Acute phase proteins in calves naturally infected with *cryptosporidium*

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Abstract

Infectious diarrhea remains one of the most important health challenges in dairy industries during the first four weeks of life, with *Cryptosporidium* infection as one of the main causes of this diarrhea. This study aimed to evaluate blood concentration of some acute phase proteins in calves naturally infected with *Cryptosporidium*. Ninety-six, 1 day to 4 week-old Holstein calves were allotted into control group (G1 n=48 healthy calves) and calves infected with *Cryptosporidium* (G2 n=48). Blood and fecal samples were collected from each calf at the same day. Enzyme Linked Immuno-Sorbent Assay (ELISA) was used to estimate serum levels of haptoglobin (Hp), serum amyloid A (SAA), and tumor necrosis factor alpha (TNFα), while gel electrophoresis was used to determine serum level of fibrinogen. Serum SAA, Hp, and fibrinogen significantly increased in infected calves, whereas there was no significant difference in serum level of TNFα between the two groups.

Key words: Acute phase protein, haptoglobin, serum amyloid A, tumor necrosis factor, fibrinogen, *Cryptosporidium*.

بروتينات الطور الحاد في العجول المصابة طبيعيا بطفيلي الابواغ الخبيئة

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الخلاصة

يمثل الإسهال الخمجي اهم التحديات الصحية التي تواجه صناعة الألبان خلال الأسابيع الأربعة الأولى من حياة العجول، ويعد طفيلي الابواغ الخبيئة احد المسببات الرئيسية لهذا النوع من الاسهال. هدفت هذه الدراسة الى تقدير المستويات المصلية لبعض بروتينات الطور الحاد في العجول المصابة طبيعيا بالأبواغ الخبيئة. وزع (96) عجلا من سلالة الهولستين ، بعمر يوم واحد – اربع اسابيع الى مجموعتين ، ضمت المجموعة الاولى (48) عجلا مصابا بالأبواغ الخبيئة فيما ضمت المجموعة الثانية (مجموعة السيطرة) (48) عجلا سليما. جمعت عينة دم وعينة براز من كل عجل في نفس اليوم. استخدمت طريقة الأدمصاص المناعي الأنزيمي (ELISA) في تقدير المستويات المصلية للهابتوكلوبين والنشواني المصلي A والعامل المنخر للسرطان، فيما استخدمت طريقة الترحيل الكهربائي على الهلام لتحديد مستويات مولد الليفين (الفبرينوجين). أظهرت المستويات المصلية للهابتوكلوبين والنشواني المصلي A والفبرينوجين ارتفاعا معنويا في العجول المصابة في حين لم يظهر فرق معنوي في مستويات العامل المنخر للورم بين المجموعتين.

الكلمات المفتاحية: بروتين الطور الحاد، الابواغ الخبيئة، الهابتوكلوبين ، النشواني المصلي A ،العامل المنخر للسرطان

Introduction

Cryptosporidium spp. is a common protozoan parasite that mainly infects the microvilli of the epithelial surface of the gastrointestinal tract, it is causing enteric disorders and diarrhea that may lead to weight loss and nutritional deficiencies (1). Acute phase proteins (APP) are blood proteins primarily synthesized by hepatocytes as part of the acute phase response (APR), which is part of the early defense or innate immune system (2). APP has been well recognized for their application to human diagnostic medicine and has been described to have value in the diagnosis and prognosis of cardiovascular disease, autoimmunity, and organ transplant and cancer treatment (3,4,5). Despite the limited number of studies conducted on animals in

this respect, many authors have shown an association between APP levels and the presence of severity of some diseases such as limb disease, dictyocaulosis and diarrhea in calves (6,7,8), Furthermore, it has been suggested that the APP can be used for haptoglobin (HP), serum amyloid A (SAA), and plasma fibrinogen (Fb) was detected during viral and /or bacterial respiratory infections in calves (9). This study aimed to estimate serum concentrations of SAA, Hp, Fb and tumor necrosis factor α (TNFα) in dairy calves infected with *Cryptosporidium* during the first 4 weeks of life.

Materials and methods

This study was conducted during the period from October 2009 to March 2010. Fecal and blood samples were collected from 120 diarrheic and 60 apparently healthy Holstein calves up to 4 weeks in age from Al-Nasr station/Wasit. Fecal samples were visually categorized into two categories: 1; a paste-like consistency, yellow color and perineum and/or tail smeared with feces, 2; watery consistency and perineum and/or tail smeared with watery feces. Modified Zehil Neelson (ZN) stain and floatation with saturated salt solution were used to detect the presence of *Cryptosporidium* oocysts in fecal samples (10). According to the results of these tests, 62 fecal samples from diarrheic calves were positive for cryptosporidiosis, from which 48 samples were chosen (24 samples with fecal score 1 and the other 24 with fecal score 2). On the other hand, 54 fecal samples from healthy calves were negative for the parasite, from which 48 samples were chosen randomly. Five ml of blood samples were collected simultaneously with fecal samples from each calve. Each blood sample was divided into two parts: one in a plane tube to obtain serum for estimation of serum levels of SAA, Hp and TNF-α; and the other in EDTA tubes for measurement of plasma concentration of Fb. According to the results of ZN stain and floatation, 40 serum and plasma samples were taken to represent calves that gave positive result for the disease, and 40 serum and plasma samples were taken to represent healthy calves. Enzyme linked immune-sorbent assay (ELISA) (Immunotech, France) was used to measure serum levels of SAA, Hp and TNF-α following the manufacturer's instructions, while Miller method (11) was used to estimate plasma concentration of Fb.

Statistical Analysis

Mean concentrations of acute phase proteins in healthy and infected calves with both fecal scores were compared using one-way ANOVA test. A value of $p \leq 0.05$ was considered to be significant.

Results

The results of this study revealed that the Infected calves with fecal score 2 showed the highest serum concentration of serum amyloid A (41.18 ± 13.3 mg/L), and different significantly from infection calves with fecal score 1 and healthy calves (27.36 ± 6.2 and 21.12 ± 2.7 mg/L respectively) (table 1). The infected calves with fecal score 1 and 2 had very closed serum concentration of Haptoglobin (0.28 ± 0.07 and 0.29 ± 0.06 g/L respectively) and both of them differed significantly from healthy calves (0.11 ± 0.02 g/L) (table 1). The highest concentration of plasma fibrinogen was recorded in infected

Table 1: Concentrations of Serum amyliod A (SAA), Haptoglobin (Hp), Fibrinogen (Fb), and Tumor necrosis factor-α (TNF-α) (Mean ± SE).

Parameters	Health calves (n=48)	Calves infected with Cryptosporidium	
		Fecal score 1 (n = 24)	Fecal score 2 (n = 24)
SAA (mg/L)	21.12 ± 2.7[a]	27.36 ± 6.2[b]	41.18 ± 13.3[c]
Hp (g/L)	0.11 ± 0.02[a]	0.28 ± 0.07[b]	0.29 ± 0.06[b]
Fb (g/L)	6.48 ± 1.4[a]	9.18 ± 2.1[ab]	10.14 ± 2.0[b]
TNF-α (pg/dl)	21.30 ± 10.2[a]	23.4 ± 8.2[a]	25.0 ± 9.2[a]

Note: Different letters within a row show significant differences

calves with fecal score 2 (10.14 ± 2.0 g/L) which differed significantly from healthy calves (6.48 ± 1.4 g/L) and significantly from infected calves with fecal score 1 (9.18 ± 2.1 g/L) (table 1).There were no significant differences in serum concentration of Tumor necrosis factor-α among the healthy calves, calves with fecal score 1, and calves with fecal score 2 (21.30 ± 10.2, 23.4 ± 8.2 and 25.0 ± 9.2 pg/dl respectively) (table 1).

Discussion

Diarrhea is one of the most important health challenges in dairy industries during the first 4 weeks of life. *Cryptosporidium* infection represents one of the most important cause of this diarrhea (10) which may not be diagnosed properly and cause heavy economic losses. One of the modern method to monitor the course and severity of the disease is to estimate the changes in the concentration of APP (12). The proteins whose concentration increase due to inflammatory factors are termed positively reacting proteins (e.g. SAA, Hp, and Fb), and those whose concentrations decrease are called negatively reacting proteins (e.g. albumin and transferrin) (13). It is not surprising that most APPs investigated in this study has been elevated in infected calves since *Cryptosporidium* is known to cause destruction of intestinal epithelia, blunting of the microvilli and mononuclear cells infiltration in the lamina properia (10). Hence the releasing of inflammatory factors causes an elevation of positively reacting proteins. (14) reported that SAA was shown to induce leukocyte migration, differentiation of neutrophils, and activation of putative receptors of neutrophils leading to increase the production of IL-8 (15). These activities increase innate host response and enhance the elimination of the pathogen. Hp main functions are to bind the free hemoglobin and remove it from the circulation, prevent iron-stimulated formation of oxygen radicals, and acts as anti-oxidant (16). Furthermore, Hp may have a role in inflammatory process as it stimulates the formation of prostaglandin E2 and potentiates the stimulatory effect of bradykinin and thrombin on PGE2 formation (17). Fibrinogen is one of the main plasma proteins. Its main function is in clot formation. In addition, It has several different roles. (18) Found inflammatory process, it primarily interacts with leukocytes through the surface receptors called integrin's, and facilitates a chemotactic response for leukocytes. One of the proposed mechanism by which Fb induce pro-inflammatory changes in leukocytes include an increase in the free intracellular calcium which results in an increase in phagocytosis, antibody mediated leukocyte toxicity and delay in apoptosis (19). TNF-α is a pro-inflammatory cytokine that is commonly produced by T-cells and other cells during infection. Numerous studies have shown that this cytokine is up-regulated in the intestine during *Cryptosporidium parvum* infection of mice (20, 21), However serum concentration of this cytokine is affected by many internal and environmental condition and this may explain the insignificant difference in its concentration between infected and healthy calves. It is obvious from the values of the four parameters that the severity of infection (as indicated by high fecal score) is accompanied by increased concentrations of investigated APP. That is why these APPs can be used as indications for the course and severity of infection.

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