

## Significance of milk feeding strategies for development of *cryptosporidiosis* and *giardiasis* in dairy calves

Veterinary Master's Thesis

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## Summary

*Cryptosporidium* and *Giardia* are protozoan zoonotic parasites known to infect a large variety of animals including cattle and humans. The parasites cause diarrhea and discomfort in the host and their presences do not only have relevance to the health and productivity of dairy herds, but are also of substantial interest in relation to public health.

Research on the effect of different feeding regimes on health and later productivity in the neonate calf has been conducted with promising results, but the effect of feeding regimes in relation to parasitic challenges have yet to be clarified.

The aim of this veterinary master's thesis was to investigate the effect of milk feeding strategies on development of Cryptosporidiosis and Giardiasis in dairy calves. Therefore, a field study with a practical useful setting, implementable in Danish dairy herds was conducted on two Danish Dairy farms.

The calves were subjected to one of two different types of milk feeding, a normal (2×3 liters a day) - or a high (2×5 liters a day or 2×4 liters for two weeks followed by 2×5 liters a day for another two weeks) feeding regime during their first 4 weeks of life.

Feces were collected from the calves once a week and clinical registrations (temperature, signs of respiratory disease and general well-being) were made. A questionnaire on management was conducted and the calves were weighed at the end of the trial period. In one herd (case-group), left over milk was weighed after every feeding.

At the laboratory the samples were scored for consistency and purified. Quantitative immunofluorescence analysis was performed using a *Cryptosporidium/Giardia* fluorescent antibody mix.

The relation between the variables was described using linear regression and T-tests. The tests were performed using Grafpad Prism 6 and "R" Statistics.

No significant effect ( $P < 0.05$ ) of the milk feeding strategy on weight gain, (oo)cyst excretion or occurrence of diarrhea were found in the dairy calves. In one herd, the test of the effect of the milk feeding strategy on weight-gain in the dairy calves was inconclusive, since the final body-weights were biased.

Though not statistically significant, a tendency towards some effect of the feeding strategy on the feces consistency was seen. Secondly, the present study weren't able to show a correlation between *Cryptosporidium* oocyst excretion and diarrhea in young calves.

The statistically insignificant results could be ascribed to the setting of the field study as well as to unintended changes, which were made to the study design.

In the light of the possible improvements of our study design, we find it highly relevant to further investigate the impact of milk feeding strategies on parasitic load, health and performance in dairy calves, with the aim to implement beneficial changes to commercial calf rearing.

## Resumé

*Cryptosporidium* og *Giardia* er zoonotiske protozoer, der inficerer en lang række dyr, herunder kvæg og mennesker. Parasitterne forårsager diarré og ubehag i værten og har ikke kun relevans for sundhed og produktiviteten i malkekvægsbesætninger, men er også relevant at diskutere i forhold til folkesundheden.

Der er blevet forsket i effekten af forskellige fodringsstrategier på sundhed og senere produktivitet hos småkalve med lovende resultater, men effekten af fodringsstrategier i forhold til parasitbyrde er endnu ikke klarlagt.

For at undersøge effekten af praktisk implementerbare mælkefodringsstrategier, på udvikling af Cryptosporidiose og Giardiasis i småkalve, blev der udført et feltstudie i to danske malkebesætninger.

Kalvene blev udsat for én af to typer mælkefodring i løbet af deres første 4 leveuger: En normal mængde (2 × 3 liter om dagen) eller en høj (2 × 5 liter om dagen eller 2×4 liter i to uger efterfulgt af 2×5 liter om dagen i endnu to uger).

Fæces blev indsamlet fra kalvene en gang om ugen og der blev foretaget kliniske registreringer af kalvene (temperatur, tegn på respiratorisk sygdom og generel trivsel). Desuden blev der udfyldt et spørgeskema om management i besætningerne og kalvene blev vejet i slutningen af forsøgsperioden. I den ene besætning (case-gruppen) blev det overskydende mælk tilbagevejet efter hver fodring.

På laboratoriet blev prøverne konsistensbedømt, oprenset og Kvantitativ immunfluorescens analyse blev udført ved hjælp af et *Cryptosporidium* / *Giardia* -fluorescens antistoftest kit.

Forholdet mellem variabler blev beskrevet ved lineær regression og T-test. De statistiske beregninger blev udført i programmerne Grafpad Prism 6 og "R" Statistics.

Der blev ikke fundet nogen signifikant effekt ( $p < 0,05$ ) af mælkefodringsstrategien på tilvæksten, (oo)cyst-udskillelsen eller forekomsten af diarré hos småkalvene. I den ene besætning var testen af effekten af mælkefodringsstrategien på tilvæksten hos småkalvene ikke konkluderende, da tilvæksten var forkert beregnet.

Selv om der ikke blev fundet en statistisk signifikant sammenhæng, sås alligevel en tendens i retning af en vis effekt af fodringsstrategien på fæceskonsistensen. Vores undersøgelser var ikke i stand til at påvise en korrelation mellem *Cryptosporidium*-oocyst-udskillelsen og diarré i småkalve.

De statistisk in-signifikante resultater kan forklares både ved, feltforsøgets opstilling på såvel som ved de uforudsete ændringer, der blev implementeret i studiedesignet undervejs.

På baggrund af de ændringer, der i et fremtidigt studie hensigtsmæssigt kan implementeres i vores studiedesign, finder vi det stadig yderst relevant at undersøge virkningen af mælkefodringen på parasitbyrden, sundhed og produktiviteten i småkalve i de danske malkekvægsbesætninger.

## **Preface**

This thesis concludes the Master's program in Veterinary Medicine at the University of Copenhagen. It has been completed with the ambition of providing more information about the significance of milk feeding strategies for development of *Cryptosporidiosis* and *Giardiasis* in dairy calves in practical useful setting, implementable in Danish dairy herds.

The master's thesis consists of two parts, a theoretical part describing the epidemiology of the parasites as well as general review of calf diarrhea and its impact on production and a second part presenting the experimental work.

The work presented here is aimed at an audience of veterinarians and other professionals working with calves in the field as well as scientific workers for further research.

## **Acknowledgements**

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### ABBREVIATIONS

- OPG oocysts per gram feces
- CPG cysts per gram feces
- LD low diet
- HD high diet
- FS Fecal score

# Significance of milk feeding strategies for development of cryptosporidiosis and giardiasis in dairy calves

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# Abstract

## 1. Introduction

*Cryptosporidium* and *Giardia* are protozoan zoonotic parasites known to infect a large variety of animals including cattle and humans. Their presence does not only have relevance to the health and productivity of dairy herds, but are also of substantial interest in relation to public health (Cacciò *et al.* 2005; Thompson *et al.* 2008).

Milk feeding intensity has in several studies been shown to correlate with weight gain, body composition and feed conversion (Raeth-Knight *et al.* 2009; Bartlett *et al.* 2006; Diaz *et al.* 2001; Quigley *et al.* 2006) but knowledge of the effect of feeding regimes on parasitic challenges is still sparse. The present Master's thesis investigates the correlation between milk feeding intensity and parasitic load, weight gain and diarrhea in Holstein dairy calves. It was a part of a larger project on neonate calf health, carried out in cooperation with Videncenter for Landbrug.

### 1.1 Objective of the study

The aim of this project was to evaluate the effect of milk feeding intensity (high versus normal amount of milk) on the occurrence of single-celled intestinal parasites (*Cryptosporidium* and *Giardia*), diarrhea and weight gain in dairy calves in a field setting, which can be easily implemented in Danish dairy herds.

Hypothesis:

Intensive feeding of young calves with high amounts of energy and protein will:

- Improves the calves' general immunity whereby the risk of clinical disease caused by protozoa.
- Reduce the intensity of infection in the herd, so that calves will excrete fewer (oo)cysts to the environment.
- Increase growth beyond what can only be attributed to increased feeding intensity.

## **1.2 Method**

The study is a case-control study performed on 73 Holsteins calves at two Danish dairy farm situated in the northern part of Jutland, Denmark.

The calves were subjected to one of two different types of milk feeding, a normal (2×3 liters a day) - or a high (2×5 liters a day or 2×4 liters for two weeks followed by 2×5 liters a day for another two weeks) feeding regime during their first 4 weeks of life.

Feces were collected from the calves once a week and clinical registrations (temperature, signs of respiratory disease and general well-being) were made.

At the laboratory the samples were scored for consistency and purified and Quantitative immunofluorescence analysis was performed using a *Cryptosporidium/Giardia* fluorescent antibody mix.

Statistical analyses were done using GrafPad Prism 6 and “R” statistics.

## **1.3 Delimitation**

73 Holstein calves from two Danish dairy herds were included in the present study in the months from 28<sup>th</sup> of January to 14<sup>th</sup> of April 2014. The herds were chosen based on the farmer’s commitment and ability to maintain the feeding regimes during the entire study period as well as on a history of diarrhea amongst the young calves.

Literature relevant to the aim of the study – basic information on the biology of the parasites, calf scours and milk feeding in relation to calf health and production was reviewed.

# **PART I**

## **2. Calf diarrhea overview**

Calf diarrhea together with bovine respiratory disease are the two most common causes of death in young dairy heifers despite being high morbidity – low mortality diseases as review by Johnson *et al.* (2011).

Calf diarrhea is often multifactorial, which makes diagnosis and treatment difficult. *C. parvum* and rotavirus are the dominant pathogens amongst diarrheic calves internationally (Johnson *et al.* 2011; Cho & Yoon 2014).

*Escherichia coli* and *Salmonella enterica* are the two most common bacterial causes of calf diarrhea. Both bacteria infect the calves via direct contact with feces from infected animals or from bedding and stable interior. Calves infected with *E. Coli* develop watery diarrhea typically within their first week of life due to villous atrophy in the small intestines and toxin production. Salmonellosis in calves is characterized by bloody and fibrinous watery or mucoid diarrhea and is often seen in calves less than three weeks old. The bacteria infect all age groups, but the symptoms are often severe in calves 10 days to 3 months old (Cho & Yoon 2014).

Rota- and corona virus are the most common viral pathogens causing diarrhea in young calves. Both agent cause malabsorptive diarrhea in 1-2 week old calves that shed large amounts of virus in their feces to contaminate the environment as reviewed by Cho & Yoon (2014).

In Danish dairy herds having rota/corona virus problems, it is common to vaccinate the cows twelve to three weeks pre partum with *Rotavec® Corona Vet.* to stimulate the production of antibodies in the cow and boost the immunity in the newborn calf (Veterinærmedicinsk Industriforening, 2014b).

Protozoan parasites are also causing enteric disease in young calves. The major species being *Cryptosporidium* and *Giardia* (thoroughly described later) and *Eimeria spp.* *Eimeria* is highly prevalent in Danish dairy young stock, yet its pathogenicity varies amongst subspecies and the infection can be both clinical and subclinical. The pathogenic *E. zuernii* and *E. bovis* can cause severe clinical symptoms such as profound badly smelling diarrhea and depression in cattle 1-6 months of age. The symptoms last about 5-12 days (Enemark *et al.* 2013; Maddox-Hyttel & Vestergaard, no year).

### **3. *Cryptosporidium* ssp.**

#### **3.1 *Cryptosporidium* introduction**

Cryptosporidiosis is caused by the unicellular eukaryotic protozoa *Cryptosporidium*, which infect and replicated in the epithelial cells of the intestines causing diarrhea and discomfort in all species of animals including humans (Fayer *et al.* 2009).

The first member of the *Cryptosporidium* genus was discovered in 1907 by E. E. Tyzzer in the ventricle of laboratory mice and thereby named *Cryptosporidium muris*. A few years later

Tyzzar discovered *C. Parvum* in the small intestines of mice and had thereby characterized the two first species within the genus (Tyzzar 1907; Tyzzar 1912).

In the later years several subspecies were discovered in many different species of animals including humans and what was earlier thought to be an opportunistic parasite is now commonly regarded as a sickness inducing pathogen with relevance to both animals and humans. (O'donoghue 1985; Levine 1980).

### 3.2 Classification and taxonomy

As reviewed by Cacciò *et al.* (2005) the taxonomy of *Cryptosporidium* was first determined on host specificity, which cause a fair bit of confusion as more than spp. share the same host spectrum. The classification of the family was made difficult by the lack of molecular methods to differentiate between phenotypes. Today we know that the family Cryptosporidiiae includes a single genus *Cryptosporidium* with 14 subspecies containing several genotypes, varying genetic differences (Table 3.1).

*C. parvum* is the major subspecies in cattle, primarily infecting younger calves (Langkjær *et al.* 2006; Santín *et al.* 2004).

Tab. 3.1 - Species of *Cryptosporidium* and major hosts according to Cacciò *et al.* (2005)

<b>Cryptosporidium</b>	<b>Major hosts</b>
<b>C. hominis</b>	Humans, monkeys
<b>C. parvum</b>	Cattle, other ruminants, humans
<b>C. andersoni</b>	Cattle
<b>C. Muris</b>	Rodents
<b>C. suis</b>	Pigs
<b>C. felis</b>	Cats
<b>C. canis</b>	Dogs
<b>C. wrairi</b>	Guinea pigs
<b>C. bailey</b>	Poultry
<b>C. meleagridis</b>	Turkeys
<b>C. gali</b>	Finches
<b>C. serpentis</b>	Reptiles
<b>C. saurophilum</b>	Lizards
<b>C. molnari</b>	Fish

Modified from Cacciò *et al.* (2005)

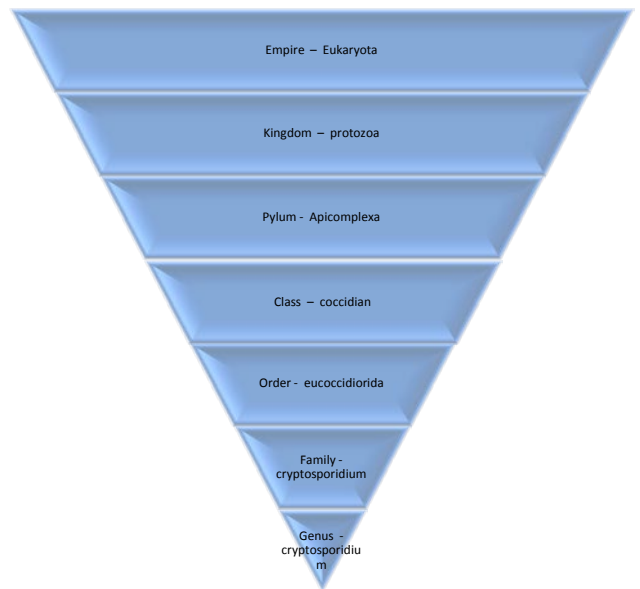


Fig. 3.1 - *Cryptosporidium* classification

### 3.3 Morphology

The Oocyst of the *Cryptosporidae* family is morphologically different from other species of *Coccidia*. It is smaller, round and measuring an average 49 nanometers in thickness. *C. Andersoni* oocysts are ovoid and 6-8  $\mu\text{m}$  in length and 5-6,5  $\mu\text{m}$  in width. The oocyst found in the feces is surrounded by a thick three layered wall containing four Sporozoites. The oocyst wall is regular except from a line forming slightly towards one pole from the midline. This is where the oocyst opens at excystation (Fayer 2008; O'handley & Olson 2006).

### 3.4 Life cycle

The *Cryptosporidium* life cycle is complex and can be divided into six stages.

The first state (i) is the environmental state, where the infective sporulated oocyst has been excreted in the feces of an infected host. The four infective Sporozoites in the oocyst are now capable of infecting a new host without further sporulation.

The Excystation (ii) is an endogenous phase, where the ingested oocyst gets in contact with the digestive enzymes and acids of the digestive tract and releases the Sporozoites. It is not entirely clear which factor initiates the opening of the Oocyst.

The cell invasion (iii) is the third stage - now the motile Sporozoites invade the intestinal epithelial cell by forming vacuoles near the site of attachment, which gradually fuse with the host cell membrane, so that the parasite is now intracellular but still extracytoplasmic. Now the sporozoite transform to a circular Trophozoite. This process takes approximately 15 minutes.

Asexual Multiplication or "Merogoni" is the fourth step (iv) in which the trophozoite undergoes asexual reproduction and forms a Meront. Merozoites from within the Meront leave the host cell to infect other host cells. There are two different types of Meronts. Only the type 2 Meronts are thought to initiate sexual reproduction, which is the fifth (v) stage; Microgamonts (male) and macrogamonts (female).

The micro- and macrogamonts enter a host cell and the microgamont penetrates the macrogamont cell wall to create an oocyst containing four sporozoites. The oocysts are either thin- or thick walled. The thick walled oocysts are excreted in the feces (Sporogony (vi)) and the thin walled oocysts rupture in the intestines to autoinfect the host (Fayer 2008).

The time passing from an oocyst having been ingested till oocysts are being excreted in the feces is called the prepatent period. The prepatent period varies between species of

*Cryptosporidium*, as well as with host species and infective dose. The prepatent period for *C. parvum* in calves is determined from 1 to 12 days and calves can shed oocysts from 2 days of age peaking around day 14 (Thompson *et al.* 2008, Fayer 2008; O’handley & Olson 2006).

### **3.5 Clinical manifestation and pathology**

Clinical signs of cryptosporidiosis include diarrhea, depression, anorexia and in rare cases, death. Diarrhea is the most prominent clinical sign and death due to dehydration, metabolic acidosis and cardio vascular collapse is rarely seen. Clinical signs can persist from 4 to 14 days with varying intensity (Langkjær *et al.* 2007, Thompson *et al.* 2008; O’Handley & Olson 2006) and show a decrease in severity of clinical signs and duration of the infections, the older the animal is when exposed (O’handley & Olson 2006).

In most well managed herds *Cryptosporidiosis* is not a major health problem but *Cryptosporidium* is often found together with other enteropathogens potentially worsening the clinical condition of the animal (O’handley & Olson 2006).

The malabsorptive diarrhea of *Cryptosporidiosis* is caused by enterocyte apoptosis leading to inflammation and atrophy of the intestinal villi, which results in loss of intestinal surface for decreased absorption of nutrients. The presence of a cholera-like toxin may also contribute to the development of diarrhea. The cattle host specific *C. andersoni*, affects the gastric glands causing hypertrophy of the gastric mucosa and epithelial lining and increases abomasal pH (Thompson *et al.* 2008; O’handley & Olson 2006).

### **3.6 Epidemiology and risk factors**

As reviewed by O’handley & Olson (2006) *Cryptosporidiosis* has both a high incidence and prevalence in dairy herds in American and in European countries. A recent Danish study revealed a *Cryptosporidium* herd prevalence of 60, 82 and 100% for cows, young calves and older calves respectively and the prevalence of calves shredding *Cryptosporidium* oocysts was 84% (Maddox-Hyttel *et al.* 2006) and Silverlås *et al.* (2009a) found a *Cryptosporidium* prevalence amongst Swedish dairy herds of 52%, 29% and 5.6% in calves, young stock and cows respectively. Both results underlining that *Cryptosporidiosis* is one of the most common causes of neonatal diarrhea in ruminants.

*Cryptosporidiosis* in cattle is most commonly seen from 1-3 weeks of age, but infections can be seen in calves as young as a couple of days old. Oocyst excretion starts 2-7 days post infection and lasts from 1-12 days (O’handley & Olson 2006; Brook *et al.* 2008; Silverlås *et al.* 2009a).

Shedding of oocysts in feces is not always correlated with clinical cryptosporidiosis and oocyst excretion can also continue after determination of diarrhea (Enemark *et al.* 2002). Cattle are known to be zoonotic transmitters of *Cryptosporidium* with a higher risk of transmission from calves under two months of age than older calves, since the zoonotic *C. parvum* is highly prevalent in that age group. Livestock fecal pollution of drinking water has also been the source of human *Cryptosporidiosis* (Thompson *et al.* 2008; O’handley & Olson 2006; Santín *et al.* 2004). The minimum infectious dose of *C. parvum* in humans is 30 oocyst, as reviewed by O’Handly & Olson (2006).

As described earlier, the oocysts are thick walled and stay infective for many months in cold and wet environments, which make a high biosecurity and hygiene highly important in minimizing infection pressure in the herd.

Risk factors include: insufficient cleaning and drying of calf pens between animals (empty periods, high pressure cleaning or use of disinfectants). *Cryptosporidium* oocysts are resistant to most commercial disinfectants hence drying is crucial and can prevent high contamination levels among calves (Johnson *et al.* 2011; Silverlås *et al.* 2009a; Silverlås *et al.* 2010).

Insufficient bedding has also been identified as a risk factor for *Cryptosporidiosis*. Studies on management risk factors suggest that deep litter bedding might be more effective on keeping infection pressure down than more frequently cleaned calf pens. The difference can be explained by less exposure to viable oocysts when these are buried in the bedding and perhaps the fact that *Cryptosporidium* oocysts are sensitive to ammonia, which will be found in higher concentrations in the deep litter (Maddox-Hyttel *et al.* 2006; Silverlås *et al.* 2009a; King & Monis 2006).

Time with the cow after calving as a risk factor for infection has been discussed. The more common perception is that the longer the calf stays with the cow, the higher the risk of infection, but Silverlås *et al.* (2006a) found the opposite to be the case. This, probably because of the higher infection pressure in the calf sheds and the low infection rate in periparturient cows.



### **3.7 Diagnostics and typing**

When diagnosing *Cryptosporidiosis* several different laboratory methods are available.

Directs light microscopy techniques are widely used in veterinary clinics to detect oocysts in fecal samples. The oocysts are either visualised using a flotation technique or a faecal smear is fixed and stained with an acid fast stain, such as Ziehl-Nilsen, followed by direct microscopy. These methods are easy and cheap and can be done quickly in the clinic. In research and in diagnostic laboratories the more expensive immunofluorescence microscopy method is commonly used for diagnostic as well as quantitative analysis enzyme-linked immunosorbent assay (ELISA), PCR and real time PCR are being used for diagnostic purposes as well as typing (O'handley & Olson 2006; Cho & Yoon 2014).

### **3.8 Treatment**

A lot of work has been done investigating therapeutic and prophylactic drug regimes on *Cryptosporidiosis* in ruminants with different outcomes (Stockdale *et al.* 2008; O'handley & Olson 2006).

Halofuginone lactate (Halocur<sup>®</sup>) is registered in Denmark for calves as treatment and prevention of *Cryptosporidium parvum* infections. Halofuginone does not cure *Cryptosporidiosis* but reduces the amount of oocysts excreted by the animal and reduces diarrhea. The efficacy of Halocur as treatment and prevention of *Cryptosporidiosis* is questionable. The importance of rehydration and electrolyte replenishment by either oral or parenteral administration, when the animal has severe diarrhea in the first stages of the infection and taking management measures to lower the infection pressure in the herd, is stressed by several workers (Veterinærmedicinsk Industriforening 2014a; O'handley & Olson 2006, Silverlås *et al.* 2009; Thompson *et al.* 2008; Stockdale *et al.* 2008). Multiple experiments on developing vaccines against *Cryptosporidium* have been done, but none proved successful in preventing infection (O'handley & Olson 2006; Thompson *et al.* 2008).

## **4. Giardia ssp.**

### **4.1 Giardia introduction**

*Giardia* is a flagellated protozoan that infects a wide variety of mammals including humans. Together with *Cryptosporidium*, *Giardia* is a major cause of waterborne gastro intestinal disease in humans and is known to be highly prevalent in cattle herds (O'handley & Olson 2006). Even

though *Giardia* rarely causes severe clinical disease in ruminants, there is reason to believe that its potential impact on production needs to be investigated further (O'handley & Olson 2006; Olson *et al.* 1995).

#### **4.2 Classification and taxonomy**

Like *Cryptosporidium*, *Giardia* has a wide host range, with some subspecies and genotypes being restricted to specific species and others known to infect a variety of species including man (Thompson *et al.* 2008).

The *Giardia* family counts 6 species. The only species known to infect humans is *G. duodenalis*, which is synonymous with *G. lamblia* and *G. intestinalis*. The species *G. duodenalis* consists of at least 7 assemblages not morphologically distinguishable from each other (Table 4.1). Genetic differences have been noted and so fare assemblage A and B are the only isolates being associated with human infections (Cassiò *et al.* 2005; Cassiò *et al.* 2009; Thompson 2009).

Tab. 4.1 - Species of *Giardia* and major hosts according to Thompson (2009)

<b>Giardia</b>	<b>Major hosts</b>
<b>G. duodenalis Assemblage A</b>	Humans, livestock
<b>G. duodenalis Assemblage B</b>	Humans
<b>G. duodenalis Assemblage C</b>	Dogs
<b>G. duodenalis Assemblage E</b>	Cattle, other hoofed livestock
<b>G. duodenalis Assemblage F</b>	Cats
<b>G. duodenalis Assemblage G</b>	Rats
<b>G. agilis</b>	Amphibians
<b>G. muris</b>	Rodents
<b>G. microti</b>	Muskrats, voles
<b>G. psittaci</b>	Birds
<b>G. ardeae</b>	Birds

Modified from Thompson (2009) & Cacciò *et al.* (2005)

### 4.3 Morphology

*Giardia* belongs to the order, Diplomonada being a teardrop-shaped flagellated protozoan with two intracellular nuclei each with four flagellates attached to it, used for propulsion. The Cysts are ovoid measuring 8-15 µm in length and 7-10 µm in width (Thompson *et al.* 2008; O’Handley & Olson 2006; Benchimol 2009).

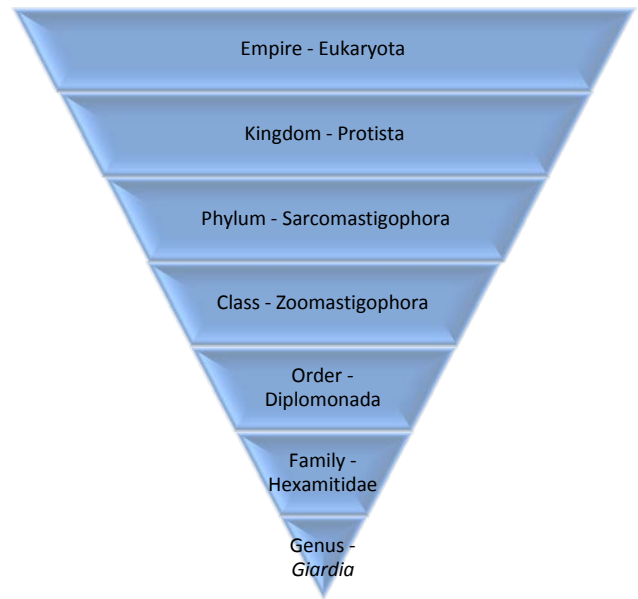


Fig. 4.1 - *Giardia* Classification

#### 4.4 Life cycle

*Giardia* has a life cycle very similar to that of *Cryptosporidium* yet more simple. When the host ingests the cyst the digestible enzymes and acids trigger excystation and releases the trophozoites. The trophozoites then undergo asexually multiplication via binary fission and colonize the small intestine adhering to the epithelium by using a specialized organelle, called the adhesive disk. The trophozoites encyst and are immediately infective, when excreted from the host (O'handley & Olson 2006; Olson *et al.* 2004; Benchimol 2009).

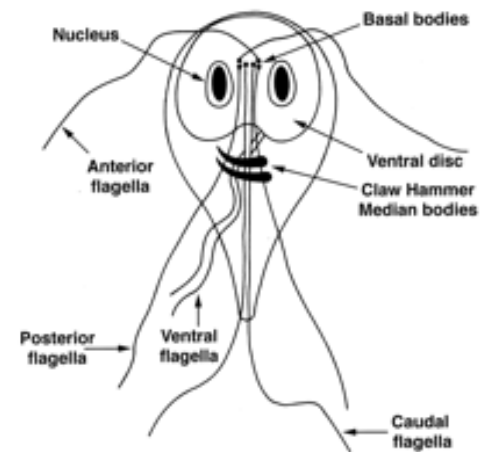


Fig. 4.2 - Trophozoite morphology of *G. duodenalis*.

(Photo from: <http://cmr.asm.org/content/13/1/35.figures-only?related-urls=yes&legid=cmr;13/1/35>)

#### 4.5 Clinical manifestation and pathology

In both dogs, cats and ruminants most infections are asymptomatic. The most common clinical signs in dogs and cats is small bowl diarrhea and in ruminants diarrhea and depression. In cattle diarrhea is often multifactorial and *Giardiasis* plays a minor role in neonate calf diarrhea, but is commonly found in dairy herds especially in older calves. In humans diarrhea is the main symptom of *Giardiasis*. The pathology of *Giardia* is not fully understood but apoptosis of the enterocytes, shortening of villi and loss of epithelial barrier function have been reported in experimental infections (Thompson *et al.* 2008; O'Handley & Olson 2006; Maddox-Hyttel *et al.* 2006).

#### 4.6 Epidemiology and risk factors

*Giardia* cysts are very stable in the environment and can stay infective for up to several months and the effective dose is low (10-100 cyst). The prepatent period of *Giardia* in cattle is 7-8 days (Cacciò *et al.* 2009; O'Handley & Olson 2006; Thompson 2009). *Giardia* is found in all age groups of cattle, but is most commonly found in older calves even though the younger calves are likely to be exposed shortly after birth. Cyst excretion is normally seen a few weeks after birth and can continue past 100 days post infection. The infection is often self-limiting but there are also indications that *Giardia* infections can be chronic in older animals. The reason for this is uncertain (O'Handley & Olson 2006; Olson *et al.* 2004). As mentioned earlier *Giardia* infections are commonly asymptomatic. The mechanism in this is uncertain, but passive immunity in some individuals has been suggested (O'Handley & Olson 2006; Thompson 2009). In a study

from 2006 estimating the prevalence of *Cryptosporidium* and *Giardia* in 50 randomly selected sow and dairy herds in Denmark, the prevalence of calves shedding *Giardia* detected in the fecal samples was 82% in younger calves and 100% in older calves. Risk factors associated with *Giardiasis* were young age and lack of empty periods in calf pens between calves (Maddox-Hyttel *et al.* 2006). These results correspond well with other work stating low immunity status and poor hygienic standards to be risk factors strongly correlated to manifestation of disease both in animals and humans (Thompson 2009; Hunter *et al* 2005). *Giardiasis* in humans is most commonly seen in children in developing countries, where the disease can range from subclinical to chronic infections, having serious impact on nutritional state and welfare of children in poor communities. Animals can act as zoonotic carriers, but the transmission is more likely to be happening by direct person-to-person transfer or via food and drinking water (Thompson 2009; Hunter *et al* 2005).

#### **4.7 Diagnostics and typing**

Light microscopy after a purification and flotation procedure is the most commonly used tool in diagnosis of *Giardia* from fecal samples. ELISA methods are also useful but expensive to use as well as PCRs and immunofluorescence microscopy, which are only used as a tools in research and diagnostic laboratories (Thompson *et al.* 2008; O’Handley & Olson 2006).

#### **4.8 Treatment**

As previously mentioned, humeral immunity in infected animals result in a self-limiting infection, but it can take over 100 days post infection till antibodies are produced. Antibodies can be transmitted through colostrum and to some extent protect the young calf from infection. Benzimidazoles, fenbendazole, albendazole and paromomycin sulfate are shown to be effective against *Giardiasis* in calves, but do not protect the animals from reinfection and must be administered frequently, which makes it not a practical approach (Olson *et al.* 2004; O’handley & Olson 2006). In Denmark, Panacur® Vet. (Fenbendazole) is registered to use as treatment of *Giardiasis* in dogs, but not in ruminants (Veterinærmedicinsk Industriforening 2014c). Cleaning and disinfection of the surroundings and maintaining a high hygienic standard are the only fully proven way of minimizing the risk of infection (Olson *et al.* 2004; O’handley & Olson 2006).

## **5. Impact on production – economic consequences**

The effects of calf diarrhea on later milk production and economic consequences in dairy herds are still not fully understood (Olson *et al.* 2004, Svensson & Hultgren 2008). There is however strong indications, that both *Giardiasis* and *Cryptosporidiosis* in cattle have a negative impact on production and therefore negatively affect the economy of the farm (Olson *et al.* 2004; de Graaf *et al.* 1999). There is no unambiguous answer to the question, if intestinal *Cryptosporidiosis parvum* in calves, affect the productivity later in life. (O’handley & Olson 2006; Olson *et al.* 2004; de Graaf *et al.* 1999).

Growth could be inhibited (de Graaf *et al.* 1999). The costs of treating the clinical signs of *Cryptosporidiosis* as well as the extra work managing an outbreak together with the obvious loss due to mortality do inevitably have impact on farm economics. Furthermore the loss is more pronounced when other enteropathogens are involved as well (O’handley & Olson 2006; Olson *et al.* 2004; de Graaf *et al.* 1999).

Studies have shown a decrease in production in cattle infected with the gastric *C. Andersoni*. As reviewed by O’handley & Olson (2006) and Olson *et al.* (2004) a correlation between the *C. andersoni* oocyst excretion and decreased weight gain (probably caused by decreased feed efficiency) in feedlot cattle have been found. Furthermore dairy cattle excreting *C. andersoni* oocyst have been shown to produce less milk than cows not excreting oocysts (O’handley & Olson 2006; Olson *et al.* 2004).

Olson *et al.* (1995) showed that in lambs, *Giardiasis* was correlated with a decreased carcass weight compared to healthy animals. Their results also indicated that a lower weight gain can be expected. These results are consistent with other work, as reviewed by de Graaf *et al.* (1999), who also show a higher mortality rate in lambs compared to calves.

The intestinal pathological changes and chronic nature of the *Giardia* infection suggests a larger impact on production than already identified (O’handley & Olson 2006).

## **6. Dairy Calf management in Denmark**

Denmark has a long history of dairy farming. There are approximately 3241 (2012 numbers) dairy farms in Denmark with an average of 149 cows per farm (Landbrugsinfo 2013). The tendency points towards still larger dairy farms and the average number of cows is not a very

accurate estimation, since hobby farming is also included in the inventory (Landbrugsinfo 2013). Calf mortality amongst all dairy breeds in Denmark is currently 6% at birth and has been mildly decreasing over the last 10 years from 7.3% in 2004. Calf mortality within the first 30 days of life is 4.7% and has been fairly consistent over the same period of time (Landbrugsinfo 2014a). From table 3, it is apparent that the most common cause of spontaneous death amongst Holstein heifer calves in Denmark is diarrhea whereas approximately the same percentages of the euthanized calves were put down because of diarrhea and pneumonia.

**Tab. 6.1 – Holstein heifer calf mortality causes in Denmark 2013. Calf disease registrations on animals 0-12 months old based on the farmers own registrations. The missing percentages are not-calf-related diseases. (Registrations collected by Jørn Pedersen, VFL. Full registration sheet available in appendix 10)**

	<i>Calf Diarrhea</i>	<i>Calf Pneumonia</i>	<i>Other calf diseases</i>	<i>Not registered</i>
<b><i>Slaughtered</i></b>	0,0%	2,0%	8,6%	45.4%
<b><i>Dead</i></b>	23,6%	10,4%	5,8%	46,2%
<b><i>Euthanized</i></b>	10,2%	10,5%	10,8%	30,0%

The table is constructed from individual registrations made by the farmers at “Dyreregistreringen”. This means, that the risk of incorrect registration is high and the results must be viewed as an indication more than actual true numbers. The data is also questionable due to the high number of mortalities not registered with a cause.

Danish dairy calves are typically kept in single pens or huts for a couple of weeks after birth and since moved to larger pens with up to 10 animals. The calves are either kept indoors or outdoors. The pens are typically bedded with straw or sawdust (Landbrugsinfo 2014b).



A



B



C



D

**Fig. 6 – Examples of calf housing in Denmark, 2014. A: Outdoor single pens, B: Indoor single pen, C: Indoor large pen, D: Outdoor large pen.**

Soon after calving, the calf is fed colostrum. It is not legal in Denmark to use a tube feeder, but none the less it is still common practice (according to own observations). Then the calves fed milk replacer or full milk, either sellable- or waste milk (mastitis- and antibiotic milk) or a combination. The milk is fed to the calf in a bucket with or without a nipple (some form of stimulation of the suckling reflex must be provided by law) or via an automatic milk feeder. The calves must also be provided roughage feed to meet its physiological needs as well as water (Retsinformation 2014a; Retsinformation 2014b). Full milk has a higher fat and protein content than milk replacer and therefore has higher energy content (Sehested *et al.* 2003). As reviewed by Sehested *et al.* (2003) calves fed ad libitum will consume 8-9 liters of full milk a day. Chwalibog (2000)'s norm for daily energy consumption for a calf 0-4 weeks of age and approximately 40-60 kg bodyweight is 1.8 FE (1 FE=7890 KJ/1.885 Mcal), which equals 3.393 Mcal. Daily protein requirements are 290 g per day (Chwalibog 2000). The 2001 NRC



guide “Nutrient Requirements for Dairy Cattle” calculates the total energy requirement of a calf from “the metabolizable energy (ME) system” on basis of energy requirements for weight gain and maintenance (table 6.2).

**Tab. 6.2 – Metabolizable energy (ME) requirements for calves fed milk or milk replacer. MEm (ME requirement for maintenance). MEg (ME requirement for gain). ME (total ME requirement). ADG (average daily gain).**

Bw (kg)	ADG (g/day)	MEm (Mcal/day)	MEg (Mcal/day)	ME (Mcal/day)
30	200	1.28	0.41	1.69
30	400	1.28	0.94	2.22
35	200	1.44	0.43	1.87
35	400	1.44	0.99	2.43
40	200	1.59	0.45	2.04
40	400	1.59	1.04	2.63
40	600	1.59	1.69	3.28
45	200	1.74	0.47	2.21
45	400	1.74	1.08	2.82
45	600	1.74	1.76	3.50

(From Quigley 2001)

Quigley (2001) formulated the equation (simplified):

$$\left(\frac{\text{Mcal pr. day}}{5.37}\right)/0.125 = \text{kg. of whole milk per day}$$

If we want a 40 kg. calf to gain 600 grams of BW/day, its energy requirements equals 4.89 kg of whole milk per day (Quigley 2001). When using the norm for daily energy consumption by Chwalibog (2000) in the Quigley (2001) equation the result is an equivalent 4.9 kg. whole milk per day.

The Danish milk feeding recommendations differ (Landbrugsinfo 2014c; Quigley 2001), but calves fed approximately 6 liters of milk a day is commonly seen (according to own observations).

## **PART II**

### **7. Introduction**

In the present study it was attempted to clarify the significance of milk feeding strategies for development of *Cryptosporidiosis* and *Giardiasis* in dairy calves in a practical useful setting, implementable in Danish dairy herds.

The hypothesis was that intensive feeding young calves high amounts of energy and protein will:

- Improves the calves' general immunity whereby the risk of clinical disease caused by protozoa.
- Reduce the intensity of infection in the herd, so that calves will excrete fewer (oo)cysts to the environment.
- Increase growth beyond what can only be attributed to increased feeding intensity.

Thus, the specific aims of the present study were to test two different feed intensity regimes in a field setting which can easily be implemented in Danish dairy herds.

### **8. Materials and methods**

#### **8.1 Farms and study design**

The study was a case-control study performed in two Danish dairy herds situated in the northern part of Jutland. A total of 73 calves (44 from Herd A and 29 from Herd B) were enrolled in the study from 21<sup>st</sup> of January to 5<sup>th</sup> of May in the winter/spring of 2014. The selection of study population was mainly based on the farmer's commitment and ability to maintain the feeding regimes during the entire study period. Information of farm management was achieved through a questionnaire completed by the investigator, interviewing the personnel handling the calves on a daily basis (appendix 2 and 3).

From birth until four weeks of age (from 21<sup>st</sup> of January till 25<sup>th</sup> of February) 22 of the 44 calves on farm A were fed a "low diet" (LD) consisting of 2 times 3 liters discharged whole milk

(antibiotic, mastitis etc.) sometimes supplemented with milk replacer, if lacking enough full milk.

The other 22 calves were fed a “high diet” (HD) consisting of 2 times 5 liters discharged whole milk and milk replacer per day from birth until four weeks of age in the trial period (from the 26<sup>th</sup> of February till the 22<sup>nd</sup> of April). The LD calves were approximately 50/50 heifer- and bull calves and the HD calves were only bull calves as the farmer did not wish to enroll his heifer calves in the HD experiment.

In herd B, only heifer calves were included in the study. The LD group, consisting of 15 calves was fed 2 times 3 liters discharged whole milk, from birth till four weeks of age in the trial period (from 8<sup>th</sup> of February till the 14<sup>th</sup> of April). The other 14 calves were fed HD of 2 times 4 liters discharged whole milk a day for two weeks followed by 2 times 5 liters of discharged whole milk a day for another two weeks, in the trial period (from 24<sup>th</sup> of March till 5<sup>th</sup> of May). At all times, the calves had access to water, calf muesli and in Herd A, hay.

At birth the farmers noted the birth weight of the calf and at the end of the trial period the calves were weighed again.

### **8.1.1 Herd A**

Herd A, a conventional dairy herd with 1238 cows in milking and 1105 calvings within the last 12 months. The calves were removed from the cow as quickly as possible after calving (within approximately 0-6 hours), fed 4 liters of quality checked colostrum with an esophageal tube and placed individually in straw bedded out door huts with contact to the neighboring calf through a 15 cm diameter hole in the wall. After approximately two weeks the calves were moved to common huts with six calves in each (see appendix 13). The calves were fed daily (morning and evening) with whole milk sometimes added milk replacer if needed. They were offered hay, muesli and water ad libitum. The farm had a calf mortality rate of 12.1 % (among calves aged 0-180 days) within the last 12 months and with the majority of deaths occurring 8-14 days of age. The herd has a history of calf diarrhea, with symptoms starting around day 7-10 after birth (see Appendix 2 and 4). In herd A, the calves were weighed at approximately 5-10 weeks of age since scheduled weighing at 5 weeks of age failed. The milk, that the HD calves did not manage to drink in herd A, was measured and the amount noted.

### 8.1.2 Herd B

Herd B, a conventional dairy herd with 546 cows in milking and 618 calvings within the last 12 months. The calves were moved from the cow as quickly as possible typically within two hours into straw bedded indoor huts and fed 4 liters of colostrum with no use esophageal tube. After approximately two weeks the calves were moved to common huts with five calves in each (see appendix 3 and 14). The calves were fed whole milk twice a day (morning and evening). The calves were offered ad libitum calf muesli and water – no hay. The farm had a calf mortality rate at 10.2 % (of calves up to 180 days of age) within the last 12 months and with the majority of deaths occurring at 8-14 days of age. The herd has a history of calf diarrhea typically occurring, when the calf is approximately 3 days old (see appendix 3 and 5). In Herd B, the calves were weighed at 5 weeks of age, as was intended for all calves in the study, but the measuring of left over milk after every feeding of the “HD” calves failed to be followed through.

**Tab. 8.1 – The final Study design of the field study performed in Herd A and Herd B to clarify the significance of milk feeding strategies for development of *Cryptosporidiosis* and *Giardiasis* in dairy calves in a practical useful setting, implementable in Danish dairy herds.**

	<b>Herd A</b>	<b>Herd B</b>
<b>Calves enrolled</b>	<b>Case:</b> 22 bull calves	<b>Case:</b> 14 heifer calves
	<b>Control:</b> 22 heifer and bull calves	<b>Control:</b> 15 heifer calves
<b>Feeding</b>	<b>Case:</b> 2×5 L full milk per day (added milk replacer if in lack of full milk)	<b>Case:</b> week 1+2: 2×4 L of full milk week 3+4: 2×5 L of full milk
	<b>Control:</b> 2×3 L full milk per day (added milk replacer if in lack of full milk)	<b>Control:</b> 2×3 L of full milk per day
<b>Samples</b>	Fecal samples once a week for 4 weeks	Fecal samples once a week for 4 weeks
<b>Milk measurement</b>	<b>Case:</b> left over milk measured after every feeding	No milk measured
<b>Calf weight</b>	Final weight measured between 5 and 10 weeks old	Final weights measured at 5 weeks old

## **8.2 Fecal sampling**

A total of 292 fecal samples were collected from the calves during the study period.

Once a week fecal samples were collected from the calves either rectally or when spontaneous defecation was observed. The samples were collected in rectal gloves or in containers with lids and transported in cooler boxes by car to the National Veterinary Institute (DTU), where they were kept at 4°C until analyzed.

The consistency of the collected fecal samples was scored in the laboratory on a five point scale:

1: normal with structure; 2: soft; 3: liquid; 4: watery; 5: watery with blood and/or mucus.

The samples were then purified and the (oo)cysts quantified and differentiated using a semi quantitative immunofluorescence analysis.

## **8.3 Health scoring**

At each herd visit i.e. once weekly, the temperature was measured and the general health of the calves was recorded based on a 3-point scale:

1: The calf already stands or rises when approached.

2: the calf rises when provoked.

3: the calf is unwilling or unable to stand.

Respiratory symptoms were recorded qualitatively.

Respiratory symptoms: If the calf was coughing, heavy breathing due to airway infection or if excessive or purulent nasal discharge was observed.

No respiratory symptoms: If none of the above mentioned signs were observed.

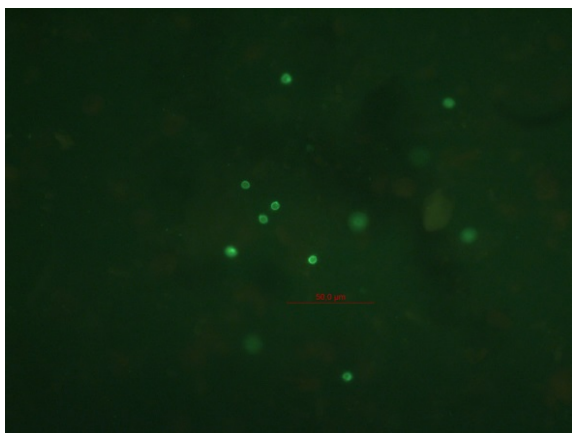
## **8.4 Quantitative immunofluorescens analysis**

The method for purification and quantification of *Giardia* cysts and *Cryptosporidium* oocysts was carried out as described by Maddox-Hyttel *et al.* (2006).

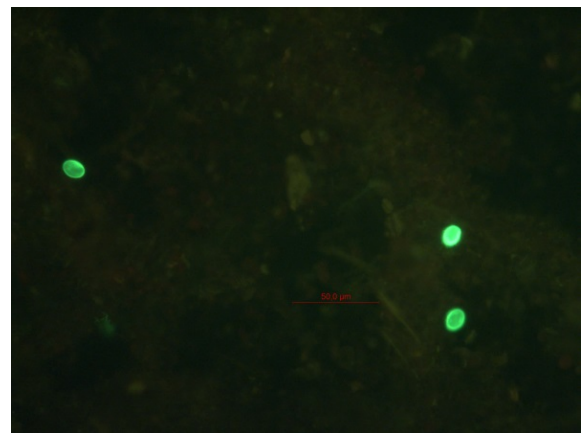
1 g. of feces was suspended with MilliQ water and Tween20 and filtered through gauze in a syringe. The sample was centrifuged and the supernatant transferred into a clean tube, which was again centrifuged and washed three times with MQ water. A volume of 2 mL was reached

and a 10 microL subsample was placed in a well on a diagnostic slide, air-dried, fixed with acetone and stained with 25 microL of anti-*Giardia*/*Cryptosporidium* fluorescein isothiocyanate (FITC)-labelled antibody mix (Crypto/*Giardia*-CEL IF test, CellLabs, Australia).

The samples were examined using an epifluorescence microscope and the (oo)cyst were identified according to morphology, quantified and converted into interpretable oocyst per gram (OPG) and cyst per gram (CPG) values, that were used in later statistical analysis (see appendix 1 and 9).



**Fig. 8.1** - Anti-*Giardia*/*Cryptosporidium* fluorescein stained *Cryptosporidium* oocyst found in calf feces April 2014 at 200x magnification.



**Fig. 8.2** - Anti-*Giardia*/*Cryptosporidium* fluorescein stained *Giardia* cysts found in calf feces April 2014 at 400x magnification.

## 8.5 Questionnaire

A questionnaire was designed to collect basic information on the farm management. Questions on calving management, feeding, cleaning and moving of animals were included in the questionnaire (appendix 2 and 3).

The questionnaires were completed on farm during on one of the weekly visits. An interview was set up with the person in charge of the calves and an objective assessment of the calf hut hygiene and climate was performed. On Farm A, this was completed by the author, on Farm B an experienced agronomist.

## 8.6 Statistical analysis

Three hypotheses were constructed:

H<sub>0</sub>1: The milk feeding strategy does not affect growth in the calves

H<sub>0</sub>2: The milk feeding strategy does not affect (oo)cyst excretion levels in the calves

H<sub>0</sub>3: There is no correlation between the feed intensity and the occurrence of diarrhea

First, the prevalence of animals with detectable numbers of (oo)cysts in their feces were calculated.

To smoothen and to properly manage the data, the (oo)cyst counts were log(10)-transformed.

A copy of the complete database can be found in appendix 7 and 8.

## 9. Results

### 9.1 Descriptive results

All calves included in the study did at some point excrete *Cryptosporidium* oocysts, which makes the overall prevalence of *Cryptosporidiosis* 100% in both herds. For *Giardia* the prevalence was 25% in herd A and 65,5% in herd B. The highest *Cryptosporidium* excretion levels were seen in their second week of life for majority of the calves. In herd A, 81,8% of the calves shedded the highest number of oocysts in week two. In herd B, 79,3% of calves shedded the higher number of *Cryptosporidium* oocysts in week two. In herd A, 45,5% of calves had their peak *Giardia* cyst excretion levels in week four and in herd B, 40% in week two and 33% in week four (appendix 7 and 8).

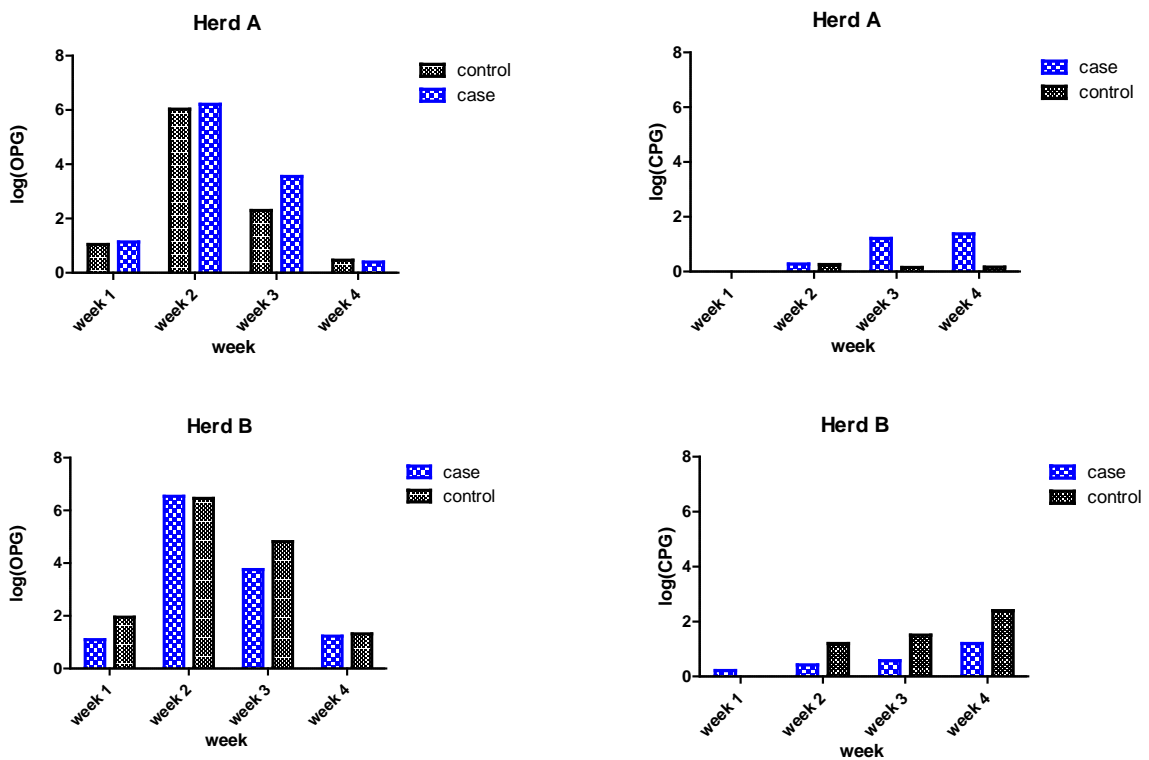


Fig. 9.1 – Mean log(10) *Cryptosporidium* (OPG) and *Giardia* (CPG) (oo)cyst excretion per week, in a 4 week period in calves from two Danish dairy herds. The case group was fed 2×5 liters of whole milk and the control group 2×3 liters of whole milk a day.

In both Herd A and B the mean oocyst excretion levels didn't differ much between the control and case groups in all four weeks. In Herd B, control group *Giardia* cyst excretion levels were markedly higher in weeks two, three and four. The opposite was the case for Herd A, in which case group animals were excreting several times the amount of cysts compared to control animals in weeks three and four.

Since the measuring of left over milk in herd B failed, the only exact milk intake (of the "HD" calves) recorded was from herd A.

In herd A we found, that all milk had been consumed from days 0-4. From days 5-17 many of the calves did not drink the entire 10 liters. The lowest mean milk intake was seen at day 11 (7463.8 grams) but did vary in quantity between the days. After day 17 most of the calves were grouped in common housing and the individual milk intake could no longer be measured. Around this time it also seemed, that most of the calves were again drinking the full 10 liters of whole milk (appendix 6).



When comparing birth weights with the milk consumption of the calves, there was a slight tendency towards the heavy calves drinking more milk than the smaller ones (Appendix 7). Furthermore when comparing oocyst excretion levels and milk intake, the numbers strongly indicate, that calves having Cryptosporidiosis failed to drink the amount of milk offered (appendix 6 and 7).

## 9.2 Statistical results

### 9.2.1 $H_01$ : The milk feeding strategy does not affect growth in the calves

The hypothesis,  $H_01$ : “The milk feeding strategy does not affect growth in the calves” was tested using a two-tailed unpaired T-test at a 5% level of significance.

#### Herd B:

With a P value  $> 0.05$  ( $p = 0.4035$ ) there was no significant difference between the means of the two groups. Fig. 9.2 illustrates the lacking differences in median values and mid 50<sup>th</sup> percentiles between the two groups. The variation in weight gain is the only parameter significantly different between the two groups – larger in the control group. In summary the  $H_0$  hypothesis could not be rejected (see Appendix 11).

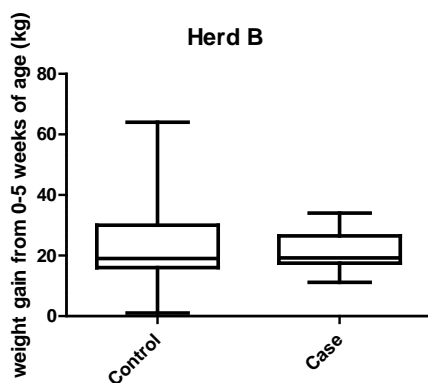


Fig. 9.2 – Box-and-Whisker Plot of the weight gain of the case- and control calves from 0-5 weeks of age in kg. Whiskers are marking the maximum and minimum of observations. The bottom and top edges of the bars are located at the sample 25<sup>th</sup> and 75<sup>th</sup> percentiles and the centered horizontal lines show the median.

Herd A:

By mistake the calves in the Herd A “HD” group weren’t weighed at 5 weeks of age. Instead alternative weights, measured at different ages were used. Based on these weights a daily weight gain was calculated by dividing the final weight by age in days (appendix 7).

This is not the optimal way of comparing weight gain between the groups.

The actual daily weight gain cannot be correctly estimated from a mean over a long period of time, since the daily weight gain must be assumed to vary substantially at during different stages of the calf’s life.

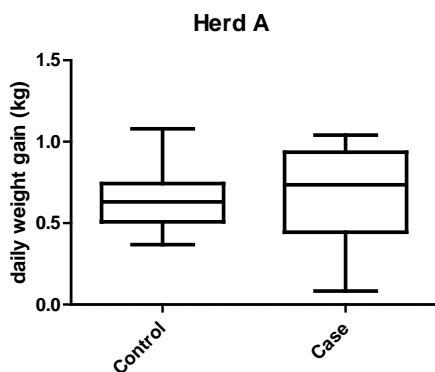
To test if the calves weighed early and the calves weighed later within the “HD” group had significantly different means and therefore could not be tested against the “LD” group, another two-tailed unpaired t-test was performed.

The case group was divided into two. Calves weighed at 48-74 days of age were tested against the calves weighed at 37-47 days of age.

The result was a P value of 0.5771. There was no significant difference in means between the two groups (Appendix 11).

On this background, the  $H_0$  hypothesis for Herd A was tested the same way as done Herd B.

With a P value of 0.5591, the t-test showed no significant differences in mean daily weight gain between the two groups (Appendix 11). Therefore we cannot reject the  $H_0$  hypothesis, claiming the milk feeding strategy does not affect growth in the calves.



**Fig. 9.3 – Box-and-Whisker Plot of the daily weight gain of the case- and control calves in kg. Whiskers are marking the maximum and minimum of observations. The bottom and top edges of the bars are located at the sample 25<sup>th</sup> and 75<sup>th</sup> percentiles and the centered horizontal lines show the median.**

It was not possible to test the hypothesis on both herds collectively, because the weight gain was measured differently between the herds.

### ***9.2.2 H<sub>02</sub>: The milk feeding strategy does not affect (oo)cyst excretion levels in the calves***

The hypothesis, H<sub>02</sub>: “The milk feeding strategy does not affect (oo)cyst excretion levels in the calves” was tested using a linear regression model at a 5% level of significance, using the program “R” statistics. The final model was corrected for the effect of “herd” on both feed groups.

Before analyzing the data, we removed calves with missing fecal samples in one or more weeks from the data set. This was done to ensure more accurate statistical calculations.

To compare (oo)cyst excretion between the groups, the total “area under curve” for every calf was calculated under the assumption, that the calves in “week 0” and “week 5” had an (oo)cyst excretion equal zero. The “area under curve” in this example, was equal to the total sum of log(10) of OPG/CPG excretion for all four weeks.

#### OPG:

First, we tested for interaction between parameters “feed” and “herd”, to establish whether or not we could use the collective data in further testing. This was done using a linear regression model. With a P value of 0.1215, there was no interaction between the two parameters, and we continued.

The results showed a significant ( $p=0.0068$ ) difference in total OPG excretion between the two herds, which would be expected due to difference in infection pressure between herds. There was no significant effect of the feeding regime on oocyst excretion ( $p=0.238$ ). Hence, the H<sub>0</sub> hypothesis could not be rejected (Appendix 12).

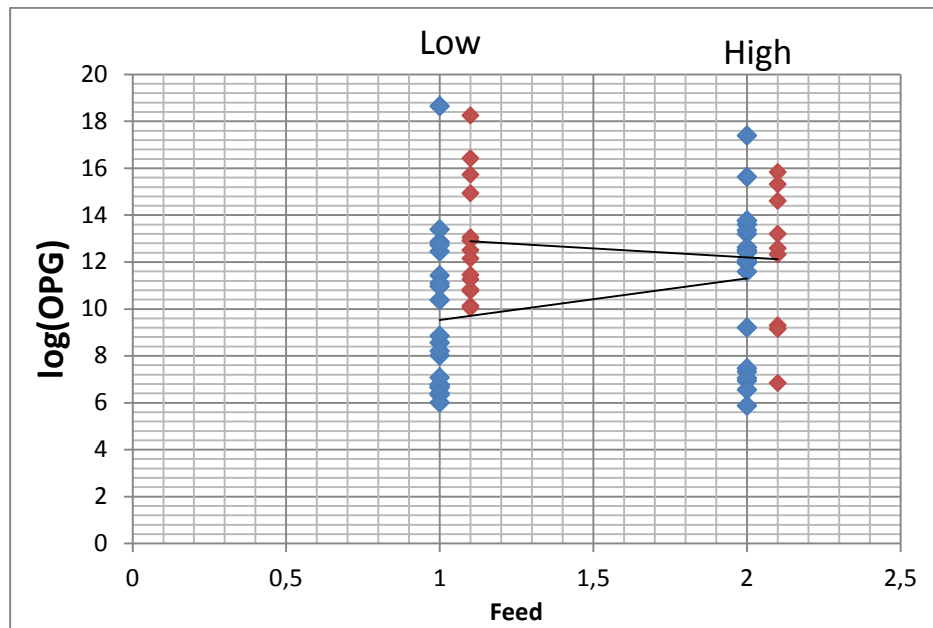


Fig. 9.4 – Linear regression plot of log. transformed oocyst excretion as a function of feed (low or high) in two Danish Dairy herds. The red dots mark Herd A and the blue dots mark Herd B. There are no significant effect of feed on log(OPG).

CPG:

As was done with the OPG counts, the interaction between the parameters “feed” and “herd” was tested. With a P value of 0.04772 a significant interaction between the parameters was proven. This meant that no significant effect of the feeding strategy on cyst excretion would be found, if doing the final regression analysis.

Therefore the  $H_0$  hypothesis could not be rejected.

When viewing Fig. 9.1 the differences in CPG levels between the groups are visualized. It is obvious that in Herd A, the “HD” calves had the highest excretion levels whereas in Herd B, the “LD” calves maintained the highest mean excretion of cysts over the weeks (Appendix 12).

**9.2.3  $H_{03}$ : There is no correlation between the feed intensity and the occurrence of diarrhea**

The hypothesis “There is no correlation between the feed intensity and the occurrence of diarrhea” was also tested using a linear regression model at a 5% level of significance.

Again the calves with missing fecal samples in one or more weeks were removed from the data set to ensure more accurate statistic calculations.

Fecal scores were grouped, so that FS 1-3=0, FS 4=1, and FS 5=2.

First, the effect of the feeding regime on the occurrence of diarrhea was tested.

There was no interaction between “herd” and “feed” ( $P = 0.5618$ ) and no significant effect ( $P = 0.0915$ ) of “feed” on the occurrence of diarrhea. Despite the statistically insignificant result, the relatively low  $P$  value indicates a tendency towards effect of the feeding strategy on the feces consistency (appendix 15).

Because it is well known that *Cryptosporidium* oocyst excretion is correlated to diarrhea in young calves, we wanted to correct the model for the effect of oocyst excretion on the occurrence of diarrhea.

To be able to do this, the OPG counts were transformed.

A mean total OPG value for all calves was calculated. Any calf with a total OPG count above this number was put in the “high excretion group” and calves with total OPG counts lower than the mean, was put in the “low excretion group”.

Again, a linear regression analysis was performed and the interaction between the factors (OPG and feed) was tested. With a  $P$  value of 0.5544, there was no interaction and we continued the analysis.

There was no effect of (high/low) OPG counts on the occurrence of diarrhea ( $P = 0.2752$ ) and no effect of the feeding on the occurrence of diarrhea when corrected for the effect of OPG excretion levels ( $P = 0.0761$ ). Which is why, we cannot reject the  $H_0$  hypothesis.

Yet, again we see a tendency towards an effect of feed on the feces consistency (appendix 15).

## 10. Discussion

In the present field study, the aim was to investigate the effect of a milk feeding strategy, practical applicable in the dairy farm, on growth, (oo)cysts excretion levels and the occurrence of diarrhea in dairy calves.

To do this, the study was done in a farm setting with all the disadvantages of a field study.

We were challenged on keeping the study design as planned.

The final weights of the calves were planned to be measured at the end of the four week trial period, but were measured over a longer time span and differently between farms.

Weighing of the left over milk at every feeding (“HD” calves) was only executed in herd A which meant, that we did not know the exact milk intake of herd B.

The sex ratio amongst the calves was not equally distributed, as first attempted and the case and control groups, that were originally set to be tested over the exact same time-period, were enrolled at different times.

All of these changes weakened the final study design and make the results less valid.

We found no statistical significant effect of the milk feeding strategy on weight gain, (oo)cyst excretion or occurrence of diarrhea in the dairy calves.

The lack of significant differences in outcomes between the feeding regimes could be explained by that fact, that both feed groups were fed relatively high amounts of milk (6 vs. 8/10 L. a day). When looking at the exact milk intake of the herd A “HD” calves, we can see that the difference in average milk consumption between the “HD” and “LD” calves was relatively small.

There was a strong tendency towards high oocyst-excreting calves drinking less milk than offered. This corresponds well with Cryptosporidiosis causing depression and lack of appetite in young calves (Langkjær *et al.* 2007, Thompson *et al.* 2008; O’Handley & Olson 2006). The healthy “HD” calves consumed close to what could be expected from calves fed ad libitum, which is 8-9 liters of full milk per day (Sehested *et al.* 2003) and were getting their daily energy requirements covered (Quigley 2001; Chwalibog 2000).

Furthermore, the “LD” calves, consuming 6 liters of full milk per day, were most likely getting their daily energy needs covered as well (Quigley 2001; Chwalibog 2000). This was not the case in the study performed by Olivett *et al.* (2012), in which the “LD” calves only received approximately 2.2 Mcal/day above maintenance requirements.

Olivett *et al.* performed a controlled trial study on 20 Holstein bull calves. The calves were fed milk replacer on a high (0.30 Mcal/kg of mean body weight after day 7) or a conventional (0.13 Mcal/kg of mean body weight) plane and inoculated with *C. parvum* oocysts. Faecal scores, health scores, oocyst counts, weight gain, dry matter intake and haematological variables were measured over a period of 21 days to evaluate the effect of the nutritional plane on performance and health after the *C. parvum* challenge. Once in the 21 days the calves were tested for co-infections with *salmonella* spp. and rotavirus. Olivett *et al.* showed that the calves receiving a higher nutritional plane had a faster resolution of diarrhea, grew faster, maintained hydration and had a greater conversion of the feed than the control calves.

We did not calculate the feed efficiency (average daily gain-to-dry matter intake ratio) as was done by Olivett *et al.* (2012).

Calculating the feed efficiency would have been difficult, since the calves were fed whole milk (with varying energy content) instead of milk replacer. In future studies, this parameter could provide important information as would testing for co-infections with other enteropathogens.

In the present study, we found no statistically significant effect of feed intensity on weight gain. In herd A, a possible reason for this is that our calculated daily weight gain is biased.

Estimating a daily weight gain from a weighing, as late as five weeks after the end of the study period, is not correct, which is why the results on herd A must be seen as invalid.

In herd B, the insignificant results can be ascribed to the flaws of the study design.

As mentioned earlier, little work on the effect of different feeding regimes on calves with a pathogen challenge has been done. Apart from the study by Olivett *et al.* (2012) one experiment, on calves challenged with coronavirus and fed milk replacer at a high versus a low nutritional plane showed a greater body weight gain in calves fed higher amounts of milk replacer than the calves fed less milk replacer (Quigley *et al.* 2006).

Studies on healthy calves also show positive effects of high intensity feeding and high protein intake. Raeth-knight *et al.* (2009) showed a correlation between intensive milk replacer feeding at a high feeding rate and increased bodyweight and hip height at the time around weaning (day 42). Yet, the differences were only maintained until 112 days post weaning. There were no differences in lactation performance and age at first calving between groups and all feeding

regimes resulted in healthy calves. Bartlett *et al.* (2006) had similar results when calves, fed a daily amount of 1.75% of their bodyweight in milk compared to 1.25%, had a greater final body weight, when slaughtered. They also showed that the final body weight increased linearly with the amount of crude protein in the milk replacer. The calves fed high crude protein also had a greater feed conversion. Similar results have also been published by Diaz *et al.* (2001).

Besides feeding intensity, there are multiple other factors influencing body weight gain in calves. Windeyer *et al.* (2014) found daily average weight gain to be influenced by: Month of birth, failure of transfer of passive immunity (a factor not included in the present study), if the calf was a twin and treatment of neonatal calf diarrhea or other diseases. The later risk factor indicating, that if a high nutritional plane is correlated with a lowered risk of clinical Cryptosporidiosis/Giardiasis, then it would indirectly affect the daily weight gain as well.

Another interesting study proved an increased growth of mammary parenchyma in heifer calves fed high levels of protein and energy (milk replacer and grain mix) from 2 to 8 weeks of age, which indicates a higher production of milk later in life (Brown *et al.* 2005).

Our study showed no significant effect of the milk feeding strategy on (oo)cyst excretion levels in the calves.

The excretion of *Cryptosporidium* and *Giardia* (oo)cysts vary significantly during the course of infection (Cacciò *et al.* 2005).

When only collecting fecal samples once a week, the (oo)cyst excretion levels measured are merely a snapshot of the current state of infection. The peak excretion can easily be missed and the data present might not be representative of the course or severity of infection in the calf.

The present study was performed in only two dairy herds. The small sample size weakens the data, because herd differences and confounding biases more easily affect the results. Later work could advantageously include daily fecal samples and more herds for improved statistical power.

The calves in this study were enrolled in the months from February to May, with the “HD” calves born and enrolled late in the sampling period – during the spring/summer months. The risk of seasonal variations as described by Trotz-Williams *et al.* (2007) cannot be rejected. Trotz-Williams *et al.* (2007) showed that calves born during the summer months in Ontario, Canada were more likely to shed *C. parvum* oocysts than calves born during winter. No seasonal



differences were found in Denmark by Maddox-Hyttel *et al.* (2006) but Williams *et al.* (2007) suggest the differences to be caused by better survival of oocysts in the bedding and thereby a larger challenge for the calves. Oocysts are sensible to drying out and survive longer in a cool damp environment screened from sunlight (King & Monis 2006). For these reasons as well as an expected higher pathogen challenge in the winter months, seasonal difference can be suspected.

Both of the dairy farms in this study fed waste milk or a waste milk/milk replacer combination to their calves. Using waste milk to feed calves is a very common practice in Danish calf rearing. The effect of low quality milk on calf health has been studied by others. As reviewed by Kesler (1981) many workers found no difference in incidences of health disorders and performance in calves fed mastitic milk compared to those fed control milks. Yet, Trotz-Williams *et al.* (2007) found that calves fed milk replacers or waste milk were twice as likely to shed *Cryptosporidium parvum* oocysts, than calves fed saleable milk.

Similar results were published by Losinger & Heinrichs (1998), who stated that feeding calves mastitic or antibiotic milk were associated with high death levels amongst pre-weaned dairy heifers.

The present study showed no statistically significant effect of the milk feeding strategy on the occurrence of diarrhea in the calves.

Olivett *et al.* (2012) showed a faster resolution of diarrhea in calves fed the “high plane nutrition diet”. Because of our experimental setup of one feces sample per week, we weren’t able to comment on the resolution of diarrhea in the calves.

To include this information will also be advantageous in further studies because of the fact, that even though positive effects of a high nutritional plane on calf performance have been suggested (Raeth-knight *et al.* 2009; Bartlett *et al.* 2006; Brown *et al.* 2005; Diaz *et al.* 2001), scientific work also show a higher incidence of diarrhea in calves on a higher nutritional plane (Quigley *et al.* 2006).

A Swedish study also states, that minimizing the occurrence of calf diarrhea is of great importance to the later performance of the calves. Svensson and Hultgren (2008) proved that calf diarrhea is associated with lowered milk yield in first lactation and also that diarrhea was correlated with later respiratory disease, which was again correlated to a lower milk yield in the

first lactation. A mean milk reduction of 344 kg was seen in the first lactation of heifers who have had health disorders as calves compared to the ones who had not (Svensson and Hultgren 2008).

In the present study, we weren't able to show a statistically significant correlation between diarrheic feces and high/low *Cryptosporidium* oocyst excretion.

A study from 2006 on Danish dairy cattle showed a correlation between *Cryptosporidium* oocysts excretion and feces consistency in young calves (Maddox-Hyttel 2006). These results are consistent with much other work (Olson *et al.* 2004; de Graaf *et al.* 1999; Silverlås *et al.* 2010b).

When commenting on the correlation between diarrhea and the feeding strategy it must also be kept in mind, that other factors could have affected the result.

In a recent American/Canadian study, risk factors of neonatal calf diarrhea were identified as: weight at enrollment in the experiment (0-7 days of age), other diseases before 2 weeks of age and an interaction between season of birth and herd-level incidence risk of neonatal calf diarrhea (Windeyer *et al.* 2014).

Furthermore, Trotz-Williams *et al.* (2007) proved the odds of diarrhea to be strongly correlated with the time the calf spent with the dam after birth. If the calf stayed more than one hour with the dam, the odds of diarrhea were 39 times higher than calves removed from the dam within an hour of birth – but no significance between oocyst shedding and time with the dam was proven.

These management risk factors on calf diarrhea as well as on *Cryptosporidium* shedding (Silverlås *et al.* 2009a) might also have affected the results of our study, since only two herds were included.

## 11. Conclusion

In the present field study, the aim was to investigate the effect of a milk feeding strategy, practical applicable in the dairy farm, on growth, (oo)cysts excretion levels and the occurrence of diarrhea in dairy calves.

To comply with the aim of making the feeding regime practical applicable in the dairy herd, the study was designed in a way that could have limited the chances of statistically significant results. Thus, the difference between the amount of milk fed in the “low” and the “high” milk feeding regime was not large enough.

Furthermore unintentional changes in the study design also limited the chances of statistically significant results and even made some results inconclusive.

In herd A, the test of the effect of the milk feeding strategy on weight-gain in the dairy calves was inconclusive, since the final body-weights were biased.

There was no statistically significant effect of the milk feeding strategy on weight-gain of the dairy calves in herd B.

There was no statistically significant effect of the milk feeding strategy on (oo)cyst excretion levels and there was no statistically significant effect of the feeding strategy on the occurrence of diarrhea amongst the calves.

Yet, the relatively low p-value indicated a tendency towards an effect of milk feeding on the occurrence of diarrhea.

Secondly, the present study was not able to show a correlation between *Cryptosporidium* oocyst excretion and diarrhea in young calves. This could also be ascribed to flaws of the study design.

In the light of the possible improvements of our study design, we find it highly relevant to further investigate the impact of milk feeding strategies on parasitic load, health and performance in dairy calves, with the aim to implement beneficial changes to commercial calf rearing.

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## 12. Appendices

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## **Appendix 1 - Quantitative immunofluorescens analysis protocol**

### **Metode til oprensning af Giardia og Cryptosporidium fra fæces**

1. I 10 ml sprøjtehylstret proppes først en oprullet bindetråd (ca. 9 cm oprullet til ringform der passer ind i 10 ml sprøjte), et stykke 5x5 cm gaze, og til sidst endnu en ring af bindetråd, og stemplet sættes i. De 'færdige' sprøjter placeres i autoklave pose og sendes til autoklaving indtil filtrering foretages.
2. Flotationsvæske (mættet salt-sukker) blandes 1:1 med MQ vand Vf: 1,13
3. Ét gram fæces afvejes i et 15 ml rør ("rør 1"). Ved brug af mindre mængde noteres dette i skema.
4. 3,5 ml 0,01% Tween 20 i MQ vand tilsættes.
5. Vortex indtil 'homogent'. Hvis der er hårde klumper kan disse opbrydes vha. (autoklaveret) træpind.
6. Fjern stemplet fra sprøjtehylstret fra pkt.1, hold bundåbningen oven over et 15 ml rør ("rør 2") og hæld fæces-suspensionen i hylstret .
7. Pres væsken igennem med stemplet. Pres stemplet hårdt ned nogle gange for at få så meget væske ud som muligt. Fjern stemplet, og sæt til side, så forurening undgås. Hvis væsken umuligt kan presses igennem kan anvendes kanyle (N.B.: ny kanyle for hver prøve) til at prikke hul i gazen
8. Tilsæt endnu 3,5 ml 0,01% Tween 20 i MQ vand til "rør 1", og vortex indtil røret er så rent som muligt og hæld over i sprøjtehylstret.
9. Filtrér. Igen presses stemplet hårdt i bund 2-3 gange for at trykke al væsken ud.  
Sprøjtehylstret smides ud.
10. Ryst røret ("rør 2") for at blande.
11. Ca. 3,5 ml flotationsvæske (pkt. 2) overføres via en pasteurpipette placeret i rør 2 ned i bunden af røret (under væsken).

12. Centrifuger 53 x g i 3 min. 3 bremse (program 1).
13. Overfør alt supernatant til 15 ml rør ("rør 3") vha. 5 ml pipette. Fyld røret helt op med MQ vand.
14. Centrifuger 1540 x g i 10 min. Mellem bremse (5) (program 2).
15. Forsigtigt suges supernatanten fra (vakuumsug) ned til 2 ml.
16. Vortex. Såfremt vortex ikke er tilstrækkeligt til at opslemme bundfaldet, skal bundfaldet opslemmes i væsken vha. en pasteurpipette. N.B.: Ny pasteurpipette for hver prøve
17. Tilsæt MQ vand til 15 ml, og ryst røret.
18. Gentag pkt. 14-17 én gang; derefter punkt 14-16 hvorefter prøven overføres til objektglas (2 vaske i alt. Man ender med pkt. 18 og 2 ml væske i røret)
19. Prøver pipetteres på objektglas (jf. pkt. 20-23) og resten sættes på køl indtil resultat foreligger. Positive prøver skal nedfryses (-20°C i 2ml Eppendorf-rør)(undtagen svineprøver med mange crypto - de skal deles så halvdelen fryses og resten sættes på køl) og nogle af dem udvælges (tilfældigt) til brug for DNA analyse.

### **Tælling:**

20. Anbring 50 µl MQ vand på en objektglasbrønd (Afhængig af mængde i pkt.21)
21. Udtag 10 µl (0,5 %) af produktet fra pkt. 20 (umiddelbart efter gentagen grundig vortex), tilsæt til vandet i brønden, og bland. NB: ved mindre mængde fæces anvendes større mængde oprenset materiale til aflæsning, jf. tabel. Sørg for at prøven dækker hele brønden. Tør ved stuetemp. eller i varmeskab (37°C) indtil præparatet er tørt (ca. 1 time i varmeskab).
22. Fiksér ved at nedsænke objektglasset i acetone i 5 min. (Acetonen kan genbruges nogle gange).
23. Lad objektglasset lufttørre.
24. Farv med FITC iflg. brugsanvisningen, dvs.: farvestof og mounting buffer tages ud af køleskab for at opnå stuetemperatur. 25 µl farvestof placeres på hver brønd. Igen sørges der

for at hele brønden dækkes.

25. Objektglassene placeres i fugtkammer, og fugtkammeret sættes i varmeskab (37°C) i 45 minutter. (Der må gerne farves længere end den halve time angivet på kittet. )

26. Sug farvestoffet fra brønden vha. en pipette ved brøndkanten, idet objektglasset holdes skråt.

Tilsæt 100 µl PBS, og sug det forsigtigt fra. Gentag PBS vask, og tilsæt derefter en dråbe (ca. 10 µl) mounting buffer fra kittet. (Efter farvestoffet og PBS er suget fra brøndene, må de ikke tørre helt ud inden tilsætning af mounting buffer).

27. Læg dækglasset på - og undgå luftbobler! Opbevar i mørke indtil aflæsning. Hvis det forventes, at der går et par timer indtil aflæsning, kan det være en god idé at forsegle med neglelak. Prøven kan opbevares i op til 24 timer i mørke ved 2-8°C.

## Appendix 2 – Questionnaire Herd A

### 1.) Kælvning

- Hvor fødes kalvene?

-I kælvningsboks med dybstrøelse. kvier og køer for sig. Disse sættes der over ca. 14 dage før forventet kælvning

- Type af underlag i kælvningsboks? dybstrøelse
- Hvor mange køer i kælvningsboksen? forskelligt, men generelt høj belægning
- Hvor tit muges/fjernes efterbyrd i kælvningsboksen ? hver 14. dag. om sommeren oftere.

### 2.) Fodringsrutiner herunder håndtering af mælk og råmælk

- Hvornår gives råmælk?

inden 3 timer men om natten 4-5 timer. 4 L råmælk gives med sonde, hvorfra der går et døgn inden næste fodring. Med mindre det er koldt og kalven er lille da gives mælk inden et døgn.

- Får kalven lov at die hos koen/hvornår bliver den taget fra?
- Måles råmælkskvaliteten – hvordan? colostrummeter.
- Findes der en råmælksbank? ja
- Fodres med mælk/mælkeerstatning/syrnet mælk. mælk fra nykælvere og lidt mælkeerstatning om aftenen.
- Hvilken temperatur er mælken ved udfodring (og hvordan måles temperaturen)? 40,5 grader om vinteren et par grader varmere.
- Hvor lang tid tager en udfodring? Og bruges mælketaxi? 10-15 minutter i en isoleret mælketaxi (billede)
- Hvilken kalveblanding anvendes (I skal tage foto af indholdsfortegnelsen)?
- Hvornår begynder tildelingen af kalveblanding (alder i uger)? med det samme.
- Har kalvene adgang til hø af en god kvalitet? også fra dag 1 det bedste hø gives til de små kalve. elektrolytvand gives til kalvene i enkeltboksene når der er drukket op

- Er det den samme person som altid passer kalvene? om formiddagen Camilla og om eftermiddagen det personale, der malker.

-Hvis ikke, hvor mange forskellige personer deltager i pasning af kalvene?

### 3.) Rengøring af skåle og trug

- Hvilken type skåle/flasker anvendes til udfodring af mælk? Metalskåle
- Hvornår rengøres skålene? 3 gange ugentligt i maskinen. De store vandtrug rengøres med vand, når dyrene får vand hvis dette er nødvendigt, dvs hvis det er for meget halm eller gødning i truget,
- Hvor tit?
- Hvordan rengøres de?

### 4.) Rengøring af hytter

- Hvor ofte? Når dyrene flyttes, dvs. 2-3-uger for enkelthytterne og 3 måneder ved de ældre kalve. Og varmekassen et par gange om ugen.
- Hvordan? Højtryksrensens og vaskes med Vircones
- Hvor længe tørrer hytterne bagefter? Oftest kun et par timer, men når der er tid (belægningsgraden er lavere) en dag.
- Hvor tit strøes der i boksene/muges? Der strøes hver dag i alle hytter (manuelt, så halmen bliver fordelt) og muges kun når dyrene flyttes. Der foretages ikke støvlevask mellem boksene og der er ingen "aldersrækkefølge" når der strøes.
- Hvilken type strøelse anvendes? Halm.

### 5) Opstaldningsforhold

- Hvilken type underlag: strøelse (type?), spalter, fast gulv.... halm
- Træk? (egen observation) ingen træk i fælleshytterne. En anelse i enkelthytterne
- Fugt? (egen observation) ingen fugt
- Kulde/varme? (egen observation) Varmt i fælleshytterne, når vejret er varmt.

## 6.) Flytning af dyr

- Hvornår flyttes kalvene i fællesbokse? 1-3 uger afhængigt af dyreflowet
- Hvor mange kalve er der i fællesboksene? 5-6 dyr.
- Er der en løbende udskiftning af kalvene i fællesboksene (de ældste flyttes når der kommer nye til)? Nej. (dog er det observeret at enkelt dyr har skiftet boks, dette er en fejl når dyrene er blevet håndteret udenfor boksen og er sat ”forkert” ind)
- Hvor længe går kalvene typisk i fællesboksene? ??
- Er vognen rengjort inden og mellem dyrene? Gøres det i bestemt rækkefølge? Ingen bestemt rækkefølge og vognen rengøres efter behov.
- Flyttes dyrene rundt mellem boksene? (enkelt og fælles) nej, ikke bevidst
- Hvordan bevæger personalet (kalvepasser) sig mellem boksene (rengøring støvler)? Ingen systematik eller rengøring
- Hvad er proceduren for evt. isolation eller aflivning af syge dyr? Ingen dyr isoleres ved sygdom og aflives kun hvis elektrolytter og medicinering ikke kan få dem på ret køl.

## 7.) Sygdom

- Har besætningen en rådgivningsaftale? (tjek op) dyrlægen kommer en gang om ugen.
- Hvornår optræder der oftest diarré hos kalvene? 7-10 dages alderen
- Er der andre sygdomsproblemer i kalveholdet end diarré – e.g. lungebetændelse? respirationslidelser
- Vaccineres med Rotavec Corona? nej
- Hvordan behandles diarré? Borgal vet (Sulfa TMP) og elektrolytter plus evt. Metacam hvis smertepåvirket.
- Hvordan behandles lungebetændelse? Resflor Gold. (floramfenicol og Flunixin meglumine) og evt NSAID
- Hvordan behandles navlebetændelse? Borgal vet. Og smertestillende
- Kalvedødelighed i besætningen (trækkes selv)
- Bruges Baycox? Nej ikke i forsøgsperioden

## Appendix 3 – Questionnaire Herd B

### 1) Kælvning

- Hvor fødes kalvene?

-I kælvningsboks med dybstrøelse.

- Type af underlag i kælvningsboks? dybstrøelse
- Hvor mange køer i kælvningsboksen? Ca. 4
- Hvor tit muges/fjernes efterbyrd i kælvningsboksen? ca. hver 8. uge

### 2.) Fodringsrutiner herunder håndtering af mælk og råmælk

- Hvornår gives råmælk?

0-2 timer efter kælvning

- Får kalven lov at die hos koen/hvornår bliver den taget fra? Den bliver taget fra hurtigst muligt
- Måles råmælkskvaliteten – hvordan? nej
- Findes der en råmælksbank? ja
- Fodres med mælk/mælkeerstatning/syrnet mælk? Mælk
- Hvilken temperatur er mælken ved udfodring (og hvordan måles temperaturen)? 38 grader
- Hvor lang tid tager en udfodring? Og bruges mælketaxi? 1,5 time om morgenen og 1 time om aftenen i mælketaxa
- Hvilken kalveblanding anvendes (I skal tage foto af indholdsfortegnelsen)? Kalvenova
- Hvornår begynder tildelingen af kalveblanding (alder i uger)? I først leveuge
  
- Har kalvene adgang til hø af en god kvalitet? Nej
- Er det den samme person som altid passer kalvene? Ja

### 3.) Rengøring af skåle og trug

- Hvilken type skåle/flasker anvendes til udfodring af mælk? Metalskåle i enkelt båse, plastik trug i fælles
- Hvornår rengøres skålene? Hvor tit? Hver morgen



- Hvordan rengøres de? Med børste og klor

#### 4.) Rengøring af hytter

- Hvor ofte? Hver 14. dag
- Hvordan? Med minilæsser eller trillebør
- Hvor længe tørrer hytterne bagefter? En uge
- Hvor tit strøes der i boksene/muges? Der strøes hver dag
- Hvilken type strøelse anvendes? Halm.

#### 5) Opstaldningsforhold

- Hvilken type underlag: strøelse (type?) Fast gulv – dybstrøelse
- Træk? (egen observation) Ja
- Fugt? (egen observation) moderat
- Kulde/varme? (egen observation) ok.

#### 8.) Flytning af dyr

- Hvornår flyttes kalvene i fællesbokse? Ved 14 dage
- Hvor mange kalve er der i fællesboksene? 5 kalve
- Er der en løbende udskiftning af kalvene i fællesboksene (de ældste flyttes når der kommer nye til)? Nej.
- Hvor længe går kalvene typisk i fællesboksene? 8 uger
- Er vognen rengjort inden og mellem dyrene? Gøres det i bestemt rækkefølge? Ingen bestemt rækkefølge og vognen rengøres efter behov.
- Flyttes dyrene rundt mellem boksene? (enkelt og fælles) Kun fra enkelt til fælles
- Hvordan bevæger personalet (kalvepasser) sig mellem boksene (rengøring støvler)? Ingen systematik eller rengøring
- Hvad er proceduren for evt. isolation eller aflivning af syge dyr? Ingen dyr isoleres ved sygdom. aflives.

## 9.) Sygdom

- Har besætningen en rådgivningsaftale? ja
- Hvornår optræder der oftest diarré hos kalvene? Dag 3
- Er der andre sygdomsproblemer i kalveholdet end diarré – e.g. lungebetændelse? Respirationslidelser og salmonella
- Vaccineres med Rotavec Corona? nej
- Hvordan behandles diarré? Elektrolytter og antibiotika
- Hvordan behandles lungebetændelse? antibiotika
- Hvordan behandles navlebetændelse? Ingen
- Kalvedødelighed i besætningen (trækkes selv)
- Bruges Baycox? Nej

## Appendix 4 - Herd A data sheets

Dansk Kvæg	Malkekvæg	Nøgletal
	Bes-nr [redacted] CHR [redacted] Kontrol dato 28.05.14 5	Udskrevet 02.06.14 14.54 Side 1  Heden & Fjorden, Kvæg 96 29 66 66 514

### Besætningsoplysninger

Dyrstatus pr.	28.05.14	Fodring, optimalt (FE)	Avl
Køer:	Ialt 1158 Årskøer 1238	Fodermiddelnavn 1. kalvs Øvrige	Gns.NTM 0 Gns.Y-indeks.køer 99 Gns.Y-indeks.kvier 103
Ungdyr:	Kvier		
Over 24 mdr.	1 43		
12 - 24 mdr.	2 206		
0 - 12 mdr.	142 503		
Dage fra kælvning	224		
Udskiftningspct.	44		

	EKM		Fedt pct.		Protein pct.	Klassificering: Kimtal 2 mdr. Celletal 3 mdr	Antal/
	Mål	Opnået	Mål	Opnået	Mål		
Afsl.fast fodning						Kimtal:Antal under 30	5
1.kalv 0 - 20 uger	27,5	28,1	-	4,24	-	" 30 - 100	
	3,29					" Over 100	
Øvrige 0 - 20 uger	35,4	35,5	-	4,03	-	Geometrisk gns	9
	3,25					Celletal:Antal leverancer til mejeri	
Sidste kontrol	26,0	26,3	4,10	4,20	3,40	under 201 38 201 - 300	52
	3,51					301 - 400 401 - 500	
2.Sidste kontrol	25,3	24,8	4,23	4,05	3,44	501 - 600 Over 600	
	3,53					Geometrisk gns.	205
3.Sidste kontrol	25,2	24,4	4,34	4,07	3,49	Pct.lev.til mejeri sidste 3 mdr.	93

### Kødproduktion

Køer	Norm	Opnået sidste		Ungtyre	Norm	Opnået sidste	
		3 mdr.	12 mdr.			3 mdr.	12 mdr.
Klassificering	3,9	3,1 *	3,2 *	Klassificering	5,2	3,8 *	6,0
Beregnet levende vægt		672	688	Beregnet levende vægt		554	929
Dage fra	-	388	393	Daglig tilvækst	1151	1135	
kælvning Ialt	-	538	106	Ialt slagtet	-	11	2

### Sundhed

Køer	Sidste 12 mdr. Norm		Sidste 3 mdr. Norm		Ungdyr	Sidste 12 mdr. Norm		Sidste 3 mdr. Norm	
Sygdom ex. klovregistr.	1670	993	348	275	Sygdomstilfælde i alt Kalve under 6 mdr Kvier over 6 mdr. Tyre over 6 mdr.	46	1487 *	11	411 *
Yverbetændelse	784	463	146	126			32 *		6 *
Fordøjelse/stofskifte	198	128	49	32					
Lemmelidelser	60	108 *	14	23 *					
Reg. v. klovbeskæring	308	285	71	93 *	Dødfødte kalve	46	172 *	11	50 *
Reproduktionslidelse	26	70 *	7	21 *	Døde 0-180 dag		11 *		5 *
	250	242	250	245					

### Reproduktion

Pr. 28.02.14	Sidste 12 mdr.		(Alle Afsl.)	Næste 12 mdr.		Sidste	Forv.næst	
	Mål	Opnået		Mål	Forventet			
Køer:Antal drægtige	813	440 *	(441)	***	711*	Kælvning ialt:	1105	1235
Pct.drægtige af påbegyndt	85	52 *	( 52)	85	74	Heraf 1.kalvs	374	175
Tomperiode,dage	91	104 *	( 73)	91	95*	Alder 1.kælvning	27,1	23,9
Start inseminering	40	40		40	46*	Ukendt drægtighedsstatus		
Insemineringspct	50	57		50	58			
Drægtigheds pct	40	25 *		40	35*	Antal køer	248	(20%)
Kvier:Antal drægtige	330	253 *	(265)	211	176*	Antal kvier	97	(41%)
Alder ved drægtighe	17,1	18,1	(18,1)	17,1	18,1			

Dansk Kvæg

Malkekvæg

Dødelighed, Kalve



Bes-nr [redacted] CHR  
 [redacted]  
 Kontrol dato 28.05.14

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 14.50

5 Heden & Fjorden, Kvæg 514

	jun 13	jul 13	aug 13	sep 13	okt 13	nov 13	dec 13	jan 14	feb 14	mar 14	apr 14	maj 14	S.12 mdr	2013
Total antal kalve 1-180 dg	455	389	383	414	422	425	426	458	478	415	409	413	424	459
Antal fødte kalve	74	60	83	113	92	101	119	97	74	85	108	93	1099	1116
Heraf kviekalve	41	34	42	67	53	55	63	48	43	46	57	56	605	618
tyrekalve	33	26	41	46	39	46	56	49	31	39	51	37	494	498
Antal dødfødte kviekalve	5		1	4	3	6	2	1	1	3	1	2	29	33
Antal dødfødte tyrekalve	1	1	3	3	1	1	2	1	2	5	7	3	30	24
Dødfødte i alt	6	1	4	7	4	7	4	2	3	8	8	5	59	57
Dødfødte i procent													5,4	5,1
Antal dødfød., 1. kalvs køer	2	1	1	1	2	2	2		2	5	3	1	22	24
Pct. dødfødte, 1. kalvs køer													6	5,8
Antal dødfød., ældre køer	4		3	6	2	5	2	2	1	3	5	4	37	33
Pct. dødfødte, ældre køer													5,1	4,7
Aflivet som spæd														
Antal indkøbte kviekalve														
Antal indkøbte tyrkalve														
Antal solgte kviekalve		11											11	12
Antal solgte tyrkalve	9	67		20	15	56	15	49	35	82	41		389	337
Antal kalve døde 1-14 dg		1	5	6	5	7	5	6	1	3	2	4	45	45
Antal kalve døde 15-30 dg	1	1	3	5	6	1	3	1		3	4	7	35	39

Antal kalve døde 31-60 dg				1	1	1	2		1	1		3	10	10	
Antal kalve døde 61-180 dg	1	2	6	6	2	1	1	2			1	3	25	32	
Antal kalve døde 1-180 dg	2	4	14	18	14	10	11	9	2	7	7	17	115	126	
Pct. kalve døde 1-180 dg													12,1	(1)	(2)

(1) Denne størrelse angiver dødeligheden i alderen 1-180 dage blandt kalve født i perioden 18 - 6 mdr. forud for beregningstidspunktet

(2) Dødelighed blandt kalve født i det angivne år. Kalvene kan være døde i perioden indtil 6 mdr. inde i det følgende år.

Dansk Kvæg

Malkekvæg

Dødelighed, Kalve

Bes-nr [redacted] CHR

Udskrevet 02.06.14  
14.50

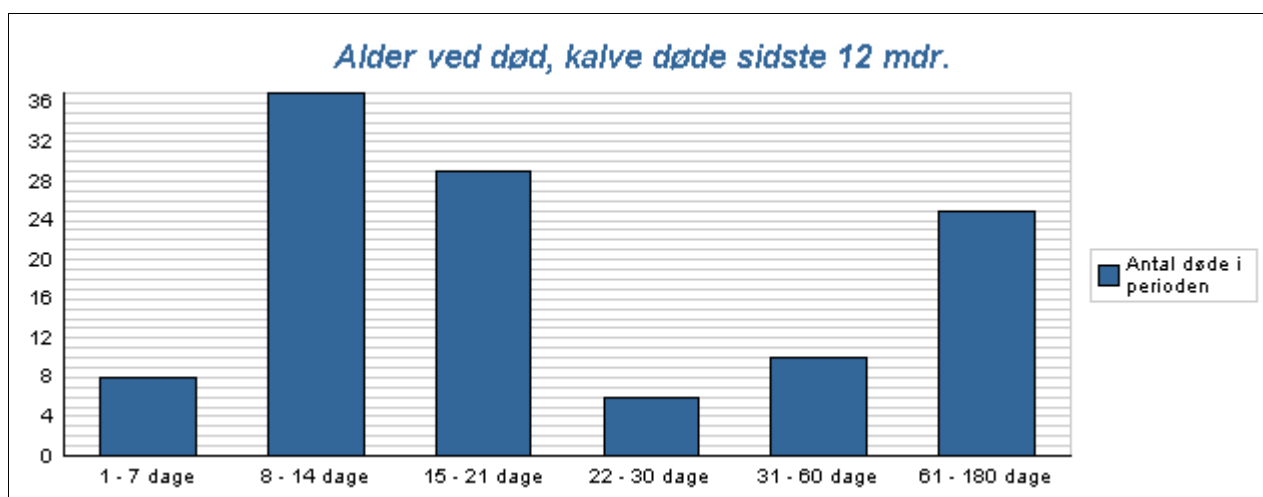
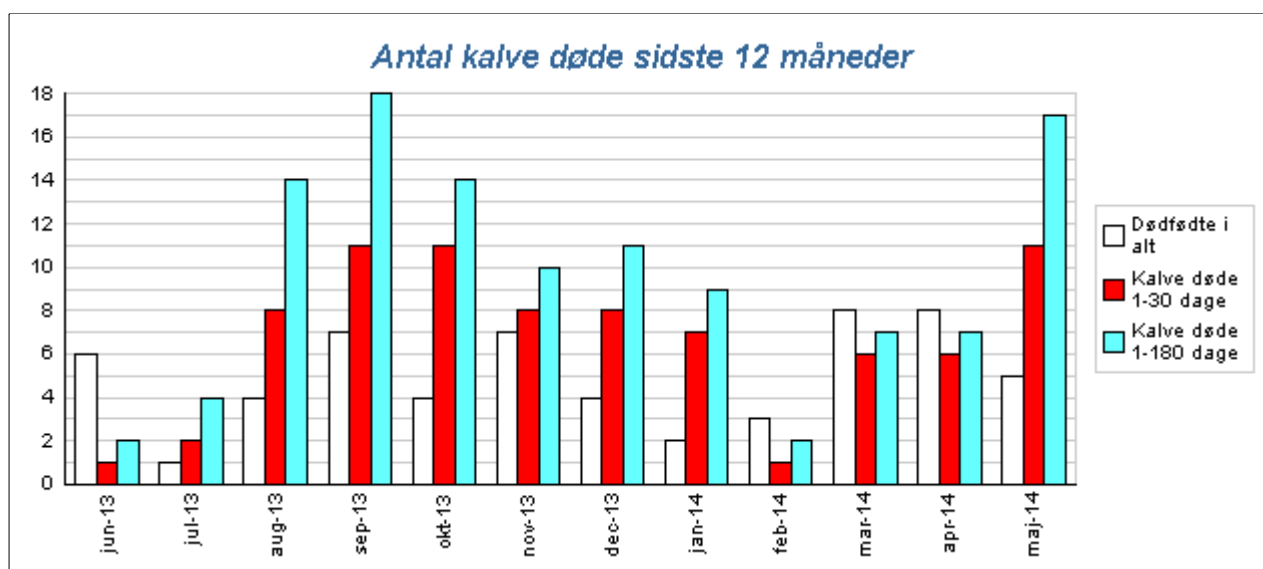
Side 2

Kontroldato 28.05.14 5

02-06-14

Heden & Fjorden, Kvæg  
96 29 66 66

514



<b>Dansk Kvæg</b>

### Malkekvæg

### Sygdomsopgørelse ungdyr

Bes-nr [redacted] CHR

Udskrevet 02.06.14 14.50

Kontroldato 28.05.14 5

Side 1

Heden & Fjorden, Kvæg  
96 29 66 66

514

	Antal tilfælde pr.måned											Sidste 12 mdr		2013	2012		
	Jul	Aug	Sep	Okt	Nov	Dec	Jan	Feb	Mar	Apr	Maj	Jun	Norm	Besæt	Tilfælde dyr	Tilfælde dyr	
<b>Fødte kalve</b>																	
Kvier	34	42	67	53	55	63	48	43	46	57	56	-	603	-	617	-	630
Tyre	26	41	46	39	46	56	49	31	39	51	37	-	499	-	498	-	604

### Kalve under 6 måneder

Diarré	14	30	58	59	39	54	54	33	28	28	37	453	432	421	160	159	
Lungebetændelse	67	75	95	97	47	40	69	120	106	102	109	***	151	785	838	632	
Navlebetændelse															5	5	
Ledbetændelse																	
Digital dermatitis																	
Klovbrandbyld						3			1			4	13	13	2	2	
Bemærk. fra slagteri																	
Andet												46	1	1	2	1	
Dødfødt	1	4	7	4	7	4	2	3	8	8	5	40	59 *	-	56	-	73
Døde 1-30 dage	4	12	18	16	14	12	9	4	12	14	16	28	137 *	141	133		
Døde 30-180 dage	2	6	7	3	2	3	2	1	1	1	6	18	35 *	-	42	-	69

### Kvier over 6 måneder

Yverbetændelse																
Fluemastitis																
Fordøjelsesforstyrel																
Digital dermatitis															1	1
Klovbrandbyld						2	2	1	1			6				
Såleblødning																
Sålesår																
Overgroet klov					1							1	1	1		
Andre hornrel.																
Hasebetændelse																
Lungebetændelse	1	1	5	5	1	2	3	2	2	3	25	5	4	3	3	
Andet																



Døde	3	2	4	9	-	20	-	25
Bemærk. fra								

#### Tyre over 6 måneder

Lungebetændelse								4	3
Fordøjelsesforstyrel									
Halelæsion									
Digital dermatitis									
Klovbrandbyld								2	2
Såleblødning								1	1

Dansk Kvæg
[Redacted]
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#### Malkekvæg

Bes-nr [Redacted] CHR

Kontrol dato 28.05.14 5

#### Sygdomsopgørelse ungdyr

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Heden & Fjorden, Kvæg  
96 29 66 66

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Overgroet klov									
Andre									
hornrelaterede	1					1		2	-
Andet								1	-

#### Afgangsårsag (døde)

Diarré	4	9	8	2	6	5	1	3	2	2	17	42 *		
Lungebetændelse	2	5	7	3	4	4	4	1	4	5	13	21	52 *	60
Andre årsager	2		1							2		1	6 *	-
Årsag uoplyst	5	11	16	12	18	9	4	6	14	16	10	133	-	-

## Appendix 5 - Herd B data sheets

Dansk Kvæg	Malkekvæg	Nøgletal
	Bes-nr [redacted] CHR Kontroldato 28.05.14 8	Udskrevet 16.06.14 07.53 Side 1

### Besætningsoplysninger

Dyrstatus pr. 28.05.14	Fodring, optimalt (FE)	Avl
Køer: Ialt 535 Årskøe 546	Fodermiddelnavn 1. Øvrig kalvs e	Gns NTM 2 Gns.Y-indeks.køer 100 Gns.Y-indeks.kvier 105
Unadvr: Kvie		
Over 24 mdr. 22		
12 - 24 mdr. 2 253		
0 - 12 mdr. 14 168		
Dage fra kælvning 200		
Udskirtningspct. 43		

### Mælkeproduktion

	EKM		Fedt pct.		Protein		Klassificering: Kimtal 2 mdr. Antal/	
	pct.	Mål	Opnået	Opnået	Mål		Celletal 3 mdr	gns.
Afsl.fast fodning							Kimtal:Antal under 30	5
1.kalv 0 - 24 uger	29,5	32,1	-	3,80	-	3,17	" 30 - 100	
Øvrige 0 - 24 uger	38,7	39,6	-		3,77	-	" Over 100	
	3,15						Geometrisk ans	11
Sidste kontrol	27,5	29,6	4,06	3,84	3,36	3,25	Celletal:Antal leverancer til mejeri	
2.Sidste kontrol	28,4	31,1	4,13	3,81	3,38	3,33	under 201 79 201 - 300	12
3.Sidste kontrol	28,2	30,5	4,20	3,89	3,39	3,34	301 - 400 1 401 - 500	
Sidste 12 mdr. 30.04							501 - 600 Over 600	
Yktr Ydelse/ko	10.352		-	3,91	-	3,31		
Mejeri i alt(Kg mælk)	5.318.432		-	3,79	-	3,32	Pct.lev.til mejeri sidste 3 mdr.	93

### Kødproduktion

Køer	Nor	Opnået sidste		Ungtyre	Nor	Opnået sidste	
						12 mdr.	3 mdr.
Klassificering	3,9	2,1 *	2,0 *	Klassificering			
Beregnet levende		602	602	Beregnet gt			
Dage fra	-	215	195	Dåglig tilvækst			
kælvning Ialt	-	192	53	Ialt slagtet	-	0	0

**Sundhed**

Køer	Sidste 12 mdr.		Sidste 3 mdr. Norm		Ungdyr	Sidste 12 mdr. Norm		Sidste 3 mdr. Norm	
	Sygdom ex. klovregistr.	804	700	164		184 *	Sygdomstilfælde i alt Kalve	19	39 *
Yverbetændelse	349	426 *	68	128 *	under 6 mdr		4 *		3 *
Fordøjelse/stofskifte	107	31	24	6	Kvier over 6 mdr. Tyre	23	56 *	5	13 *
Lemmelidelser	31	104 *	6	37 *	over 6 mdr.	19	53 *	4	4
Reg. v.	161	130	34	13			11 *		1 *

**Reproduktion**

	Sidste 12 mdr. (Alle mdr)			Næste 12		Sidste		Forv.næ
	Køer: Antal drægtige	450	337 *	(338)	536	432 *	Kælvning ialt:	
Pct. drægtige af påbegyndt	79	85	64 *	(64)	85	Heraf 1.kalvs	221	244
Tomperiode dage	90	93	(73)	90	76	Alder 1.kælvning	***	23,1
Insemineringspct	50	71		50	75	Ukendt drægtighedsstatus		
Drægtigheds pct	40	37 *	(218)	40	41	Antal køer	101	(20%)
Kvier: Antal drægtige	232	215 *	(218)	148	152 *	Antal kvier	41	(16%)
Alder ved drægtighe	16,1	15,0	(15,0)	16,1	15,0			

Dansk Kvæg

Malkekvæg

Dødelighed, Kalve

Bes-nr [redacted] CHR

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Side 1

07.53

Kontroldato 28.05.14 8

Heden & Fiorden, Kvæg

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	jun 13	jul 13	aug 13	sep 13	okt 13	nov 13	dec 13	jan 14	feb 14	mar 14	apr 14	maj 14	S.12 mdr	2013	2012								
Total antal kalve 1-180 dg	127	128	141	142	163	157	166	168	170	157	152	140	151	149	150								
Antal fødte kalve	39	59	62	69	57	51	62	53	48	46	37	52	635	626	623								
Heraf kviekalve	22	28	28	38	29	26	44	24	28	19	23	26	335	321	331								
tyrekalve	17	31	34	31	28	25	18	29	20	27	14	26	300	305	292								
Antal dødfødte kviekalve				1	1	3	2	2	1	1	2	2	15	12	20								
Antal dødfødte tyrekalve		3	5	8	5	4	2	3	3	5		3	41	44	27								
Dødfødte i alt		3	5	9	6	7	4	5	4	6	2	5	56	56	47								
Dødfødte i procent													8,8	8,9	7,5								
Antal dødfød., 1. kalvs køer		2	1	1	2	4	1	3		1	1	1	17	15	18								
Pct. dødfødte, 1. kalvs køer													7,6	7,1	9,3								
Antal dødfød., ældre køer		1	4	8	4	3	3	2	4	5	1	4	39	41	29								
Pct. dødfødte, ældre køer													9,5	9,9	6,8								
Aflivet som spæd																							
Antal indkøbte kviekalve														1									
Antal indkøbte tyrkalve																							
Antal solgte kviekalve														1	16	8	29	10	19		11	94	25
Antal solgte tyrkalve	21	17	29	23	20	19	12	19	21	20	14	19	234	239	234								
Antal kalve døde 1-14 dg	1	3	4	3	5	3	6	5	1				31	35	42								
Antal kalve døde 15-30 dg			3		1	2			6	2	2		1	17	11	20							

Antal kalve døde 31-60 dg	1									1		2	3	7
Antal kalve døde 61-180 dg		1						1	1			3	1	14
Antal kalve døde 1-180 dg	2	4	7	3	6	5	6	12	4	3	1	53	50	83
Pct. kalve døde 1-180 dg												10,2		14,8
												(1)	(2)	(2)

(1) Denne størrelse angiver dødeligheden i alderen 1-180 dage blandt kalve født i perioden 18 - 6 mdr. forud for beregningstidspunktet

(2) Dødelighed blandt kalve født i det angivne år. Kalvene kan være døde i perioden indtil 6 mdr. inde i det følgende år.

Dansk Kvæg

Malkekvæg

Dødelighed, Kalve

Bes-nr [redacted] CHR

Udskrevet 16.06.14  
07.53

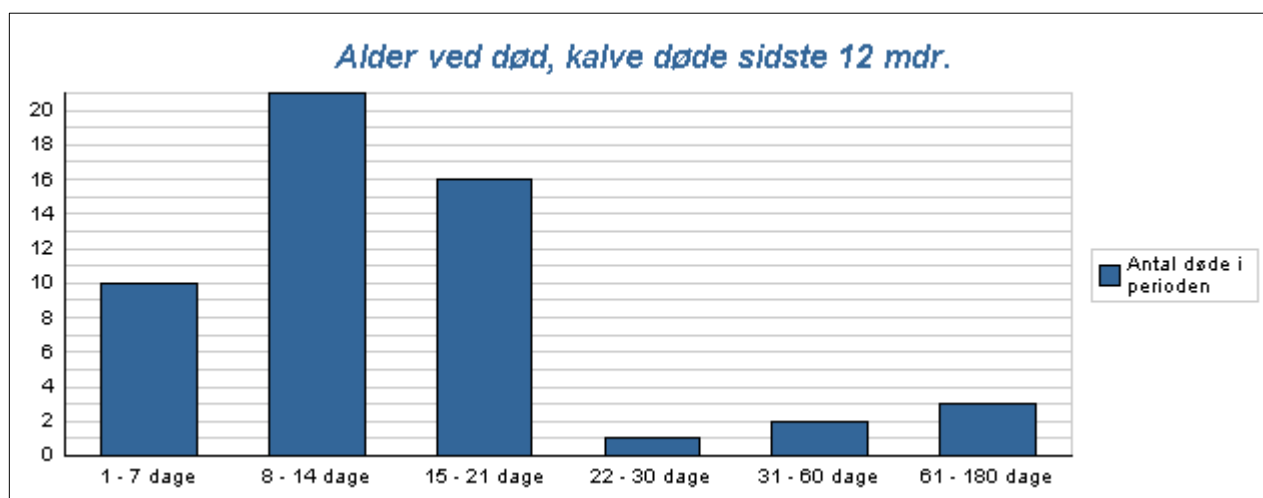
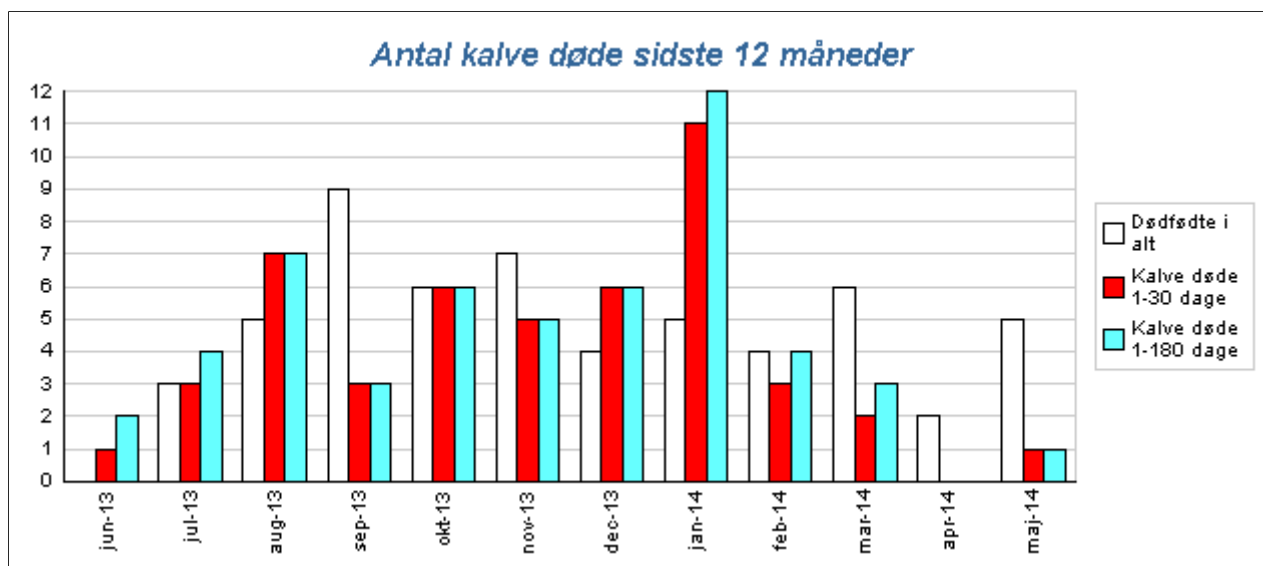
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Kontroldato 28.05.14 8

16-06-14

Heden & Fjorden, Kvæg  
96 29 66 66

514



Dansk Kvæg

**Malkekvæg**

**Sygdomsopgørelse ungdyr**

Bes-nr [redacted] CHR

Udskrevet 16.06.14 14.21

Kontrol dato 28.05.14 8

Side 1

Dansk Kvæg, Henrik Læssø

87 40 50 00

9921

	Antal tilfælde pr.måned Jul 13 - Jun 14												Sidste 12 mdr		2013	2012		
	Jul	Aug	Sep	Okt	Nov	Dec	Jan	Feb	Mar	Apr	Maj	Jun	Norm	Besæt	Tilfælde dyr	Tilfælde dyr		
<b>Fødte kalve</b>																		
Kvier	28	28	38	29	26	44	24	28	19	23	26	14	-	335	-	321	-	330
Tyre	31	34	31	28	25	18	29	20	27	14	26	13	-	300	-	305	-	292

**Kalve under 6 måneder**

Diarré	1													7	8	8		
Lungebetændelse	2	17			2									23	35	31	1	1
Navlebetændelse																		
Ledbetændelse																		
Digital dermatitis																		
Klovbrandbyld																		
Bemærk. fra slagteri																		
Andet		1	2	1			1			1	1		19	9	9	9	5	5
Dødfødt	3	5	9	6	7	4	5	4	6	2	5	3	23	56 *	-	56	-	45
Døde 1-30 dage	3	7	3	6	5	6	11	3	2		1		14	48 *		46		62
Døde 30-180 dage	1						1	1	1				5	5	-	4	-	21

**Kvier over 6 måneder**

Yverbetændelse																			1				
Fluemastitis																							
Fordøjelsesforstyrrel																							
Digital dermatitis																							
Klovbrandbyld																							
Såleblødning																							
Sålesår																							
Overgroet klov																							
Andre hornrel.											1							2	2				
Hasebetændelse											1							3					
Lungebetændelse																				1	1		
Andet	1	1	3		1															1	1	-	16





## Appendix 6 – Actual daily milk intake of case group calves in Herd A

dag1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
10000	10000	10000	10000	10000	10000	10000	8538	8551	6637	2710	6750	7330	10000	10000	10000	10000	10000	10000	10000	10000 x
10000	10000	10000	10000	10000	9666	7114	8517	8636	7707	6439	5980	9058	9330	10000	4500	7180	6020	6800	8520	9200 x
10000	10000	10000	10000	10000	5302	3894	6450	8087	6760	7984	8206	8206	3579	7600	7370	7380	6080	4530	9160 x	
10000	10000	10000	10000	10000	5588	4941	7690	4458	5630	6470	4798	6614	6010	9196	7030	7654	8328	8980 x		
10000	10000	10000	10000	10000	10000	10000	10000	10000	9436	10000	8975	10000	10000	10000	10000	10000	10000 x			
10000	10000	10000	10000	10000	10000	10000	10000	9726	8890	8590	7500	8592	7724	7420	8909	10000	10000 x			
10000	10000	10000	10000	10000	10000	10000	10000	8052	6018	6120	8836	8460	8736	7381	10000	10000 x				
10000	10000	10000	10000	8885	7993	9064	9404	4723	52200	7500	4530	8186	6844	8234	8670	8402	8290 x			
10000	10000	10000	10000	10000	10000	10000	10000	10000	7870	10000	10000	10000	10000	10000	10000	10000 x				
10000	10000	10000	10000	10000	10000	10000	7518	8436	6900	7006	9794	9846	10000	9703	10000	10000	10000	10000	10000 x	
10000	10000	10000	10000	10000	9413	9270	8522	5492	7860	7740	7640	9556	8670	10000	10000	10000 x				
10000	10000	10000	10000	5618	7583	8558	8224	7870	8130	6806	5123	8670	6934	8386	10000	5830	9218 x			
10000	10000	10000	10000	10000	9777	10000	10000	6370	5515	6203	6230	7840	7020	5870	8760	7435	9566 x			
10000	10000	10000	10000	10000	10000	10000	6186	6599	6696	7924	8490	6029	7950	10000	10000	10000	10000	10000	10000	10000 x
10000	10000	10000	10000	10000	10000	10000	9257	9244	8450	8858	8855	6848	9900	10000	10000	10000	10000	10000	10000	10000 x
10000	10000	10000	10000	10000	10000	10000	7900	4553	8460	7064	6362	7920	8852	8524	10000	10000	10000	10000	10000	8852
10000	10000	10000	10000	10000	10000	10000	9021	10000	7936	8910	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000 x
10000	10000	10000	10000	10000	10000	10000	6710	7085	8520	9100	10000	10000	9664	9676	10000	10000	10000	10000	10000	10000 x
10000	10000	10000	10000	10000	10000	10000	10000	10000	6810	9644	6612	7299	9924 x							
10000	10000	10000	10000	10000	10000	10000	8380,81	7806,952	9855,143	7463,762	7930,333	8410,1	8587,65	8801,474	9337,105	9118,789	7721			
10000	10000	10000	10000	10000	10000	10000	8592,238	8592,238	9623,381	9623,381	9290 x									
gn snit g/pr dag																				







UGB3	T	Færes	Luft	Alm	x synstelt	C synstelt	C stribe	C brand	ØF6 syn	ØF6 synb	log	grupper	x synstelt	G synstelt	G stribe	G brand	CPG	log CPG	grupper	UGB4	T	Færes	Luft	Alm	x synstelt	C synstelt	C stribe	C brand	ØF6 syn	ØF6 synb	log	grupper	G synstelt	G stribe	G brand	CPG	log CPG	grupper								
24-02-2014	39	4	0	0	1	21	80													09-03-2014	39,4	3	0	0	3			15	7300	0,3	ØF5061	3														
03-03-2014	40,1	2	0	0	1															10-03-2014	39,9	2	0	0	1			0	0	0	0	1														
03-03-2014	38,6	2	0	0	1															30-03-2014	39,7	2	0	0	1			0	0	0	0	1														
03-03-2014	39,7	2	0	0	1															01-03-2014	39,1	2	0	0	1			0	0	0	0	1														
03-03-2014	39,7	2	0	0	1															10-03-2014	MINUS	2	0	0	2			0	0	0	0	1														
17-03-2014	39,2	3	0	0	1															24-03-2014	38,6	2	0	0	1			0	0	0	0	1														
24-03-2014	39,4	2	0	0	1															31-03-2014	40,1	1	0	0	3			0	0	0	0	1														
24-03-2014	38,9	2	0	0	1															01-04-2014	39	2	0	0	1			1	200	0,2	230103	2														
31-03-2014	38,5	2	0	0	1															07-04-2014	38,7	2	0	0	1			8	1600	0,3	20412	3														
31-03-2014	38,1	2	0	0	1															10-04-2014	39,4	2	0	0	1			372	7400	0,4	ØF5173	4														
31-03-2014	38,4	2	0	0	1															17-04-2014	38,6	2	0	0	1			0	0	0	0	1														
31-03-2014	38,4	2	0	0	1															24-04-2014	40,5	2	1	1				1438	287600	0,5	ØF709	4														
31-03-2014	38,4	2	0	0	1															01-05-2014	39,4	2	0	0	1			0	0	0	0	1														
07-04-2014	38,8	1	0	0	1															07-04-2014	39,4	2	0	0	1			0	0	0	0	1														
07-04-2014	39,2	1	1	1	1															14-04-2014	38,6	2	0	0	1			0	0	0	0	1														
07-04-2014	38,8	2	0	0	1															14-04-2014	39,6	4	0	0	3			0	0	0	0	1														
																				21-04-2014																										
																				28-04-2014																										
14-04-2014	39,2	1	0	0	1															28-04-2014	38,8	2	1	1				0	0	0	0	1														
14-04-2014	38,6	1	0	0	1															28-04-2014	39,5	2	1	1				0	0	0	0	1														
22-04-2014	40,4 MINUS	1	1	1																28-04-2014	39,4 MINUS	1	1	1				0	0	0	0	1														
22-04-2014	39,4 MINUS	0	1	1																28-04-2014	39,3	3	0	0	1			7	1400	0,3	ØF6128	3														
22-04-2014	39,9 MINUS	1	1	1																28-04-2014	39,4	2	0	0	1			5	1000	0	3	3														
22-04-2014	38,6	2	0	0	1															28-04-2014	38,5	1	0	0	1			0	0	0	0	1														
28-04-2014	38,8	2	0	0	1															05-05-2014	39,1	1	0	0	1			0	0	0	0	1														
28-04-2014	39,2	2	0	0	1															05-05-2014	39,2	2	0	0	1			2	400	0,2	ØF0206	2														
28-04-2014	38,5	2	0	0	1															05-05-2014	38,4	2	0	0	1			2	400	0,2	ØF0206	2														
28-04-2014	38	2	0	0	1															05-05-2014	38,2	2	0	0	1			1	200	0,2	ØF0103	2														
28-04-2014	1	2	0	0	1															05-05-2014	39,1	2	0	0	1			0	0	0	0	1														
28-04-2014	2	2	0	0	1															05-05-2014	39,6	2	0	0	1			0	0	0	0	1														
28-04-2014	39,2	2	0	0	1															05-05-2014	39,6	2	0	0	1			0	0	0	0	1														
28-04-2014	38,2	2	0	0	1															05-05-2014	38,9	2	0	0	1			1	200	0,2	ØF0103	2														
28-04-2014	39,2	2	0	0	1															05-05-2014	38,9	2	0	0	1			1	200	0,2	ØF0103	2														
28-04-2014	38,2	2	0	0	1															05-05-2014	38,9	2	0	0	1			1	200	0,2	ØF0103	2														
28-04-2014	38,2	2	0	0	1															05-05-2014	38,9	2	0	0	1			1	200	0,2	ØF0103	2														
28-04-2014	38,2	2	0	0	1															05-05-2014	38,9	2	0	0	1			1	200	0,2	ØF0103	2														

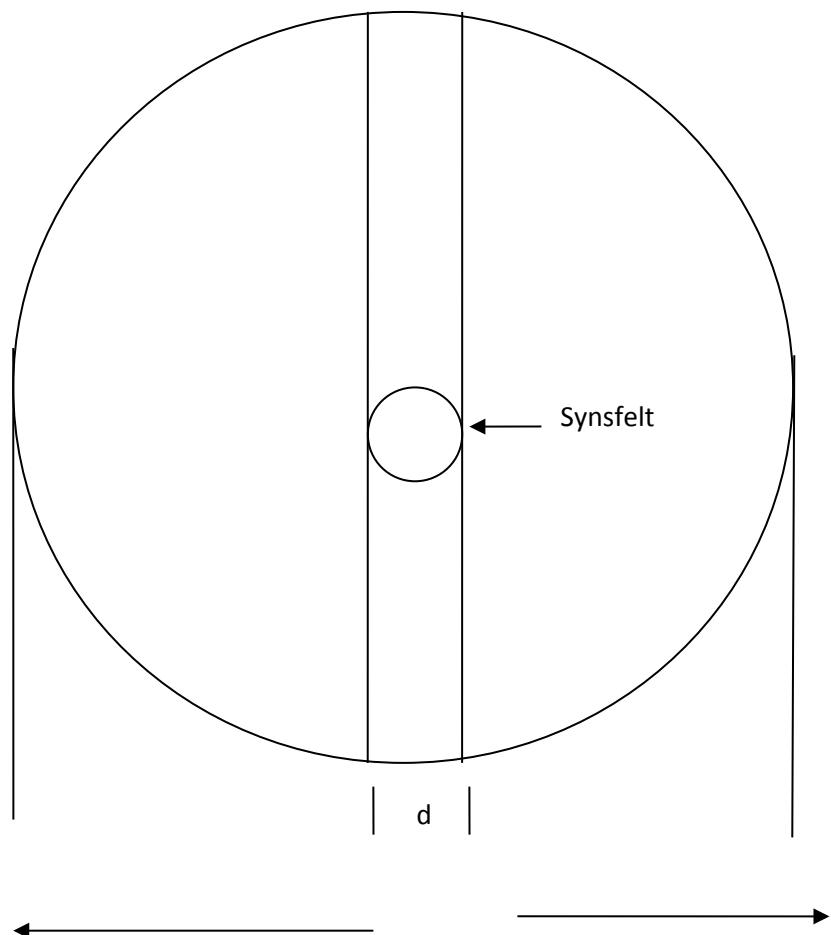
## Appendix 9 - FITC staining quantification

**A** = areal af hele prøven = Total antal oocyster i brønden

**a** = areal af synsfeltet = Antal oocyster i et synsfelt

**D** = n d

**n** = antal synsfelter i bredden



### Synsfelt tælling

$$A = \Pi R^2 = \Pi (D/2)^2 = \Pi D^2/4$$

$$A = \Pi (nd)^2/4 = \Pi n^2 d^2/4$$

$$a = \Pi d^2/4$$

$$A = n^2 a$$

### Kolonne tælling

**a<sub>k</sub>** = areal af midterkolonnen  $\approx n d^2$  = antal oocyster i midterste kolonne

$$A = \Pi n a_k/4$$

## Appendix 10 - Afgangsårsager kvier by Jørn Pedersen 2014

DH kviekvæ afgået i 2013 (kviekvæ = hundyr som ikke har kvævel)																								
Afgangskode	ærd	alder	afgået	afgang	lav	eller mærkev	Verets	Andre/UK	Reprodukt-	Verbetæ	Indlæses-	lemmeid	sygdom-	me	Diare	kvævel	Andet	Forhøjet	Tempera	Uheld/Til-	skade-	Alder (Aug 2008)	Parabter	Ikke
															(kvæ)	(kvæ)	(kvæ)	celletal	ment	Malkværd	konst		kvise	registre-
Slåget	DH	01år	152	546	00	00	26	92	00	07	11,2	4,6	0,0	2,0	8,6	0,0	0,7	0,0	15,1	0,0	0,0	0,0	0,0	45,4
Slåget	DH	12år	545	677	03	03	17	47,1	0,7	0,4	4,7	2,1	0,1	0,2	6,0	0,0	0,5	0,0	2,6	0,8	0,1	0,1	32,5	
Slåget	DH	23år	554	625	08	03	12	50,6	0,7	0,2	1,8	0,3	0,0	0,1	4,0	0,1	0,2	0,0	0,7	1,0	0,1	0,1	37,7	
Slåget	DH	≥3år	314	433	13	00	03	33,4	1,0	0,0	1,6	1,0	0,0	0,0	3,2	0,0	0,0	0,0	1,0	0,6	0,0	0,0	56,7	
Døde	DH	01år	11362	540	00	00	67	00	0,0	1,6	0,3	2,4	23,6	10,4	5,8	0,0	0,0	0,0	2,5	0,2	0,0	0,0	46,2	
Døde	DH	12år	1007	540	00	01	120	0,0	0,2	2,4	1,6	2,2	0,7	6,5	7,2	0,0	0,0	0,0	20,7	0,3	0,0	0,0	46,2	
Døde	DH	23år	449	472	00	00	125	0,7	0,7	2,0	3,3	2,7	1,6	2,4	6,7	0,0	0,0	0,0	13,6	0,2	0,9	0,9	52,8	
Døde	DH	≥3år	75	107	00	00	40	1,3	0,0	0,0	0,0	2,7	0,0	1,3	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	89,3	
Aflivet	DH	01år	1532	706	00	01	61	00	0,0	3,5	7,3	7,7	10,2	10,5	10,8	0,0	0,1	0,0	13,7	0,1	0,0	0,0	30,0	
Aflivet	DH	12år	331	734	03	00	45	0,0	0,0	2,1	10,9	4,5	0,6	4,2	4,8	0,0	0,3	0,0	39,6	0,0	0,9	0,9	27,2	
Aflivet	DH	23år	122	689	00	00	66	1,6	0,8	2,5	9,0	3,3	0,8	2,5	8,2	0,0	0,0	0,0	29,5	0,0	2,5	2,5	32,8	
Aflivet	DH	≥3år	3	667	00	00	00	0,0	0,0	0,0	33,3	33,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	33,3	

## Appendix 11 – Statistical calculations H<sub>0</sub>1

### Herd B

#### Result sheet of Two sided unpaired t-test on weight gain 0-5 weeks of age

Control	Case
30,	18,7
64,	19,0
46,	13,5
56,	11,2
24,	19,5
16,	26,3
17,	19,0
20,	28,0
15,	23,0
19,	14,0
16,	27,0
22,	23,0
15,	19,0
17,	34,0
1,	

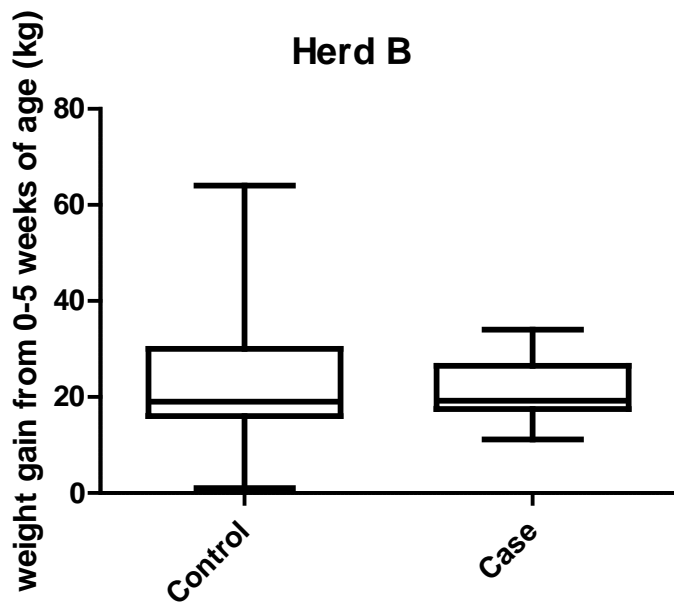
Table Analyzed	Data 1
Column A	Control
vs	vs
Column B	Case

Unpaired t test	
P value	0,4035
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.8487 df=27

How big is the difference?	
Mean ± SEM of column A	25.20 ± 4.410 N=15
Mean ± SEM of column B	21.09 ± 1.675 N=14
Difference between means	4.114 ± 4.848
95% confidence interval	-5.834 to 14.06
R square	0,02598

F test to compare variances	
F,DFn, Dfd	7.425, 14, 13
P value	0,0009
P value summary	***
Are variances significantly different?	Yes





Herd A

**Result sheet of two sided unpaired t-test on daily weight gain of calves weighed at different timed in the Herd A case group:**

<u>calves weighed at 48-74</u> <u>daysof age</u>	<u>calves weighed at 37-47 days</u> <u>of age</u>
0,6122449	0,2340426
0,4081633	0,6521739
0,08333334	0,4444444
0,9932432	0,4888889
0,7142857	0,4444444
0,9852941	0,3111111
	0,8522727
	0,9186047
	0,9166667
	0,837500
	1,000000
	0,6666667
	0,7567568
	1,040541
	0,7567568
	1,040541

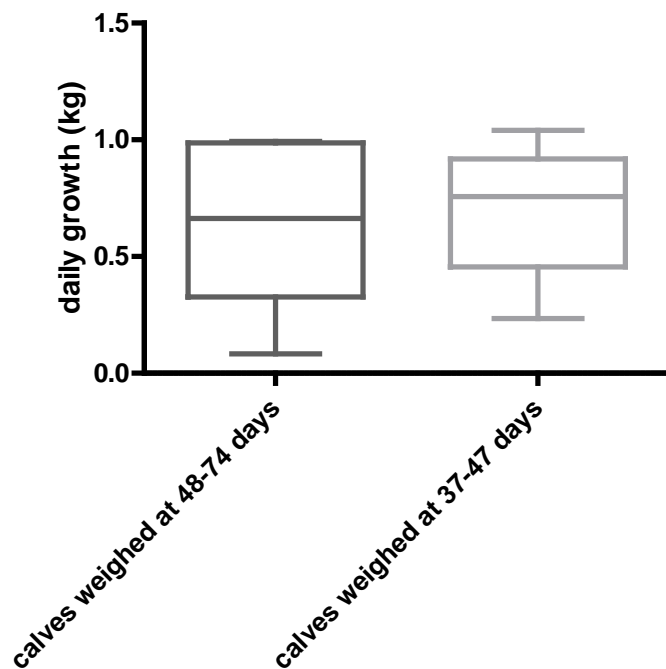
Table Analyzed Data 3  
 Column A calves weighed at 48-74 days  
 vs vs  
 Column B calves weighed at 37-47 days

Unpaired t test  
 P value 0,5771  
 P value summary ns  
 Are means signif. different? (P < 0.05) No  
 One- or two-tailed P value? Two-tailed  
 t, df t=0.5668 df=20

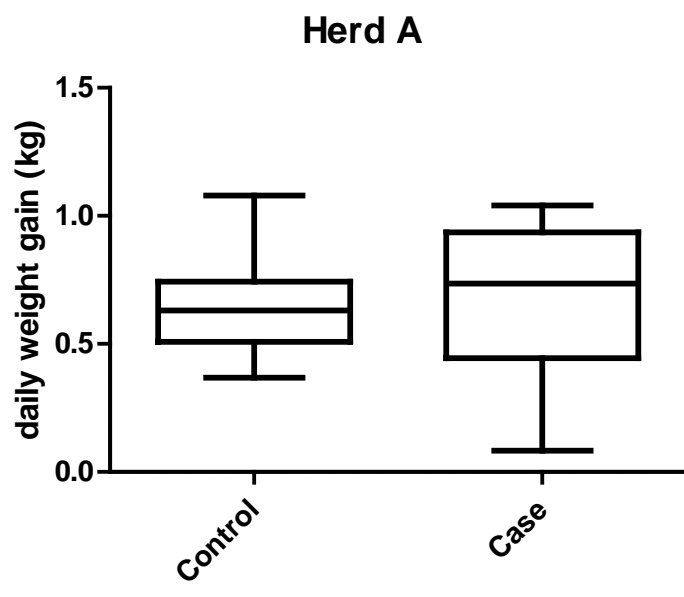
How big is the difference?  
 Mean ± SEM of column A 0.6328 ± 0.1430 N=6  
 Mean ± SEM of column B 0.7101 ± 0.06489 N=16  
 Difference between means -0.07733 ± 0.1364  
 95% confidence interval -0.3619 to 0.2072  
 R square 0,01581

F test to compare variances  
 F,DFn, Dfd 1.822, 5, 15  
 P value 0,3380  
 P value summary ns  
 Are variances significantly different? No

### Herd A case group comparison







## Appendix 12 – Statistical calculations H<sub>0</sub>2 “R”

### OPG

opg feed herd

1	18.645975	0	0
2	6.323335	0	0
3	8.013994	0	0
4	8.551160	0	0
5	6.392416	0	0
6	6.644439	0	0
7	12.445055	0	0
8	13.388240	0	0
9	11.425799	0	0
10	6.765013	0	0
11	8.845818	0	0
12	8.197287	0	0
13	10.370498	0	0
14	6.641494	0	0
15	11.091913	0	0
16	12.859368	0	0
17	10.949895	0	0
18	12.721981	0	0
19	5.996599	0	0
20	6.730168	0	0
21	7.072573	0	0
22	13.587501	1	0
23	11.580006	1	0
24	7.314684	1	0

25 7.463983 1 0  
26 6.932240 1 0  
27 11.953223 1 0  
28 13.758405 1 0  
29 12.493041 1 0  
30 13.200682 1 0  
31 9.200719 1 0  
32 12.479101 1 0  
33 5.860937 1 0  
34 13.343950 1 0  
35 7.042379 1 0  
36 13.755152 1 0  
37 11.980167 1 0  
38 12.616189 1 0  
39 12.068878 1 0  
40 15.625071 1 0  
41 17.390360 1 0  
42 12.401191 1 0  
43 6.547529 1 0  
44 16.408465 0 1  
45 10.127639 0 1  
46 10.765801 0 1  
47 13.045665 0 1  
48 10.821917 0 1  
49 14.932188 0 1  
50 11.257222 0 1  
51 12.900868 0 1  
52 18.238685 0 1  
53 12.495924 0 1

```

54 15.718967 0 1
55 11.446254 0 1
56 10.043766 0 1
57 12.144829 0 1
58 12.315797 1 1
59 9.291822 1 1
60 9.134699 1 1
61 14.595893 1 1
62 15.314980 1 1
63 15.832846 1 1
64 6.837563 1 1
65 12.583074 1 1
66 13.196376 1 1

```

```

model1<-lm(opg~herd+feed+herd:feed)
model2<-lm(opg~herd+feed)
model3<-lm(opg~feed)
summary(model1)

```

```

Call:
lm(formula = opg ~ herd + feed + herd:feed)

```

```

Residuals:
    Min     1Q   Median     3Q    Max
-5.4389 -2.7603  0.2367  2.0487  9.1187

```

```

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  9.5273    0.6703  14.214 <2e-16 ***
herd         3.3547    1.0598   3.166  0.0024 **
            feed     1.7725    0.9371   1.892  0.0632 .
herd:feed   -2.5320    1.6125  -1.570  0.1215
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

Residual standard error: 3.072 on 62 degrees of freedom
Multiple R-squared:  0.1537,    Adjusted R-squared:  0.1127
F-statistic: 3.753 on 3 and 62 DF, p-value: 0.01526

```

```

summary(model2)
Call:
lm(formula = opg ~ herd + feed)

```

Residuals:

Min	1Q	Median	3Q	Max
-6.3057	-2.7146	0.3379	2.2818	8.6812

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	9.9647	0.6167	16.159	< 2e-16 ***
herd	2.2611	0.8080	2.798	0.00681 **
feed	0.9175	0.7714	1.189	0.23877

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 3.107 on 63 degrees of freedom  
Multiple R-squared: 0.12, Adjusted R-squared: 0.09211  
F-statistic: 4.297 on 2 and 63 DF, p-value: 0.01781

summary(model3)

Call:

lm(formula = opg ~ feed)

Residuals:

Min	1Q	Median	3Q	Max
-5.6777	-2.6049	0.4859	1.9558	7.7768

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	10.8692	0.5525	19.67	<2e-16 ***
feed	0.6695	0.8062	0.83	0.409

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 3.269 on 64 degrees of freedom  
Multiple R-squared: 0.01066, Adjusted R-squared: -0.004798  
F-statistic: 0.6896 on 1 and 64 DF, p-value: 0.4094

anova(model2,model1)

Analysis of Variance Table

Model 1: opg ~ herd + feed

Model 2: opg ~ herd + feed + herd:feed

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	63	608.19				
2	62	584.93	1	23.26	2.4655	0.1215

anova(model3,model2)

Analysis of Variance Table

Model 1: opg ~ feed

Model 2: opg ~ herd + feed

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	64	683.78				



2 63 608.19 1 75.599 7.831 0.006806 \*\*  
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**CPG**

cpg feed herd

1	0.000000	0	0
2	0.000000	0	0
3	0.000000	0	0
4	0.000000	0	0
5	0.000000	0	0
6	0.000000	0	0
7	9.259308	0	0
8	0.000000	0	0
9	0.000000	0	0
10	0.000000	0	0
11	0.000000	0	0
12	0.000000	0	0
13	0.000000	0	0
14	0.000000	0	0
15	0.000000	0	0
16	0.000000	0	0
17	0.000000	0	0
18	0.000000	0	0
19	3.237292	0	0
20	0.000000	0	0
21	0.000000	0	0
22	4.301030	1	0
23	0.000000	1	0
24	0.000000	1	0
25	11.314387	1	0
26	10.581096	1	0
27	6.880745	1	0
28	4.214844	1	0
29	0.000000	1	0
30	0.000000	1	0
31	0.000000	1	0
32	0.000000	1	0
33	6.556303	1	0
34	3.623249	1	0
35	0.000000	1	0
36	0.000000	1	0
37	0.000000	1	0
38	0.000000	1	0
39	0.000000	1	0
40	2.301030	1	0
41	0.000000	1	0
42	0.000000	1	0
43	13.165427	1	0
44	0.000000	0	1

```

45 7.870638 0 1
46 12.388356 0 1
47 9.444669 0 1
48 3.869232 0 1
49 0.000000 0 1
50 0.000000 0 1
51 2.778151 0 1
52 8.915927 0 1
53 2.301030 0 1
54 10.465145 0 1
55 3.944483 0 1
56 0.000000 0 1
57 0.000000 0 1
58 2.301030 1 1
59 0.000000 1 1
60 9.294093 1 1
61 6.623249 1 1
62 2.602060 1 1
63 2.602060 1 1
64 0.000000 1 1
65 0.000000 1 1
66 2.301030 1 1

```

feed, herd

```
model1<-lm(cpg~herd+feed+herd:feed)
```

```
model2<-lm(cpg~herd+feed)
```

```
model3<-lm(cpg~feed)
```

```
summary(model1)
```

Call:

```
lm(formula = cpg ~ herd + feed + herd:feed)
```

Residuals:

```

  Min    1Q  Median    3Q   Max
-4.4270 -2.8608 -0.5951  0.5078 10.3046

```

Coefficients:

```

              Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.5951    0.7891   0.754 0.45362
herd         3.8319    1.2477   3.071 0.00316 **
feed         2.2657    1.1032   2.054 0.04422 *
herd:feed    -3.8346    1.8984  -2.020 0.04772 *
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

Residual standard error: 3.616 on 62 degrees of freedom

Multiple R-squared: 0.1402, Adjusted R-squared: 0.09856

F-statistic: 3.369 on 3 and 62 DF, p-value: 0.02401

```
summary(model2)
```

```
Call:
```

```
lm(formula = cpg ~ herd + feed)
```

```
Residuals:
```

```
  Min   1Q Median   3Q   Max  
-4.404 -2.228 -1.258  1.174 10.937
```

```
Coefficients:
```

```
      Estimate Std. Error t value Pr(>|t|)  
(Intercept)  1.2576    0.7350   1.711  0.0920 .  
herd         2.1756    0.9631   2.259  0.0274 *  
feed         0.9708    0.9195   1.056  0.2951  
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 3.703 on 63 degrees of freedom  
Multiple R-squared:  0.08358, Adjusted R-squared:  0.05449  
F-statistic: 2.873 on 2 and 63 DF, p-value: 0.06397
```

```
summary(model3)
```

```
Call:
```

```
lm(formula = cpg ~ feed)
```

```
Residuals:
```

```
  Min   1Q Median   3Q   Max  
-2.860 -2.128 -2.128  1.294 10.305
```

```
Coefficients:
```

```
      Estimate Std. Error t value Pr(>|t|)  
(Intercept)  2.1278    0.6457   3.295  0.00161 **  
feed         0.7322    0.9422   0.777  0.43995  
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 3.82 on 64 degrees of freedom  
Multiple R-squared:  0.009348, Adjusted R-squared: -0.006131  
F-statistic: 0.6039 on 1 and 64 DF, p-value: 0.4399
```

```
anova(model2,model1)
```

```
Analysis of Variance Table
```

```
Model 1: cpg ~ herd + feed
```

```
Model 2: cpg ~ herd + feed + herd:feed
```

```
  Res.Df  RSS Df Sum of Sq  F Pr(>F)  
1     63 864.05  
2     62 810.70  1   53.349 4.08 0.04772 *  
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(model3,model2)
```

Analysis of Variance Table

Model 1: cpg ~ feed

Model 2: cpg ~ herd + feed

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	64	934.04				
2	63	864.05	1	69.992	5.1032	0.02735 *

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Appendix 13 – Herd A calf housing**

**Single pens:**



**Common pens:**





**Appendix 14 - Herd B calf housing**

**Single pens:**



**Common pens:**



## Appendix 15 – Statistical calculations H<sub>0</sub>3 “R”

### Effect of feed on diarrhea

	fec	feed	herd
1	3	0	0
2	0	0	0
3	3	0	0
4	0	0	0
5	0	0	0
6	0	0	0
7	3	0	0
8	0	0	0
9	0	0	0
10	0	0	0
11	0	0	0
12	0	0	0
13	2	0	0
14	0	0	0
15	0	0	0
16	1	0	0
17	1	0	0
18	0	0	0
19	0	0	0
20	0	0	0
21	0	0	0
22	0	1	0
23	0	1	0
24	1	1	0
25	0	1	0
26	0	1	0
27	1	1	0
28	0	1	0
29	0	1	0
30	0	1	0
31	0	1	0
32	0	1	0
33	0	1	0
34	0	1	0
35	1	1	0
36	0	1	0
37	0	1	0
38	3	1	0
39	1	1	0
40	0	1	0
41	1	1	0
42	0	1	0
43	0	1	0
44	1	0	1

```
45 1 0 1
46 1 0 1
47 1 0 1
48 0 0 1
49 0 0 1
50 0 0 1
51 1 0 1
52 0 0 1
53 0 0 1
54 1 0 1
55 0 0 1
56 0 0 1
57 1 0 1
58 0 1 1
59 0 1 1
60 0 1 1
61 0 1 1
62 0 1 1
63 0 1 1
64 0 1 1
65 0 1 1
66 0 1 1
```

```
feed, herd
model1<-lm(fec~herd+feed+herd:feed)
model2<-lm(fec~herd+feed)
model3<-lm(fec~feed)
```

```
summary(model1)
```

```
Call:
```

```
lm(formula = fec ~ herd + feed + herd:feed)
```

```
Residuals:
```

```
Min 1Q Median 3Q Max
-0.6190 -0.5000 -0.3636 0.4702 2.6364
```

```
Coefficients:
```

```
Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.6190 0.1743 3.552 0.000737 ***
herd -0.1190 0.2756 -0.432 0.667214
feed -0.2554 0.2436 -1.048 0.298569
herd:feed -0.2446 0.4193 -0.583 0.561759
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.7986 on 62 degrees of freedom
Multiple R-squared: 0.0612, Adjusted R-squared: 0.01578
F-statistic: 1.347 on 3 and 62 DF, p-value: 0.2673
```

```
summary(model2)
```

```
Call:
```



```
lm(formula = fec ~ herd + feed)
```

```
Residuals:
```

```
  Min    1Q  Median    3Q   Max
-0.6613 -0.4366 -0.3233  0.5072  2.6767
```

```
Coefficients:
```

```
      Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.6613    0.1577   4.194 8.73e-05 ***
herd        -0.2247    0.2066  -1.088  0.2809
feed        -0.3380    0.1972  -1.714  0.0915 .
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.7944 on 63 degrees of freedom
Multiple R-squared:  0.05605, Adjusted R-squared:  0.02608
F-statistic:  1.87 on 2 and 63 DF, p-value: 0.1625
```

```
summary(model3)
```

```
Call:
```

```
lm(formula = fec ~ feed)
```

```
Residuals:
```

```
  Min    1Q  Median    3Q   Max
-0.5714 -0.5714 -0.2581  0.4286  2.7419
```

```
Coefficients:
```

```
      Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.5714    0.1345   4.249 7.1e-05 ***
feed        -0.3134    0.1962  -1.597  0.115
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.7956 on 64 degrees of freedom
Multiple R-squared:  0.03833, Adjusted R-squared:  0.0233
F-statistic:  2.551 on 1 and 64 DF, p-value: 0.1152
```

```
anova(model2,model1)
```

```
Analysis of Variance Table
```

```
Model 1: fec ~ herd + feed
```

```
Model 2: fec ~ herd + feed + herd:feed
```

```
  Res.Df  RSS Df Sum of Sq   F Pr(>F)
1     63 39.760
2     62 39.543  1  0.21706 0.3403 0.5618
```

```
anova(model3,model2)
```

```
Analysis of Variance Table
```

```
Model 1: fec ~ feed
```

```
Model 2: fec ~ herd + feed
```

```
  Res.Df  RSS Df Sum of Sq   F Pr(>F)
```

1 64 40.507  
2 63 39.760 1 0.74657 1.1829 0.2809

**Effect of feed on diarrhea corrected for OPG**

fec feed opgscore  
1 3 0 1  
2 0 0 0  
3 3 0 0  
4 0 0 0  
5 0 0 0  
6 0 0 0  
7 3 0 1  
8 0 0 1  
9 0 0 1  
10 0 0 0  
11 0 0 0  
12 0 0 0  
13 2 0 0  
14 0 0 0  
15 0 0 0  
16 1 0 1  
17 1 0 0  
18 0 0 1  
19 0 0 0  
20 0 0 0  
21 0 0 0  
22 0 1 1  
23 0 1 1  
24 1 1 0  
25 0 1 0  
26 0 1 0  
27 1 1 1  
28 0 1 1  
29 0 1 1  
30 0 1 1  
31 0 1 0  
32 0 1 1  
33 0 1 0  
34 0 1 1  
35 1 1 0  
36 0 1 1  
37 0 1 1  
38 3 1 1  
39 1 1 1  
40 0 1 1  
41 1 1 1  
42 0 1 1  
43 0 1 0

```

44 1 0 1
45 1 0 0
46 1 0 0
47 1 0 1
48 0 0 0
49 0 0 1
50 0 0 1
51 1 0 1
52 0 0 1
53 0 0 1
54 1 0 1
55 0 0 1
56 0 0 0
57 1 0 1
58 0 1 1
59 0 1 0
60 0 1 0
61 0 1 1
62 0 1 1
63 0 1 1
64 0 1 0
65 0 1 1
66 0 1 1

```

```

model1<-lm(fec~opgscore+feed+opgscore:feed)
model2<-lm(fec~opgscore+feed)
model3<-lm(fec~feed)

```

```
summary(model1)
```

```
Call:
lm(formula = fec ~ opgscore + feed + opgscore:feed)
```

```
Residuals:
```

```

  Min   1Q  Median   3Q   Max
-0.7500 -0.4210 -0.2857  0.2500  2.7143

```

```
Coefficients:
```

```

      Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.4211    0.1832   2.299  0.0249 *
opgscore     0.3289    0.2709   1.214  0.2292
feed        -0.2211    0.3119  -0.709  0.4812
opgscore:feed -0.2432    0.4092  -0.594  0.5544
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

Residual standard error: 0.7984 on 62 degrees of freedom
Multiple R-squared:  0.06182, Adjusted R-squared:  0.01642
F-statistic: 1.362 on 3 and 62 DF, p-value: 0.2628

```

```
summary(model2)
```

```
Call:
```

```
lm(formula = fec ~ opgscore + feed)
```

```
Residuals:
```

```
  Min    1Q  Median    3Q   Max
-0.6921 -0.4698 -0.3298  0.3079  2.6702
```

```
Coefficients:
```

```
      Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.4698    0.1629   2.883 0.00538 **
opgscore     0.2224    0.2020   1.101 0.27516
feed        -0.3623    0.2009  -1.804 0.07605 .
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.7942 on 63 degrees of freedom
Multiple R-squared:  0.05647, Adjusted R-squared:  0.02652
F-statistic: 1.885 on 2 and 63 DF, p-value: 0.1602
```

```
summary(model3)
```

```
Call:
```

```
lm(formula = fec ~ feed)
```

```
Residuals:
```

```
  Min    1Q  Median    3Q   Max
-0.5714 -0.5714 -0.2581  0.4286  2.7419
```

```
Coefficients:
```

```
      Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.5714    0.1345   4.249 7.1e-05 ***
feed        -0.3134    0.1962  -1.597  0.115
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.7956 on 64 degrees of freedom
Multiple R-squared:  0.03833, Adjusted R-squared:  0.0233
F-statistic: 2.551 on 1 and 64 DF, p-value: 0.1152
```

```
anova(model2,model1)
```

```
Analysis of Variance Table
```

```
Model 1: fec ~ opgscore + feed
```

```
Model 2: fec ~ opgscore + feed + opgscore:feed
```

```
  Res.Df  RSS Df Sum of Sq   F Pr(>F)
1     63 39.742
2     62 39.517  1  0.22517 0.3533 0.5544
```

```
anova(model3,model2)
```

```
Analysis of Variance Table
```

```
Model 1: fec ~ feed
```

```
Model 2: fec ~ opgscore + feed
```

```
  Res.Df  RSS Df Sum of Sq   F Pr(>F)
```

1 64 40.507  
2 63 39.742 1 0.76445 1.2118 0.2752

## Appendix 16 - Herd A case group milk intake

Kalvefodrings-skema  
CHR: 30000 EJER: Bøje Pedersen

MORGEN:      AFTEN:      (sæt kryds)

Dato	CKR-nr	Mælk tildelt	Mælk tilbagevejet	Kommentar
4	3/3	15633 5L 237g	524g	
	—	15634 5L 0	384g	
	—	15635 5L 125g	0	
	—	15636 5L 137g	342g	
8	4/3	15633 5L 0	1840g	
	—	15634 5L 0	2886g	
	—	15635 5L 0	0	
	—	15636 5L 226g	150	
9	5/3	15636 5L 265	725	
	—	15633 5L 365	1097	
	—	15634 5L 0	1483	
	4/3	15633 5L 897	552	
11	—	15634 5L 769	595	
	—	15635 5L 3068	1630	
	—	15636 5L 1842	2570	
	—	15642 5L 343	0	
	—	15643 5L 847	268	

Kalvefodrings-skema  
CHR: 30000 EJER: Bøje Pedersen

MORGEN:      AFTEN:      (sæt kryds)

Dato	CKR-nr	Mælk tildelt	Mælk tilbagevejet	Kommentar
11	7/3	15633 5L 1352	2011	
	—	15634 5L 312	1981	
	—	15635 5L 3075	3031	
	—	15636 5L 2314	2745	
	—	15638 5L 1610	3027	
	—	15643 5L 357	1650	
12	8/3	15633 5L 2530	4760	
	—	15634 5L 341	3220	
	—	15635 5L 1400	2150	
	—	15636 5L 1220	1090	
	—	15638 5L 1480	1530	
	—	15642 5L 250	340	
	—	15643 5L 372	564	
12	9/3	15634 5L 1210	<del>2810</del> 2810	
	—	15635 5L 1125	<del>788</del> 788	
	—	15636 5L 2140	3402	
	—	15638 5L 486	3290	
—	15633	0	3250	
—	15643	0	596	VFL - køceg faktureret 11/3 14



CHR: 30000 EJER: Bøje Pedersen

MORGEN: AFTEN: (sæt kryds)

Dato	CKR-nr	Mælk tildelt	Mælk tilbagevejet	Kommentar
10/3	15634	5L 292	650	
	15635	5L 1070	2170	
	15636	5L 3270	1100	
	15643	5L 2937	2340	
	15633	5L 0	2670	
	15638	5L 0	486	
	15641	5L 0	274	
	15647	5L 0	587	
11/3	15635	5L 676	1340	
	15636	5L 1940	1590	
	15638	5L 2944	0	
	15642	5L 1164	784	
	15643	5L 2440	2340	
	15645	5L 632	1850	
	15647	5L 730	0	
	15648	5L 2011	1950	
	15649	5L 2142	2240	
		15634	0	670
	640	0	564	
	641	0	1110	

Kalvefodrings-skema

CHR: 30000 EJER: Bøje Pedersen

MORGEN: AFTEN: (sæt kryds)

Dato	CKR-nr	Mælk tildelt	Mælk tilbagevejet	Kommentar	
12/3	15635	5L 734	1060		
	15636	5L 3042	2160		
	15642	5L 2952	1030		
	15643	5L 1030	1470		
	15645	5L 174	1390		
	15647	5L 924	554		
	15648	5L 2021	1312		
	15649	5L 1547	870		
	15641	5L 0	1460		
13/3	15644	5L 0	2130		
	15634	5L 1000	4500	Fodret med sande morgen	
	15635	5L <del>3201</del> 646	3220		
	15636	5L 646	2740		
	15640	5L 310	715		
	15642	5L 1070	2810		
	15643	5L 2250	3220		
	15647	5L 748	3760		
		15638	0	100	
		15641	0	2500	
	15645	0	3100		



### Kalvefodrings-skema

CHR: 30000 EJER: Bøje Pedersen

MORGEN: AFTEN: (sæt kryds)

Dato	CKR-nr	Mælk tildelt	Mælk tilbagevejet	Kommentar
13/3	15648	5L 346	1045	
—	15649	5L 792	650	
—	15651	5L 223	0	
14/3	15634	5L 1670	1150	
—	15635	5L 1000	1400	
—	15636	5L 1390	<del>2600</del> 2600	
—	15643	5L 484	1330	
—	15645	5L 354	<del>2640</del>	
—	15647	5L 1090	1050	
—	15648	5L 3038	1440	
—	15649	5L 226	1550	
—	15641	5L 0	1408	
—	15642	5L 0	1164	
15-3	15634	5L 986	2994	<i>Handwritten note</i>
—	15635	5L 1610	1020	
—	15636	5L 804	0	
—	15641	5L 476	1800	

### Kalvefodrings-skema

CHR: 30000 EJER: Bøje Pedersen

MORGEN: AFTEN: (sæt kryds)

Dato	CKR-nr	Mælk tildelt	Mælk tilbagevejet	Kommentar
15/3	15648	5L 1540	0	
—	15643	5L 2270	886	
—	15645	5L 206	0	
—	15647	5L 960	1300	
—	15648	5L 3194	<del>2920</del> 3450	<i>Handwritten note</i>
—	15649	5L 1170	960	
16/3	15634	5L 1670	1530	
—	15635	5L 1260	1360	
—	15636	5L 1110	1860	
—	15641	5L 1430	1150	
—	15642	5L 630	634	
—	15643	5L 786	980	
—	15647	5L 630	1730	
—	15648	5L 2830	2900	
—	15649	5L 1870	0	
—	15651	5L 1640	1990	
—	15656	5L 1920	2100	



### Kalvefodrings-skema

CHR: 30000 EJER: Bøje Pedersen

MORGEN:      AFTEN:      (sæt kryds)

Dato	CKR-nr	Mælk tildelt	Mælk tilbagevejet	Kommentar
<del>15-3</del>	15652	5L 0	672	
<del>15-3</del>	15645	5L 0	154	
<del>15-3</del>	15656	5L 0	2100	
17-3	15656	5L 1720	3727	fak. til VFL
<del>16-3</del>	15652	5L 728	3086	
<del>16-3</del>	15651	5L 224	4261	
<del>16-3</del>	15649	5L 1730	1464	
<del>16-3</del>	15648	5L 4300	3564	
<del>16-3</del>	15647	5L 140	304	
<del>16-3</del>	15643	5L 1330	0	
<del>16-3</del>	15642	5L 1710	909	
<del>16-3</del>	15636	5L 1200	1146	
<del>16-3</del>	15635	5L 916	3004	
<del>16-3</del>	15634	5L 1480	0	
17/3	15641	5L 0	1091	
—	15655	5L 0	743	
—	15657	5L 0	979	

23

24	18-3	15655	5L 280	476	
		15654	5L 1200	3194	
		15652	5L 751	2650	
		15651	5L 785	3012	
		15649	5L 1947	2930	
		15648	5L 2660	2700	
		15645	5L 297	0	
		15643	5L 378	1220	
		15635	5L 1770	3700	
		15636	5L 482	1190	
		15634	5L 800	0	
		15656	5L 0	1540	
24	19-3	15655	5L 634	916	15633 } 15634 } 15635 } 15636 } 15638 } Fælles boks 19/3
		15656	5L 296	2640	
		15657	5L 124	1940	
		15652	5L 294	3010	
		15651	5L 2470	1300	
		15648	5L 3250	2641	
		15643	5L 1710	0	15640 } 15641 } 15642 } 15643 } 15644 } Fælles 19/3
		15635	5L 840	0	
		15636	5L 1020	0	
		15653	5L 0	3048	
	15649	5L 0	1330		
		5L			
		5L			



25	20/3	15655	5L 636	506	
		15656	5L 614	8024	
		15659	5L 1020	2270	
		15654	5L 354	1060	
		15653	5L 420	2496	
		15652	5L 556	1520	
		15651	5L 2160	0	
		15649	5L 2170	896	
		15648	5L 2500	Død <sup>2/5</sup>	
		15657	5L 0	1090	
24	21-3	15655	5L 485	660	15645
		15656	5L 380	1700	15647
		15659	5L 1045	1870	15649
		15654	5L 2310	2872	15650
		15653	5L 1240	3150	15651
		15651	5L 1120	1860	
		15649	5L 1270	834	
		15652	5L 0	1510	
24		15647	5L 0	1330	
	22/3	15649	5L 1856	2000	
		15651	5L 1630	2500	
		15652	5L 716	3255	
		15653	5L 3798	2500	
		15654	5L 3542	4700	
		5L			

27	22/3	15655	5L 252	2900	
		15656	5L 238	910	
		15659	5L 0	1480	
	23/3	15656	5L 476	1000	
		15655	5L 100	0	
		15654	5L 4500	2200	
		15653	5L 2000	2500	3 liter sonde morgen
		15651	5L 140	1100	
		15652	5L 0	2050	
		15659	5L 0	900	Factura til VFL.
24	24/3	15653	5L 1500	2350	35 liter med sonde morgen
		15654	5L 1500	Død <sup>2/5</sup> Aften	— 17 —
		15651	5L 1725	840	
		15649	5L 2840	1330	
22	25/3	15651	5L 434	0	
		15649	5L 782	0	
		15653	5L Død morgen	0	
22	26/3	15659	5L 336		
		15660	5L 1160	2030	
	27-3	15659	5L 324	0	
		15660	5L 356	0	
		<del>15660</del>	5L	2768	
		-11 2	5L	5030	
		-11-3	5L	2826	
			5L		

22	28-3	15660	5L	920	2468	
		Hyte 2	5L		2184	
29-3	15660	5L	812	1760		
	15661	5L	630	1920		
	Hyt 1	5L	2000	0		
	Hyt 2	5L	1418	0		
	15656	5L	0	1200		
30-3	15656	5L	1000	348		
	1560	5L	1634	1069		
	15661	5L	500	689		
	Hold 1	5L	1460	0		<u>Small adjustment to V/L.</u>
	Hold 2	5L	426	0		
31-3	15659	5L	129			
	15660	5L	76			
	15656	5L	80			
	15661	5L	710			
		5L				
		5L				
		5L				
		5L				
		5L				
		5L				
		5L				