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FACULTY OF HEALTH AND MEDICAL SCIENCES

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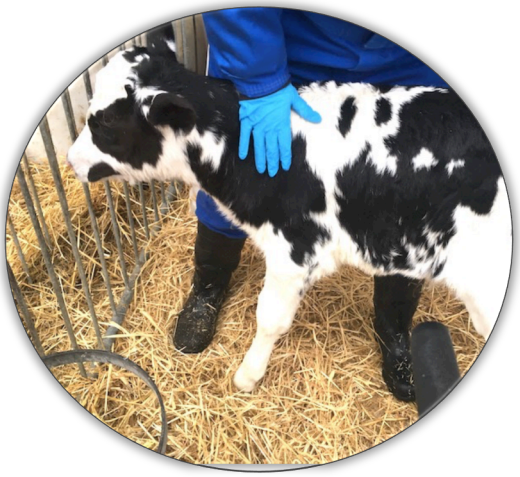
Association between serum IgG Level and clinical signs of gastrointestinal disease in newborn Danish dairy calves



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Preface and acknowledgement

This Master thesis study was conducted as a part of the 'Robust Calves' project carried out in collaboration between the University of Copenhagen, Aarhus University, Technical University of Denmark and the private farmers advising company SEGES. The project is funded by the Danish Cattle Levy Fund and the Danish Milk Levy Fund and was initiated to enhance collaboration and sharing of data and knowledge in research performed on calf health by the involved institutes. The overall aim of the Robust Calves-project is to shed light on the different factors of calf management that result in robust calves and further on healthy and high-producing cows.

The aim of this master thesis was to investigate the potential of reducing the occurrence of gastrointestinal disease by preventing failure of passive transfer. This was done by use of multivariable generalised mixed regression to see the association between IgG levels and predicted probability of clinical signs of gastrointestinal.

To the coordinating research group, I have greatly appreciated the availability and valuable feedback from my supervisor Liza Rosenbaum Nielsen. I would like to direct thanks to Anne Marie Michelsen and Masja Feline Reipurth Søndergaard for organising the many herd visits, during which I have learned a lot. The same goes for all the professional instructions, patience and hospitality of my Co-supervisor Nina Dam Otten, Henrik Læssøe Martin and Bodil Højlund Nielsen. Furthermore I would like to thank everyone in the team for all of their help and input throughout the process.

Special thanks also goes to Matt Denwood, Carsten Thure Kirkeby, Søren Saxmose Nielsen and Nina Breinegaard for their helpful advice about statistical methods, to the farmers enrolled in the project for giving access to their herds and being open to provide background information and insights and to my friends and family for supporting me during the process.

Abstract

Passive transfer of maternal immunoglobulin via colostrum is essential for good protection against pathogens in newborn calves. A lack of maternally derived immunoglobulin has been shown to increase morbidity and mortality in young calves. Failure of passive transfer (FPT) has been studied and examined in many ways as a risk factor for disease susceptibility. Gastrointestinal (GI) disease is one of the biggest health problems for calves in the first week of life. The aim of the research, reported in this thesis, was to investigate the influence that immunoglobulin measured in serum and FPT has on the probability of GI disease, estimated by clinical signs of diarrhoea. The association to fever was invested as well.

This study was based on a cross-sectional sample of 250 calves (n=250) from 9 different herds in Southern Jutland and Zealand, Denmark. The herds were all enrolled in a project called 'Robust Calves' run by the University of Copenhagen, Aarhus University, Technical University of Denmark and the private farmers advising company SEGES. The data were collected during September-December 2018 from 1-10 day old calves. Serum immunoglobulin-G (IgG) levels were measured in calf serum as a measure of passive transfer status. Using a standard protocol, clinical examination scores were used to determine which calves showed signs of GI disease. Environmental information was scored as well. To map the herd-level differences the farmers' answers to a series of questions from BioSecure®, an online questionnaire about biosecurity and information on treatment strategies were collected. Multivariable generalised mixed effects regression was used to predict probability of GI-disease as well as fever. The IgG level was tested on two different scales, one continuous and one dichotomous with a cut-point of 10 g/L IgG in serum to distinguish between FPT and sufficient passive transfer. The model was further tested on an extended sample size including 102 more calves (n=352) from 13 other herds enrolled in the 'Robust Calves'-project where less background information was available.

The results could only confirm an impact of IgG level predicted probability of disease in the extended sample size. Here a significant association between continuous IgG levels and GI disease and between dichotomous FPT and fever was found. There was a large variation in GI disease prevalence between herds suggesting that herd-specific factors such as management and hygiene and treatment strategies may have more important roles in the probability of disease than the respective IgG levels.

Resumé

Maternel immunitet spiller en stor rolle for nyfødte kalves immunforsvar. Hvis kalven får for få antistoffer via råmælken kaldes dette for 'Failure of passive transfer' eller FPT. Denne tilstand er blevet belyst i mange studier og er associeret med øget risiko for sygdom og dødelighed. Et af de største helbredsproblemer hos de helt unge kalve er diarré som følge af mavetarmsygdom. Målet med dette speciale er at belyse betydningen af antistofniveauet målt ved immunoglobulin G (IgG) i serum og FPT for forekomst af mavetarmsygdomme, målt ved kliniske tegn på diarré i den første leveuge. Betydningen for forekomst af feber blev også undersøgt.
Dette studie blev baseret på 250 kalve (n=250) fra 9 malkekvægs besætninger fordelt i Jylland og på Sjælland. Alle besætningerne blev rekrutteret igennem 'Robuste kalve' projektet, som bliver drevet i samarbejde mellem Københavns Universitet, Aarhus Universitet, Danmarks Tekniske Universitet og den private rådgivningsorganisation for landbruget, SEGES. Dataindsamlingen foregik I september-december 2018 på kalve i aldersgruppen 1-10 dage. Blodprøver blev taget til bestemmelse af serum IgG niveau og der blev udført kliniske undersøgelser for tegn på sygdom og miljøscoringer. For at kortlægge risikofaktorer på besætningsniveau blev landmændenes besvarelser på BioSecure ® spørgeskemaet, et online spørgeskema om biosecurity og management anvendt til at se forskelle imellem besætningerne og den besætningstilknyttede dyrlæge blev spurgt ad om behandlingsstrategier. Multivariabel mixed logistisk regression blev anvendt til at undersøge sandsynligheden for sygdomsforekomst af mavetarmsygdomme og feber. Niveauet af IgG blev testet i modellen på to forskellige skalaer; en kontinuert og en dichotom konverteret med FPT defineret ved <10 g/L IgG og værdier derover antaget som et tilstrækkeligt niveau. Modellerne blev herudover testet på en udvidet stikprøve med yderlige 102 kalve (n=352) fra andre besætninger, som var del af 'Robuste kalve' projektet, men hvor færre baggrundsinformationer var tilgængelige.

Resultaterne kunne kun bekræfte associationen mellem IgG niveau og forventet sandsynlighed for diarré eller feber-forekomst I den udvidede stikprøve. Her var der en signifikant sammenhæng imellem kontinuerte IgG målinger og diarré samt imellem FPT og feber. Der var stor forskel på diarré forekomst imellem de forskellige besætninger, som tyder på at besætningsspecifikke faktorer som management og hygiejne, og behandlingsstrategier muligvis har en større indflydelse på risikoen for diarré end IgG niveauet.

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Abbrevations:

FPT	Failure of passive transfer
GI	Gastrointestinal
IgG	Immunoglobulin Class G
HOL	Danish Holstein
RDM	Danish Red
CROSS	Crossbreed of Danish Holstein and Belgian Blue
ELISA	Enzyme-linked immunosorbent assay
RID	Radial Immunodiffusion
AIC	Akaike information criterion
BIC	Bayesian information criterion

Introduction

Background and Purpose

Neonatal calf health is an important element in the life cycle of raising dairy cattle. Healthy calves have better chances of living in good welfare and spare the farmer of additional working hours for special treatment and care. In further perspective, they are also more likely to become productive and robust cows and less likely to cause economic loss. A weak immune system with insufficient amounts of maternally derived antibodies has long been recognised as a predisposing risk factor for disease occurrence (Weaver et al. 2000). One of the main well-known health hazards in the first week of a calf's life is gastrointestinal (GI) disease, which also has been identified as a major risk factor for mortality (Svensson et al. 2006). The purpose of this study was to see if there is a potential to get healthier calves and lower the occurrence of GI disease by means of enhancing passively derived immunity in commercial Danish dairy herds.

Newborn calf immunity

Newborn calves have a naïve immune system. They are not yet able to produce their own antibodies, which are essential for the calf's resilience against pathogens. Therefore they depend on maternal immunoglobulin supplied by so-called passive transfer of immunity through the first colostrum feeding (Barrington & Parish 2001). The uptake of immunoglobulin through the gut wall into the blood stream is only possible for a limited time after birth. Quick and sufficient administration of good quality colostrum is therefore a key factor for successful passive transfer of immunity (Michanek et al. 1989; Godden 2008). Other factors such as dystocia, parity of the dam and season have also been shown to affect the success of passive transfer (Furman-Fratczak et al. 2011; Beam et al. 2009) If the calf is left with only a low amount of maternally derived immunoglobulin, this is termed failure of passive transfer (FPT) and can have great consequences for the future life of the calf. These consequences include a higher risk of illness and death and lower growth rate and productivity later in life (Furman-Fratczak et al. 2011; Denise & Robison 1989; G. Arthur Donovan et al. 1998)

There is little to do about the amount of maternal immunoglobulin absorbed by the calf, once the gut wall has closed. Plasma transfusion of immunoglobulin has been tried to alleviate a deficiency, but it does not appear to be working well (Boccardo et al. 2016). FPT is not a disease in itself but can be considered a piece in a puzzle that eventually can lead to disease. In a study

by Windeyer et al. (2014) the status of passive transfer was shown to have a good negative predictive value for mortality, but a poor positive predictive value. It means that high levels of IgG are associated with a much lower risk of death, but low levels of IgG not necessarily mean the calf is determined to die early. This goes well in line with the assumption that passive transfer is not the only causative factor, but rather a predisposing state making calves more vulnerable to disease. It also implies that there might be something to gain by reducing the number of calves with FPT and appropriately managing calves that are in a high-risk group because of FPT.

Other factors in disease manifestations

To find out why some calves with low maternal immunity get sick and others do not, management procedures and environmental factors should be taken into account (Weaver et al. 2000; Barrington & Parish 2001). The spread of pathogens leading to disease between animals is often facilitated by contact with infected faeces and thus biosecurity and pathogen pressure play a key role (Maunsell & Donovan 2008). Housing type, milk feeding volume and differences in predisposition of dairy breeds and beef crossbreeds have also been suggested as important risk factors for infectious diseases. Additionally, veterinary advice and initiation of different types of treatment can influence the severity and outcome of illness and has to be considered as well (Svensson et al. 2003).

Association of passive immunity and GI disease in calves

A lot of research has been done on the effect of low maternally derived immunity on general morbidity and mortality, without distinguishing between different types of diseases. The aim of this thesis was to look at the specific relation between measures of passive immunity and GI diseases and to investigate whether the overall risk of disease associated with FPT also includes a higher risk for GI diseases. A couple of newer studies have looked at this particular relation. There are many different designations used for GI diseases, including Neonatal Calf Diarrhoea (NCD), enteritis or scours. They are all characterised by causing clinical symptoms of diarrhoea, but can involve different pathogens and have different grades of severity. Some studies found a significant association between FPT and GI disease (Lora et al. 2018; Furman-Fratczak et al. 2011), while others did not find a significant link (Meganck et al. 2015). A study by Boccardo et al. (2017) looked at the consequences of calves with FPT suffering from diarrhoea and found passive transfer status to be a good predictor of case fatality of neonatal diarrhoea. This indicates that calves with FPT would have a more severe degree of diarrhoea in general.

Methods of measuring passive transfer status

How successful the transfer of maternal immunity has been can be measured by different methods. The methods all depend on collection of calf serum but differ in what they measure. The most direct measure is the quantification of immunoglobulin class G, which is generally used to define FPT (Godden 2008). The direct measurement of IgG has to be done in a laboratory and can be done by either Radial Immunodiffusion (RID), which has historically been the gold standard, or by a newer Enzyme-linked immunosorbent assay (ELISA) method (Weaver et al. 2000; Gelsinger et al. 2015). The ELISA method detects the calf's serum IgG by means of anti-bovine antibodies and then calculates the amount by the absorbance level of a conjugated marker enzyme (Bethyl Laboratories). Alternatively, indirect measures by optical refractometry that can be done on site can also be used. The concept of refractometry is to classify a liquid by how it breaks the incoming light and thereby estimating the content of solids within the liquid. This can be used to estimate the serum total protein amount, which is known to be correlated with the serum immunoglobulin content (Tyler et al. 1996). In recent years 'Brix' refractometers have also emerged as an on-farm tool. They work with the same concept but report in a different unit, the so-called brix-percentage (Brix%), calibrated to correlate with IgG content (Deelen et al. 2014).

Classification of FPT

When using either the indirect or direct methods, there have been established several cut-points to categorize calves with a dichotomous outcome of either having sufficient maternally derived immunity or FPT. The commonly recommended IgG cut-point is 10g/L serum and the recommended correlated values for total protein are 5.2 g/dL serum and 8.4 for Brix refractometry (Calloway et al. 2002; Tyler et al. 1996; Deelen et al. 2014). The methods and respective common cut-points are summarised in **Table 1**.

Method	Unit	Cut point
Radial Immunodiffusion	IgG g/L	10
ELISA	IgG g/L	10
Refractometer	Serum Total Protein g/dL	5.2
Brix	Brix %	8.4

Table 1 Methods of measuring passive immunity in calf serum and the corresponding recommended cut points to categorise failure of passive transfer found in literature.

The universal applicability of recommended cut points is debatable, as there is a lot of variation in cut points between different studies as well as discussion about whether they can be transferred from one setting to another (Buczinski et al. 2018) It is also worth mentioning that even though the amount of IgG is commonly accepted as a measure of maternal immunity, looking only at these specific proteins neglect taking into account other immunologically relevant colostrum components like cytokines and leucocytes (Chase et al. 2008). All these differences in analysis and categorisations between studies have to be kept in mind when drawing conclusions about FPT.

Objective

The objective of this study was to investigate the association between serum IgG levels and FPT and the probability of GI disease during the first 10 days of age in Danish dairy calves. The association with fever was tested separately for comparison and as an indicator of systemic disease. The investigation was based on statistical analysis of the association of serum immunoglobulin G content on the occurrence of GI disease in 250 calves aged 1-10 days from 9 commercial dairy herds in Denmark and it was further tested on an extended sample size with an additional 102 calves from 13 different herds.

Material and Methods

Study design

This study was designed as a cross-sectional study with 6 herds in the area of Jutland and 3 herds in the area of Zealand involved. The herds were chosen based on their connection to two weaning calf producers that had been enrolled in the Robust Calves project beforehand.

Calves involved

The sample that was used for base of descriptive analysis, termed the initial sample size, counted 250 calves (n=250), aged 1-10 days. They came from 9 different herds and were Scandinavian Holstein (HOL) breed or Red Danish (RDM) (n=210), though some were crossbred with Belgian Blue (CROSS) (n=40). The sample was chosen based on the herds and further defined by certain exclusion criteria. It was attempted to use a conservative exclusion strategy, to keep the sample size as large and representative as possible, while optimizing the conclusive value of observations by minimizing the risk of confounding. Calves aged 0 and 11 days were excluded on the base of the biological effect of age on IgG levels, which resulted in exclusion of 5 observations. Furthermore, calves with extraordinary high values of Brix% >11 and IgG >45 g/L were excluded on the notion of measurement uncertainty. This excluded further 3 observations. A graphic overview of the 258 calves before exclusion can be found in the appendix p.52

Extended sample size

For the Multivariable generalised mixed effects regression an extended sample size that included 102 more calves from 13 other herds, enrolled in a different part of the 'Robust Calves' project, was generated (n=352). These additional observations do not show in the descriptive part and in the herd level analysis because of the limitations in available information and in the extent of this thesis. The purpose of including them was to gain an impression of how extending sample size affects the conclusive value. The same rules of exclusion were applied for the extended sample size, which resulted in additional exclusion of 1 more calf with IgG-measures above 45 g/L. Furthermore 20 calves from 7 herds with below 5 observations were also excluded as it was considered too few observations per herd. A graphic overview of the 381 calves before exclusion can be found in the appendix p.54

Calf level analysis

Clinical signs and individual environmental scores as well as blood samples for immune status evaluation were collected as a base for statistical analysis of disease outcome.

Clinical examinations

Clinical examinations were performed following a protocol (see appendix p.45) developed in collaboration between the Robust Calves project participants to score several clinical indications that suggest either respiratory, GI or other diseases. Two veterinarians engaged in the Robust Calves project were responsible for scoring the calves (One on Zealand and one in Jutland). The calves' housing and environment were also scored on different parameters and noted at the time of clinical examination. All information was recorded on tablet an uploaded to a project database ('EasyOn') developed by Aarhus University.

Drinking tray hygiene

One of the environmental scores noted was the drinking tray hygiene. This was used as a marker for hygiene and an increased biosecurity risk. A drinking tray could be scored as either clean (0) or dirty (1) indicating the water to be unclear and/or with slimy coating and/or the presence of biofilm or manure.

Definition of disease

Two categories of sick calves (GI disease and fever) were defined for the purpose of being able to investigate relations between IgG-levels and clinically ill calves. These two categories were defined based on clinical signs, as listed in Table 2.

	Min. One of the following indications:
GI disease	-Faecal sample was watery and/or had heavy amounts of mucus or fresh or coagulated blood -At least 25 % of the calfs' surface is soiled
Fever	Rectal temperature >39.5

Table 2 Criteria for the disease definitions of GI disease and Fever used for analysis within the study

Sample collection

Blood samples for IgG extraction were collected by jugular puncture into 10 ml EDTA vacuum tubes. They were centrifuged at 4500 RPM for 4 minutes with an EIKEMEYER® standard table top centrifuge, after which the serum was extracted into 5 ml Eppendorf tubes and cooled

until frozen later for transport to the Foulum research facility, where IgG was quantified by Bovine IgG ELISA quantitation set and by Brix refractometry. The ELISA results were used as the default measure in statistical analysis within this thesis as they are the most direct measure of IgG levels and passive immunity.

Definition of FPT

For the statistical analysis, the information on IgG levels was used to create a new dichotomous variable for FPT. The cut point of 10 g/L IgG was used for this, so that calves below this point would be categorised as having FPT.

Herd level analysis

The information collected on herd level was used as a descriptive tool for a better understanding of the farm effect on individual calf variables. For the sake of anonymity, the herds are represented by herdlabels A-I. An attempt to map some of the key differences between farm management practices has been made by including a series of questions on treatment strategies and a biosecurity of the farms involved in this thesis.

Information on treatment strategies

The veterinarian clinic used by each of the respective farms was contacted after the herd visits in order to gather knowledge on treatment procedures and herd diagnoses. The aim was to get a picture of possible confounding because of treatment differences on herd level.

Biosecurity questionnaire

The online BioSecure® questionnaire (www.biosecure.dk) was developed in collaboration between the University of Copenhagen, Danish Technological Institute and the farmer advising companies SEGES and Sagro with the purpose of evaluating calf management practices on farm in relation to biosecurity. It was used on every farm enrolled in the Robust Calves project to provide knowledge on the biosecurity and management status of the sampled animals. The questionnaire had to be filled out electronically by the person responsible for calf handling at each respective farm. Because it is a very long questionnaire, covering many sections and specific questions, only selected questions are presented in this context. These are picked based on the criteria that they are related to the very early life of the calves and might have a causal relation to health status other than passive immunity status. Since there were only 9 farms enrolled, the sample size limits the possibility to draw conclusions at farm-level. A few of the

questions were only related to heifer calves, but were still included as an example of factors worth considering.

Statistical methods

Data extraction and descriptive analysis

The information provided by the clinical and environmental scores was extracted from the database and analysed by use of the statistical software R (version 3.5.2). Analysis of Variance (ANOVA) was used for univariate analysis, to look at potential confounding effects of IgG-levels of Herd, Breed and Sex. Linear regression was used to estimate correlation between measurement methods and to see whether there was an effect of age alone or age and herd combined on IgG-levels.

Statistical modelling

Multivariable generalised mixed effects regression modelling was used to investigate the relation between disease status and IgG levels combined with other relevant factors for the two defined diseases outcomes; GI-disease and Fever. The model was used on the initial 250-calf sample and additionally also on the extended sample size, as described previously, to see if the results were affected by sample size. The primary priority of the model was to investigate the impact of the maternal immunity on disease outcome. IgG level was therefore included as a fixed effect in two variants on either continuous or dichotomous scale using a FPT cut point. This resulted in two versions of the model per disease and an additional two versions for the extended sample size. A number of fixed effects were chosen on the base of assumed relevance and then tested in the model. The initial fixed effects were: Breed – either purebred HOL/RDM or CROSS, Age on registration, Sex and Drinking tray hygiene. To account for potential cluster-effects of calves within the same herd, herd was added to the model as a random effect.

To optimise the model, the initial fixed effects were tested in the model by backwards selection. This was based on the initial sample size by use of the Akaike information criterion (AIC) and Bayesian information criterion (BIC) that estimate the relative quality of information provided by the data. The continuous variables of Age and IgG level were transformed using the poly() function in R, creating orthogonalised variables of first and second degree to allow for a quadratic fit of the curve. The transformation was only kept if the quadratic variable was significant on a 0.05 significance level. Figure 1 shows a graphic overview of how the different final models were built.

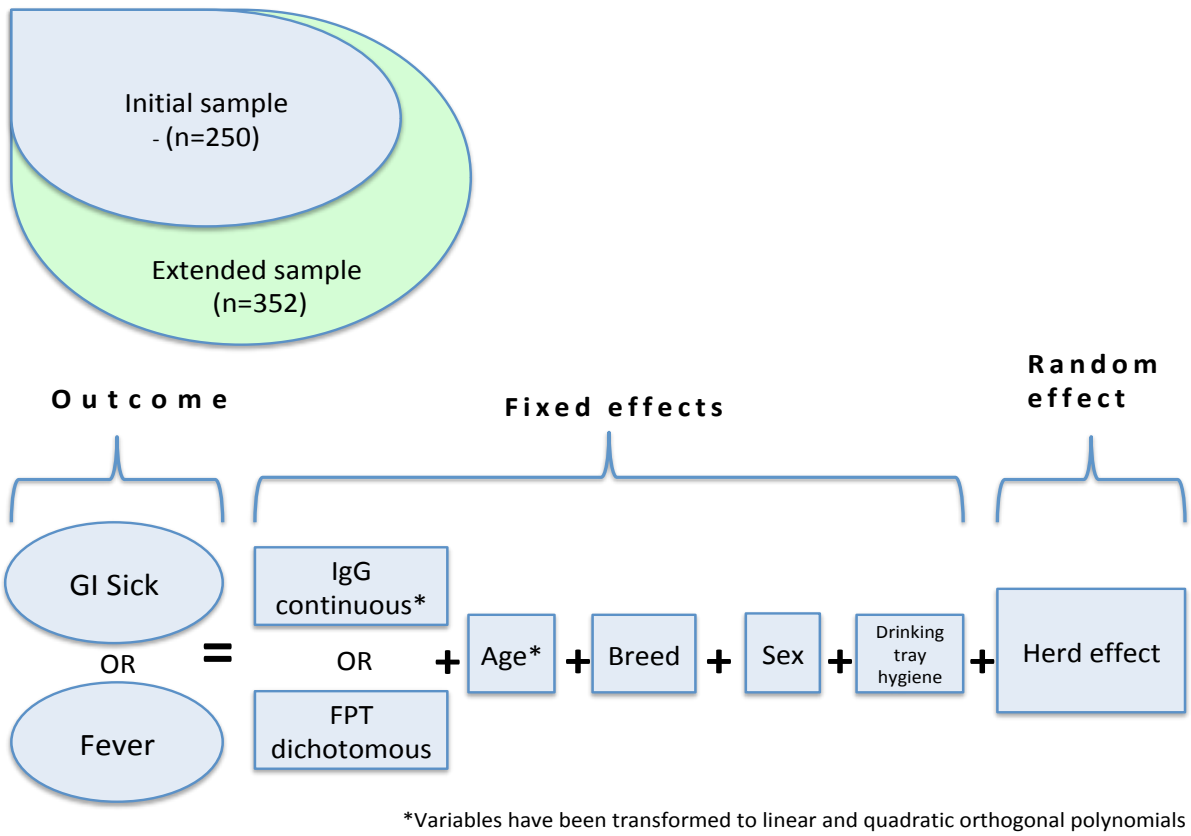

Figure 1 Overview of the elements used to build the final disease probability prediction models with the outcome of gastrointestinal disease and fever.

Noise characterisation

To test the final model for influence of IgG value tendencies on GI disease and fever on the results in this particular study, different curve fittings were tried out. This was done by a natural spline (ns()) function which allowed for the curve to have more flexibility and observe tendencies. Natural splines are piecewise interpolations with a number of different cubic functions that are connected at boundary knots and linear at the ends. The more knots that are allowed, the more the graph will fit the exact pattern of the data.

The biological hypothesis of IgG effect that was investigated in this thesis is based on the assumption a linear or quadratic relationship, or in other words a relatively simple declining probability of disease with increasing IgG levels. The alternative curve fittings are sample specific and have more complex functions fitting the line closer to the observations. They are thus not fit to be used as a base for conclusion on the population level. However, they can be helpful in clarifying what stands in the way of finding a potentially simpler connection, by characterising the sample specific noise.

Results

IgG levels, passive transfer status and disease prevalence

The passive transfer status and mean IgG values for calves that were scored either GI Sick or healthy or having fever (Yes/No) is summarized in Table 3. A higher proportion of GI sick calves had FPT compared to the healthy ones. The same tendency showed for calves with fever. The fraction of calves that were categorized as having FPT and were GI sick was 13 % points higher than the total average of 30% calves with FPT. The fraction of calves that had fever and FPT was 15% points higher than the total average of calves with FPT. The Mean IgG values were -3.4 g/L lower for GI Sick calves and -1.4g/L for calves with fever compared to the total average of 16.9 g/L. The extended sample size had very similar tendencies. An overview hereof can be found in the appendix (p58).

Variable	Score	Calves total	FPT (%)	Mean IgG g/L
GI disease	Sick	40	17 (43%)	13.5
	Healthy	207	57 (28%)	17.5
Fever	Yes	29	13 (45%)	15.5
	No	221	62 (28%)	17.1
TOTAL		**250**	**75 (30%)**	**16.9**

Table 3 Overview of 250 calves from 9 Danish dairy herds categorised by gastrointestinal disease and fever occurrence with failure of passive transfer (FPT) defined by <10 g/L IgG and Mean IgG- levels

The prevalence of disease and measures of passive immunity across the investigated variables were summarized in Table 4. The range of prevalence was biggest within the Herd variable compared to the other 3 variables. GI disease occurrence deviated relatively little from the total average of 16% in the first three variables, Sex, Breed and Drinking tray hygiene with a maximum ±3% points for breed. For Fever occurrence in the same three variables there was a maximum deviation of ±6% points for breed. Herd differences for GI disease and Fever differ up until ±19% points for GI disease in Herd F and ±12% points for Fever in Herd G and H. The prevalence of FPT likewise stayed relatively closer to the total average in the first three variables, deviating by max ±8% points for the variable Drinking tray hygiene, while deviating up until ±25% points between Herds for Herd G. The mean IgG values also had the biggest range between herds (min:14.1;max:21.6), while being close to the total average of 16.9 g/L in the other three variables(min:16.5;max:17.3).

Variable	Score	Calves total	GISick (%)	Fever (%)	FPT (%)	Mean IgG g/L
Sex	Heifer	133	19 (14%)	12 (9%)	33 (25%)	17.3
	Bull	117	21 (18%)	17 (15%)	42 (36%)	16.5
Breed	HOL/RDM	210	35 (17%)	22 (10%)	64 (30%)	16.9
	CROSS	40	5 (13%)	7 (18%)	11 (28%)	17.3
Drinking tray hygiene	0 (clean)	173	27 (16%)	20 (12%)	56 (32%)	17.2
	1 (dirty)	65	10 (15%)	7 (11%)	14 (22%)	16.9
Herd						
	A	18	6 (33%)	4 (22%)	5 (28%)	21.6
	B	21	3 (14%)	3 (14%)	5 (24%)	19.0
	C	43	7 (16%)	9 (21%)	16 (37%)	13.8
	D	35	2 (6%)	5 (14%)	14 (40%)	16.6
	E	40	4 (10%)	2 (5%)	5 (13%)	20.2
	F	40	14 (35%)	4 (10%)	14 (35%)	12.8
	G	11	3 (27%)	0 (0%)	6 (55%)	14.1
	H	23	0 (0%)	0 (0%)	6 (26%)	18.5
	I	19	1 (5%)	2 (11%)	4 (21%)	19.5
TOTAL		**250**	**40 (16%)**	**29 (12%)**	**75 (30%)**	**16.9**

Table 4 Overview of disease and failure of passive transfer (FPT) prevalence defined by <10g/L serum IgG and mean IgG levels of Calves defined in groups of variables: Sex, Breed, Drinking tray hygiene score and herd of 9 Danish dairy herds (A-I)

A graphic overview of the IgG level difference of healthy and sick calves in the two disease categories can be seen in **Figure 2** and **Figure 3**, where it is evident that even though the overall mean IgG level for sick calves is lower, this tendency was not visible within all herds. Some even showed an opposite effect with high IgG levels in calves that were categorised sick or febrile.

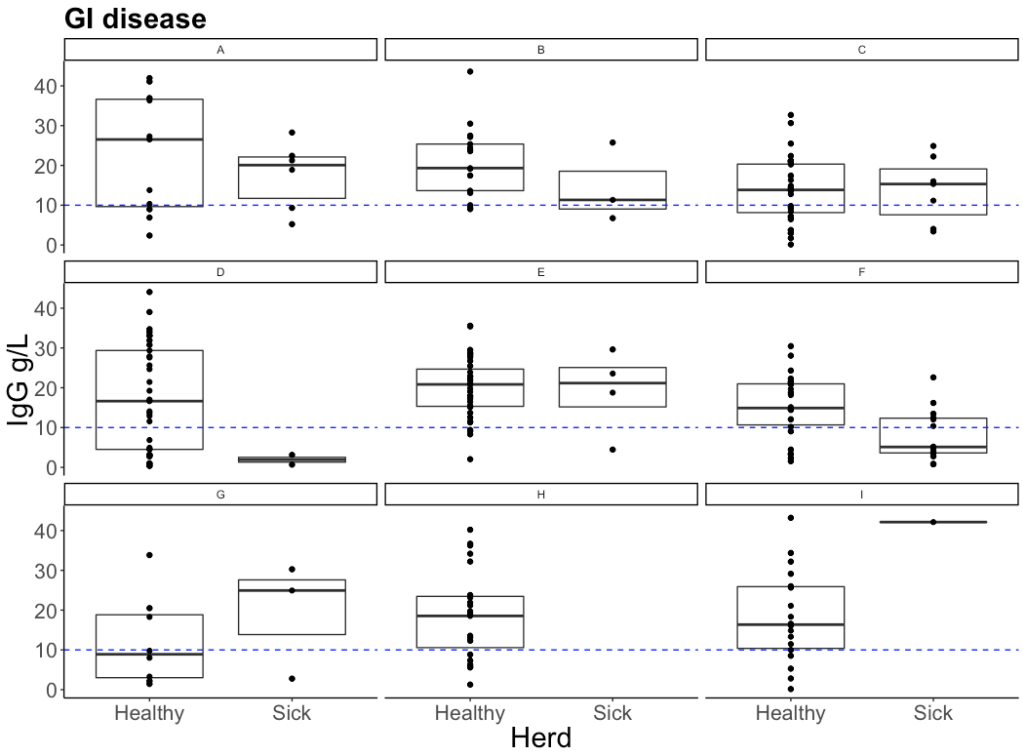

Figure 2 Boxplots of the IgG level distribution at herd level in 250 calves from 9 Danish dairy herds (A-I) with 3 not available observations removed, divided into two groups categorised as either GI sick or GI healthy. The dashed blue line indicates the 10 g/L IgG cut point for categorisation of failure of passive transfer.

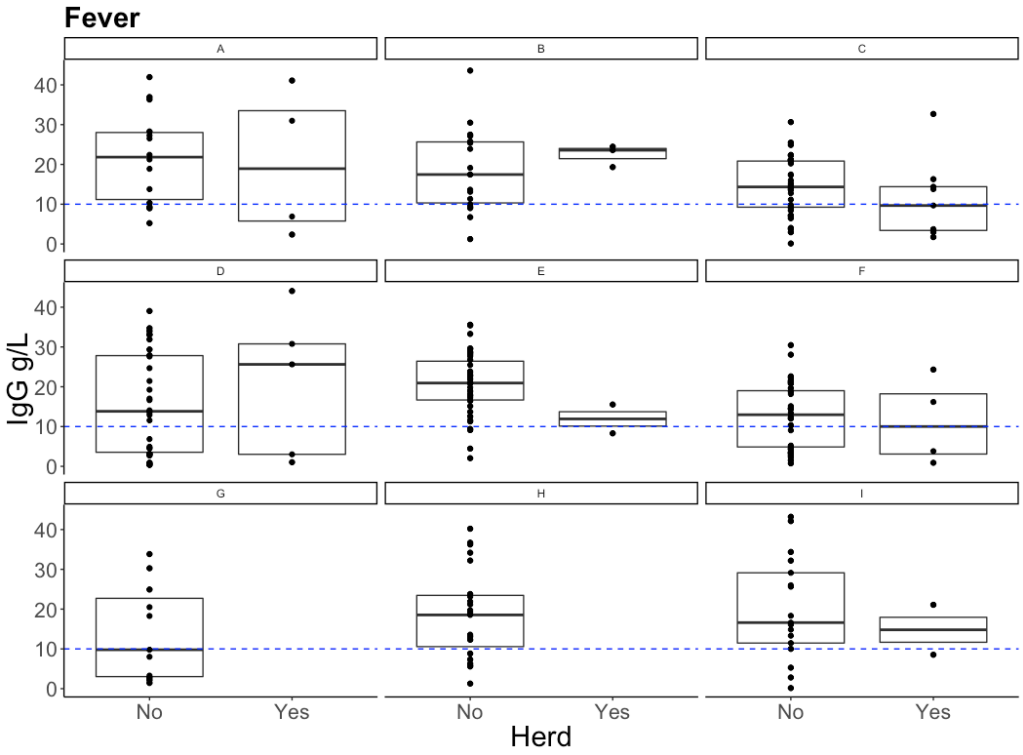

Figure 3 Boxplots of the IgG level distribution at herd level in 250 calves from 9 Danish dairy herds (A-I), divided into two groups of calves categorised by whether they had fever (yes/no). The dashed blue line indicates the 10 g/L IgG cut point for categorisation of failure of passive transfer.

Effect of herd and age on mean IgG

There was a significant effect of herd alone on IgG confirmed by analysis of variance (P=0.0197). Linear regression of the IgG level as a function of age alone pointed towards a declining correlation, but this was not significant (P=0.067, see appendix p. 55)

To take age and herd-effect into account simultaneously, a combined linear model with IgG as a function of herd+age was built. **Figure 4** shows a graphic illustration of this function. It shows that that the estimated IgG lines have different levels, which substantiates the significant finding of Herd-effect on IgG levels. The herds were held up against herd A, the herd with the highest mean of 21.6 IgG g/L, as a reference value. In this comparison the Herd C and F were significantly lower (P=0.010 and P=0.004). The estimates and corresponding p-values of the combined linear model can be found in the appendix(p.57) The effect of Age was even less significant in this model (P=0.326). The graphic illustration also shows that the herd-specific slopes are varying and in some cases even have a positive incline (Herd C, F and G), which explains how a general declining effect of age on IgG during the first 10 days of life becomes less significant in this model and cannot be confirmed on this base.

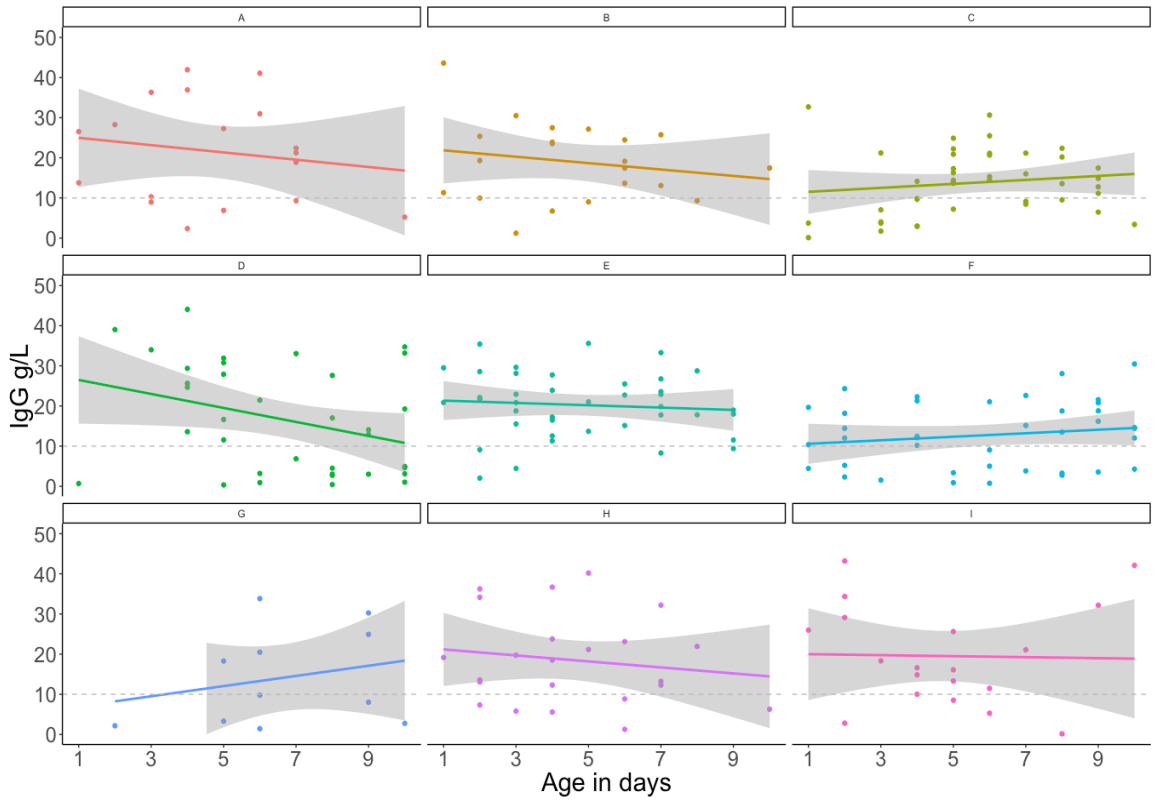

Figure 4 Graphic illustration of the the linear function of Herd+Age on IgG levels of 250 calves from 9 Danish dairy herds (A-I), the dashed grey line indicates the cut-point value of 10 g/L IgG used to categorise failure of passive transfer

Effect of Sex and Breed on mean IgG

The distribution levels of IgG on Sex and Breed was analysed by analysis of variance. These were not significantly different (P-values 0.82 for breed and 0.55 for Sex – see appendix p. 56 for graphic illustrations)

Correlation of ELISA and Brix measurements

A simple linear model estimated the correlation between the two methods of measurement, ELISA and Brix refractometry. Brix% is shown as a function of IgG values in **Figure 5**. The p-value for the slope was highly significant <0.001 and the correlation was good (Adjusted R^2=0.75). The predefined cut point of 10 g/L IgG was used to find the correlating value of Brix-percentage in our dataset. The correlating value was 7.9 Brix% which is indicated on the graph. For the further analysis the direct IgG measurements derived by ELISA were used.

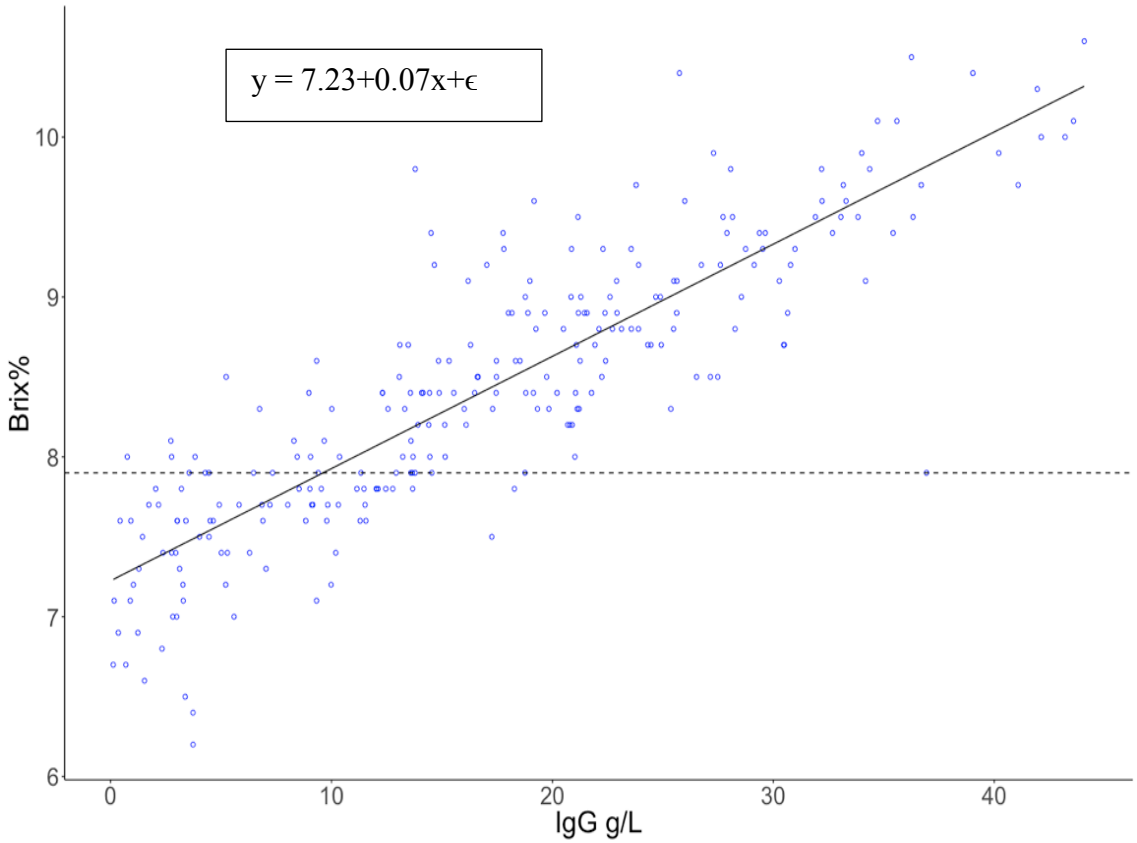

Figure 5 Graphic illustration of the linear correlation of IgG and Brix% measures derived by ELISA and Brix refractometry methods respectively. The fitted line is shown along with the corresponding equation. The grey dashed line indicates the cut-point value of 7.9 Brix% correlated to the 10 g/L IgG used to categorise failure of passive transfer

Herd level descriptive analysis

Biosecurity

BioSecure® questions were selected with the purpose of pointing out some of the relevant differences between the enrolled farm management practices. The questions and answers were translated and summarised in **Table 5**.

Question			
Calving facilities inside	Only individual calving pens 1 (C)	Only group calving pens 6 (A,D,E,G,H,I)	Individual and group calving pens 2 (B,F)
Are some calves born outside?	Yes, some of them 2 (A,I)	No 7 (B,C,D,E,F,G,H)	
Are calving pens also used for sick animals	Yes 1 (G)	No 8 (A,B,C,D,E,F,H,I)	
How are heifer calves fed colostrum	Colostrum is administered and the calf is not allowed to suckle the dam 1 (D)	Colostrum is administered and the calf can also suckle the dam 7 (A,B,C,E,F,H,I)	Calves only get Colostrum by suckling the dam 1 (G)
Is the udder always clean when the calf is allowed to suckle the dam?	Yes, always 1 (G)	Usually 6 (A,B,C,E,F,I)	No 1 (H)
Do you use a colostrum bank	Yes, freezer 6 (B,C,E,F,G,I)	Yes, fridge and freezer 1 (A)	No 2 (D,H)
How often is colostrum from dams with mastitis used?	Regularly 1 (I)	Rarely 5 (A,B,C,E,G)	Never 3 (D,F,H)
Do dams get vaccinated against certain pathogens?	Yes 1 (D)	No 8 (A,B,C,E,F,G,H,I)	
Do heifer calves get milk replacer?	Yes 8 (B,C,D,E,F,G,H,I)	No, never 1 (A)	

Do heifer calves get whole milk, of the same quality that is delivered to the dairy?	Yes, during the entire milk feeding period 4 (E,C,B,G)	Yes, sometimes 1 (F)	No, never 4 (A,D,H,I)
Is the whole milk heat treated/pasteurized?	Yes, during the entire milk feeding period 2 (D,E)	No, never 7 (A,B,C,F,G,H,I)	
How often do heifer calves receive milk with elevated cell counts?	Daily 5 (A,D,F,H,I)	- 4 (B,C,E,G)	
How many litres of milk do heifer calves get a day under normal weather conditions during week 1?*	4 -5L 2 (F,H)	6-7L 6 (A,B,D,E,G,I)	8L 1 (C)

* ***The answers have been changed after a follow-up on the initial questionnaire, because the question had been misunderstood at first.***

Table 5 Summary of the included answers to the BioSecure® questionnaire with counts of how many herds chose the same respective answer and indication of how the herds were distributed by included herdlabels in brackets from 9 Danish dairy herds.

Treatment background

The herd-affiliated veterinarian was contacted after the herd visits took place and asked to answer the following questions on treatment status and herd diagnoses of each of the 9 respective farms:

1. *Does the farm have explicit problems of diarrhoea in calves <14 days of age?*
2. *What relative threshold does the given farm have for treatment of sick calves? (Low/Medium/High)*
3. *Are there herd diagnoses for GI diseases and/or diarrhoea for calves <14 days of age?*
4. *Which prescribed medications does the farmer administrate for GI sick calves?*
5. *How old is the calf typically when treatment is initiated?*

The answers can be found in Table 6.

Question:	*1*	*2*	*3*	*4*	*5*
Herd A	Yes	High	Yes	Tylosin	6 days
Herd B	Yes	Low	Yes	Parofor*	4-5 days
Herd C	No	Medium	Yes	Parofor*	5 days
Herd D	No	Low	Yes	Norodine/Metacam	5 days
Herd E	No	Low	Yes	Dihydrostreptomycin	7 days
Herd F	Yes	High	Yes	Clamoxyl/Metacam	5-10 days
Herd G	No	Low	Yes	Norodine/Metacam	3-14 days
Herd H	No	Low	Yes	Parofor	5-8 days
Herd I	Yes	High	Yes	Synolox/Metacam	7-14 dage

**Treatment was phased out during sampling period*

Table 6 Overview of herd affiliated veterinarians' answers to questions (1-5) about treatment status and herd diagnoses for calves in 9 enrolled Danish dairy farms(A-I).

Multivariable statistical analysis

Multivariable generalised mixed effects regression modelling was performed to analyse the effect of IgG level and chosen fixed and random variables on disease outcome. Whether fixed effects were kept in the model was evaluated by their effect on the AIC and BIC value outcome. The AIC and BIC values turned out to be consistently higher when taking Breed into the model for both disease outcomes, suggesting that the model would be better without it. Breed was thus excluded from the final model. The effect of Sex did increase the AIC and BIC value when tried in the GI-models but did the opposite in the Fever models, so it was only excluded from the GI-model.

The drinking tray hygiene score did show conflicting results when included in the model and could in some cases not be fitted. It was therefore considered whether there could be a problem of overlapping with the herd-effect, meaning that the drinking tray hygiene was really correlated to the specific herd and thus could not be used as a calf level measure. A proportional calculation of the drinking trays scored dirty on herd level revealed that there were indeed great differences in drinking tray scoring between herds (see appendix p. 58). It was thus decided to leave the variable out and rely on the random herd effect to provide the information to account for biosecurity risks on farm level.

The continuous variables, IgG and Age, were transformed to fit a quadratic function when put into the model, to allow the curve to bend for a better fit. The transformation was performed by the poly() code in R, creating orthogonalised polynomial variables of specified degree one (linear) and two (quadratic). The orthogonalised transformation counteracts the high correlation of variables in different polynomial degrees derived from the same original variable. This is useful when they are supposed to be in the model at the same time, but it also means that their estimates cannot be directly interpreted. The P-values still relate to the original variable. The transformed variables were only kept if the quadratic variable was significant in either sample size, indicating that the quadratic function was useful for the model fitting. If the quadratic variable was not significant, the non-transformed original variable was used instead because it could be directly interpreted. The marginal and conditional R^2 values, herd variance and variable estimates with p-values, Odds Ratio and corresponding 95% confidence intervals are summarised in the respective result tables. The transformed continuous values are shown without Odds Ratio and corresponding 95% confidence intervals, as these are not directly interpretable. Some observations were excluded because of missing values. The final number of included observations is noted in the results. Graphic illustrations of the predicted probability curve of the models isolating the effect of either continuous age or IgG with predetermined conditions within the other variables are found alongside the result tables.

The continuous and dichotomous scaled IgG model was tried on the two sample sizes and with the prediction outcome of either GI Sick or Fever positive calves. A key to the eight different model versions can be found in Table 7.

Model	Outcome	IgG scale	Sample size	Graphic illustration	Result table
1	GI disease	continuous	Initial	**Figure 6**	Table 8
1.e	GI disease	continuous	Extended	**Figure 7**	Table 9
2	GI disease	dichotomous	Initial	**Figure 8**	**Table 10**
2.e	GI disease	dichotomous	Extended	Appendix p.59	
3	Fever	continuous	Initial	**Figure 9**	Table 11
3.e	Fever	continuous	Extended	Appendix p.60	
4	Fever	dichotomous	Initial	**Figure 10**	**Table 12**
4.e	Fever	dichotomous	Extended	**Figure 11**	**Table 13**

Table 7 Overview of the eight different generalised mixed regression model versions. An individual number was assigned to each combination of the varying model conditions

Model 1, n=247, GI disease with continuous IgG scale						
Marginal R^2		0.04				
Conditional R^2		0.11				
Random effect	***Variable***	***Variance***		***SD***		
	Herd	***0.59***		***0.77***		
Fixed effects	***Variable***	***Estimate***	***se***	***p-value***	***OR***	***CI 95%***
	Intercept	-1.30	0.44	***		
	IgG g/L	-0.04	0.02	0.068	0.97	0.93-1.00
	Age_linear	4.71	2.77	0.090		
	Age_quadratic	3.50	2.79	0.209		
: significant <0.05 **:significant<0.01 *:significant<0.001*						

Table 8 Model 1 Results of generalised mixed regression with continuous IgG level for probability prediction of GI disease based on 250 calves from 9 Danish dairy herds

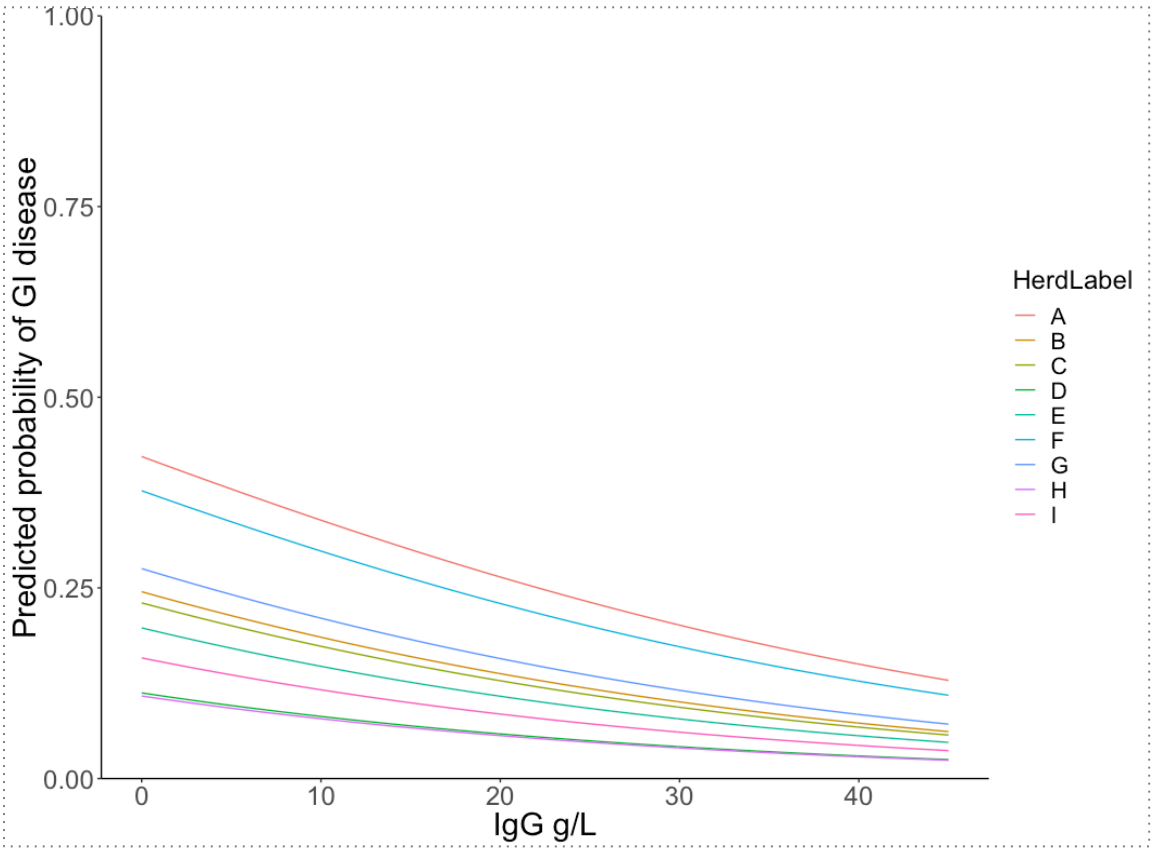

Figure 6 Model 1 : Graphic illustration of the effect of IgG level on predicted probability of GI disease based on 250 calves from 9 Danish dairy herds (A-I). The Age is set to mean age on day of registration and sampling (5 days)

Model 1.e, n=349, GI disease with continuous IgG scale						
Marginal R^2		0.05				
Conditional R^2		0.13				
Random effect	***Variable***	***Variance***	***SD***			
	Herd	***0.52***	***0.72***			
Fixed effects	***Variable***	***Estimate***	***se***	***p-value***	***OR***	***CI 95%***
	Intercept	-1.08	0.32	***		
	IgG g/L	-0.03	0.02	0.046*	0.97	0.94-1.00
	Age_linear	7.49	2.68	0.005**		
	Age_quadratic	5.46	2.67	0.041*		
: significant <0.05 **:significant<0.01 *:significant<0.001*						

Table 9 Model 1.e: Results of generalised mixed regression with continuous IgG level for probability prediction of GI disease based on 352 calves from 22 Danish dairy herds

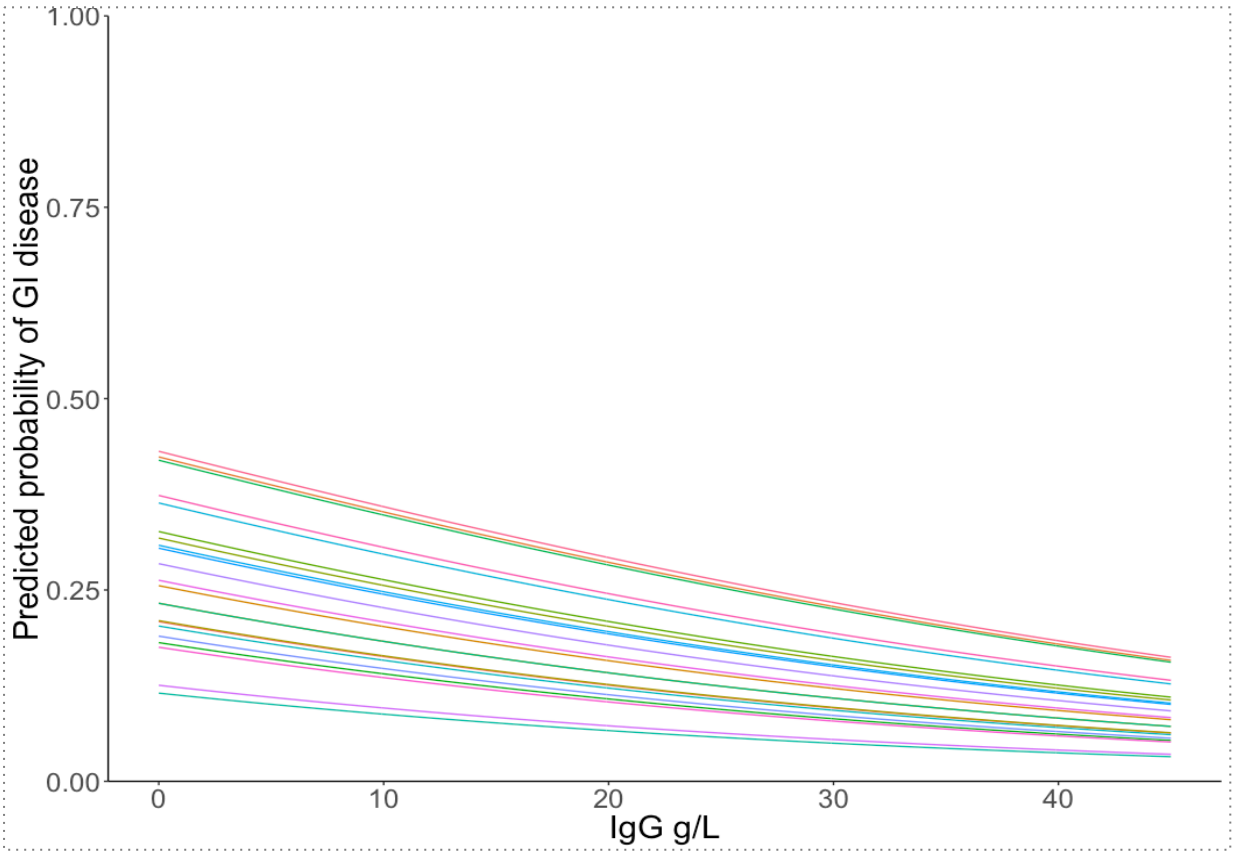

Figure 7 Model 1.e : Graphic illustration of the effect of IgG level on predicted probability of GI disease based on 352 calves from 22 Danish dairy herds. The Age is set to mean age on day of registration and sampling (5 days)

Model 2, n=247, GI disease with dichotomous IgG scale						
Marginal R^2	0.03					
Conditional R^2	0.10					
Random effect	***Variable***	***Variance***	***SD***			
	Herd	0.60	0.77			
Fixed effects	***Variable***	***Estimate***	***se***	***p-value***	***OR***	***CI 95%***
	Intercept	-2.10	0.37	***		
	FPT +	0.63	0.38	0.095	1.88	0.88-3.98
	FPT -	0			1	
	Age_linear	4.85	2.76	0.079		
	Age_quadratic	3.53	2.77	0.203		
: significant <0.05 **:significant<0.01 *:significant<0.001*						

Table 10 Model 2: Results of generalised mixed regression with dichotomous IgG level (cut point of <10g/L IgG categrosing failure of passive transfer) for probability prediction of GI disease based on 250 calves from 9 Danish dairy herds

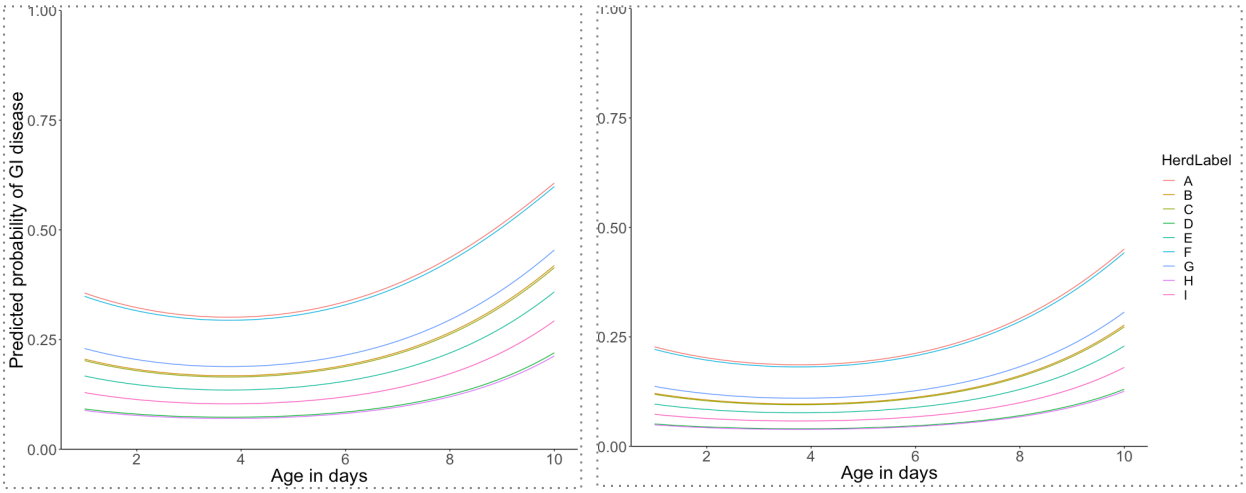

Figure 8 Model 2: Graphic illustration of the age effect on predicted probability of GI disease based on 250 calves from 9 Danish dairy herds (A-I). The graph is set to the precondition of calves having failure of passive transfer (serum IgG<10 g/L) on the left and adequate passive transfer on the right.

GI disease prediction models

Association between GI disease and IgG on continuous scale

Model 1) The model on initial sample size showed no significant association of any variables other than the intercept (see table Table 8). The quadratic IgG variable was not significant and was thus excluded, the quadratic age variable was kept because it became significant when extending the sample size. The IgG variable was close to being significant (P=0.068). The prediction curve displayed in **Figure 6** gives the impression that increasing IgG levels resulted in lower probability of GI disease, though it is not significant. There was a profound difference between marginal and conditional R^2 indicating a considerable variation in baseline risk on herd-level.

Association between GI disease and IgG on dichotomous scale

Model 2) The dichotomous model showed no significant p-values of any variables other than the intercept (see **Table 10**). The FPT variable got relatively close to being significant (P=0.095) and so did the linear age variable (P=0.079). The different relative level of the herd curves in **Figure 6** illustrates that within this model the calves that do have FPT do generally had higher risk of GI disease, but this is tendency is not significant. The shape of the slope in **Figure 8** also suggests that the effect of increasing age also increased the probability of GI disease. The same large difference between marginal and conditional R^2 indicates that the herd effect accounted for a large part of the disease probability prediction.

Results of GI disease models with extended sample size

When the same models were tested on the extended sample size the continuous IgG variable became significant (P=0.046) in Model 1.e (shown in Table 9) and so did the linear and quadratic age variables. The estimate for the continuous IgG variable was -0.03 with an OR of 0.97 and a corresponding 95% confidence interval of [0.94-1.00]).

The dichotomous IgG variable did not become significant in the extended sample size in Model 2.e either, but the linear and quadratic age variables did. The corresponding table and figure of model 2.e can be found in the appendix p.59.

Model 3, n=250, Fever with continuous IgG scale						
Marginal R^2	0.05					
Conditional R^2	0.07					
Random effect	***Variable***	***Variance***	***SD***			
	Herd	***0.17***	***0.41***			
Fixed effects	***Variable***	***Estimate***	***se***	***p-value***	***OR***	***CI 95%***
	Intercept	-1.89	0.64	***		
	IgG g/L	-0.01	0.02	0.511	0.99	0.95-1.03
	Age	-0.06	0.08	0.471	0.94	0.80-1.10
	Sex:Bull	0.56	0.41	0.169	1.75	0.81-4.90
	Sex:Heifer	0			1	
: significant <0.05 **:significant<0.01 *:significant<0.001*						

Table 11 Model 3: Results of generalised mixed regression with continuous IgG level for probability prediction of fever based on 250 calves from 9 Danish dairy herds

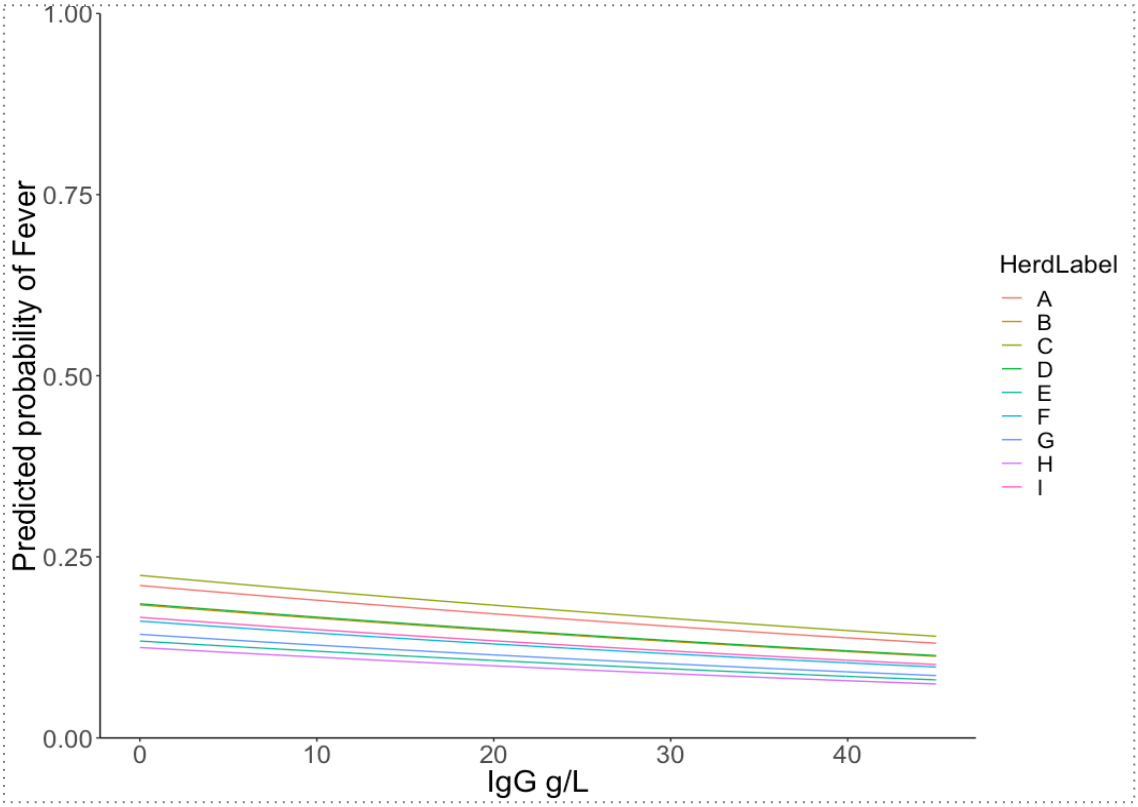

Figure 9 Model 3 : Graphic illustration of the effect of IgG level on predicted probability of fever based on 250 calves from 9 Danish dairy herds (A-I). The Age is set to mean age on day of registration and sampling (5 days) and Sex is set to bull calves

Model 4, n=250, Fever with dichotomous IgG scale						
Marginal R^2	0.02					
Conditional R^2	0.03					
Random effect	***Variable***	***Variance***	***SD***			
	Herd	***0.14***	***0.38***			
Fixed effects	***Variable***	***Estimate***	***se***	***p-value***	***OR***	***CI 95%***
	Intercept	-2.29	0.54	***		
	FPT +	0.66	0.41	0.109	1.94	0.85-4.36
	FPT-	0			1	
	Age	-0.06	0.07	0.475	0.94	0.81-1.10
	Sex:Bull	0.50	0.41	0.224	1.65	0.74-3.81
	Sex:Heifer	0			1	

: significant <0.05 **:significant<0.01 *:significant<0.001*

Table 12 Model 4: Results of generalised mixed regression with dichotomous IgG level (cut point of <10g/L IgG categorising failure of passive transfer) for probability prediction of fever based on 250 calves from 9 Danish dairy herds

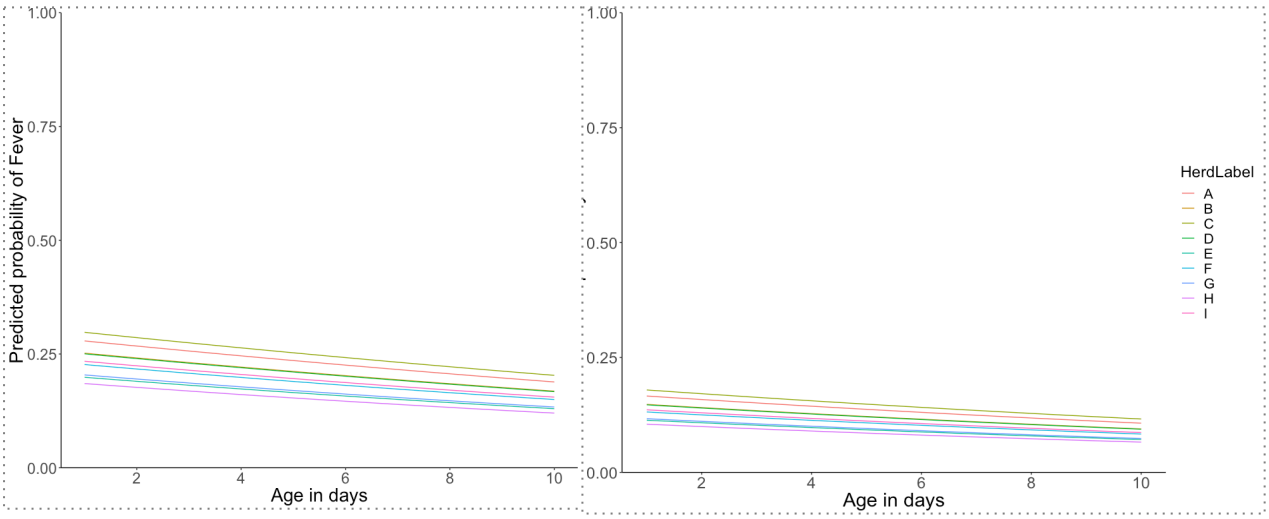

Figure 10 Model 4: Graphic illustration of the Age effect on predicted probability of Fever based on 250 calves from 9 Danish dairy herds (A-I). The graph is set to the precondition of bull calves having failure of passive transfer (serum IgG<10 g/L) to the left and adequate passive transfer to the right.

Model 4.e, *n = 352, Fever with dichotomous IgG scale*						
Marginal R^2		0.02				
Conditional R^2		0.05				
Random effect	***Variable***	***Variance***	***SD***			
	Herd	***0.33***	***0.57***			
Fixed effects	***Variable***	***Estimate***	***se***	***p-value***	***OR***	***CI 95%***
	Intercept	-1.97	0.44	***		
	FPT +	0.72	0.33	0.026*	2.06	1.08-3.94
	FPT -	0				
	Age	-0.05	0.06	0.451	0.95	0.84-1.08
	Sex:Bull	0.13	0.32	0.696	1.13	0.60-2.15
	Sex:Heifer	0				

: significant <0.05 **:significant<0.01 *:significant<0.001*

Table 13 Model 4.e Results of generalised mixed regression with dichotomous IgG level (cut point of <10g/L IgG categorising failure of passive transfer) for probability prediction of fever based on 352 calves from 22 Danish dairy herds

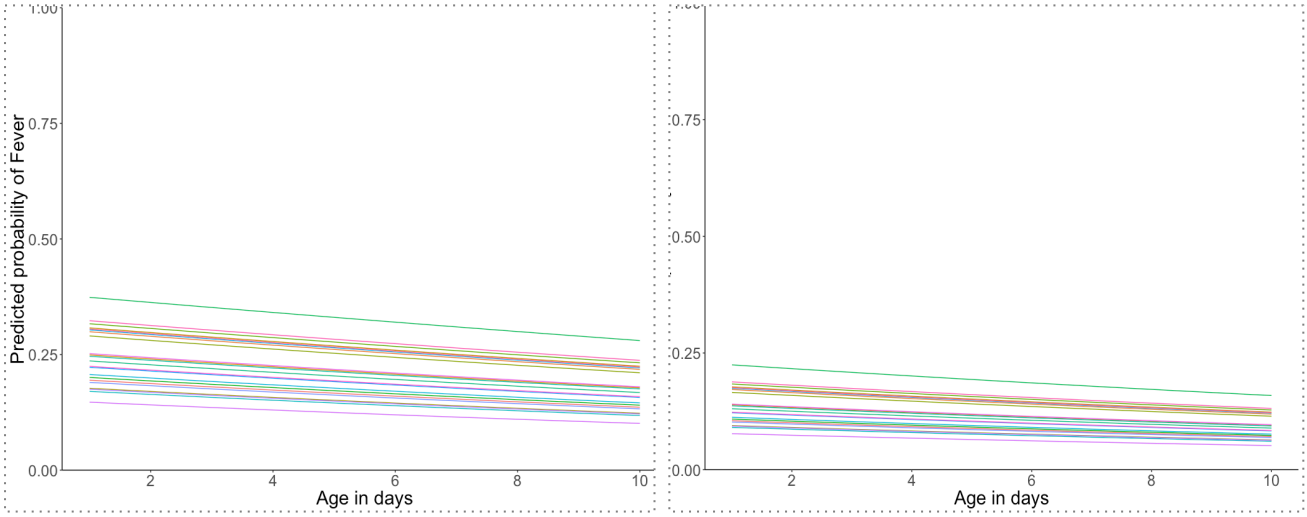

Figure 11 Model 4.e: Graphic illustration of the age effect on predicted probability of Fever for 352 calves from 22 Danish dairy herds. The graph is set to the precondition of bull calves having failure of passive transfer (serum IgG<10 g/L) to the left and adequate passive transfer to the right.

Fever prediction models

Association between Fever and IgG on continuous scale

Model 3) The fever model on initial sample size only had a significant p-value for the intercept (see Table 11). The quadratic IgG variable was almost significant (P=0.069) but was excluded from the model. The original IgG variable that was used instead does not come close to being significant at all (P=0.511). The graphic illustration in **Figure 9** also does not have a very steep slope. This suggests that probability of fever was not very affected by continuous IgG level. The effect of Sex did not show significance.

Association between Fever IgG on dichotomous scale

Model 4) In the dichotomous fever model on initial sample size the FPT variable was not significant (P=0.100) (see **Table 12**). Neither were any of the other included variables except for the intercept. The marginal and conditional R2 were lower than in the model with continuous IgG, indicating that the variables in this model had less explanatory value. In the graphic illustrations the change of the FPT outcome from true to false (**Figure 10**) did result in a lower level of overall risk, but as mentioned this was not significant. The slight decrease in the slope indicates that increasing age lowered the probability of fever

Results of fever models with extended sample size

With the extended sample size the continuous IgG variable was still not significant in model 3.e but the dichotomous IgG variable in the model 4.e was (**Table 13**) The effect of having FPT suggested an increase in probability of fever with an estimate of 0.72 (P=0.026) and an OR of 2.06 an 95% confidence interval of: [1.08-3.94]. The model still had a very low Marginal and Conditional R^2 though. The corresponding result table and figure of model 3.e can be found in the appendix p.60

Noise characterization within the sample

A natural spline function was used to observe tendencies of a probability prediction curve that had more flexibility than the investigated continuous IgG models. This sort of intentional over fitting was done for the purpose of examining noise within the data influencing the results. The graphic illustrations of these functions for model 1 and 3 be found in the appendix p.61.

Model 1) In the GI model 1 with continuous IgG levels it appeared that a more complex curve would not decline steadily, but instead plateaus on relatively high values of IgG, before eventually going down. This suggests that there might be calves with relatively high IgG values that skew the overall assumed decline in probability because of high IgG values. The illustration of this model can be found in the appendix p.61

Model 3) In the fever model 3 the curve had a strong tendency of becoming U-shaped (see appendix p.61), meaning that both very low and very high values of IgG do result in higher risk of disease.
It suggested that the continuous IgG variable might be better fitted with a quadratic function. This explains how the effect of continuous IgG levels shifted from almost being significant in the quadratic variable to a quite high p-value of 0.51 of the untransformed variable in the final model. It indicates that the linear model is losing a lot of information and that the even slope is a consequence of extreme tendencies in both ends of the scale.

Discussion

The objective of this study was to determine the association between the IgG level on either continuous or dichotomous scale with a set cut point of 10 g/L IgG indicative for FPT on the probability of calves showing clinical signs of GI disease or fever. The hypothesis behind the investigation was that calves with lower amounts of maternally derived immunity would be less resilient against infections resulting in higher risk of developing disease.

Association between IgG and GI disease

Based on the results of this study it was only tentatively possible to conclude that a lower level of IgG is associated with a higher predicted probability of calves showing clinical signs of GI disease during the first 10 days of life with multivariable generalised mixed effects regression. There was no significance of IgG on either continuous scale or dichotomous scale detected with the initial sample size of 250 calves from 9 Danish Dairy herds, which indicates that there must be other important determinants of GI disease in calves. In the descriptive results it was found that IgG levels are not consistently lower in the GI sick calves across all herds in the initial sample size. There were some calves categorized as having GI disease with very low IgG values while others had relatively high values (Figure 2). These tendencies were further highlighted in the noise characterization with alternative curve fittings, shedding light on the mathematical reasons why the model could not conclude a clearer effect of IgG levels with the data available. The lack of findings in the initial sample size are in line with some other studies that reported passive transfer to be a poor predictor of GI disease (Windeyer et al. 2014; Meganck et al. 2014) These studies did however only look at the effect of a dichotomous categorised FPT definition.

There was a significant effect of the continuous scale IgG level variable when tested on the extended sample size of 352 calves from 22 herds. The change in significance from the initial to the extended sample size shows the influence of sample size and underline the importance of having enough observations to be able to reject a Null-hypothesis of no association. The result from the extended sample size model 1.e implied an estimated effect of -0.03 decrease in probability for every increase of 1 unit in IgG g/L and an OR of 0.97 with a 95% confidence interval of [0.94-1.00]). This would mean that an increase of 1 unit in IgG g/L resulting in an OR of 0.97 compared to a reference of 1. It is not a big shift for an increase of only 1 unit in IgG level and the confidence interval is very close to 1 as well. If it were seen in the context of a

bigger increase, like a calf potentially getting 20g/L serum IgG instead of 10 g/L, the OR would decrease to 0.74. The increase would thus have to be of a certain size so that the effect would be noteworthy. The result of the extended sample size continuous IgG model support the findings of other studies that looked at the relation between IgG and GI disease did find a significant connection (Lora et al. 2018; Furman-Fratczak et al. 2011) These studies looked at IgG on either continuous scale or a differentiated FPT categorisation with 4 intervals which allows for a more detailed interpretation.

The effect of herd was considerably high, as can be in the difference between marginal and conditional R_2, suggesting that the herd effect plays a relatively bigger role than the fixed effects in the risk of disease. This underlines the complex nature of disease manifestation with many underlying factors involved (Meganck et al. 2015) and implies an important role of management and prevention practices (Lorenz et al. 2011).

Association between IgG and fever

Based on the results of this study an association between low levels of IgG and higher predicted probability of fever in calves during the first 10 days of life could only be confirmed partly.
When testing the association by multivariable generalised mixed effects regression, having lower IgG levels on continuous scale did not show an increase in the probability prediction of fever in either sample size

The dichotomous scaled model only showed a significant effect of FPT for the extended sample size of 352 calves from 22 herds. Having FPT in the extended sample size resulted in an OR of 2.06 with a 95% confidence interval of: [1.08-3.94]. This would mean that a calf with fever had approximately twice the odds of being from the FPT group. Finding studies documenting a relationship between IgG levels and fever was difficult, which could very well be because fever is only a symptom and not a disease in itself. The relationship could thus be underlying to a lot of other investigations of morbidity and specific diseases. One study was found that had fever as a criteria for systemic disease, for which it found a significant association to passive transfer status (G Arthur Donovan et al. 1998). In the descriptive part of the study the IgG levels are not consistently lower in calves with fever than in calves without fever across herds. The alternative curve fittings revealed a skew in the data, caused by febrile calves with very high IgG values.

Noise within the sample and limitations of probability curves

Adding more flexibility to the prediction curves in the probability models can reveal some interesting patterns in the dataset that was otherwise lost by the investigation of an assumed simple linear or quadratic relationship (Durrleman & Simon 1989). Especially the probability curves of the continuous IgG fever model seemed to be limited by the restriction of being linear. It could thus be argued that exclusion of the quadratic variables due to lack of significance should be revised. However, including it would result in a graph that shows a higher probability of fever for both very low and very high levels of IgG. This has to be seen in context of the dataset, which is based on relatively few observations of fever sick calves (29 out of 250 calves). So this tendency could very well be over fitted and not representative reality. The investigation of the continuous IgG relation with GI disease also shows that the lack of significance could be caused by some calves with relatively high IgG levels hat still are at high risk of getting GI disease. This poses the question whether it's worth to look at the high IgG values in calves with clinical signs of GI disease or fever that skew the trend and ask how they came about and whether there are confounding factors that could be corrected for.

Calf level confounders

On calf level the difference in age at registration of the animals has been taken into account, which turned out to be an important factor to account for as it became highly significant when tested on the extended sample size of the GI disease models. The registrations were only done once per calf without any follow-up meaning that changes in disease status prior or after might have been missed. The effect of Sex was only improving the fever model but did not show a big effect, while breed seemed to be even less important, despite some previous studies showing an effect (Svensson et al. 2003). The marginal $R^{2,}$ indicating the percentage of what the fixed effect explains, is not very high compared to the conditional R^2 in any of the models. It is thus likely that other variables, which could provide valuable information, have not been included. One candidate variable to consider could be the season, or weather conditions on registration day. Another could be which pathogens are present in the calf's environment and what pathogen pressures the calves is under (Torsein et al. 2011). As a part of the part of the 'Robust Calves' project, samples for specific pathogen identification and estimation of pathogen load have been taken during the herd visits. The results of these samples could have been good to take into account, but they were not analysed in time for inclusion in this thesis. This type of information might overlap with herd level confounders if all calves within a herd are exposed to the same environment.

Herd level confounders

On herd level, the differences in management and biosecurity might have caused confounding due to a lot of factors not corrected for that might be relevant in risk of disease development (Maunsell & Donovan 2008). The disease prevalence varied a lot between herds, with some farms having 0% calves categorised as being sick, while others had up to 33% sick. Besides the mentioned potential of differences in pathogen presence in herds, the answers to the biosecurity questionnaire and questions about treatment also revealed some differences in management factors that could play a role in the observed variation. Some farms replied that they had individual calving pens, while others had group calving pens or let their cows calve outside. Their risk of being exposed to pathogens might have been affected by these differences in calving facilities (Pithua et al. 2009) dam vaccination protocols and as well as hygiene around feeding and pasteurisation practices of colostrum and milk (Armengol & Fraile 2016; Godden 2008). Whether they were fed whole milk or milk replacer and the quantity they were given per day might have affected the nutrition status and disease susceptibility of calves (Lorenz et al. 2011) Additionally, the fact that all farms had herd diagnoses of diarrhoea problems and different treatment strategies with medication that the farmer administrates himself could have potentially been an important confounder. This could be true on both calf and herd level as some of the farms may have treated all calves with antibiotics, according to the herd veterinarians. It creates uncertainty whether the clinical registrations give an accurate picture of how many calves were or would become sick. The typical start of treatment of calves was within the age span of our registrations. This means that calves that were registered at higher age could potentially have already been treated and thus the clinical signs may be masked.

Even though the herd effect was included as a random effect, looking at a single farm would mean more homogeneity in the preconditions of the calves. This could have helped in determining a more precise effect of the IgG level, but of course it would also have the downside of being only applicable to that one farm and set of given preconditions. Another problem with correcting for management and treatment is also that there are differences within each farm e.g. when a farm has both individual and group calving pens or uses milk for feeding from cows with mastitis that could transmit pathogens (Villarroel et al. 2007) on some days only. To address these issues it could be considered to include as many management and treatment factors as possible on calf level even though this might mean a more complicated study design.

Clinical and environmental registrations and disease definitions

Fever was easier defined than GI disease, since it is based on only one objective measure of temperature by thermometer. It can however still be masked by administration of anti-inflammatory medication. Registration of clinical signs that were the foundation of the GI disease definition in this study had a greater risk of being biased by the assessment of the individual observer. This uncertainty could also be true for the environmental score of drinking tray hygiene. The consistency of faeces and the size of soiled fur area as indicators for GI disease are measures depending on an evaluation that is not as easily quantified. The soiled area furthermore is an indirect measure and does not ensure that the calf has or has had diarrhoea, as it can have gotten soiled from other sources. The clinical registrations were focussed on getting an overall picture of health status, but for the purpose of this study it might have been beneficial to focus more on specific GI related signs. A way to do this could be to include hydration status or creating more options in the scoring of faecal samples. This could be differentiating between watery, bloody or mucous consistency.

Confounding of IgG values by Age, Breed and Sex and Herd

In this study there was no significant confounding effect detected in the investigation of age, breed and sex. However, this might not be transferable to other settings based on previous examples of significant findings in literature (Villaroel et al. 2013). Like with the cut points for classification, there seems to be no consensus in recommendations of age at measurement. Several studies use samples of calves aged 1-7 days to study passive immunisation (Windeyer et al. 2014; Filteau et al. 2003). Within these studies the age effect was tested and did not show any effect on the mean IgG level. Based on the biological background knowledge, it is worth considering age as a possible confounding factor. At first, the calf must have time to fully absorb the colostral-supplied immunoglobulins before they will show in the bloodstream. This will result in a peak level, expected to occur at approximately 24 hours after birth. From thereon the serum immunoglobulin content will gradually decline due to biological degradation until the calf is about 2-4 weeks of age before onset of endogenous production (Burton et al. 1989; Husband et al. 1972). This would mean that measures on day 7 or later would be relatively lower than on day 1. However, according to some studies looking further into it there seems to be only a slight decreasing slope and a good correlation between repeated measurements over the first week in the same calf allowing to compare calves across the first 2 weeks or even longer (Hancock 1983; Furman-Fratczak et al. 2011). There was a significant difference in IgG level on herd-level,

indicating that some might have more successful colostrum management strategies than others (Godden 2008).

Reliability of ELISA and Brix methods

The values of IgG and Brix% measured by different methods show an overall good association. But there are also some values that stray from the linear correlation. Within both methods, it is important to be critical whether the measurements are really reliable and represent what we want them to. The advantage of indirect measuring by either serum total protein or Brix % is, that it is cheaper and easily done with a handheld refractometer on site, but with the downside that it has limitations to accuracy (Topal et al. 2018). Looking at the Brix% it is important to remember that it is an indirect measure and that refractometry does not distinguish between which proteins they measure. A weakness in conclusive value of high measurements has been pointed out, due to the fact that they can be confounded by dehydration or infection status, causing a rise in albumin and acute phase proteins. This concern has been validated for indirect testing by refractometry only, while dehydration seems to actually be connected to lower IgG values by direct testing (Fecteau et al. 2013). The ELISA measurements do have the benefit of being a more direct and presumably valid measure of maternally derived IgG, but can also show false results if something goes wrong in the dilution process (Bethyl Laboratories). Double analysis by the ELISA method was introduced quite late in the trial period and could also be a good practice to add to future studies. Only 3 calves with extreme high values were excluded, because they diverted too much from the rest of the measurements and were not considered reliable. Looking at our models however, it is clear that there were still some sick calves with high IgG and Brix% values that were not in line with what was expected to see based on previous studies an literature. Even though the model could probably be better without these calves, there was not found enough reason to exclude them. More information about infection or hydration status and studies of possible confounders of the IgG measures derived by ELISA would be needed to get clarity on the reliability of high values.

Correlating cut points

The values derived by Brix refractometry had a correlating cut point of 7.9 Brix% to the standard 10 g/L IgG derived by ELISA. This is 0.5%-points lower than the recommended Brix cut point found in the literature (Deelen et al. 2014). This indicates that recommended cut points, especially for indirect measures should be used with caution. This is not very surprising based on the great variety of cut points found in other studies (Buczinski et al. 2018).

Furthermore, the variety might also mean that there is not one particular minimum or cut point value, which is perfect for distinguishing whether a calf has a sufficient passive transfer or not. This goes for indirect as well as direct measures. Considering the herd variety in disease prevalence and the corresponding mean IgG levels it is apparent that some farms with considerably low IgG status manage to have a low disease status as well. The best cut point for these respective farms may lie lower than for others, because they somehow are able to make up for low IgG status by management or other factors.

Use of continuous or dichotomous scales

This poses the question whether the use of cut points is a good idea or whether it is preferable to the continuous scale. The recommended cut points have been criticised for being either too low or too high. A range of other cut points, have been proposed in other studies, pointing towards a general problem with determining and transferring cut points for both direct and indirect methods, as there might be a different optimal level in different settings and for different outcome investigations. Using a cut point to create a dichotomous scale for classification of FPT can have some advantages over the use of a continuous IgG scale, though. It is easier to interpret and draw conclusions when there is a point that clearly defines what is insufficient. For instance, farmers and veterinarians can use cut points as a quantitative tool to evaluate if management practices are working well in terms of providing calves with passive immunity. It can also be used to determine which calves are in a high-risk group because of FPT and thus more susceptible to disease. The downside to this dichotomization however is that it could be an overly simplified categorisation that leads to invalid conclusions. Some argue that there is not a big change in biological effect when a certain minimum level has been reached, which would justify the use of classification (Weaver et al. 2000). Another approach suggested is thus to use a scale of different intervals to differentiate between complete and partial FPT and sufficient or very good passive transfer, which could be used as a compromise allowing more detailed categorisations (Furman-Fratczak et al. 2011).

Conclusions

The initial investigation did not demonstrate any significant association between serum IgG level on either continuous or dichotomous scale and fever or clinical signs of GI disease in the 250 calves from 9 Danish dairy herds. Extending the sample size by 102 calves from 13 other herds led to the finding of some significant associations, though.

The results of the extended sample size showed a significant decrease in predicted probability of GI disease with increasing IgG levels on continuous scale. The identified association implied that a substantial increase in IgG levels was needed before an effect on OR would be notable. When the continuous IgG measures were transformed into a dichotomous variable, differentiating only between FPT (<10 g/L IgG) and sufficient passive transfer, the significance for GI disease was lost. It suggested that the dichotomous categorisation might not be a good tool for interpretation of GI disease probability prediction.

The extended sample size also showed a significant increase in predicted probability of Fever with the occurrence of FPT on dichotomous scale. This connection was not found with the continuous scaled IgG. Few observations of febrile calves with some of them having unusually high IgG values presumably caused these conflicting results. The lack of an approximate linear relation in the study specific sample led to questioning the foundation of the investigated association. Accuracy and possible confounders in analysing methods for passive transfer status could be an issue. Further investigation and double analysis should thus be considered.

The effect of herd had more explaining value for the predicted probability of disease than the other included factors, suggesting that herd specific management and biosecurity as well as treatment and pathogen presence may have played a more important role than the respective IgG levels. For future studies a larger sample size could be recommended to get more information on the relation between IgG and diseased animals, as well as correcting for the stated concerns.

Perspective

Based on the study presented in this thesis, testing calves for FPT on farms does not seem to be an effective tool in predicting which would be in high risk of developing GI disease within the first 10 days of life. Monitoring overall serum IgG values on herd-level might still be useful to evaluate whether there is a potential for improvement of colostrum management. This could be used as part of a preventative strategy of GI disease, but other measures should be taken into account too.

It should be considered that health status could be a potential confounder of some methods of measuring passive transfer and that this relation should be clarified in the course of finding valid associations.

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Appendix

Clinical Protocol

Overview of the clinical protocol and the relevant sections for registrations within this study:

Robuste kalve – Klinisk protokol

I protokollen gennemgås de enkelte scores – både hvordan de skal udføres og hvordan de skal scores. Hvor ikke andet er angivet, er billederne i protokollen taget af Mari Reiten (AU), Kristoffer Eriksen, (SEGES) eller projektdeltagere (Mette Bisgård Petersen (KU), Per Spleth (SEGES), Henrik Læssøe Martin (SEGES) eller Bodil Højlund Nielsen (AU)).

Hvor ikke andet angivet er scorerne udarbejdet til projektet i samarbejde mellem projektdeltagere.

Scores og beskrivelser passer til registreringssystemet EasyOn, som vil blive benyttet til indtastning.

AU, Foulum 31. august 2018

Observation	Nummer/ *Number*	Type	Aldersgruppe/*Group*				
			Malkekvæg 1 uge *Dairy 1 week*	Malkekvæg 3 uger *Dairy 3 week*	Malkekvæg 3 måneder *Dairy 3 months*	Slagtekalve 2 uger e. inds *Veal 2 weeks*	Slagtekalve 3 mdr gamle *Veal 3 months*
Boks_ID *Pen ID*	1	Miljø	X	X	X	X	X
Antal kalve i boks *Number of calves in pen*	2	Miljø	X	X	X	X	X
Opstaldning *Type of pen*	3	Miljø	X	X	X	X	X
Opstaldning sted *Location of pen*	4	Miljø	X	X	X	X	X
Vandforsyning *Access to water supply*	5	Miljø	X	X	X	X	X
Vandtildeling, hygiejne *Water, cleanliness*	5a	Miljø	X	X	X	X	X
Fodertildeling, hygiejne *Cleanliness, food provision*	6	Miljø	X	X	X	X	X
Adgang til hudpleje *Access to grooming*	7	Miljø		X	X	X	X
Belægningsgrad, areal *Stocking density, area*	8	Miljø	X	X	X	X	X
Andel af areal med tørt, rent leje *Clean and dry bedding in resting area*	9	Miljø		X	X	X	X
Næse flåd *Nasal discharge*	10	Klinisk	X	X	X	X	X
Øre-/hovedholdning *Ear drop and/or head tilt*	11	Klinisk	X	X	X	X	X
Øjenflåd *Eye discharge*	12	Klinisk	X	X	X	X	X
Navle *Navel/umbilical region*	13	Klinisk	X	X			
Led *Joints*	14	Klinisk	X	X	X	X	X

Vægtbæring, halthed ***Weight bearing, lameness***	15	Klinisk	**X**	**X**	**X**	**X**	**X**
Hårlag ***Hair coat***	16	Klinisk	**X**	**X**	**X**	**X**	**X**
Hårtab, bagparti ***Hair loss, hindquarter***	17	Klinisk	**X**	**X**	**X**	**X**	**X**
Hudforandring ***Skin anomalies***	18	Klinisk		**X**	**X**	**X**	**X**
Huld ***Body condition***	19	Klinisk	**X**	**X**	**X**	**X**	**X**
Tilsmudsning, hele kalven ***Cleanliness***	20	Klinisk	**X**	**X**	**X**	**X**	**X**
Hoste ***Coughing***	21	Klinisk	**X**	**X**	**X**	**X**	**X**
Vægt/båndmål ***Girth measurement***	22	Klinisk	**X**	**X**	**X**	**X**	**X**

Temperatur ***Temperature***	23	Klinisk	**X**	**X**	**X**	**X**	**X**
Gødningsprøve ***Faeces sample***	24	Prøve	**X**	**X**	**X**	**X**	**X**
Gødning ***Faeces***	25	Klinisk	**X**	**X**	**X**	**X**	**X**
Næsesvaber ***Nasal swab***	26	Prøve	**X**	**X**	**X**	**X**	**X**
Blodprøver ***Blood sample***	27	Prøve	**X**	**X**	**X**	**X**	**X**

5a. Hygiejne, vandtildeling

Kilde: Welfare Quality

Der kigges på vandtildeling i kalvens boks (drikkekop, drikketrug, spand eller andet) og renheden vurderes visuelt. Både frisk og/eller indtørret skidt/foderrester samt gødning på indersiden af beholderen og vandets/væskens klarhed.

Cleanliness, water provision: *Examine the water points in the calf pen (trough, reservoir, bowl or alike) and visually score the cleanliness. Presence of old and/or fresh dirt/food residues and manure as well as staining of water.*

Score	Beskrivelse	Billedeksempel
0	**Rene** Ren skål evt. med lidt friske foderrester, evt klart eller mælketilblandet/elektrolyt-blandet vand *No or small amount of fresh food residues in the trough/water. Milk mxed with milk or electrolytes*	   
1	**Beskidt/gødning/uklart vand** Uklart vand og/eller slimet/fedtet belægning og eller biofilm/gødning *Unclear water and/or slimy/greasy coating and/or biofilm/manure*	 

20. Tilsmudsning, hele kalven – Cleanliness

Kalven skal stå op og betragtes over hele kroppen dog uden hoved og benene nedenfor forknæ/has. Med tilsmudsning menes friske eller indtørrede kager/stænk/områder af skidt og/eller fugt Det er det samlede areal af alle beskidte områder, der scores. Bærer kalven dækken tjekkes kalven under dette og er den mere beskidt under dækkenet (hvis den lige har fået den på fx) scores kalven. Ellers scores tilsmudsningen af kalven uden på dækkenet.
Calf must be standing. All of the body is examined except from the head and the legs distant to the hokcs/knees. Soiled means fresh and/or dried cakes/stenches and/or moisture on shoulders, belly, sides and/or hindquarter/tail. The total area of all soiled areas is scored. Calves with rugs are checked under the rug. If it is more soiled beneath the rug, it is scored without the rug.

Score	Beskrivelse	Billedeksempel
0	**Ren** Under 2 håndflader (minus fingre) tilsmudset *Less than the area of 2 palms soiled in total*	
1	**Moderat tilsmudset** Areal svarende til sammenlagt 2 håndflader *Area of in total 2 palms soiled*	
2	**Svært tilsmudset** Mindst 25% af kalvens overflade er tilsmudset *At least 25% percent of the calfs' surface is soiled*	

23. Temperatur – *Rectal temperature*

Temperaturen måles med rektalt termometer. Angives i celsius med 1 decimal.

Body temperature measured rectally. Numeric value with one digit.

24. Gødningsprøve – *Faeces sample*

Gødningsprøve udtages ved at indføre en finger rektalt og fremprovokere peristaltik ved forsigtigt at massere væggen i rektum. Hvis stimulering ikke er nok forsøges det at indsamle med fingeren. Frisk afsat gødning (fra pgl. kalv) fra boksen bruges, hvis der ikke kan hentes nok ved rektal manipulation. Der skal gerne minimum være en mængde der svarer til 2 tsk.

Fresh faecal samples should be collected, preferably from the rectum. Gently pass a gloved, lubricated finger through the anus and massage the rectal wall to stimulate rectal evacuation. If feces are not produced, collect feces with finger. If freshly passed, faeces can be collected off the ground. The amount needs to at least 2 tablespoons.

25. Gødning (diarré) - *Faeces*

Ved udtagning af afføringsprøve vurderes gødningens konsistens.

Scoring of the texture of the faeces when sampling from the calf

Score	Beskrivelse	Billede eksempel
0	Normal Pastaagtig konsistens, formbar *Semi-formed, pasty*	
1	Blød, ikke vandig, evt moderat slimtilblanding Blød, ikke formbar, men mulig at holde i hænderne. Evt. med slim tilblanding på max 25% af volumen *Loose, not formed, but stays in the hands. And/or with mucus* *(max 25% of volume)*	
2	Vandig og/eller kraftig slimtilblanding og/eller blodig Vandig, meget lidt tekstur, løber mellem fingrene, og/eller slimtilblanding der udgør mere end 25% af mængden, og/eller slimhinde afstødning og/eller tilblanding af frisk eller koaguleret blod (~'kaffegrums'). Helt frisk blod på overfladen af udtaget prøve scores ikke som blodtilblanding – skyldes udtagning. *Watery, sifts through fingers, and/or heavy amounts of mucus,* *and/or fresh or coagulated blood. Fresh blood on the surface is* *from sampling – not scored as pathological.*	

Figures before exclusion of observations

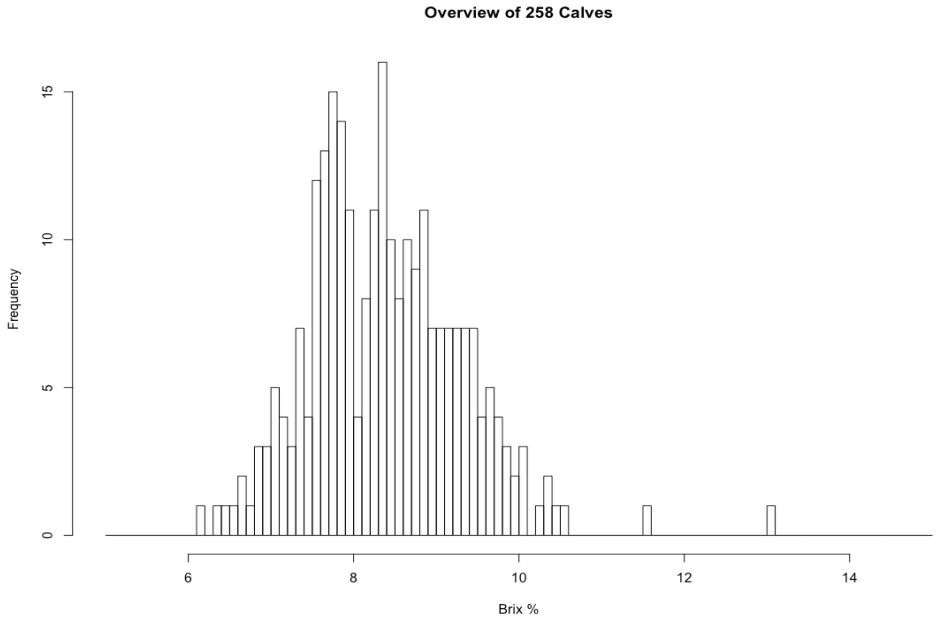

Figure 13 Frequency distribution histogram illustrating the distribution of serum Brix% values of 258 calves from 9 enrolled herds, before exclusion of 8 calves to create the initial samplesize used for analysis.

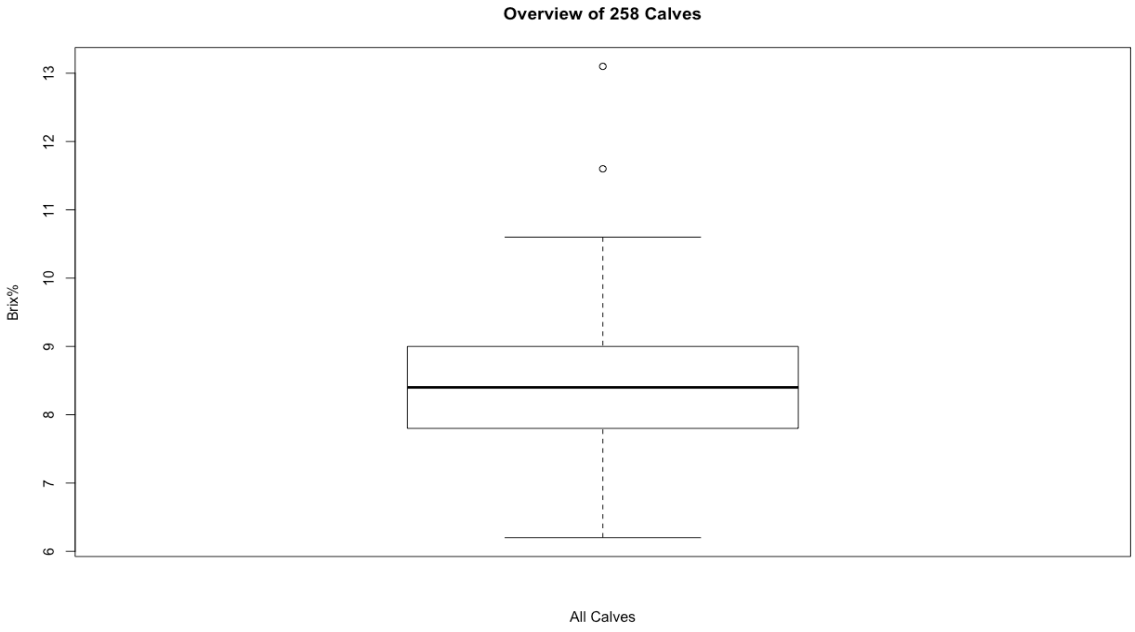

Figure 12 Boxplot illustrating the distribution of serum Brix% values of 258 calves from 9 enrolled herds, before exclusion of 8 calves to create the initial samplesize used for analysis

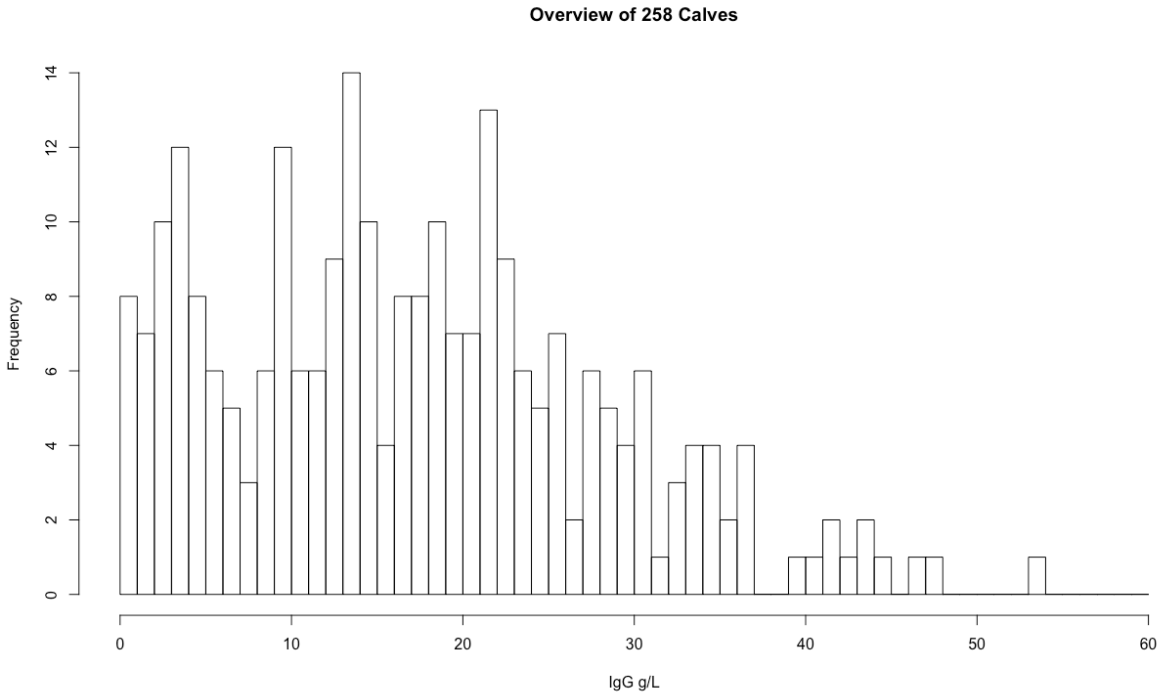

Figure 15 Frequency distribution histogram illustrating the distribution of serum IgG g/L values of 258 calves from 9 enrolled herds, before exclusion of 8 calves to create the initial samplesize used for

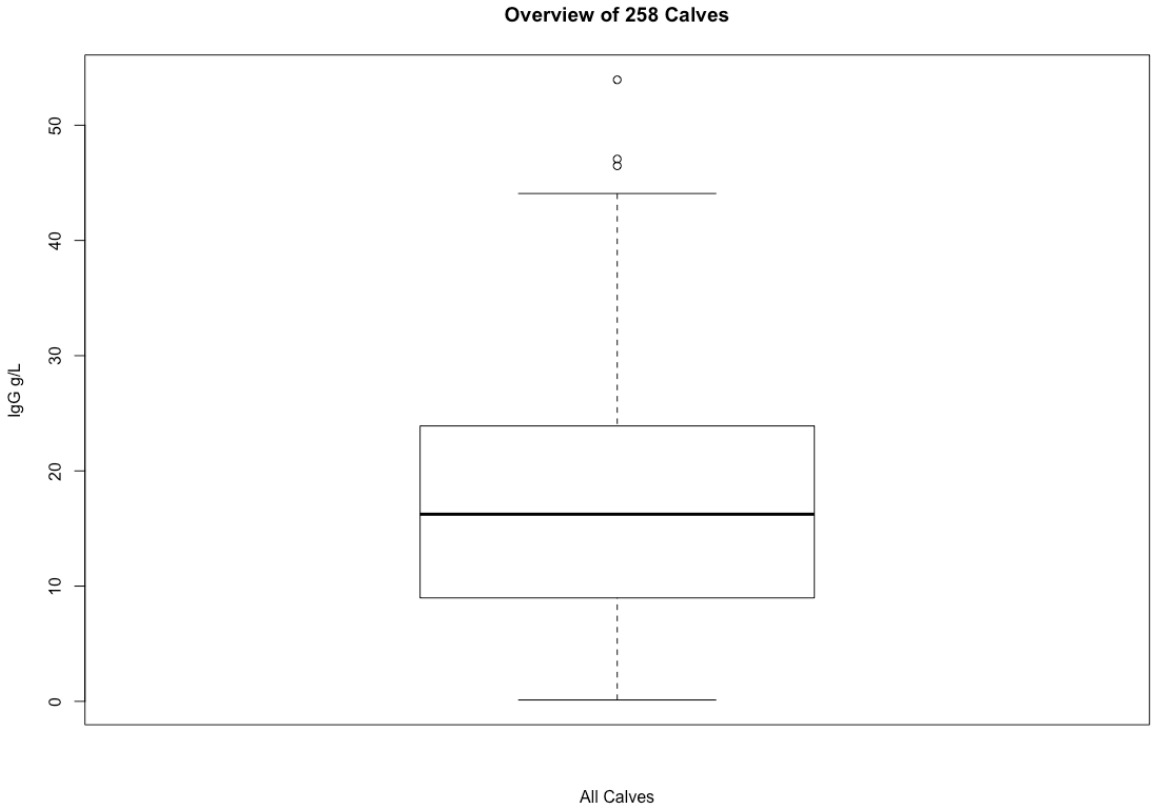

Figure 14 Boxplot illustrating the distribution of serum IgG g/L values of 258 calves from 9 enrolled herds, before exclusion of 8 calves to create the initial samplesize used for analysis

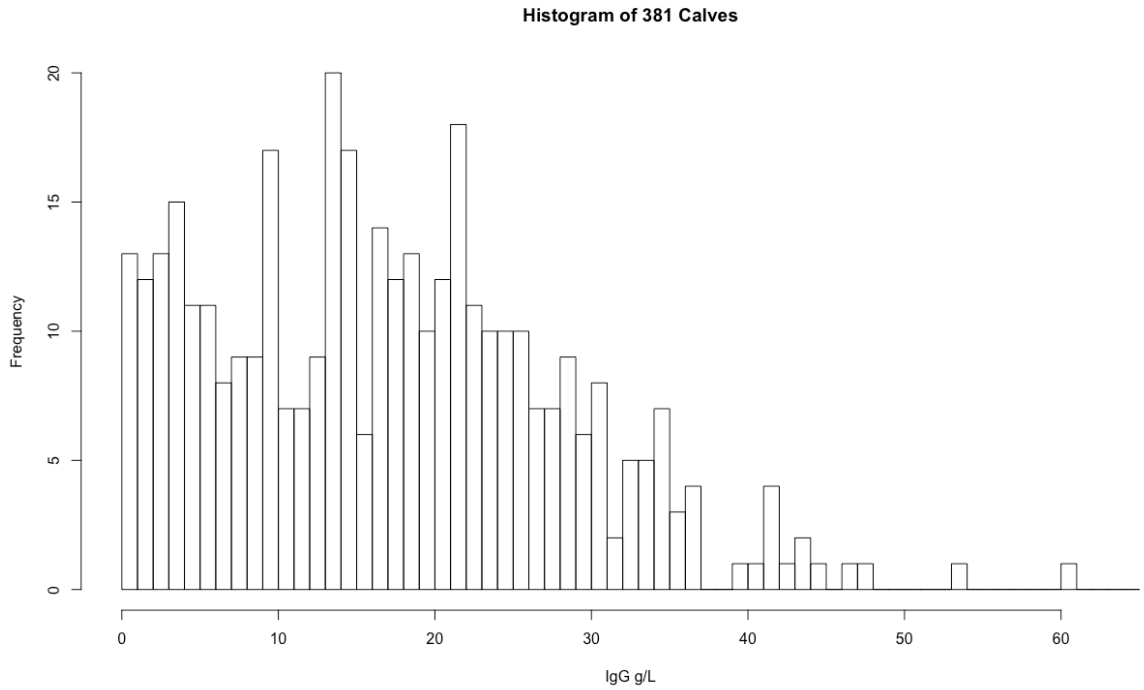

Figure 16 Frequency distribution histogram illustrating the distribution of IgG g/L values of 381 calves from 9 enrolled herds, before exclusion of 29 calves to create the extended samplesize used for

Illustration of Univariate analysis of Age, Sex and Breed impact on IgG levels

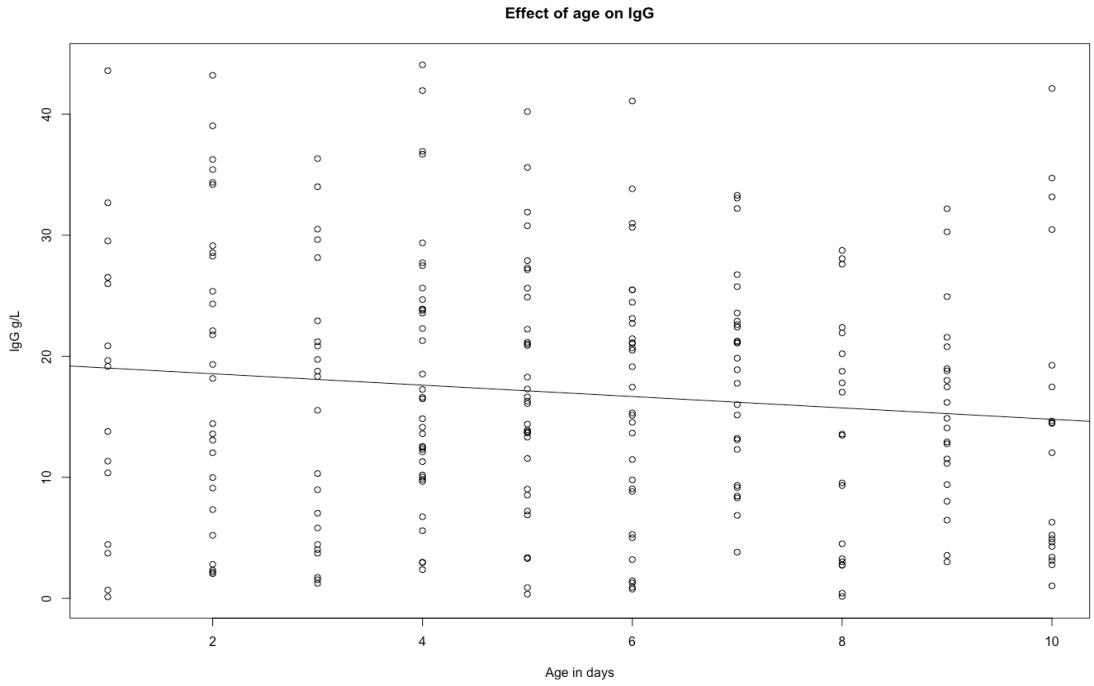

Figure 17 Illustration of all IgG g/L measurements in the initial samplesize (n=258) grouped by age in days on sampling. A trendline estimated by linear regression was added (P=0.067)

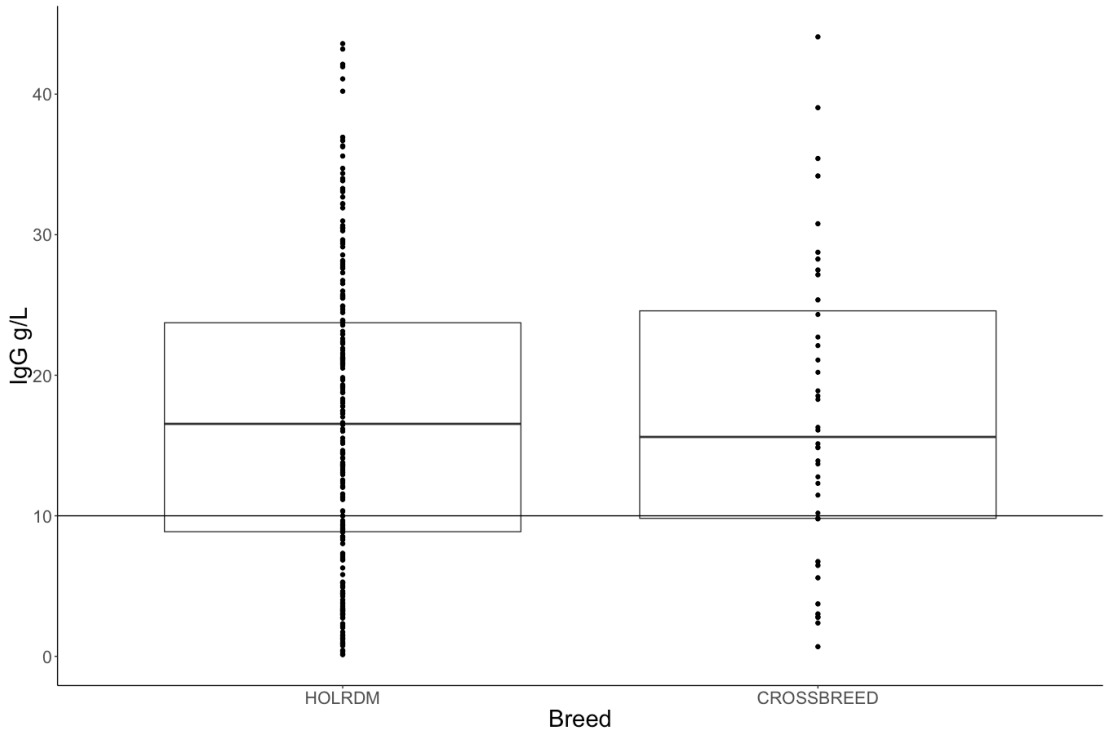

Figure 19 Boxplots illustrating the distribution of purebred dairy calves (HOL/RDM) and crosbred calves' serum IgG g/L values in the initial sample size (n=258). The difference investigated by ANOVA had a p-value of 0.82. The grey line indicates the cut point value used

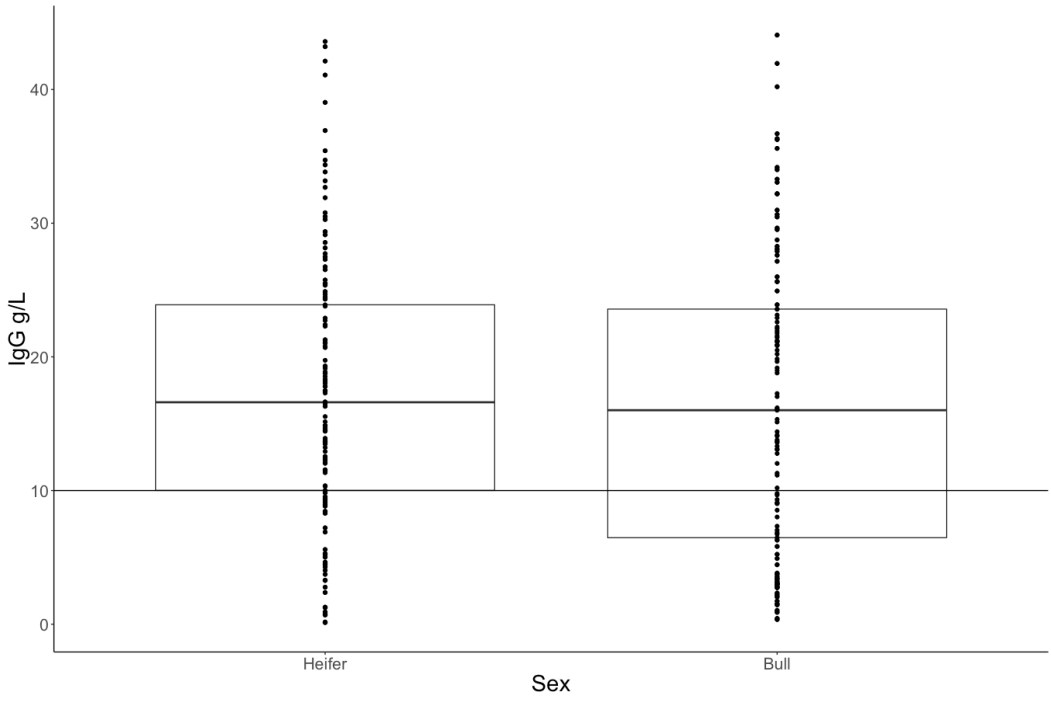

Figure 18 Boxplots illustrating the distribution of Heifer and Bull calves' serum IgG g/L values in the initial sample size (n=258). The difference investigated by ANOVA had a p-value of 0.55. The grey line indicates the cut point value used to categorize FPT <10 g/L IgG.

Table of p-values for linear effect of age on herdlevel

	Estimate	Pr(>t)
(Intercept)	22.8	<0.001***
Age	-0.26	0.326
Herd A	Ref.	
Herd B	-2.61	0.431
Herd C	-7.60	0.010*
Herd D	-4.47	0.142
Herd E	-1.34	0.648
Herd F	-8.53	0.004**
Herd G	-6.99	0.081
Herd H	-3.10	0.340
Herd I	-2.06	0.544

Table 14 Overview of the variable estimates and corresponding p-values in the linear function of Herd+Age on IgG levels based on 250 calves of 9 Danish dairy herds (A-I)

Extended sample size results and illustrations

Variable	Score	Calves total	FPT (%)	Mean IgG g/L
GI disease	Sick	67	27 (40%)	14.0
	Healthy	282	82 (29%)	17.4
Fever	Yes	48	22 (45%)	15.1
	No	304	88 (29%)	17.0
TOTAL		**352**	**110 (31%)**	**16.8**

Table 15 Extended sample size Overview of FPT status defined by <10 g/L serum IgG and Mean IgG-level for 352 calves from 22 danish dairy herds categorised by gastrointestinal disease and Fever occurrence

Herd	Drinking tray scored clean	Drinking tray scored dirty	% dirty
A	15	3	17%
B	21	0	0%
C	39	0	0%
D	25	10	29%
E	30	9	23%
F	8	26	76%
G	9	2	18%
H	12	11	48%
I	14	4	22%

Table 16 Overview of drinking tray hygiene scores of 250 calves from 9 Danish dairy herds (A-I)

Model 2.e, n = 349, GI disease with dichotomous IgG scale						
Marginal R^2	0.05					
Conditional R^2	0.12					
Random effect	***Variable***	***Variance***	***SD***			
	Herd	0.54	0.73			
Fixed effects	***Variable***	***Estimate***	***se***	***p-value***	***OR***	***CI 95%***
	Intercept	-1.73	0.26	***		
	FPT +	0.49	0.31	0.109	1.63	0.89-2.98
	FPT -	0				
	Age_linear	7.69	2.67	0.004**		
	Age_quadratic	5.45	2.66	0.040*		
: significant <0.05 **:significant<0.01 *:significant<0.001*						

Table 17 Model 2.e: Results of generalised mixed regression with dichotomous IgG level (cut point of <10g/L IgG categrosing failure of passive transfer)for probability prediction of GI disease based on 352 calves from 22 Danish dairy herds

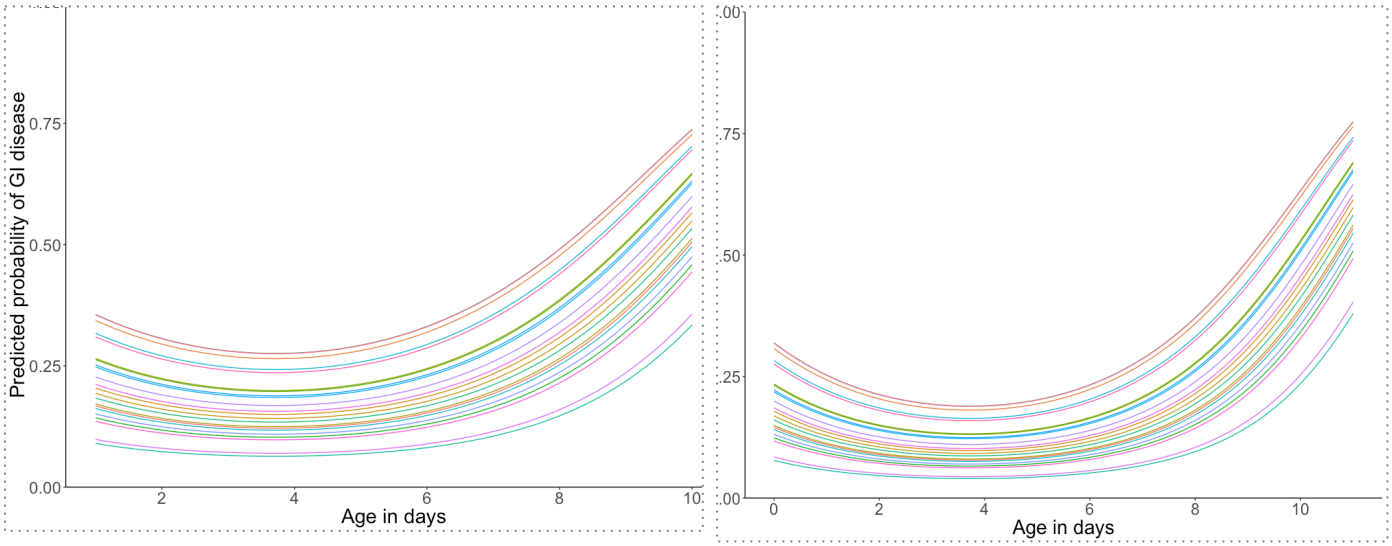

Figure 20 Model 2.e: Graphic illustration of the age effect on predicted probability of GI disease based on 352 calves from 9 Danish dairy herds .The graph is set to the precondition of calves having failure of passive transfer (serum IgG<10 g/L) on the left and adequate passive transfer on the right.

Model 3.e, n=352, Fever with continuous IgG scale						
Marginal R^2	0.01					
Conditional R^2	0.05					
Random effect	***Variable***	***Variance***	***SD***			
	Herd	***0.34***	***0.59***			
Fixed effects	***Variable***	***Estimate***	***se***	***p-value***	***OR***	***CI 95%***
	Intercept	-1.39	0.50	***		
	IgG g/L	-0.02	0.02	0.232	0.98	0.95-1.01
	Age	-2.20	0.06	0.407	0.95	0.84-1.07
	Sex:Bull	0.15	0.32	0.628	1.17	0.62-2.21
	Sex:Heifer	0				
: significant <0.05 **:significant<0.01 *:significant<0.001*						

Table 18 Model 3.e: summarized results of generalised mixed regression with continuous IgG level for probability prediction of fever based on 352 calves from 22 Danish dairy herds

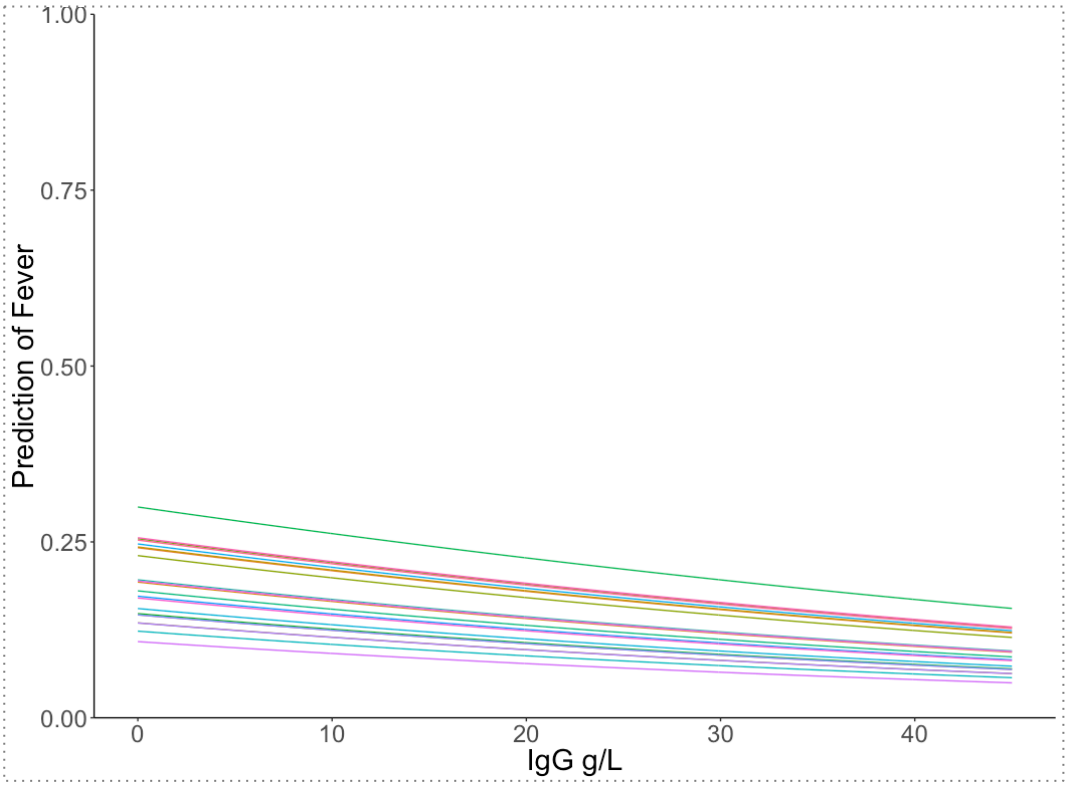

Figure 21 Model 3.e: Graphic illustration of the effect of IgG level on predicted probability of Fever for 352 calves from 22 Danish dairy herds. The graph is set to the precondition of bull calves at mean age of day of registration and sampling (5 days)

Alternative curve fittings for detection of noise and unu

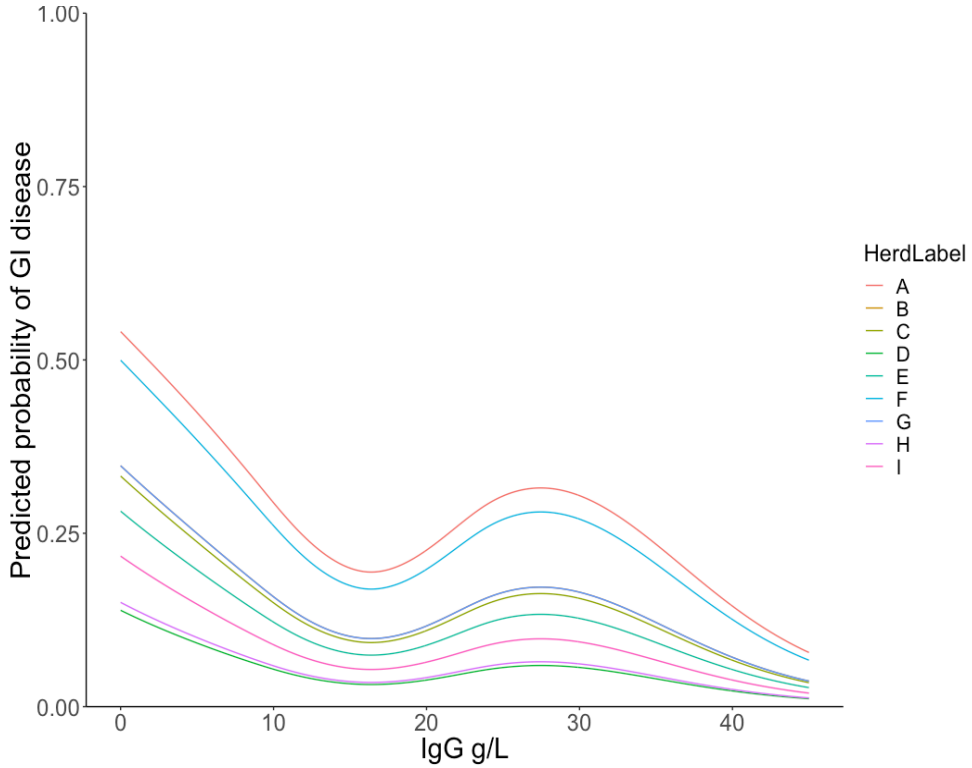

Figure 23 Model 1 Graphic illustration for noise characterisation of IgG effect fitted with natural splines with 4 degrees of freedom on predicted probability of GI disease based on 250 calves from 9 Danish dairy herds (A-I). The Age is set to mean age on day of registration and sampling (5 days)

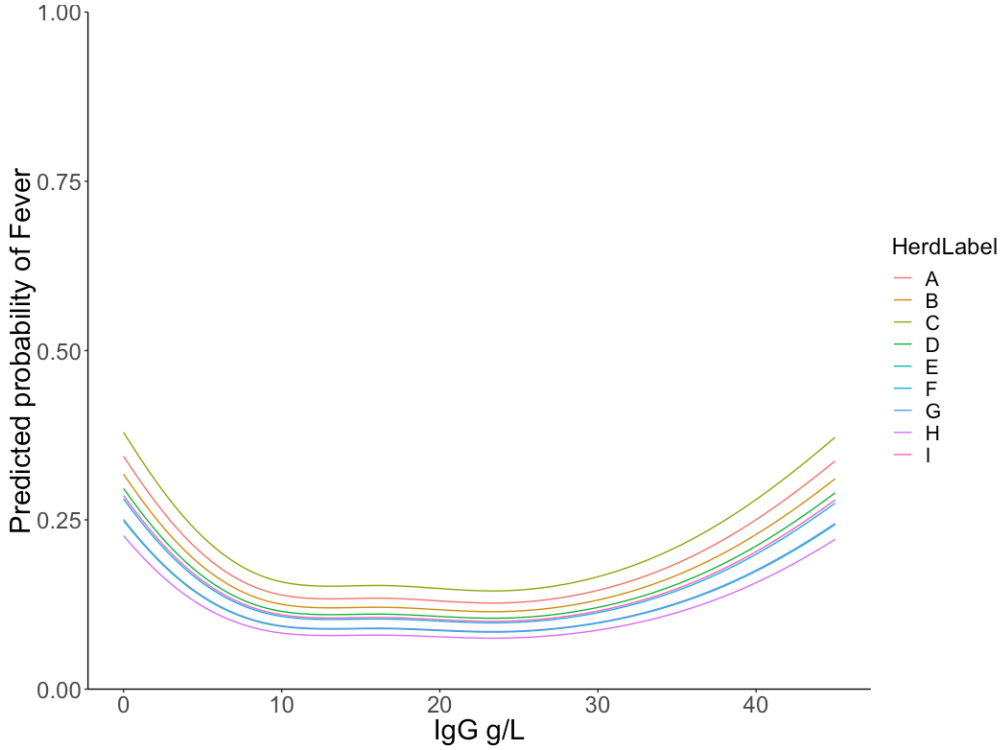

Figure 22 Model 3 Graphic illustration for noise characterisation of IgG effect fitted with natural splines with 4 degrees of freedom on predicted probability of Fever based on 250 calves from 9 Danish dairy herds (A-I). The Age is set to mean age on day of registration and sampling (5 days)