Comparative Immunology, Microbiology and Infectious Diseases 54 (2017) 45–50

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Full length article

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

Tarmo Niine[a,*], Kristel Peetsalu[a], Mauri Nieminen[b], Antti Oksanen[c], Timo Soveri[d], Toomas Orro[a]

[a] Department of Clinical Veterinary Medicine, Institute of Veterinary Medicine and Animal Science, Estonian University of Life Sciences, Tartu, 51014, Estonia
[b] Natural Resources Finland, FI-99910 Kaamanen, Finland
[c] Finnish Food Safety Authority Evira, Production Animal and Wildlife Health Research Unit (FINPAR), Elektroniikkatie 3, FI-90590 Oulu, Finland
[d] Department of Production Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Paroninkuja 20, FI-04920 Saarentaus, Finland

ARTICLE INFO

Keywords:
Semi-domesticated reindeer
Giardia infection
Acute phase response
Serum amyloid A

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.

1. Introduction

Reindeers (*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 reindeers (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.

* Corresponding author.
E-mail address: tarmo.niine@emu.ee (T. Niine).

http://dx.doi.org/10.1016/j.cimid.2017.08.001
Received 20 April 2017; Received in revised form 8 August 2017; Accepted 16 August 2017

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 22nd 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 114–127 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 min and at 21 °C for 15 min 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 micro-haematocrit 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 200× magnification were ranked as: none (no cysts/oocysts found), low (1–5 cystskk/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" 9th–14th May, n = 16; "middle birth period" 15th–17th May n = 17; "late birth period" 18th–22nd 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 (0–22 days of age). For every protein, area under the curve (AUC) was calculated for the period using the trapezoidal rule:

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

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