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Changes in biochemical analytes in calves infected by nematode parasites in field conditions☆

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ABSTRACT

Parasitic infections caused by nematodes are a major problem in bovines that resulting in losses in animal health and production. Thus, the aim of this study was to evaluate alterations in selected serum biochemical analytes in calves naturally infected with gastrointestinal (GI) and pulmonary nematodes without clinical signs. For this, samples of feces and blood of 86 calves were collected. Fecal egg counts (FEC) were determined using the modified McMaster technique with a sensitivity of 50 eggs per gram of feces (EPG). Positive nematode FEC was processed for coproculture using pooled samples to identify Strongylidae infective larvae (L3). First stage-larvae (L1) of *Dictyocaulus viviparous* were identified by a modified Baermann method. The biochemical analytes determined were: acute phase proteins such as haptoglobin and paraoxonase type 1; the enzymes acetylcholinesterase; butyrylcholinesterase; the lipid profile (triglycerides and total, HDL, and LDL-cholesterol); serum iron profile (iron and unsaturated iron-binding capacity); total protein and albumin; pancreatic profile (amylase and lipase); and minerals (phosphorus and calcium). The calves were divided into four groups according to the results of EPG and the modified Baermann method. Group 1: healthy control animals ($n = 16$); Group 2: calves with only GI parasites ($n = 51$): This group was sub-divided into sub-groups according to the EPG threshold: 2a—GI parasites with low EPG ($n = 23$), and 2b—GI parasites with high EPG ($n = 28$). Group 3: animals with only lungworms ($n = 5$), and Group 4: calves with lung + GI parasites ($n = 14$). The more prevalent genera in all coprocultures were: *Cooperia* spp., *Haemonchus* spp., *Oesophagostomum* spp., and *Trichostrongylus* spp. The nonparametric Kruskal–Wallis test was used to compare the groups and Dunn's post-test was used for multiple comparisons as the data was not normally distributed ($P < 0.05$). The haptoglobin concentration increased in calves with GI and pulmonary parasites. A significant increase in acetylcholinesterase was observed in calves infected with lungworms. Cholesterol, triglycerides, HDL, and LDL concentrations decreased but lipase concentration increased in calves with GI parasites. Therefore, this paper provides an overview of the biochemical effects produced by nematode parasites in calves in field conditions. These findings in calves without any evident clinical signs of disease could provide an indication of GI parasites and lungworm infection, especially in an endemic area for these parasites.

☆ The data regarding the nematode parasites prevalence was originally presented at the 42nd Brazilian Congress of Veterinary Medicine: Cezaro, M.C., Cury, J.R.L.M., Oliveira, R.M., Schmidt, E.M.S., 2015. Ocorrência de infecção por *Dictyocaulus viviparus* em bezerros no município de Botucatu, SP. In: 42° Congresso Brasileiro de Medicina Veterinária e 1° Congresso Sul-Brasileiro da ANCLIVEPA, Curitiba, 0204-0208 PR, Brazil.

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1. Introduction

Parasitic infections caused by nematodes in cattle are a major health problem around the world. Disease caused by nematodes both in clinical and subclinical presentations result in major losses to animal health and production (Charlier et al., 2009). In Brazil, the distribution of these parasites is favored by the predominance of tropical and subtropical climates. Estimated economic losses caused by gastrointestinal (GI) nematodes are around 7.11 billion dollars/year in Brazil (Grisi et al., 2014).

The most frequent GI nematodes in cattle in São Paulo State in southeastern Brazil are: *Haemonchus* spp. and *Ostertagia* spp., parasites of the abomasum; *Cooperia* spp., *Trichostrongylus* spp., and *Strongyloides* spp., parasites of the small intestine and *Oesophagostomum* spp., parasite of the large intestine. Additionally, the lung nematode *Dictyocaulus viviparous* has been reported in ruminants in São Paulo State (Oliveira, 1988; Gonçalves et al., 2000; Borges et al., 2001; Landim et al., 2001). These species of gastrointestinal nematodes are reported to be present worldwide (Keyyu et al., 2005; Holland et al., 2000; Jiménez et al., 2010), while, *D. viviparous* is most frequently described in temperate climates (Ploeger., 2002; Wapenaar et al., 2007; Lat-Lat et al., 2010). For all these parasites, the definitive host is infected by ingestion of infective larvae (L3) from contaminated pasture (Anderson, 2000) with the infection, in general being caused by mixed nematode species.

A variety of pathological effects occur during infection with these parasites such as anemia, weight loss, anorexia, dehydration, diarrhea, and submandibular edema (*bottle jaw*) among others (Taylor et al., 2007; Hogg et al., 2010), and when the lung parasite is also present, there could be coughing and tachypnea (Anderson, 2000; Silva et al., 2005). The clinical presentations of cattle with these parasitic infections differ by multiples factors, including the age of the animal. Calves are highly susceptible, because of the immature immune system, thus, in their first grazing season they commonly display clinical signs (Höglund et al., 2001). However, in many cases, individuals with a high parasite burden may not show any clinical signs. These subclinically infected calves are potential contaminators of pasture for the other animals (Taylor et al., 2007). Additionally, there is a decrease in production and weight gain, resulting in delayed development and furthermore this condition also adversely affects the animal's welfare (Gibbs, 1992).

We hypothesized that calves infected with GI and/or pulmonary nematodes but without clinical signs have changes in selected biochemical analytes related to inflammation, lipid and iron metabolism, pancreatic function and Ca–P metabolism, which could be used as tools to raise the possibility of infection despite the absence of clinical signs. Therefore, the aim of this study was to evaluate a panel of various serum analytes in calves naturally infected with GI and pulmonary nematode but without clinical signs. For this purpose the concentrations of selected acute phase proteins: haptoglobin (Hp), and paraoxonase-1 (PON-1), the enzymes acetylcholinesterase (AChe) and butyrylcholinesterase (BChe), a lipid profile (cholesterol, triglycerides, HDL, and LDL), a serum iron profile: iron and unsaturated iron-binding capacity (UIBC), total protein and albumin, a pancreatic profile (amylase and lipase) and minerals (phosphorus and calcium) were determined.

2. Material and methods

2.1. Animals

The study population comprised 86 crossbreed (Holstein × Girolanda) calves from 2 to 24 months old. The animals belonged to two small private farms in the municipalities of Botucatu and Manduri, São Paulo State, in the southeastern region of Brazil. The calves were monitored for 12 months (from September 2014 to August 2015). Blood and feces samples were collected every three months during the same week for both farms. This study was approved by the Faculty's Animal Experimentation Ethics Committee of the São Paulo State University—FMVZ, UNESP, Botucatu (18/2015—CEUA).

The calves were monitored clinically in both farms by weekly veterinary inspections, including a general visual inspection, evaluation of body condition score, oral mucous membranes examination, feces visual inspection, and rectal temperatures measurements. The animals were considered to be healthy if they did not show any evident clinical signs at the inspection and had rectal temperatures less than 39.5 °C. The animals were vaccinated for foot and mouth disease, and brucellosis according to current legislation in the Animal Health National Programs in Brazil (MAPA, 2009).

2.2. Fecal testing

Samples of feces were collected directly from the rectum of each animal and stored in a labeled plastic bag. Feces were transported at 4 °C to the Laboratory of Animal Parasitic Diseases of the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine and Animal Science (FMVZ), Botucatu, São Paulo State, Brazil for analysis. Fecal egg counts were determined using the modified McMaster technique with a sensitivity of 50 eggs per gram of feces (EPG) (Gordon and Whitlock, 1939). Depending on the EPG results, the animals were divided into subgroups, according to the threshold defined by Vercruysse and Claerebout (2001) and Antonello et al. (2010). Positive nematode egg feces were processed for coproculture (Roberts and O'Sullivan, 1950). In brief, coprocultures were prepared by mixing approximately 2 g of feces from each EPG positive animal to make farm pools which were macerated with distilled water, sterilized wood shavings, and incubated at 27 °C for seven days. One hundred larvae were counted under a microscope and the results were expressed as the proportion (%) of L3 recovered. Identification of Strongylidae infective larvae (L3) and the percentage of L3 were determined according to Ueno and Gonçalves (1998), Amarante, (2011) and Van Wyk and Mayhew (2013), using pooled samples. Fecal first larval stage (L1) of *D. viviparus* was determined by a modified Baermann method described by Rugai et al. (1954).

2.3. Blood analysis

Blood samples were collected from the jugular vein in plain tubes with gel separators, which were allowed to clot at room temperature for 30 min. After centrifugation (1500 × *g* for 5 min) sera were stored in Eppendorf microtubes at −20 °C.

2.3.1. Biochemical profile

Serum haptoglobin concentrations were measured via a hemoglobin binding assay previously validated for use in bovine (Eckersall et al., 1999). Serum PON-1 was determined using p-nitrophenyl acetate as substrate in an automated clinical chemistry analyzer (Olympus AU2700, Olympus Diagnostica GmbH) using an adaptation of a previously described assay (Tvarijonaviciute et al., 2012).

The Ache and BChe concentrations were determined using previously described method (Tecles and Cerón, 2001) adapted to an automated analyser (Olympus AU400, Olympus Diagnostica GmbH).

Total serum cholesterol, triglycerides, HDL and LDL; total protein, albumin; amylase, lipase, calcium, and phosphorus were measured using an automated analyzer (Olympus AU600, Olympus Diagnostica GmbH), following the instructions of the manufacturer using Olympus commercial kits.