Giardia and *Cryptosporidium* infections in neonatal reindeer calves: relation to the acute phase response

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Abstract

This longitudinal observational study was conducted to investigate the spontaneous effect of *Giardia* and *Cryptosporidium* infections on acute phase response (APR) in reindeer calves (*Rangifer tarandus tarandus*) in Finnish Lapland.

Serum (n = 609) and faecal samples (n = 366) were collected from 54 reindeer calves aged zero to 33 days. The samples were analysed for *Giardia*, *Cryptosporidium*, acute phase proteins (APP) and γ-globulins.

Linear regression models were used to investigate associations of early *Giardia* infection (before 12 days of life) with the response of APPs and acquiring of passive immunity.

Giardia was detected in 100% and *Cryptosporidium* in 23% of calves. There was a negative association between early *Giardia* infection and γ-globulin concentrations (p = 0.032) and a positive association with serum amyloid A (SAA) concentrations (p = 0.042). The results suggest a protective effect of colostrum against *Giardia* infection and that early infection may induce activation of APR.

Key words: Semi-domesticated reindeer, Giardia infection, acute phase response, serum amyloid A

1. Introduction

Reindeer (*Rangifer tarandus tarandus*) are semi-domesticated ruminants that live in the harsh Arctic environment and calve seasonally. Ensuring survival and good health of a calf is crucial to successful reindeer husbandry. A very important element of a calf's survival is the transfer of maternal immunity from the hind. The neonate gets almost all of its first immunoglobulins (Ig) from colostrum. As observed in other domesticated ruminants, female reindeer (hind) have syndesmochorial placentation, which prevents the transfer of Ig from hind to calf through the placenta. As a result, ingestion of Ig after birth (in colostrum) is important for the calf survival. The lowest level of Ig serum concentration occurs when the calf is 20 days old, which makes calves more susceptible to infections during this period [1]. Pathogenic infections during the neonatal period can have a negative impact on growth and development [2].

The acute phase reaction (APR) is an immunological reaction triggered by inflammatory processes following tissue damage. The specific proteins that increase in concentration during an APR are termed positive acute phase proteins (APP) [3]. Serum amyloid A (SAA) and haptoglobin (HP) act as positive APR markers in reindeer exposed to *Escherichia coli* lipopolysaccharide, and SAA seems to be the more sensitive APR marker of the two [4]. In reindeer, SAA concentrations peak around the second week of life while HP continues to rise until 3-4 weeks of life [5]. Higher SAA concentrations at the second week of life were negatively associated with daily weight gain at 4 months of age, suggesting that activation of APR early in life may influence negatively immunological development of new-born reindeer [5]. Concentrations of another APP, fibrinogen (FIB) increase in clinically affected reindeer [6] and red deer (*Cervus elaphus*) [7]. Albumin (ALB) is considered to be an important APP in ruminants, the concentration of which decreases during APR [3].

In dairy calves, *Giardia* stimulate the production of IgG2 and IgA antibodies [8]. These antibodies do not bind to *Giardia* very strongly in calves and simultaneously inflammation-related genes in the jejunum are down-regulated [9]. This may partly explain why there are no clinical signs of *Giardia* infection and why the infection is chronic in nature [10]. Dairy calves on average start to shed *Giardia* cysts at 31 days of age,

which suggests colostrum is of passive protective value against the parasite infection [11]. Similar interactions relevant for the early life of reindeer calves may occur for pathogens including *Giardia* and *Cryptosporidium* and for innate and passive immunity.

The aim of this study was to determine if spontaneously occurring *Giardia* and *Cryptosporidium* infections in neonatal reindeer have significant impact on the innate immune response.

2. Materials and methods

2.1 Animals

The study population comprised 54 semi-domesticated reindeer calves (28 male and 28 female) from initially born 56 calves between 9th and 22th May 2004 in the Kaamanen experimental herd of the Reindeer Herders' Association (total land area 48 km^2), in Finnish Lapland. Reindeer hinds were treated with ivermectin in the previous autumn. Weighing was performed using a digital scale (Adam Equipment Co Ltd, Milton Keynes, UK) immediately after birth, at 20 or 21 days of age, and 114 to 127 days of age.

2.2 Sample collection

In total, 609 serum samples, 210 EDTA samples and 366 faecal samples were collected from 54 calves. Blood samples were collected into 10 ml vacuum tubes (BD Vacutainer, New Jersey, USA) at 7 time points from the time of birth: day 0, 4-6, 8-10, 12-14, 16-17, and 20-22 from all calves. Sampling was planned so that all the calves would be sampled within 3-5 days after the previous sampling during first weeks of life. In addition, blood was collected from a subgroup (n = 51) at 23-33 days. EDTA blood samples were collected into 2 ml EDTA-coated tubes (BD Vacutainer, New Jersey, USA) when calves were 0-1, 4-6, 12-14, 20-22 and 23-33 days of age.

Samples were stored at 6°C for 30 minutes and at 21°C for 15 minutes before serum separation. Serum was separated by centrifuging and as divided into aliquots and stored at -18°C for further analysis. EDTA

samples were analysed for FIB on the day of collection. Because of technical difficulties, approximately half of the EDTA samples from age groups 0-1 and 23-33 were analysed for FIB.

Faecal samples were collected simultaneously with blood samples directly from the rectum into disposable latex gloves and stored at 6°C and then at -18°C until further analysis.

2.3 Sample analysis

Sample total protein concentration was determined using a modified spectrophotometry method [12] in a clinical chemistry analyser (KONE Pro, Konelab, Thermo Clinical Labsystems Oy, Vantaa, Finland). ALB was measured using the bromocresol green method in a clinical chemistry analyser (Accent-200 Albumin II Gen, PZ Cormay S.A., Poland). γ-globulins were measured by serum protein electrophoresis of agarose gel using a Paragonw electrophoresis system (Beckman Coulter, Inc., Fullerton, CA, USA). γ-globulin fraction relative size (%) to the all proteins in the agarose gel was used to calculate γ-globulin serum concentrations (g/l) when serum total protein concentrations in the sample were 100%.

The concentration of SAA was measured using an indirect ELISA test (Phase BE kit, Tridelta Ltd., Ireland) according to the manufacturer's instructions for cattle.

HP was measured using a modified method based on the ability of HP to bind to haemoglobin [13] with modifications to the original protocol using tetramethylbenzidine (0.06 mg/ml) as the substrate and microtitration plates [14]. Lyophilized aliquots of acute phase bovine serum were used as standards. Standards were calibrated using samples provided by the European Union concerted action on standardization of animal APPs for cattle (number QLK5-1999-0153).

FIB concentration was measured using a heat precipitation method [15]. EDTA blood samples were centrifuged for 5 min in a microhaematocrit centrifuge 15000 times/min. From each sample 2 capillaries were prepared. Capillaries were placed in a water bath (56°C) for 3 min to precipitate the FIB in the plasma. After 3 min of centrifugation, the heights of the FIB and serum column were measured (mm) and transformed into concentrations (g/l) by dividing the height of the FIB column by the height of the serum

column and multiplying the result by 100. The final figure was the average of the results from two capillaries prepared from a single sample.

Faecal samples were analysed for *Giardia* cysts and oocysts of *Cryptosporidium* using an immunofluorescent staining method (Crypto/Giardia Cel, Cellabs Pty Ltd., Sydney, Australia) according to manufacturer's instructions. The numbers of cysts and oocysts in the samples per visual field at 200x magnification were ranked as: none (no cysts/oocysts found), low (1-5 cysts/oocysts), medium (6-30 cysts/oocysts) and high (>31 cysts/oocysts).

2.4 Statistical analyses

Previous studies demonstrated that dairy and beef calves that were naturally infected with *Giardia* started shedding cysts during the second week of life [11,16]. To investigate the association between serum proteins and APP concentration and early *Giardia* infection a new variable was constructed – "early *Giardia* infection". Calves were considered to be of the early infection group if they had a faecal sample positive for *Giardia* at ≤12 days of age (n = 21).

Logistic regression analysis was used to determine if γ-globulin and APP (SAA, HP, FIB or ALB) concentrations during first (age 0-1) and second samples (age 4-6) had an effect on the onset of early *Giardia* infection. The outcome variable was "early *Giardia* infection" and explanatory variables were γ-globulins and total protein concentrations from the first or second sample. Birth period was added as a three level categorical variable ("early birth period" 9$_{th}$-14$_{th}$ May, n = 16; "middle birth period" 15$_{th}$-17$_{th}$ May n = 17; "late birth period" 18$_{th}$-22$_{nd}$ May, n = 23) to control for a possible confounding effect of birth period.

A linear mixed model was constructed to establish if APP (SAA, HP, FIB and ALB) or γ-globulin concentrations changed over the study period (0-33 days). Protein concentrations were used as response variables and age groups as a 7-level categorical variable (age groups: 0-1, 4-6, 8-10, 12-14, 16-17, 20-22 and 23-33 days of age), regarded as a fixed explanatory variable. Calf was included as a random factor and isotropic spatial exponential covariance structure was used to model correlation between repeated samples within reindeer calves. Statistical difference was evaluated between every consecutive age group and

Bonferroni corrections were used for controlling multiple comparison bias. Logarithmical transformations of γ-globulin, SAA and HP data were used.

Linear regression models were used to determine if "early infection" was associated with protein or APP concentration levels through the study period (0 to 22 days of age). For every protein, area under the curve (AUC) was calculated for the period using the trapezoidal rule:

$$AUC = \sum [(t_i - t_{i-1})f_{i-1}] + [0.5(t_i - t_{i-1})(f_i - f_{i-1})],$$

Where t_i = time of observation, t_{i-1}= previous time of observation, f_i = APP concentration at the time, and f_{i-1} = APP concentration at previous time. AUC was used to summarize changes in serum proteins and APP concentrations over the study period. Because the sampling periods were not equal for all calves (difference of up to 2 days), AUC values were divided by period days (day AUC) in order to allow comparison of AUCs between calves with different sample periods.

Average protein AUCs were used as outcome variables in regression models. Predictor variables were "early *Giardia* infection" (2-level categorical variable), *Cryptosporidium* infection (2-level categorical variable), and other protein (γ-globulins, SAA, ALB, HP and FIB) day AUC values. A birth period categorical variable with three levels was included in all models and a manual step-wise backward elimination procedure was used. The variables used in the multiple regression models were checked for collinearity using a threshold of 10 for the variance inflation factor (VIF), which none of the components exceeded [17]. FIB average AUC was initially added to γ-globulin and SAA models, but it was not statistically significant and was consequently removed from all models. HP day AUC was non-significant in the SAA model and was also excluded.

Linear regression models were used to investigate association between daily weight gain (DWG) in the short-term (birth to 20-21 days) and long-term (birth to 114-128 days). Predicting variables were serum proteins (total protein, γ-globulins) and APPs (SAA, HP, FIB) day AUCs, sex and birth period and early *Giardia* infection and *Cryptosporidium* infection. A manual step-wise backward elimination procedure was used.

Normality scatter plots of model residuals were used for evaluating the linear regression model assumptions.

Basic data management was done using Excel 2010 (Microsoft, Redmond, USA). Data was analysed using Stata/IC 13.1 for Windows (StataCorp LP, Texas, USA). Statistical significance level was set as $p \leq 0.05$. Coefficient plot figures were made using Stata software package coefplot [18].

Results from calves with complete data (n = 48) were used in statistical analysis (48 calves from 54 initially included in the study).

3. Results

3.1 Clinical signs

During the calving season, 9 calves in the study were diagnosed with diarrhoea at the time of sample collection. Calves were diagnosed with diarrhoea if their faeces were thin and watery. Six calves had diarrhoea at the age of 9 to 16 days and one calf had diarrhoea at the age of 31 days. Two calves experienced diarrhoea for two consecutive sampling times (at the age of 9 and 13 in one calf and 13 and 17 days in the second) and both had *Cryptosporidium* in faecal samples at the later sampling times. All calves except one (diarrhoea once at the age of 10 days) belonged to the late *Giardia* infection group.

3.2 Weight gain

The median (±SD) weight of calves at birth (0), 3 weeks of age, and 21 weeks of age was 6.4 kg (±0.65; range 4.5-7.6 kg), 15.9 kg (±2.07; range 12-21 kg), and 49.5 kg (±5.24; range 39-61 kg) respectively. The daily weight gain from birth to 3-4 weeks of age was 0.382 kg/d (±0.047; range 0.290-0.500 kg/d) and from birth to approximately 4 months of age was 0.364 kg/d (±0.037; range 0.281-0.435 kg/d). No significant associations were established between weight gain in early and late-term (respectively up to 33 and 112 days of age) with early *Giardia* infection, *Cryptosporidium* infection, protein concentrations at different age groups or average protein AUCs during 0-22 days. Male calves gained more weight (0.039 kg/d, 95% CI: 0.021-0.058; $p < 0.001$) in the long term (from birth to 112 days) than females.

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187 *3.3 Giardia and Cryptosporidium infections*

188 All the calves in the study from which a faecal sample was collected were *Giardia* positive. The faecal
189 sample of one calf was positive on day 0. At 2 weeks of age, more than 60% of calves in the study were
190 infected with *Giardia*. The infection rate sharply increased after 2 weeks of age (Fig. 1). During the first 10
191 days 38.9% calves had a low infection level. During the entire study 67% of calves had at least one sample
192 for which the cyst count was high. By the age of 16 days 83% (45/54) calves had already at least one
193 positive sample. 12 calves (22%) had at least one positive *Cryptosporidium* faecal sample during the study,
194 but the overall prevalence remained relatively low (Fig. 2). Only one calf had 2 positive *Cryptosporidium*
195 samples (at day 13 and 17). That calf also had diarrhoea at the later sample time. Six of the
196 *Cryptosporidium*-positive calves were from the early *Giardia* infection group and 6 from the late *Giardia*
197 infection group, 32% and 21% respectively.

198

199 *3.4 γ-globulin and APP concentrations changes over time*

200 Average γ-globulin concentrations were higher in the first two days (15.51 ± 4.76 g/l; n = 48).
201 Concentrations subsequently decreased, being lowest at the age 23-33 days (2.84 ± 0.56 g/l; n = 45) (Fig. 3).
202 There was significant decrease in average concentrations between every consecutive sample ($p < 0.01$). Four
203 calves had γ-globulin concentrations below 10 g/l during the 24 h period after birth (all females). Three of
204 them belonged to the early *Giardia* infection group and one to the late *Giardia* infection group (one with the
205 lowest value, 3.57 g/l). None of those calves had diarrhoea episodes during the remainder of the study
206 period.

207 Calf serum SAA levels started at a very low level after birth (0.31 ± 0.38 mg/dl; n = 48) and increased up to
208 8-10 days (6.59 ± 4.10 mg/dl) of age (change from first to second sample time and from second to third
209 sample time $p < 0.01$). They peaked at 12-14 days of age (8.09 ± 4.99 mg/dl) and then began to decrease
210 until the end of study period, but without statistically significant change (Fig. 3).

Median HP serum concentration levels were lowest at birth and at 4-6 days and then increased at 8-10 days and peaked at 12-14 days of age. Concentrations decreased again at 23-33 days (Fig. 4). ALB concentrations were lowest at birth, then increased and stabilised by the end of the third week of a calf's life (Fig. 4.). FIB concentration was lowest on the first day, briefly increased and peaked between 4-6 and 12-14 days before decreasing (Fig. 4).

3.5 Associations between overall passive immunity and APP response with early Giardia infection

The logistic regression models for the onset of early *Giardia* infection did not indicate significant associations with γ-globulin and APP (SAA, HP, FIB or ALB) concentrations at the first (age 0-1 days) and second sampling times (age 4-6 days).

The multiple regression model was used to determine whether the early *Giardia* infection was associated with overall γ-globulin concentrations during the study period (Fig. 5). Early *Giardia* infection ($p = 0.032$) and ALB average AUC ($p < 0.001$) were negatively associated, whereas SAA average AUC ($p = 0.002$) and HP average AUC ($p = 0.017$) were positively associated with γ-globulin overall concentration.

Similar models were used to evaluate factors associated with overall SAA response during 0-22 days of age (Fig. 6). Early *Giardia* infection ($p = 0.042$), average γ-globulin AUC ($p = 0.001$) and ALB ($p = 0.015$) were positively associated with SAA overall response.

Identical multiple regression models as described were constructed for HP, FIB, and ALB overall response, but there were no significant associations with parasite infections or average protein AUCs.

4. Discussion

This study describes *Giardia* and *Cryptosporidium* infection in semi-domesticated reindeer calves. *Giardia* and *Cryptosporidium* were found from wild reindeer faecal samples in Norway [19] and an epidemiologic study on reindeer in northern Finland and Norway was unsuccessful in detecting *Cryptosporidium* infection

[20]. The role of *Giardia* as a pathogen in ruminants is still uncertain, although its importance as a potential zoonotic organism should not be underestimated [21].

In the harsh Arctic climate it is unlikely that *Giardia* or *Cryptosporidium* can survive in soil over winter [22] because of physical damage from freeze-thaw cycles. They could survive in open water and in animals however or be transmitted by humans. In sheep it was established that *Giardia* shed from ewes reach peak levels at around parturition [23]. The same phenomenon could apply also to reindeer. It was demonstrated in cattle that *Giardia* infection can become chronic and persist for long time (over 7 months) [11,24]. It is unknown how long infection persisted in this study because sampling ceased when the animals were 33 days old. The greatest sources of *Giardia* infection of calves were probably the hinds and subsequently other calves.

A direct fluorescence antibody test for detecting *Giardia/Cryptosporidium* antigens from faeces is both sensitive and specific (over 90%), but is also sensitive to the concentration of oocysts before detection [25-27]. Some of the faecal samples could have been false negatives for *Giardia* and *Cryptosporidium* due to freezing of samples, which can damage the parasites and mask detection of very low numbers.

In this study the majority of *Cryptosporidium* infections were detected after 2 weeks of age. It is possible that colostrum provided sufficient protection to prolong the initiation of shedding, as was demonstrated in dairy calves [28].

Both *Giardia* and *Cryptosporidium* establish infection from very low levels (<10 oocysts/cysts) [29], which could mean that once an animal becomes infected the entire herd is quickly infected, being exacerbated by the decrease in γ-globulin levels.

In this study, all the calves from which faecal samples were collected became infected with *Giardia*. Although dairy calves are kept under very different conditions, reindeer calves did appear to shed *Giardia* from an earlier age [9]. *Giardia* cysts were detected in faeces of neonatal dairy calves in the third week of life [30]. The concentrations of γ-globulins during the first 3 weeks of life were not negatively associated with the early infection. γ-globulin concentrations decreased as progressively more calves became infected, but the time of birth did not seem to contribute significantly to shedding of *Giardia* cysts during the first 12

days of the calf's life. This finding suggests that maternal antibodies provided some protection against *Giardia* infection. However, high γ-globulin concentrations at the first week of age were not associated with early *Giardia* infection, suggesting that late *Giardia* shedding calves may have developed an early humoral immune response, resulting in inhibition of *Giardia* shedding and higher overall γ-globulin response recorded in this group. The antibody interaction with a parasite's life cycle was demonstrated in murine *Giardia* infection models, which may indicate reduction of cyst shedding in infected animals [31].

At the same time, the innate immune system appeared to have responded to *Giardia* infections because there was positive association between SAA overall response and an early infection. The differences between *Giardia* infection groups were more evident at the end of second and at the beginning of the third week of age (Fig. 3). This supports the theory that early *Giardia* infection calves were not able to mount an immune response early and more severe infection pressure at the time when passive protective immunity declined quickly (as seen in Fig. 3) resulted in more pronounced activation of APR.

SAA levels in this study were comparable with those of a previous study on reindeer [5]. In both studies SAA concentrations peaked at around 2 weeks of age and were comparable with concentrations in dairy calves after birth [32]. In our previous study, reindeer calves with higher SAA at the second week of life had lower weight gain at 4 months of age [5]. Our research group has recently established the same phenomenon in lambs [33] and beef calves [34]. Those findings support the hypothesis that the second week of life in neonatal ruminants is important for immunological development and adaptation to the environment. Similarly, in the present study it could be speculated that calves infected sooner had weaker immune responses and were more susceptible to negative environmental factors, resulting in a lower growth rate. However, no evidence for this was forthcoming. Either the infection pressure from *Giardia* was insufficiently strong or it affected all the calves similarly. Overall, our results indicate that early *Giardia* infection cannot be related to the impaired adaptation or immunological development of reindeer calves. Higher *Cryptosporidium* infection rates at the time could potentially stimulate a more severe immunomodulatory effect, but there were only mild and rare infections established in this study.

HP and FIB were without significant positive associations with early *Giardia* infection, underlining the weak inflammatory stimulus of *Giardia* infection. A positive association with γ-globulin serum levels in early life and SAA concentrations supports the hypothesis that proteins from colostrum are transferred to the calf, as was demonstrated for lambs with SAA and FIB [35].

5. Conclusions

This study describes *Giardia* and *Cryptosporidium* infections in the neonatal period of reindeer calves. The early *Giardia* infection (before 12 days if age) was positively associated with lower overall γ-globulin intake/response and with higher overall SAA response, indicating interaction between host humoral and innate immune systems and *Giardia* infection.

Conflict of interest statement

None of the authors has any financial or personal relationship with organisations or people that could influence the content or conclusions reached in this the study.

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Fig. 1. *Giardia* positive faecal samples. The age of calf for first positive faecal sample and number samples collected in a day. Left hand y-axis shows no. of faecal samples collected on given day. Right hand y-axis shows % of calves that had had at least one positive *Giardia* sample collected by that age. All the animals that had faecal sample collected tested positive (n = 54): in total 312 samples were collected.

Fig. 2. *Giardia* and *Cryptosporidium* positive faecal samples (%) at given date. Left hand y-axis shows how many of the calves were born by a given date. All the calves were born between 9th May and 22th May 2004. Right hand y-axis shows the % of how many of the collected faecal samples tested positive for *Giardia* and *Cryptosporidium*. Number on top of bar is the total no. of faecal samples collected on a specific date. The percent of calves born (54 = 100%) by given date presented in grey background.

Fig. 3. Mean (±SEM) γ-globulin and serum amyloid-A (SAA) concentrations in serum of reindeer calves in early and late *Giardia* infection groups (from 0-22 days of age n = 19 and n = 29 respectively, at 23-33 age of age n = 19 and n = 26 respectively) during study period (0-33 days of age). Filled area represents time period where protein AUCs were calculated and used for studying differences in overall protein responses between early and late *Giardia* infection groups. Significant changes in protein concentrations after birth are presented in main text.

Fig. 4. Mean (±SEM) albumin (ALB), haptoglobin (HP) and fibrinogen (FIB) concentrations in serum of reindeer calves during study period (0-33 days of age). Sample size for ALB and HP at 0-22 days of age and at 23-33 days of age n = 48 and n = 45 respectively. For FIB at 0-1 days, 4-22 days and 23-33 days of age n = 21, n = 48 and n = 23 respectively.

* Significant difference from previous age group ($p < 0.05$)

** Significant difference from previous age group ($p < 0.01$)

Fig. 5. γ-globulin AUC regression model coefficient plot (age 0 to 22 days). Model confidence intervals (CI) are presented as horizontal bars. Point estimates for variables are shown on top of the bars. AUC was calculated for each animal (n = 48) using a trapezoidal method for 6 time points and averaging for number of days (20-22 days of age).

1 Compared to late *Giardia* infection group (n = 29)

2 Middle birth period (15-17 May) compared to early birth period (9-14 May; n = 11)

3 Late birth period (18-22 May) compared to early birth period (9-14 May; n = 11)

Fig. 6. Serum amyloid A (SAA) AUC regression model coefficient plot (age 0 to 22 days). Model confidence intervals (CI) are presented as horizontal bars. Point estimates for variables are shown on top of the bars. AUC was calculated for each animal (n = 48) using a trapezoidal method for 6 time points and averaging for number of days (20-22 days of age).

1 Compared to late *Giardia* infection group (n = 29)

2 Middle birth period (15-17 May) compared to early birth period (9-14 May; n = 11)

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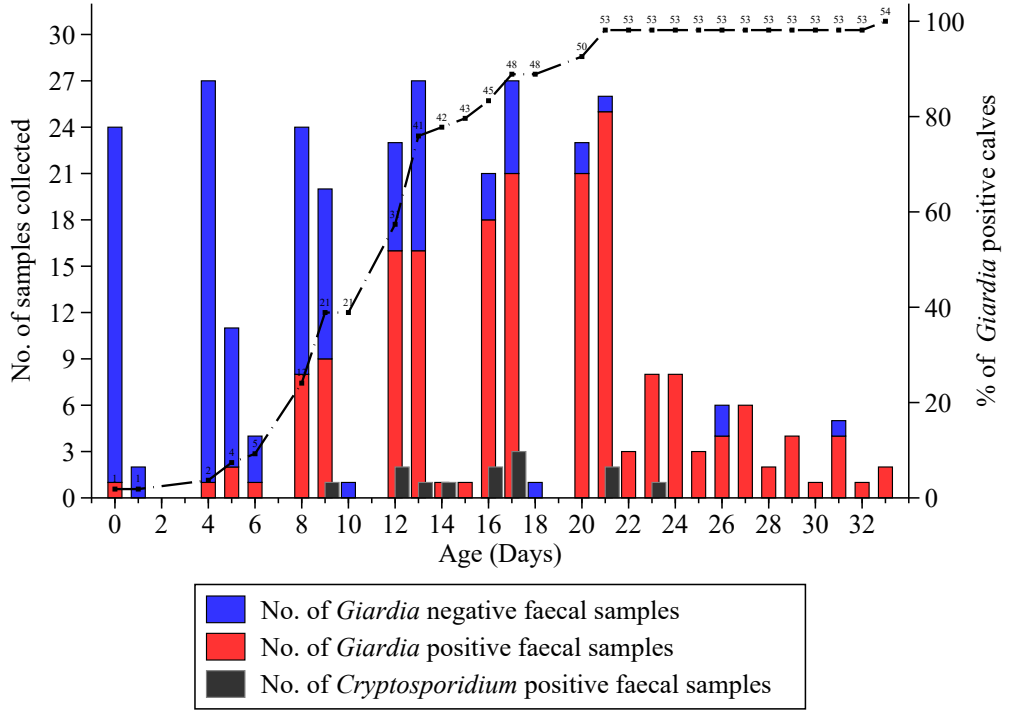
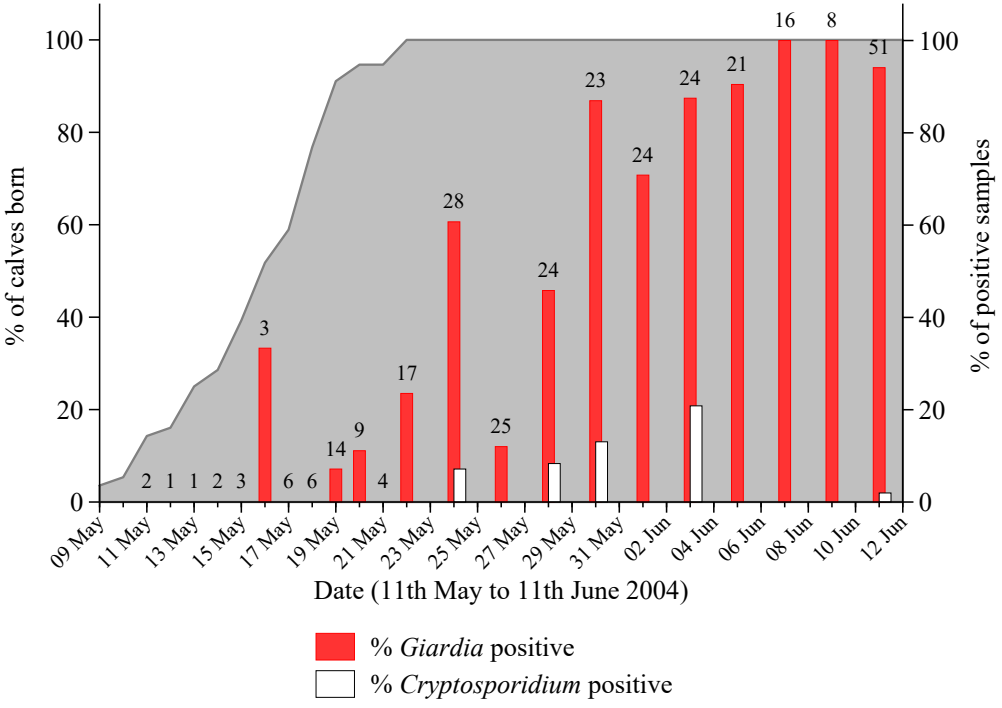
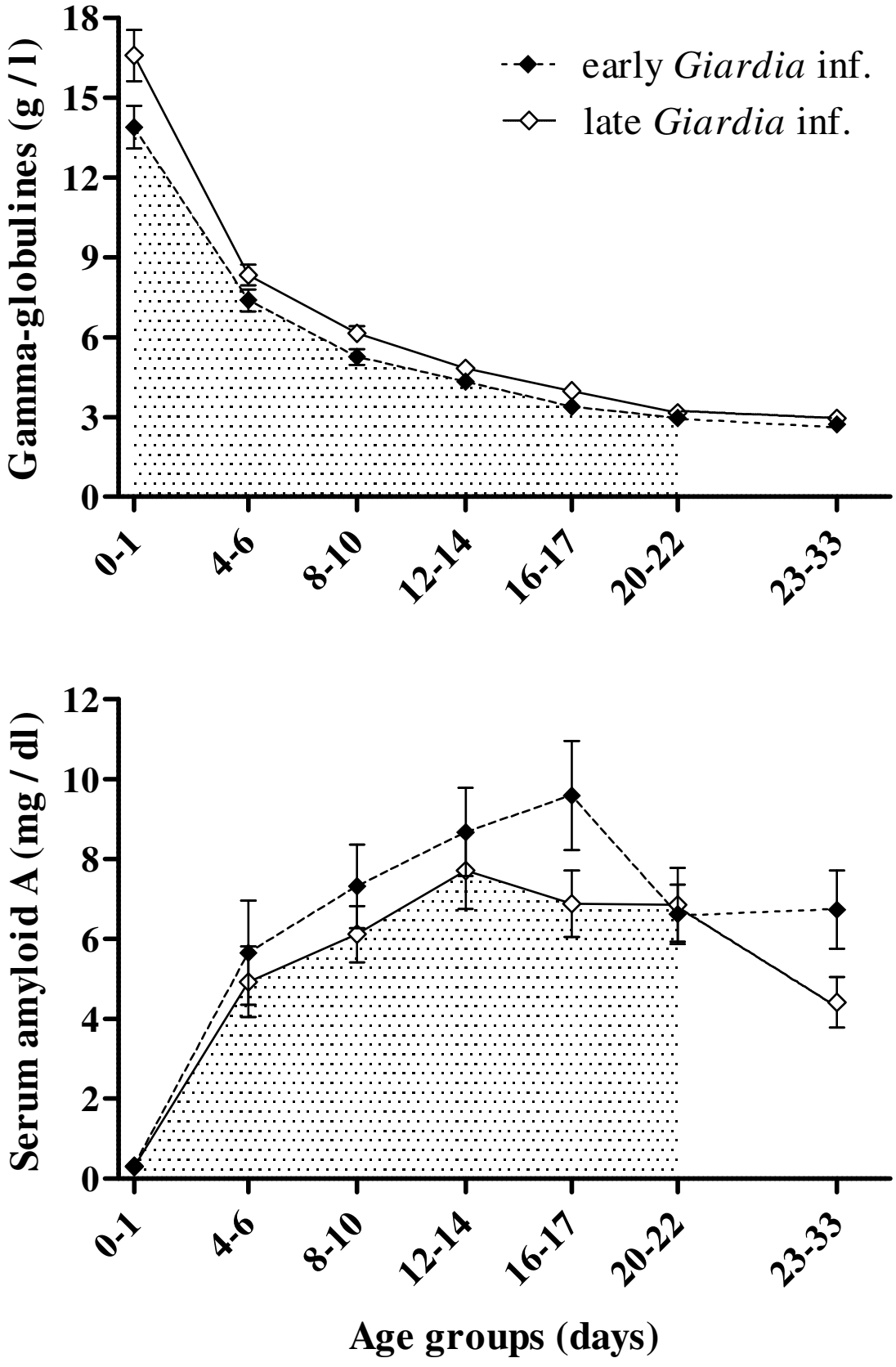
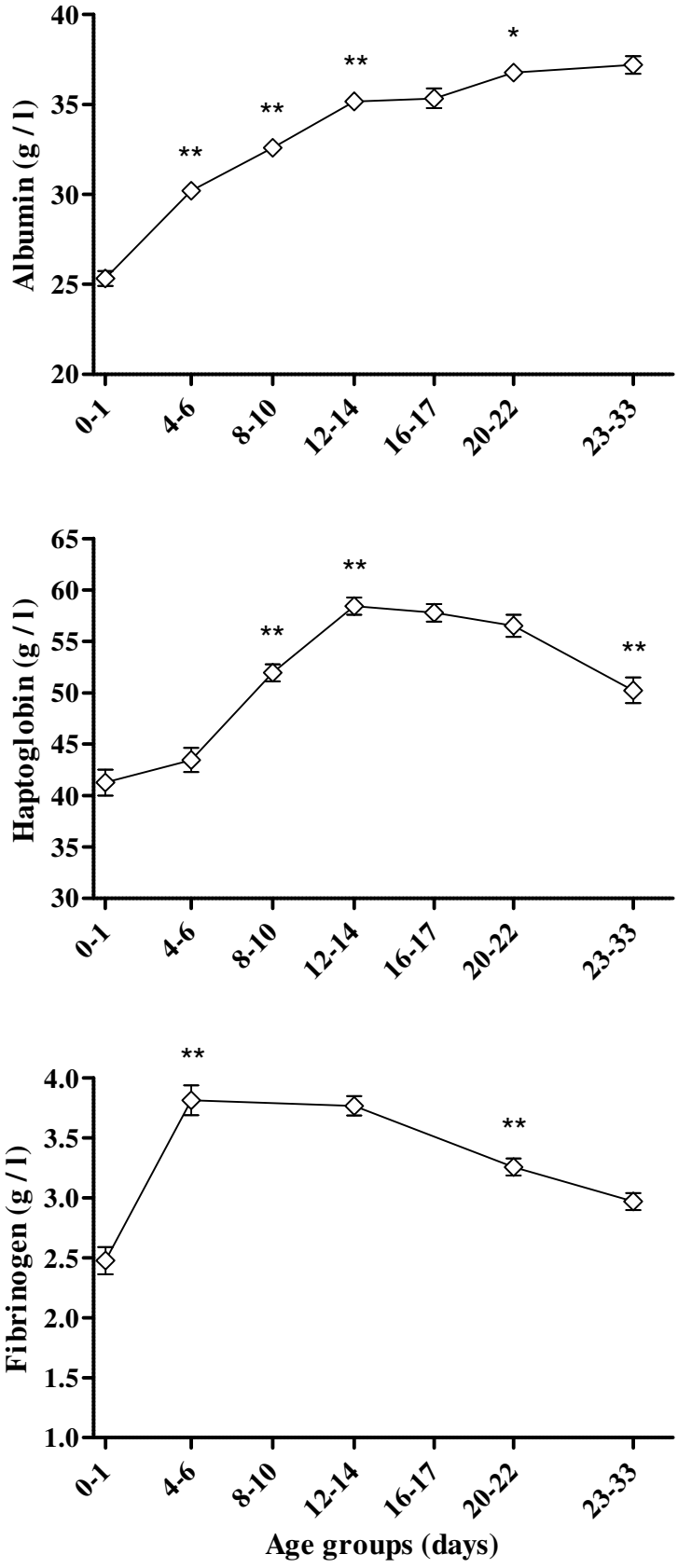
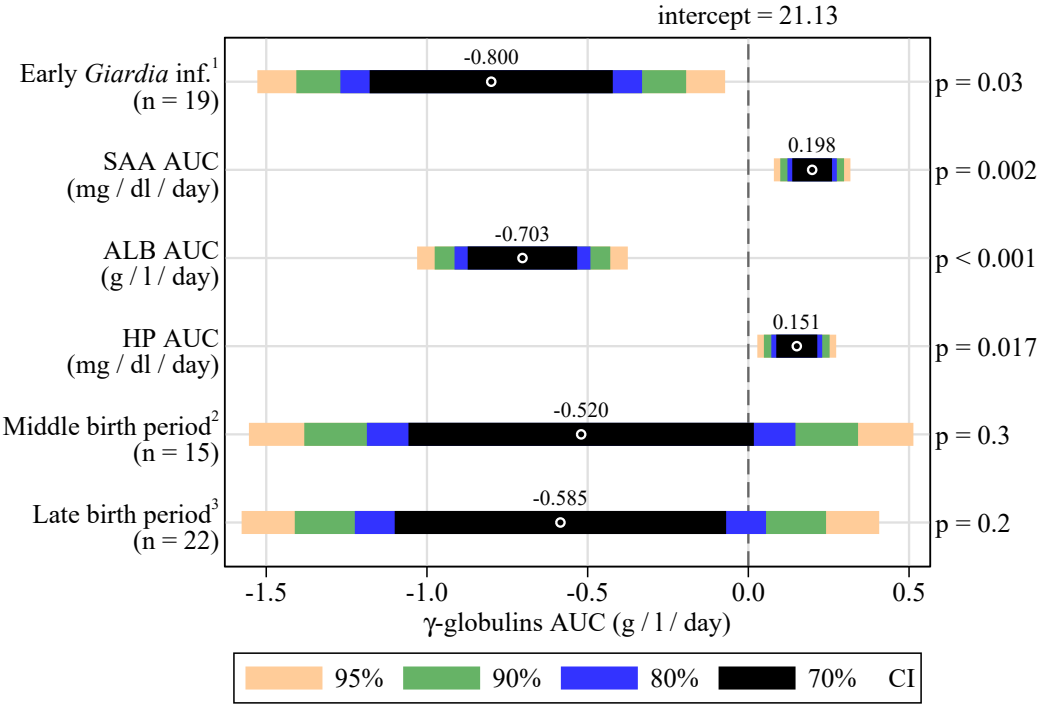
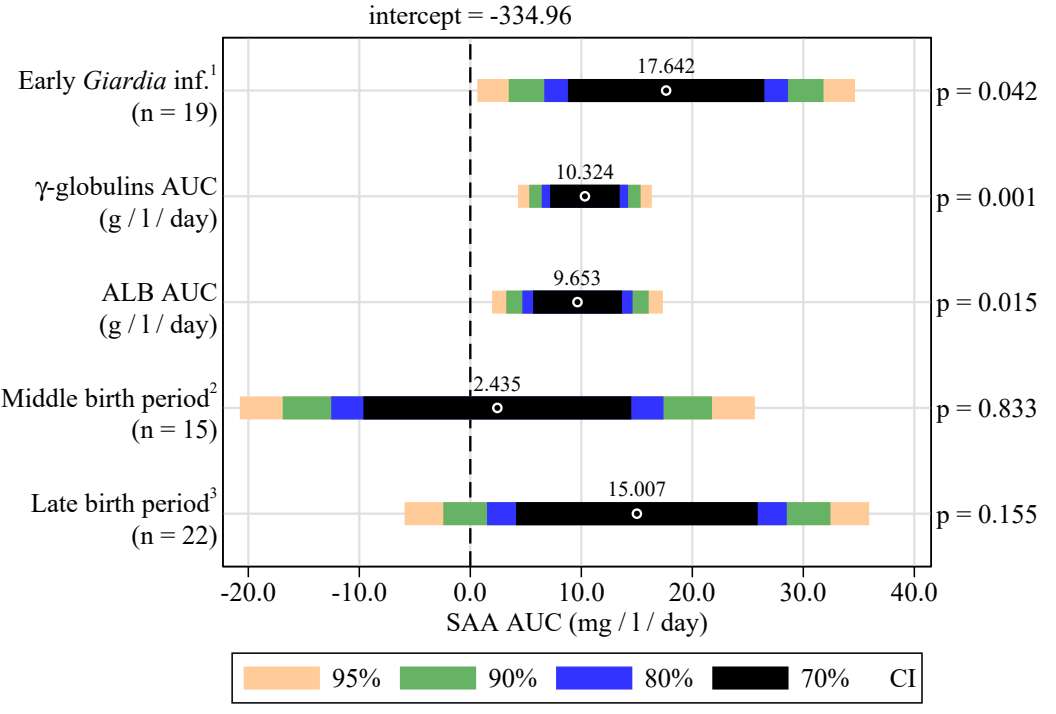

No. of samples collected
% of Giardia positive calves
Age (Days)
No. of Giardia negative faecal samples
No. of Giardia positive faecal samples
No. of Cryptosporidium positive faecal samples


% of calves born
% of positive samples
100
80
60
40
20
0
2
1
1
2
3
3
6
6
14
9
4
17
28
25
24
23
24
24
21
16
8
51
09 May
11 May
13 May
15 May
17 May
19 May
21 May
23 May
25 May
27 May
29 May
31 May
02 Jun
04 Jun
06 Jun
08 Jun
10 Jun
12 Jun
Date (11th May to 11th June 2004)
% Giardia positive
% Cryptosporidium positive


Gamma-globulines (g / l)
18
15
12
9
6
3
0
early *Giardia* inf.
late *Giardia* inf.
0-1
4-6
8-10
12-14
16-17
20-22
23-33
Serum amyloid A (mg / dl)
12
10
8
6
4
2
0
0-1
4-6
8-10
12-14
16-17
20-22
23-33
Age groups (days)


Albumin (g / l)
40
35
30
25
20
**
**
**
*
0-1
4-6
8-10
12-14
16-17
20-22
23-33
Haptoglobin (g / l)
65
60
55
50
45
40
35
30
**
**
**
Fibrinogen (g / l)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
**
**
Age groups (days)


intercept = 21.13
Early Giardia inf.[1] (n = 19)
-0.800
p = 0.03
SAA AUC (mg / dl / day)
0.198
p = 0.002
ALB AUC (g / l / day)
-0.703
p < 0.001
HP AUC (mg / dl / day)
0.151
p = 0.017
Middle birth period[2] (n = 15)
-0.520
p = 0.3
Late birth period[3] (n = 22)
-0.585
p = 0.2
-1.5
-1.0
-0.5
0.0
0.5
γ-globulins AUC (g / l / day)
95%
90%
80%
70%
CI


intercept = -334.96
Early *Giardia* inf.[1]
(n = 19)
γ-globulins AUC
(g / l / day)
ALB AUC
(g / l / day)
Middle birth period[2]
(n = 15)
Late birth period[3]
(n = 22)
17.642
10.324
9.653
2.435
15.007
p = 0.042
p = 0.001
p = 0.015
p = 0.833
p = 0.155
-20.0
-10.0
0.0
10.0
20.0
30.0
40.0
SAA AUC (mg / l / day)
95%
90%
80%
70%
CI