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Intestinal pathogens, diarrhoea and acute phase proteins in naturally infected dairy calves

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ABSTRACT

In this study, the association between *Eimeria* spp. related signs and innate immune response in dairy calves was examined. Calves ($n = 100$) aged 15–60 days were clinically examined and faecal samples, blood samples and deep nasopharyngeal swabs obtained. The samples were analysed for intestinal pathogens, acute phase proteins and WBC count, and respiratory tract pathogens, respectively.

Diarrhoea was diagnosed in 32.6% (23.3–43.0%, 95% CI) of calves. An association between the pathogenic *Eimeria* spp. and diarrhoea was detected by multiple correspondence analysis. *Eimeria* related signs (diarrhoea, presence of pathogenic species and total oocyst count) were combined resulting a four level variable. Calves with weak signs of eimeriosis had decreased haptoglobin concentrations ($p = 0.02$) and increased fibrinogen concentrations ($p = 0.048$) compared to no signs. Increased haptoglobin and fibrinogen concentrations were associated with respiratory tract infection and umbilical infection. Serum amyloid A and WBC counts showed no association with signs of eimeriosis or clinical diagnoses.

1. Introduction

Eimeriosis is caused by pathogenic *Eimeria* spp. and is considered a universal problem of dairy and beef calves. Clinical eimeriosis (coccidiosis) manifests itself as moderate to severe, and sometimes bloody, diarrhoea, inappetence and lethargy [1]. Chronic or subclinical eimeriosis reduces growth rate and can result in considerable economic losses [2,3]. As the mere presence of *Eimeria* spp. is not conclusive for them being the etiologic agents of diarrhoea, an oocyst count of pathogenic species of 500 oocyst per gram of faeces (opg) in calves with simultaneous signs of diarrhoea has been used to indicate eimeriosis for research purposes [4,5]. The oocysts are formed during gamogony, which with second merogony is the most disruptive stage for the intestine in the *Eimeria* life cycle [1]. The histopathological lesions during gamogony in the ileum include oedema, congestion, haemorrhage, mucosal ulceration, blunting of mucosal ridges and ulceration of the *lamina propria* [6].

An effective response of the local immune system against the *Eimeria* sporozoites has been recorded for *in vitro* models, where macrophages reduce the numbers of sporozoites [7]. One indicator of the innate immune response is the synthesis of the acute phase proteins (APPs) that are synthesised in the liver at the beginning of the acute phase of the inflammatory response initiated by infection, neoplasia or tissue destruction [8]. The initial location or cause of the inflammation cannot be determined with APP measurements, but the magnitude of the increase correlates with the extent and acuteness of the inflammation [9]. Most studies concentrate on the changes in haptoglobin (Hp), serum amyloid A (SAA) and fibrinogen (Fb), the major acute phase proteins in cattle [10]. In calves, research has concentrated on experimental and natural respiratory infections, where the association with the disease and the APPs has been confirmed [11,12]. Association between diarrhoea and increased APP concentrations or intestinal pathogens and increased APP concentrations has also been studied, but the results are inconclusive [13,14]. As severe tissue destruction occurs during eimeriosis, the acute phase response would be expected to follow [7].

The aim of the study was to explore possible associations between the various severities of eimeriosis and APPs under natural

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conditions while controlling the effects of inflammatory diseases and other intestinal pathogens on the acute phase response.

2. Material and methods

2.1. Animals and clinical examination

The study included 100 calves of 15–60 days of age from 20 free or tie-stall farms visited between April and June 2010. Inclusion criteria were: farms with more than 40 milking cows located near the Production Animal Hospital University of the Helsinki in southern Finland volunteering to participate in the study. Exclusion criteria were: farms that used prophylactic rotavirus vaccination or metaphylactic coccidiosis treatment (toltrazuril). Farmers reported no use of antimicrobials prior to the visit.

Two veterinarians with similar instructions performed the sample collections and clinical examinations. All the calves aged 15–60 days of age during the sampling visit were included in the study, and examined once. The calves were clinically examined for respiratory inflammation, assessed for diarrhoea, palpated for umbilical defects and examined for joint lesions in carpal, hock and fetlock joints. Two faecal samples were collected from the rectum of each calf into plastic bags, blood samples were collected from the jugular vein by vacuum venipuncture into EDTA and plain serum tubes, and a deep nasopharyngeal swab was also obtained.

All examinations and sample collections were performed on commercial family-owned farms with minimal stress to the calves, according to guidelines of the Council for International Organizations of Medical Sciences (International Guiding Principles for Biomedical Research involving Animals).

2.2. Faecal samples: handling and examination

After the collection the faecal samples were sent with cooling element packs to either the Finnish Food Safety Authority Evira on the day of the farm visit, or stored refrigerated and sent in weekly batches to the Estonian University of Life Sciences.

Eimeria oocysts were concentrated, floated, and counted in a reading chamber constructed from glass slides as previously described [15–17] and the species were identified according to Levine [18]. The presence of *Cryptosporidium* spp. and *Giardia* spp. was examined using fluorescein staining (fluorescein isothiocyanate antibody, Crypto/Giardia Cel, Cellabs, Pty Ltd, Australia) at the Estonian University of Life Sciences. *Cryptosporidium* species were identified at the Finnish Food Safety Authority Evira using a restriction fragment length polymorphism PCR [19] after a positive finding by modified Ziehl Neelsen staining. All samples underwent bacteriological culture; salmonella was detected with ISO 6579:2002 [20]. Rotavirus and bovine coronavirus (BCV) were detected from faecal samples using ELISA (Duo Digestive Kit, Bio-X, Jemelle, Belgium).

For nine calves not all the pathogens were analysed; from five calves no faecal sample was obtained, for three calves only presence of rotavirus, coronavirus and salmonella was analysed and for one calf only presence of *Eimeria* spp., *Cryptosporidium* spp. and *Giardia* spp. was analysed. The observations of these calves were excluded in the analysis concerning all the detected intestinal pathogens.

2.3. Acute phase proteins and haematology

Blood samples were analysed for white blood cell (WBC) count, fibrinogen (Fb), Hp and SAA. EDTA samples were stored in refrigeration until the WBC count was performed using an automatic counter (scil Vet ABC, scil animal care company GmbH, Viernheim, Germany) and Fb was determined using the heat precipitation method [21] within 28 h after the sampling. Serum was separated by centrifugation and stored in a freezer at −20 °C until Hp and SAA analyses were performed. Hp analysis was performed with the haemoglobin-binding assay [22], modified by Alsemgeest et al. [23] by using tetramethylbenzidine (TMB) (0.06 mg/ml) as a chromogen instead of o-dianisidine. The analysis for SAA was performed using a commercial sandwich ELISA kit (Phase SAA assay, Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland) according to the manufacturer's instructions.

2.4. Deep nasopharyngeal swabs: handling and examination

The deep nasopharyngeal swabs were collected using a sterile 27 cm cotton swab, guarded with a silicon tube (Medical Wire Equipment Ltd., Corsham, England; [24]). The swabs were rinsed in NaCl and in mycoplasma F-medium [25] and the media were cold transported to the laboratory within 24 h. Samples were examined for aerobic and anaerobic respiratory tract bacteria, bovine respiratory syncytial virus, and bovine corona virus as described in Autio [26].

2.5. Statistical methods

To account for multicollinearity associated with the correlation of diarrhoea caused by different pathogens, a multiple correspondence analysis (MCA) was performed to obtain an overall view of the associations between the findings for pathogens in faecal samples and the presence of diarrhoea. The idea of the multiple correspondence analysis is to carry out cross-tabulations of multiple categorical variables simultaneously and explore relationships within a set of categorical variables, resulting in two-dimensional scatterplot summary. In the summary, clusters of points are associated with each other, and clusters furthest from the inter-section of the axes have the strongest internal association [27]. Test values were computed for evaluating variables' correlation with model dimensions (axes). The test values were considered to be the standardised coordinates and were interpreted as the number of standard deviations from the centre of gravity of the analysis. Absolute test values higher than the threshold value of 1.96 were considered to be statistically significant. For the MCA, the *Cryptosporidium* findings from immunofluorescence staining were used. The MCA was conducted with XLSTAT (Version 2010.4.01, Addinsoft) software.

The associations between the eimeriosis related signs, the acute phase protein concentrations and clinical signs were examined using a linear mixed model. Based on clinical examination, calves were categorised having the signs of: diarrhoea (pasty-like or watery faeces), respiratory infection (temperature >39.5 °C, respiratory rate >40/min, and increased respiratory sounds), umbilical infection (enlarged, warm or tender umbilicus), joint lesion (swelling, abrasions or heat in the joint or on the skin close to the joint) or healthy (none of the previous conditions applicable). Calves having simultaneously signs of diarrhoea and any other signs were placed into the category of the other sign, considered the primary sign, so each calf had only one condition or was categorised as healthy. The final variable "Signs of diseases" was generated by combining all the individual signs. For forming eimeriosis severity categories three criteria related with *Eimeria* infection were assessed: presence of diarrhoea, total oocyst count >500 opg in faecal sample and presence of pathogenic *Eimeria* species (*E. bovis* or *E. zuernii*). If no criteria was met no evidence of eimeriosis was recorded (category 1), if one of the criteria was met the evidence of eimeriosis was considered weak (category 2), if two criteria were met the evidence was considered moderate (category 3), and if all three criteria were met the evidence were considered strong (category 4).