Intestinal lesions precede the onset of clinical signs, making medical treatment challenging (H.-C. Mundt et al., 2003). *Eimeria* spp. are found in approx. 95% of Danish dairy farms (Enemark et al., 2013).

Milk feeding intensity has been shown to correlate with weight gain and feed conversion (Bartlett et al., 2006; Diaz et al., 2001)

3 Own Studies

3.1 Aims of the study

The purpose of the study was to test the effect of increased milk-ratio on the shedding of *Eimeria*-oocysts, diarrhea and growth.

The following hypotheses were tested:

1) Intensive feeding with high amounts of energy and protein will:
    a. Reduce the excretion of oocysts.
    b. Increase growth (weight-gain).
    c. Improve the general health of the calves and thereby reduce the risk of clinical manifestation of eimeriosis (diarrhea).
2) There is a correlation between the excretion levels and diarrhea.
3) There is a correlation between the excretion levels and growth rate.

3.2 Herds and Study Period

Two conventional dairy herds in Jutland, Denmark, were monitored in the period between 16th of January, until the 18th of June 2014. Both herds were composed of a mixture of Red Danish Dairy Cattle and Danish Holstein Dairy Cattle.

In both herds, clinical signs in the calves were dominated by diarrhea and respiratory symptoms, though respiratory symptoms were more prominent in Herd 2. Preparital cows were moved to common calving pens approx. 2 weeks prior to parturition. The calving pens were straw bedded and calves were removed from the cows immediately (maximum 6 hours) post-partum. The calves were moved to individual pens with straw-bedding and possibility of rostral contact with one or two other calves of the same age. The neonates were fed 4 liters of fresh and/or frozen colostrum, which had been checked with a colostrometer. At approx. three weeks of age, the calves were moved to

common pens, housing 5-8 calves of the same age. These groups were kept during the study period and no mixing of the groups was observed. All calves were allowed ad-libitum access to water, hay/silage and muesli. Milk-feeding with whole-milk, topped up with milk replacer, was conducted in the morning and evening. The following data refer to the last twelve months, prior to the termination of the sampling-phase.

Herd 1 consisted of 1238 milking cows with 1105 calvings per year with 12.1% calf mortality in the first 180 days of life, while only few (4.3%) died in the first 14 days of life. *Streptococcus uberis* was endemic in the herd, which was also classified as a "*Salmonella Dublin* – Class 2" herd since April 2013, meaning that *S. Dublin* had been isolated from the milk-delivery tank. In herd 1, the calves were placed in a heated "drying-cabinet" before being placed in the individual pens. A total number of 44 calves were followed throughout the study period: 22 calves in each group (test and control, respectively). Calves in Herd 1 were fed and weaned, so that 50% of them (control-group) were assigned 6 liters of milk (3 liters, twice daily) until weaning at xx days of age, while 50% of the calves (treatment-group) were assigned 10 liters of milk (5 liters, twice daily) from their 3rd week of life, until weaning.

Herd 2 consisted of 399 milking cows with 458 calvings per year. The calf mortality in the first 180 days of life was 4.8%, most of which (4.1%) occurred in the first 14 days of life. No *S. uberis* or *S. dublin* had been detected.In Herd 2, 25 calves were followed, 13 receiving standard rations (control group; 3 liters, twice daily) and 12 receiving increased rations (test group; 5 liters, twice daily). Calves in Herd 2 were escalated rapidly, with 13 calves (treatment-group) being stepped up to 10 liters of milk (5 liters, twice daily) already during the first week of life, while 12 calves (control-group) were assigned 6 liters of milk (3 liters, twice daily) throughout the period, until weaning.

All calves were followed from their 2nd to 13th week of life. Calves were weighed on inclusion (week 1) and again at the termination (week 13 +/- SD) of the study. Weekly stool samples were taken from all calves. Samples were taken rectally or collected from completely fresh dung. All samples underwent quantitative *Eimeria*-analysis with a modified McMaster method and microscopic species-differentiation, conducted without prior sporulation.

Table 2: *Overview of the study design*

<table>
<tr><th colspan="2"></th><th>Herd 1</th><th>Herd 2</th></tr>
<tr><td>Calves</td><td>Test</td><td>22 bull calves</td><td>12 heifer calves</td></tr>
<tr><td></td><td>Control</td><td>22 calves of mixed sex</td><td>13 heifer calves</td></tr>
<tr><td>Feeding</td><td>Treatment</td><td colspan="2">2x5 litres per day, full milk (added milk replacer if not enough full milk)</td></tr>
<tr><td></td><td>Control</td><td colspan="2">2x3 litres per day, full milk</td></tr>
<tr><td>Sampling</td><td>Treatment</td><td colspan="2" rowspan="2">Sampling and registration taken once per week from 2nd to 13th week of life.</td></tr>
<tr><td></td><td>Control</td></tr>
<tr><td>Weighing</td><td>Treatment</td><td rowspan="2">Weight at birth and at 83-99 days</td><td rowspan="2">Weight at birth and at 74-106 days</td></tr>
<tr><td></td><td>Control</td></tr>
</table>

3.3 Faecal sampling and clinical examination

Samples were collected from the calves once per week from two weeks of age, until the age of 13 weeks. These samples were collected rectally or, if defecation was observed, from the ground. The samples were stored in a cooler until they could be analyzed at DTU-VET within 14 days.

On each visit to the farm, each calf was rated for overall general appearance; 1: the calf is standing or stands up when the pen is approached, 2: the calf can be made to stand up or if standing, shows signs of discomfort (drooping ears, low activity level etc), 3: the calf will not or cannot stand up, even when provoked.

Calves that showed respiratory symptoms were registered. Symptoms included excessive or purulent nasal discharge, spontaneous coughing or increased respiratory rate due to respiratory infection.

Rectal temperature was registered weekly for each calf, with a digital thermometer. This was done before rectal exploration, whenever possible.

The calves were weighed on electronic scales at birth and again at the termination of the study (age 12-14 weeks). The same scale was used within each herd, throughout the study.

3.4 Laboratory Methods

3.4.1 Faecal scoring

Fecal samples were scored according to the following scale: 0: “firm”, 1: “normal”, 2: “soft, 3: “liquid without structure”, 4: “watery”, 5: “watery with blood/mucus (phlegm)”. Categories 3, 4 and 5 were designated as “diarrhea”, while categories 0, 1 and 2 represented normal values. The evaluations were carried out in the laboratory by the same operant (the author).

3.4.2 McMaster Method

A “Modified McMaster-Method”, with a sensitivity of 5 oocysts per gram was used (Henriksen & Korsholm, 1975, 1984).

Initially, 4.0g of feces were weighed off and mixed with 36ml of water. If less than 4.0g of feces were available, the amount of water was decreased correspondingly, thus obtaining a mixture with a fixed ratio of feces pr. ml. Thus, the mixture contained 10% feces, by weight.

When completely dissolved, the suspension was agitated and immediately 10.0ml were poured through a single layer of gauze into a Sarstedt centrifuge tube, separating the liquid from coarse particles, thus producing a fixed volume of known concentration. This filtrate contained the oocysts from 1.0g of feces. The filtrated suspension was subsequently centrifuged for 10min at 110G.

The supernatant was discarded and the remaining sediment was mixed with flotation media up to a total volume of 6.0ml (1g feces/6.0ml). This suspension was allowed to totally dissolve, then agitated and applied to the specially made, disposable McMaster slide. The counting chamber had a fixed volume of 0.6ml, corresponding to 10% of the content of the Sarstedt tube. In this way, upon microscopy, the total number of oocysts in the corresponding amount of feces could be determined, as the liquid suspension in the chamber was known to include the sediment from 0.1g of feces. The

total count in the chamber could therefore be multiplied with 10 in order to reach the true OPG. Results were approximated to the nearest 100 OPG.

The chamber was scanned for *Eimeria* spp. oocysts using 100x magnification.

3.4.3 Species identification and differentiation

Differentiation was only carried out in samples with total OPG>1000.

Subspecies were identified through measurement of width/length and the shape of the oocyst, the color composition as well as the presence/absence of micropyles, using the identification key. The first 200 samples were analyzed under supervision of laboratory staff. When in doubt, oocysts resembling a known pathogen (E. bovis or E. zuernii) were counted as such. Differentiation was carried out using 400 x magnification, by the author.

3.5 Statistics and Data Management

Oocyst excretion and diarrhea-scores were studied using an unpaired t-test, comparing accumulated values (over 12 weeks) for each calf.

For analysis, oocyst excretion was divided into three categories; low/none (<1000OPG), moderate (1000-5000OPG) and massive (>5000OPG). Species differentiation was only carried out in samples exceeding 1000OPG. OPG-values over 1000OPG are considered pathological.

Log(x) transformed oocyst counts (OPG) were analyzed, using a two-sided t-test.

Growth rate was analyzed using a two-sided t-test.

For analysis of diarrhea score, field-values were condensed to three categories (translated values). Field-values 0, 1 and 2 were translated to 0 (normal faeces), field-value 3 was translated to 1 (diarrhea) and field values 4 and 5 were translated to 2 (profuse diarrhea). Total diarrhea-score per calf, over time, was calculated as well as total diarrhea-scores of all calves within a population within weeks of life.

Correlations were examined by using two-sided Gaussian correlation analysis with a 95% confidence interval.

4 Results

4.1 Oocyst excretion

From a total of 69 calves (44 calves from Herd 1 and 25 calves from Herd 2), sampled weekly for 13 weeks, 48 calves (70%) excreted detectable Eimeria spp. oocysts at least once. In Herd 1, 24 out of 44 calves (55%) excreted oocysts, while in Herd 2, 24 out of 25 calves (96%) shed Eimeria spp. oocysts.

In Herd 1, 13 of 22 test calves (59%) were shedding oocysts, while 11 of 22 control calves (50%) were shedding oocysts at some point during the study period. In herd 2, the test group had 11 shedding calves out of 13 total (92%) and in the control group all calves shed Eimeria spp. oocysts at some time during the study (100%).

From all samples where Eimeria spp. differentiation was carried out (sample OPG>1000), E. bovis and E. zuernii were detected in samples from 5 out of 44 calves in Herd 1 (11%) and 21 out of 25 calves in Herd 2 (84%).

In Herd 1, 10 out of 528 samples (1.9%), had moderate/massive (>1000) OPG-levels. In Herd 2, 63 out of 300 samples (21%) had moderate/massive OPG-levels.

Table 3: *A) Occurrence of shedding calves (OPG>0). B) Division of all samples into categories (<1000OPG, 1000-5000OPG and >5000OPG).*

		A	B		
		Shedders (%)	Low/none (%)	Moderate (%)	Massive (%)
Herd 1	Total	54.5	98.1	1.1	0.8
	Treatment	59.1	99.2	0.0	0.8
	Control	50.0	97.0	2.3	0.8
Herd 2	Total	96.0	79.0	15.3	5.7
	Treatment	91.7	82.6	13.9	3.5
	Control	100	75.6	16.7	7.7

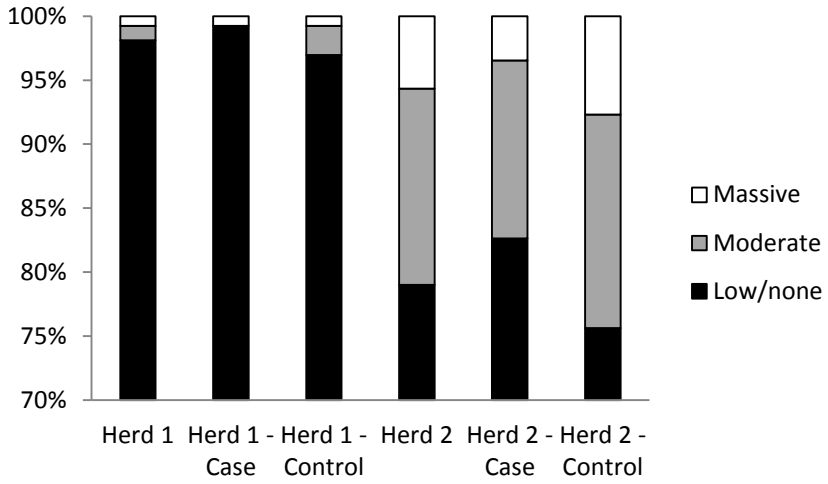


Figure 5: *Categorization of oocyst levels in fecal samples (n= xxx) from calves from two Danish dairy herds. The calves were sampled weekly from birth until 13 +/- SD weeks of life.*

Three different *Eimeria spp.* were detected in Herd 1, while seven *Eimeria spp*. were detected in Herd 2 (Table 4). In addition, a single calf in Herd 2 was found to excrete E. pellita *spp*. oocysts, but these were not included in the statistics, as the level of excretion was <1000OPG.

***Table 4**: Average OPG of* Eimeria *spp. in all samples in the herds (OPG>1000).*

	E. Bovis	*E. zuernii*	*E. auburnensis*	*E. cylindrica*	*E. ellipsoidalis*	*E. subspherica*	*E. wyomingensis*
Herd 1	4	40	-	-	70	-	-
Herd 2	180	200	4	40	390	13	4

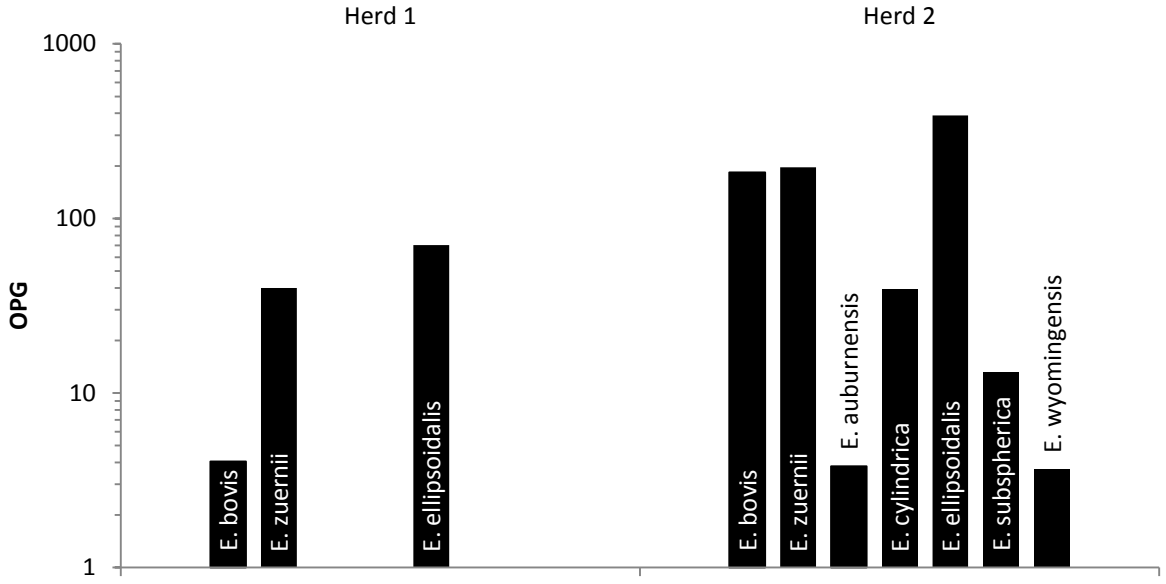


***Figure 6**:* Eimeria spp*. encountered in calves from two Danish dairy herds, and their average oocysts per gram (OPG)-values in all fecal samples (n=xx).*

The mean oocyst concentration of the positive samples in the study was 2500OPG, ranging from 100OPG to 29000OPG. In Herd 1, the mean was 1800OPG (100-19800OPG), compared to 2700 (100-29000OPG) in Herd 2.

The total OPG (accumulated from 12 samples) was calculated per calf. In Herd 1, the total OPG was similar between the treatment- and control-group, the difference being insignificant (p=0.98, n=44). In Herd 2, there was a more prominent difference between the treatment- and control-groups, the difference being borderline-significant (p=0.05, n=25). Applying log(10) transformation to the values did not produce a significant result (p=0.98/n=44 and p=0.056/n=25 in Herd 1 and 2 respectively).

Studying the OPG-data, a biphasic oocyst excretion pattern was observed. In Herd 1, there were peaks in total excretion of oocysts at approx. 8 and 12 weeks of life. In Herd 2, peaks were apparent at 7 and 11-12 weeks of life.

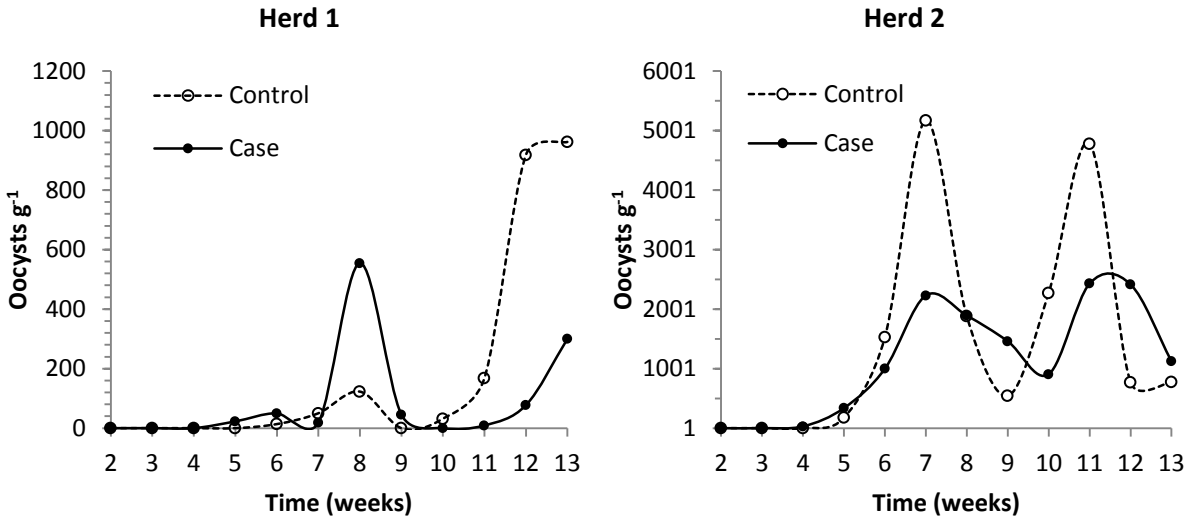


***Figure 7**: Average OPG per sample, per week of life. Note the different scales on the Y-axis.*

Refining the analysis, concentrating on the pathogenic spp. in pathological concentrations only (E. bovis and E. zuernii and OPG>1000), significant differences were observed. In this case, there was a significant difference in OPG between the treatment- and control-calves. Herd 1 had no treatment-calves excreting the pathogenic spp. in pathological concentrations, while 5 calves in the control-group did (23%). In herd 2, only 7 treatment-calves (58%) excreted E. bovis/zuernii in levels exceeding 1000OPG, compared to 11 calves in the control-group (85%). This was a significant difference (p=0,0025, n=25).

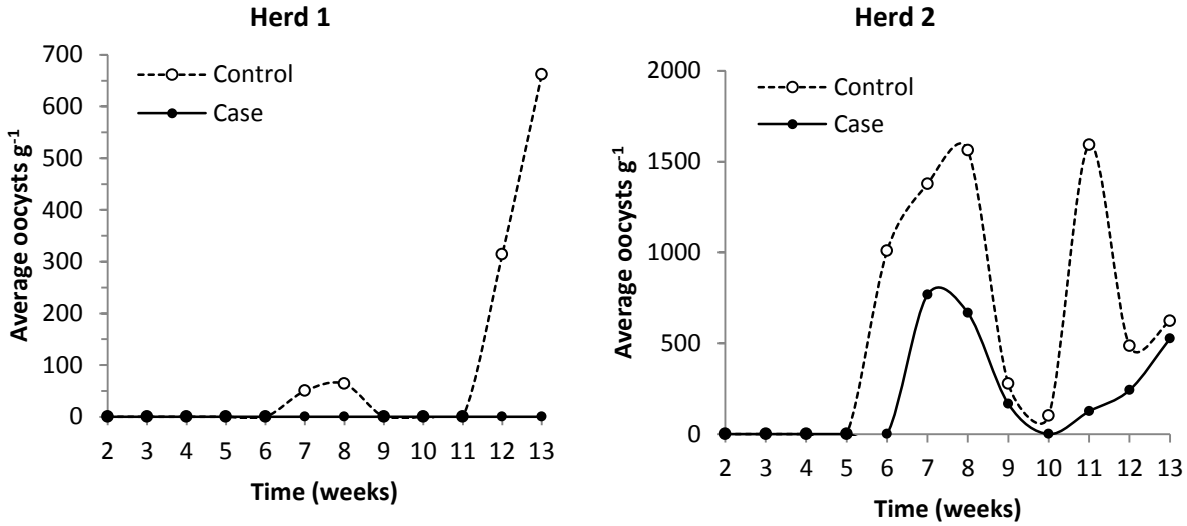


***Figure 8**: OPG (E. bovis and E. zuernii <1000OPG). Average per calf per week of life. Note the different scales on the Y-axis.*

4.2 Diarrhea score

Of the 69 calves studied, 66 calves (96%) had diarrhea at some time during the study. In Herd 1, 43 out of 44 calves (98%) had diarrhea and in Herd 2, 23 out of 25 calves (92%) had diarrhea.

Accumulated, translated diarrhea scores pr. calf were compared. In Herd 1, the treatment-group had higher scores than the control-group (p=0.02, n=44). In contrast, in Herd 2, the treatment-group had

significantly lower scores than the control-group (p=0.004, n=25). Doing the same for field-scores gave the same general result in both herds (p=0.004/n=44 and p=0.01/n=25 respectively).

Looking only at field data from age 6 weeks and onwards, there was a clear difference between the treatment- and control-groups. In Herd 1, the treatment-group had a higher score (p>0.001, n=44). In Herd 2, the control-group had a higher score, the difference being significant (p=0.006, n=25).

Over time, looking at the diarrhea-score, the treatment-group of Herd 1 had peaks in weeks 7-8 and 10-11. In Herd 2, the control-group had a peak in week 7.

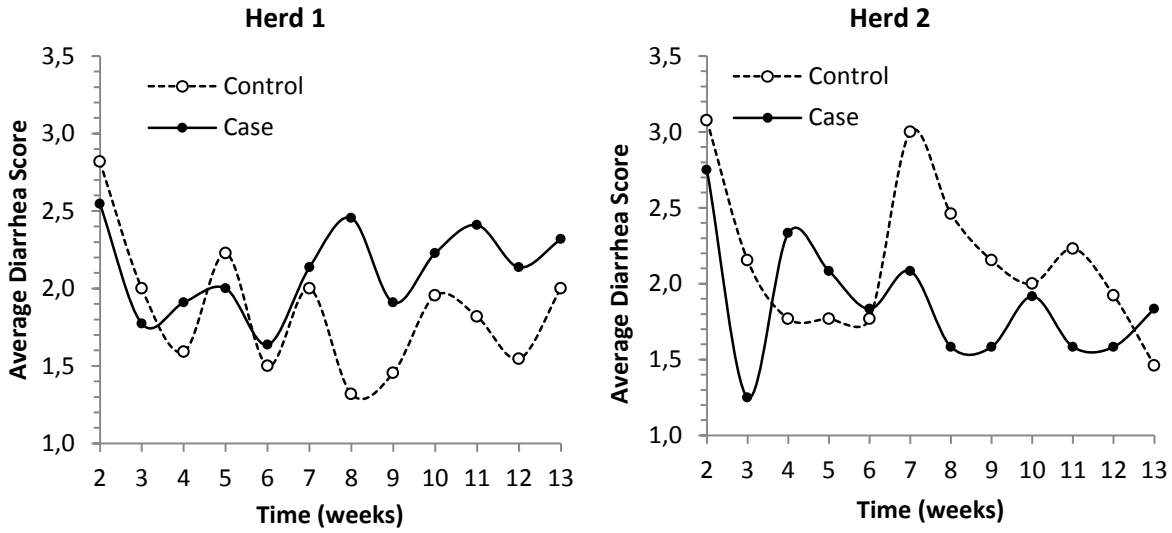


Figure 9: Average field diarrhea score as function of age.

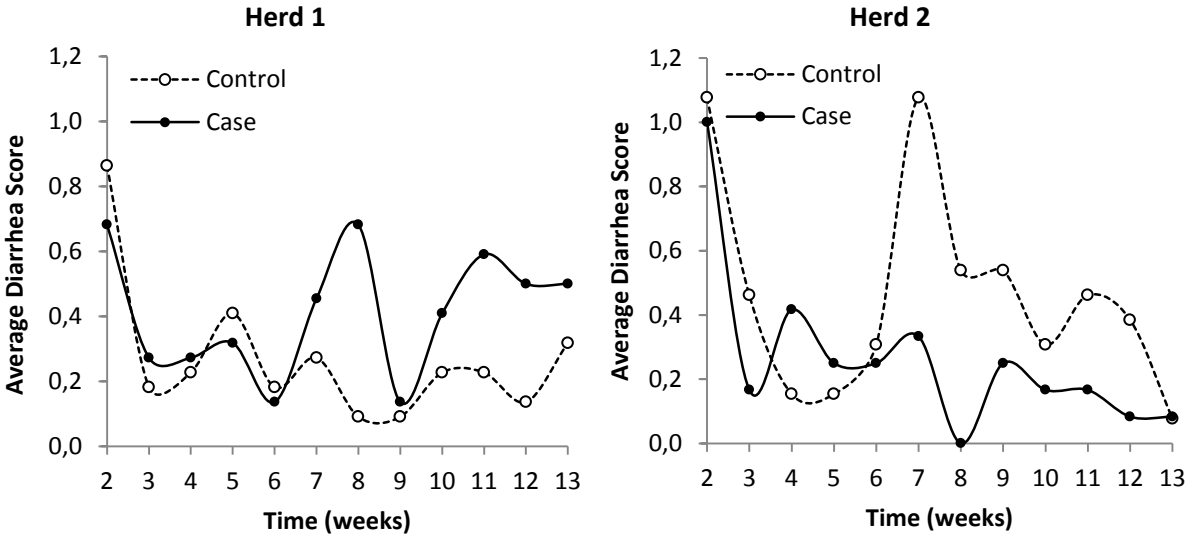


Figure 10: Average diarrhea score (translated) as function of age.

4.3 Growth rate

In general, a higher growth rate was observed in the treatment-populations, than in the control-populations. In Herd 1, the treatment-calves gained 80g more per day on average and the difference

between the two groups was significant (p=0,048, n=44). In herd 2, the treatment-calves gained 130g more per day on average. The difference was also significant (p=0,017, n=25).

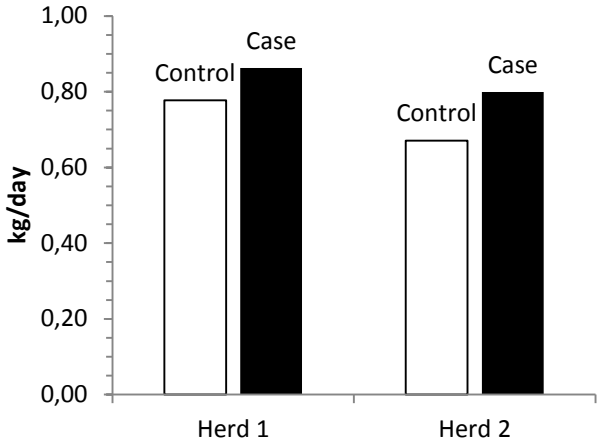


Figure 11: Growth rate of treatment-/control-groups in the two herds.

4.4 Rectal temperature

There was no significant difference in average rectal temperature, between the treatment- and control-group in Herd 1 (p=0.053, n=44). In Herd 2, the average rectal temperature was significantly higher in the control-group than in the treatment-group (p=0.02, n=25). See Figure 12.

Pooling all observations, in Herd 1, rectal temperatures were significantly lower (p=0.014, n=528) in the control group, than in the treatment group. In contrast, in Herd 2, rectal temperatures were significantly higher in the control group, than in the treatment group (p<0.0001, n=300).

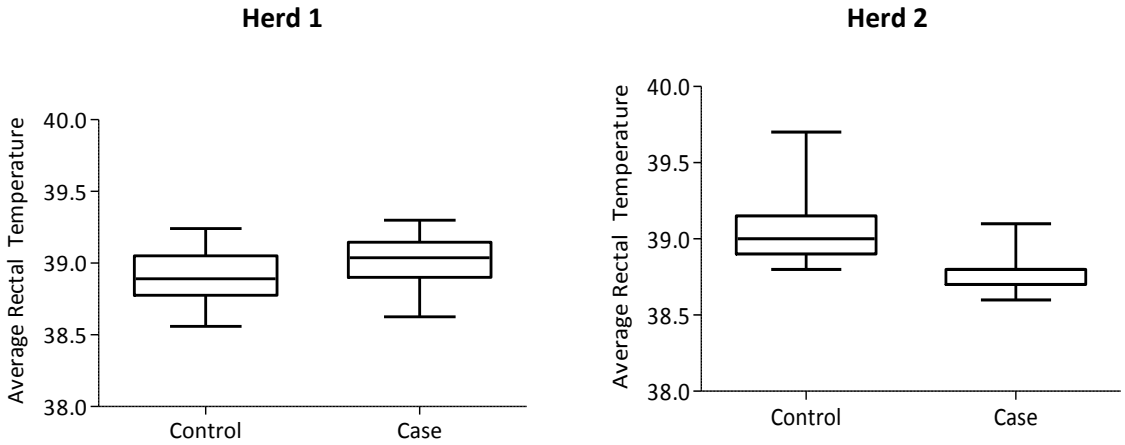


Figure 12: Rectal temperatures in Treatment- and Control-groups.

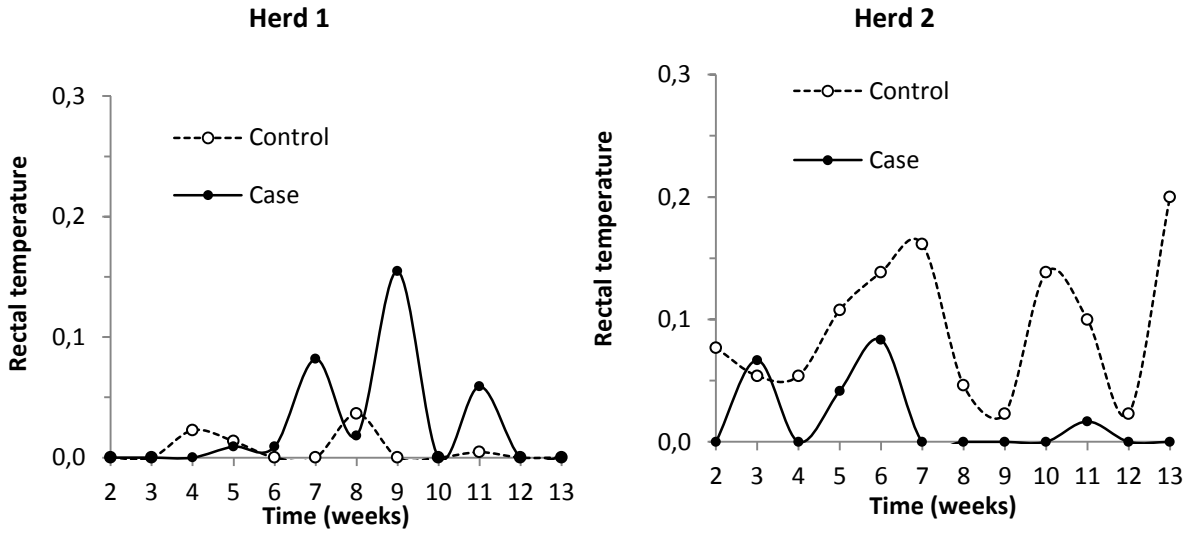


Figure 13: *Rectal temperatures in Treatment- and Control-groups. Only temperatures exceeding 39,5°C were registered.*

When studying rectal temperatures exceeding 39,5°C (hyperthermia), a pattern was observed in Herd 2, with a peak at weeks 5-7 and again at approx. weeks 9-12. In Herd 1, no obvious, common peaks were observed.

4.5 Respiratory symptoms

Studying the total respiratory-score of individual calves in Herd 1 and Herd 2, there was no difference between the treatment- and control-calves (p=0.70/n=44 and p=0.13/n=25 respectively).

Excluding data from the first 5 weeks of life did not make a difference (p=0.77/n=16 and p=0.20/n=16 in Herd 1 and Herd 2, respectively).

4.6 General appearance

Looking at the general appearance in Herd 1 and Herd 2, there was no difference between the treatment- and control-groups, whether looking at accumulated scores over time, per calf (p=0.23/n=44 and p=0.61/n=25 for Herd 1 and 2 respectively) or when comparing all scores individually (p=0.13/n=528 and p=0.60/n=300 for Herd 1 and 2 respectively). The development of symptoms over time is shown in Figure. No pattern is observed in Herd 1, but in Herd 2 there is a decrease in general appearance at approx. week 11.

4.7 Correlations

A correlation analysis was performed between the following parameters: Accumulated log(OPG) per calf in weeks 1-13, accumulated log(OPG) per calf in weeks 6-13, accumulated log(OPG) of pathogenic spp. >1000OPG only, per calf in weeks 1-13 and in weeks 6-13, accumulated diarrhea field scores per calf in weeks 1-13 and weeks 6-13, accumulated diarrhea transformed scores per calf in weeks 1-13 and weeks 6-13 and finally daily weight gain pr. calf.

This was done for each of the following populations; all calves in study (n=69), all calves in Herd 1 (n=44), all calves in Herd 2 (n=25), all treatment-calves (n=34) and all control-calves (n=35).

No significant correlations were discovered. Only four r^2-values higher than 0.3 were encountered: In Herd 2 (treatment- and control-groups pooled), diarrhea field values in weeks 6-13 were mildly correlated with daily weight gain (r^2=0.37) and translated diarrhea values in weeks 6-13 were also mildly correlated with daily weight gain (r^2=0.39).

Pooling all treatment-groups, there was a slight correlation between accumulated log(OPG) per calf in weeks 1-13 and accumulated log (OPG) per calf in weeks 6-13 on one hand, with accumulated diarrhea field values in week 6-13 on the other (r^2=0.31 and r^2=0.32 respectively).

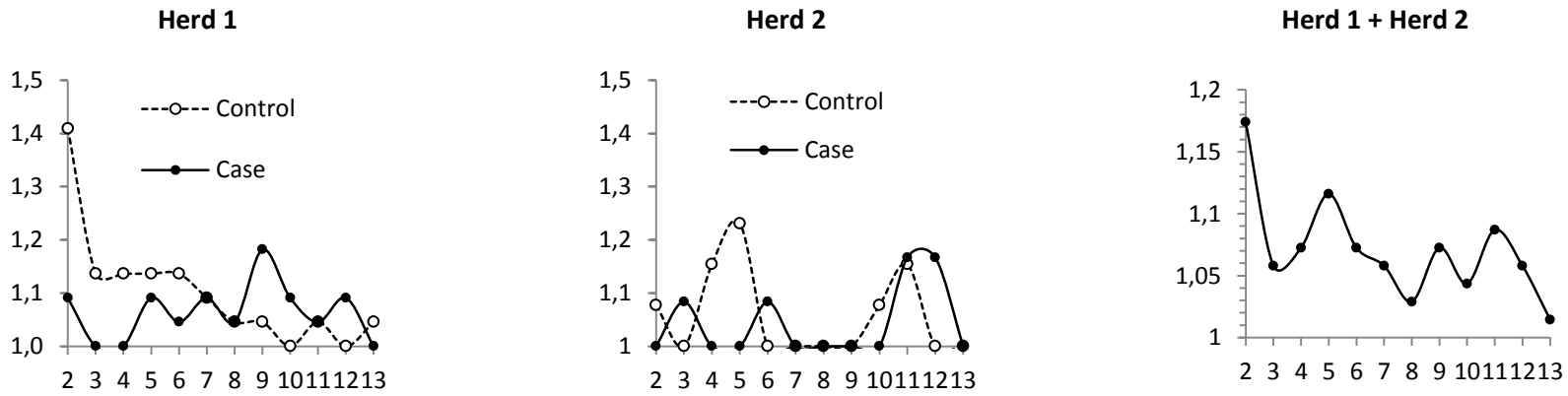


Figure 14: *General appearance of the calves as function of weeks of life. Note that higher numbers reflect decreased general appearance.*

5 Discussion

In this study, the aim was to examine the effect of increased milk-feed on the excretion of oocysts (OPG), growth rate and clinical manifestation of eimeriosis. Since the study was carried out as a field-study, there were many factors that proved to be out of our control. These factors eventually caused bias in numerous ways and effected the validity of our conclusions. These are further described in chapter 5.9.

Hvad skylder Bøje? Hvilke sygdomme passer hans symptomer (almen/luftveje/rektal) på (Salmonella)?

5.1 Oocyst excretion

In Herd 1, 55% of the calves excreted Eimeria spp. oocysts. In Herd 2, the proportion was 96%. Compared to the findings of (Enemark et al., 2013), this places Herd 1 below the national average of 61%, while Herd 2 is well above average. Pooling all calves, the prevalence was 70%, bringing the prevalence quite close to the national average.

Looking at the pathogenic spp., E. bovis and E. zuernii, 11% of the calves in Herd 1 and 84% of the calves in Herd 2 were found to be infected. Again, comparing with the findings of (Enemark et al., 2013), we had anticipated finding these spp. in both herds, although both Herd 1 and Herd 2 are quite far from the national average of 41.5%.

The OPG-levels in Herd 1 proved to be too low for significant results to be achieved. It seems that Herd 1 simply does not have sufficient parasitic load of Eimerias spp. to be suited for the study. OPG-data were inconstant and OPG-levels low. The increased feeding in the treatment-group seemed to have a negative effect on most health-parameters in this herd. A possible explanation for this is that diarrhea observed is caused by other pathogens than Eimeria spp. Salmonella

In Herd 2, sufficient parasitic load was observed, in order to obtain statistically significant results.

5.2 Correlations (OPG-Diarrhea / OPG-Growth Rate)

Patterns in rectal temperature and OPG in Herd 2 suggest that they are correlated and that the peaks in rectal temperature are caused by Eimeria spp. infection. The fact that no such pattern is apparent in Herd 1, suggests that the peaks in rectal temperature are caused by other factors than Eimeria spp. infection. In Herd 2, the peaks in OPG generally occur approx. one week later than the corresponding peak in rectal temperature.

5.3 Margins of Error

5.3.1 Sample Management

5.3.2 Bias

A number of biases were unintentionally introduced during the project.

Selection bias:

Firstly, the manager of “Herd 1”, decided not to include his heifers in the study, since he was afraid of the increased protein-intake having a negative effect of their milk-production, later in life. Therefore, the treatment-group of “Herd 1” only includes bull calves. Therefore, in “Herd 1”, we are comparing a group with mixed genders on one hand, with a group of bulls on the other.

Conversely, midway through the field study, we discovered that the bull-calves of “Herd 2” had to be excluded from the study, as they were sold within the study period. This resulted in a sample population of “Herd 2”, consisting of heifers only.

The calves were intended to enter treatment- or control-groups on basis of their date of birth, calves being born one week being selected for the treatment-group, while the calves born the following week would enter the control-group, alternating through the whole study. This was intended to randomize the selection in respect to discreet management changes, changes in weather (winter/spring) etc. Due to miscommunication, many calves had to be excluded from the study. The exclusion of the calves was not decided by factors believed to have an effect on the final result, but nonetheless resulted in a selection bias, as the control-group of Herd 1 consisting of calves born in calendar weeks 4 and 5, while the treatment-group was born in weeks 9-12. Thus, we are comparing a group born in the dead of winter with a group born in early spring. This is not considered to have been a problem in Herd 2.

At some time during the study, the sanitation procedures in Herd 2 were changed/improved. Instead of pressure-washing the pens in situ, the grates were taken elsewhere, thus minimizing aerosol-contamination of the pens. At approximately the same time, the initial procedure of only pressure-washing/air-drying the pen walls was changed to now include chalking of the walls. This is believed to have happened somewhere in the middle of the study, thus (hopefully) nulling out the effect.

Measurement bias:

The evaluation of symptoms (general appearance and respiratory symptoms) was carried out by three different observers, using a subjective evaluation. This is not considered to have resulted in bias, as scoring criteria were quite simple and the technique was discussed on beforehand. Furthermore, random checks were made throughout the study, to ensure grading was uniform.

6 Perspectives

The data obtained in this study have not been analyzed to their full potential. Examining the correlation between clinical symptoms and weight gain on one hand, with OPG can be done in different ways. Different criteria could be utilized to discover additional correlations, such as the effect of other Eimeria spp. than E. bovis and E. zuernii. The transmission of disease within each group of calves might prove more conclusive than pooling all calves in the treatment-groups.

Furthermore, collecting data daily, over a shorter period (i.e. week 5-9 of life) would significantly harmonize the scattered data obtained through this study.

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8 Appendix

8.1 Questionnaire – Herd 1

Kælvning

Q: Hvor fødes kalvene?

A: I kælvningsbokse med dybstrøelse. Kvier og køer for sig. Placeres i kælvningsbokse 14 dage før forventet kælvning.

Q: Type af underlag i kælvningsboksen?

A: Dybstrøelse.

Q: Hvor mange køer i kælvningsboksen?

A: Forskelligt (generelt blev der observeret høj belægning).

Q: Hvor tit muges/fjernes efterbyrd i kælvningsboksen?

A: Hver 14. dag. Hyppigere om sommeren.

Fodringsrutiner, herunder håndtering af mælk og råmælk

Q: Hvornår gives råmælk?

A: Inden 3 timer, men om natten 4-5 timer. 4 liter råmælk gives med sonde, hvorefter der går et døgn inden næste fodring. Medmindre det er koldt og kalven er lille. I så fald gives mælk før.

Q: Får kalven lov at die hos koen? Hvornår bliver den taget fra?

A: Kalven tages fra, ligeså snart det er muligt. Det tilstræbes at den ikke skal nå at die.

Q: Måles råmælkskvaliteten?

A: Ja, med colostrometer.

Q: Findes der råmælksbank?

A: Ja

Q: Fodres med mælk, mælkeerstatning eller syrnet mælk?

A: Fodres med mælk fra nykælvere, suppleret med mælkeerstatning.

Q: Hvilken temperatur har mælken? Hvordan sikres denne?

A: 40,5 grader ved ophældning i isoleret mælketaxa, et par grader varmere om vinteren.

Q: Hvor lang tid tager en udfodring?

A: 10-15min

Q: Hvilken kalveblanding anvendes og hvornår tildeles den?

A: Kalvene tildeles kalveblanding fra dag 1. ”Elitekalv”.

Q: Har kalvene adgang til hø af god kvalitet?

A: Ja, fra dag 1. Det bedste hø gives til de små kalve. Elektrolytvand gives til kalve i enkeltbokse når mælken er drukket op.

Q: Er det samme person der altid passer kalvene?

A: Samme person om formiddagen, men dem der har aftenmalkningen tager sig også af kalvene om aftenen.

Rengøring

Q: Hvilken type skåle/flasker anvendes til udfodring af mælk? Hvordan rengøres de?

A: Metalskåle maskinvaskes 3 gange i ugen. De store vandtrug rengøres med vand, hvis der observeres halm/gødning i truget.

Q: Hvor ofte rengøres hytter?

A: Ved flytning af kalvene, dvs. hver 3. uge for enkelthytter og hver 3. måned for fælleshytter.

Q: Hvordan vaskes hytterne?

A: Med højtryksrens og Vircon-S (der observeredes kraftig aerosol-forurening af de omkringliggende hytter)

Q: Hvor længe tørrer hytterne mellem holdene?

A: Oftest kun et par timer, men et døgn tilstræbes.

Q: Hvor tit strøes/muges i hytterne?

A: Der strøes manuelt hver dag i alle hytter. Der muges kun ved rengøring af hytterne (der er ingen støvlevask mellem hytterne og der er ingen ”aldersrækkefølge” mellem boksene)

Q: Hvilken type strøelse?

A: Halm

Opstaldningsforhold (egne observationer)

Træk: Ingen træk i fælleshytterne. Lidt træk i enkelthytter.

Fugt: Ingen fugt.

Kulde/Varme: For varmt i fælleshytterne når solen skinner

Flytning af dyr

Q: Hvornår flyttes kalvene i fællesbokse?

A: 1-3 uger, afhængig af belægningsgrad. 3 uger tilstræbes (det blev observeret at kalvene flyttes direkte til "tørreboks" efter kælvning. Denne så ud til at blive godt rengjort efter brug, hver gang).

Q: Hvor mange kalve er der i fællesboksene?

A: 5-6

Q: Hvilke andre sundhedsproblemer end diarre findes der i besætningen?

A: Lungebetændelse (besætningen er kendt med Salmonella)

Q: Er der en løbende udskiftning af kalve i fællesboksene (alders-rulle)?

A: Nej. Kalve i fællesbokse holdes sammen i gruppe.

Q: Er vognen rengjort inden og mellem dyrene?

A: Nej. Den rengøres efter behov.

Q: Hvad er proceduren for evt isolering/aflivning af syge dyr?

A: Der bliver ikke isoleret (ingen sygeboks). Kalve bliver behandlet i boksen og hvis de skrænter helt, bliver de aflivet der.

Sygdom

Q: Har besætningen en rådgivningsaftale?

A: Dyrlægen kommer ugentlig

Q: Hvornår ses diarre hyppigst?

A: i 7-10 dages alderen

Q: Vaccineres med Rotavec Corona?

A: Nej

Q: Hvordan behandles diarre?

A: Borgal vet (Sulfa-TMP) og elektrolytter, evt Metacam hvis smerte.

Q: Hvordan behandles lungebetændelse?

A: Resflor Gold (floramfenikol og Flunixin Meglumin)

Q: Hvordan behandles navlebetændelse?

A: Borgal vet og smertestillende.

Q: Bruges Baycox?

A: Nej. Ikke i forsøgsperioden

8.2 Questionnaire – Herd 2

Kælvning

Q: Hvor fødes kalvene?

A: I kælvningsboks. Køer flyttes derhen 2 uger før kælvning, kvier 3 uger

Q: Type af underlag i kælvningsboksen?

A: Halm

Q: Hvor mange køer i kælvningsboksen?

A: 2-7

Q: Hvor tit muges/fjernes efterbyrd i kælvningsboksen?

A: Efterbyrde fjernes ikke. Muges ud 3x årligt

Fodringsrutiner, herunder håndtering af mælk og råmælk

Q: Hvornår gives råmælk?

A: Max 6 timer hvis født sen aften

Q: Får kalven lov at die hos koen? Hvornår bliver den taget fra?

A: Kalven når at die, hvis den bliver født aften/nat. Fjernes straks hvis muligt

Q: Måles råmælkskvaliteten?

A: Ja. Med colostrometer

Q: Findes der råmælksbank?

A: Ja. På frost. Alle kalve får 4 liter

Q: Fodres med mælk, mælkeerstatning eller syrnet mælk?

A: Mælkeerstatning, celletalsmælk og råmælk

Q: Hvilken temperatur har mælken? Hvordan sikres denne?

A: 39 grader. Måles ved blanding i mixer/mælketaxa

Q: Hvor lang tid tager en udfodring?

A: Ca 20min

Q: Hvilken kalveblanding anvendes og hvornår tildeles den?

A: Elitekalv 1. Derudover Musli eller sojaskrå/skaller/mineraler fra dag 1

Q: Har kalvene adgang til hø af god kvalitet?

A: Godt hø indtil uge 8. Adgang til ensilage fra ca 3 ugers alder

Q: Er det samme person der altid passer kalvene?

Nej. Der har været personaleudskiftning og dårlig standard

Rengøring

Q: Hvilken type skåle/flasker anvendes til udfodring af mælk? Hvordan rengøres de?

A: Trug og skåle rengøres efter behov. Vaskes m sæbe ved flytning mellem bokse. Vand hældes i skåle 30omin efter udfodring

Q: Hvor ofte rengøres hytter?

A: Enkeltbokse rengøres efter hver kalv (ca 3 uger). Fællesbokse efter hvert hold.

Q: Hvordan vaskes hytterne?

A: Bokse højtryksrenses og strøes med hydralkalk. Fællesbokse hydralkalkes og vægge kalkes bagefter.

Q: Hvor længe tørrer hytterne mellem holdene?

A: Enkelthytter tørrer ca 1 uge mellem kalve (minimum 2 dage). Fællesbokse 2-3 dage.

Q: Hvor tit strøes/muges i hytterne?

A: Strøes 2x per uge

Q: Hvilken type strøelse?

A: Halm

Opstaldningsforhold (egne observationer)

Træk: Minimalt træk. Alle kalvebokse er undertag i stalden og der er gardiner på siderne af bygningen. Lukkede gavle.

Fugt: Tørt miljø, men dybstrøelsen i fællesboksene er for våd ud mod fodergangen

Kulde/Varme: Fint

Flytning af dyr

Q: Hvornår flyttes kalvene i fællesbokse?

A: 14-21 dage

Q: Hvor mange kalve er der i fællesboksene?

A: 5-8 kalve

Q: Hvilke andre sundhedsproblemer end diarre findes der i besætningen?

A: Luftvejssymptomer

Q: Er der en løbende udskiftning af kalve i fællesboksene (alders-rulle)?

A: Nej (ej hellere observeret)

Q: Er vognen rengjort inden og mellem dyrene?

A: Nej

Q: Hvad er proceduren for evt isolering/aflivning af syge dyr?

Ingen procedure. Skrænter/aflives i fællesboksen

Sygdom

Q: Har besætningen en rådgivningsaftale?

A: Ja. Modul 2. Dyrlægebesøg hver 14. dag

Q: Hvornår ses diarre hyppigst?

A: 10 dages alder

Q: Vaccineres med Rotavec Corona?

A: Ja. Køer vaccineres 4 uger før kælvning

Q: Hvordan behandles diarre?

A: Synulox og elektrolytter (hydrafeed) i tre dage

Q: Hvordan behandles lungebetændelse?

A: Engangsbehandling med Suprevo

Q: Hvordan behandles navlebetændelse?

A: Noromox

Q: Bruges Baycox?

A: Er udleveret, men har ikke været brugt i lang tid