

Calves with Cryptosporidiosis: Acute Phase Proteins and Biochemical Parameters

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ABSTRACT

Background: *Cryptosporidium* is a major intestinal pathogen that causes diarrhea, intestinal damage, and significant economic losses in calves. An infection can lead to systemic inflammatory and acute-phase responses, causing changes in serum biochemical parameters. Therefore, the aim of this study was to evaluate serum haptoglobin and ceruloplasmin concentrations in calves naturally infected with *Cryptosporidium*, along with selected biochemical parameters.

Materials, Methods & Results: The study included a total of 30 calves, comprising 20 calves presenting with watery diarrhea, tenesmus, and dehydration, and 10 clinically healthy calves serving as controls. *Cryptosporidium* infection was confirmed by examination of fecal samples using an immunochromatographic rapid diagnostic test and modified acid-fast (mAF) staining. Serum samples were analyzed to determine haptoglobin (HP) and ceruloplasmin (CP) concentrations, together with selected oxidative stress markers and biochemical parameters. Compared with clinically healthy controls, infected calves exhibited significantly increased serum HP and CP levels, along with alterations in oxidative stress and biochemical parameters. These findings suggest that *Cryptosporidium* infection is associated with a systemic acute-phase response in calves, characterized by increased HP and CP concentrations and concurrent biochemical alterations.

Discussion: In calves infected with *Cryptosporidium*, CD4+ T cells mediate immune responses involving both cellular and humoral mechanisms against the parasite. In response to the body's cellular and humoral immune response, increased levels of certain cytokines lead to APP production in the liver. Albumin, haptoglobin, and ceruloplasmin are diagnostically important in ruminants. This study demonstrated that haptoglobin and ceruloplasmin levels were elevated in comparison with the control group. The reason for this is likely the tissue damage caused by the parasite. Albumin, a negative APP, is produced in the liver. This study has shown that albumin levels in diseased animals are lower than those in healthy animals. Oxidative stress observed in bacterial and viral diseases stems from an imbalance between oxidants and antioxidants. The study showed that the oxidant-antioxidant system was disrupted, and when the control group was compared to the *Cryptosporidium*-infected group, MDA concentrations increased while antioxidant activity decreased. Changes occur in cholesterol, LDL-C, and HDL-C levels during lipolysis of adipose tissue. In this study, although VLDL-C concentrations were significantly higher than those observed in the control group, the reductions in cholesterol, HDL-C, and LDL-C levels may be associated with enteropathy or inflammatory processes. It has been reported that parasitic infections affect various blood parameters in animals. In this study, the increase in AST and ALP activity may be due to liver and intestinal damage, the elevation in serum urea and creatinine levels may be reduced renal perfusion, and the increase in total protein may be due to globulin synthesis. In conclusion, the findings indicate that *Cryptosporidium* infection in calves leads to a distinct acute-phase response accompanied by increased haptoglobin and ceruloplasmin concentrations and significant changes in various biochemical parameters. These findings highlight the systemic effects of *Cryptosporidium* infection and its potential impact on the metabolic and inflammatory status of affected calves.

Keywords: calf, diarrhea, cryptosporidiosis, acute phase proteins, lipid profile.

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INTRODUCTION

Neonatal calf diarrhea not only causes significant economic losses, but approximately 30% of calves with diarrhea are infected with *Cryptosporidium* [1]. The causative agents of cryptosporidiosis cause damage to the intestinal epithelium and, consequently, impaired absorption. Transmission occurs via the fecal-oral route [30]. Cryptosporidiosis is a zoonotic disease characterized, particularly in calves, by profuse watery diarrhea, dehydration, loss of appetite, and tenesmus [28].

An acute-phase response to *Cryptosporidium* and other parasitic causes stimulates the production of acute-phase proteins (APP) in the liver [27]. In cattle (*Bos taurus*), ceruloplasmin (CP) and haptoglobin (HP) are particularly important. Acute-phase proteins are key biomarkers for distinguishing between bacterial and viral infections and for assessing prognosis [8,27]. It has been shown that diarrhea can cause changes in lipid concentrations [25]. Free radicals are produced as a consequence of the body's normal use of oxygen and cause oxidative injury to proteins, nucleic acids, and lipids [20,35]. The antioxidant system functions by reducing the harmful effects of free radicals [34]. During stress and infection, the balance between oxidants and antioxidants is disrupted, which can lead to an increase in free radicals and tissue damage [3,20].

There are numerous studies on biochemical alterations in neonatal calves affected by diarrhea [6,16,37]. However, only a few studies [11,19] have examined acute-phase protein levels in calves with cryptosporidiosis. Thus, this study aims to evaluate serum HP, CP, and certain biochemical parameters in calves with cryptosporidiosis.

MATERIALS AND METHODS

Animals and study design

The study included a total of 30 calves, comprising 20 calves presenting with watery diarrhea, tenesmus, and dehydration, and 10 clinically healthy calves. Etiological diagnosis was performed on fecal samples using a rapid immunochromatographic test kit (BoviD-5 Ag Test Kit®)¹.

Detection of Cryptosporidium oocysts

Fresh fecal samples collected directly from the rectum were examined for *Cryptosporidium* oocysts using the modified acid-fast (mAF) staining method

(Figure 1). Briefly, 5-10 mL of homogenized fecal sample was diluted with tap water and centrifuged at $1,008 \times g$ for 3-5 min (Hasvet LC-04B centrifuge)². Smears prepared from the resulting sediment were stained using the mAF method and examined under a light microscope (Nikon Eclipse E600 Microscope)³ at 40× magnification [21].

Biochemical analysis

Blood samples were taken from the jugular vein, transferred into tubes anticoagulant-free, and centrifuged at $1,008 \times g$ for 10 min. Following separation, the serum was maintained at -20°C (Vestel CD 6001E)⁴ until analysis. Serum ceruloplasmin concentrations were determined spectrophotometrically (UV-1201, Shimadzu)⁵. Total protein, aspartate aminotransferase (AST), albumin, creatinine, urea, and alkaline phosphatase (ALP) were measured colorimetrically (Epoch, BioTek)⁶ using commercial test kits (IBL)⁷ according to the methods described by Colombo and Ricterich [12] and Skinner *et al.* [33]. The serum globulin level was calculated by subtracting the serum albumin level from the serum total protein level. Serum levels of triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) were determined using a colorimetric method (Epoch, BioTek)⁶ using commercial kits (DDS)⁸. The Friedewald formula [15] was applied to estimate very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations: $\text{LDL-C (mg/dL)} = \text{Total cholesterol} - \text{HDL-C} - (\text{Triglyceride}/5)$ When the triglyceride concentration was $< 400 \text{ mg/dL}$, VLDL-C was calculated as: $\text{VLDL-C (mg/dL)} = \text{Triglyceride (mg/dL)}/5$ Whole-blood glutathione (GSH) levels were determined spectrophotometrically (UV-1201, Shimadzu)⁵ according to the method described by Beutler *et al.* [7], whereas serum malondialdehyde (MDA) levels were measured according to the method described by Yoshioka *et al.* [38].

Statistical analysis

All statistical analysis were conducted using SPSS version 20.0. Data distribution was assessed for normality using the Shapiro-Wilk test. The variables that followed a normal distribution were compared between groups using the independent samples *t*-test, while the Mann-Whitney U test applied to variables that did not follow a normal distribution. Results with a $P < 0.05$ were accepted as statistical significance.

RESULTS

Clinical findings

Clinical examinations of calves aged 1 to 10 days with *Cryptosporidium* infection revealed varying degrees of dehydration. A decrease in rectal body temperature was also observed in the infected calves.

Biochemical findings

Serum haptoglobin and ceruloplasmin levels were significantly higher in *Cryptosporidium*-infected calves than in control calves ($P < 0.001$).

In the present study, calves infected with *Cryptosporidium* exhibited significantly higher globulin, ALP, and MDA levels than those in the control group ($P < 0.01$).

When serum AST, triglyceride, cholesterol, and VLDL levels were compared between calves with *Cryptosporidium* infection and the controls, a statistically significant increase was observed in the infected group ($P < 0.001$).

Serum urea and creatinine levels ($P < 0.001$) and total protein levels ($P < 0.05$), were significantly higher in *Cryptosporidium*-infected calves than in control calves.

In the present study, serum albumin ($P < 0.05$), GSH ($P < 0.05$), cholesterol ($P < 0.001$), HDL-C ($P < 0.05$), LDL-C ($P < 0.01$), and albumin-to-globulin ratio ($P < 0.01$) levels were significantly lower in *Cryptosporidium*-infected calves than in the control group.

In the group with *Cryptosporidiosis*, total bilirubin levels were higher than in the healthy control group, but the difference was not statistically significant ($P > 0.05$) [Tables 1 & 2].

Fecal examination findings

Microscopic examination of fecal smears prepared using the modified acid-fast (mAF) staining method revealed *Cryptosporidium* spp. oocysts measuring approximately 4-6 μm in diameter exhibiting a characteristic round/spherical morphology and staining bright pink-red in color. The oocysts were clearly distinguishable from the surrounding fecal material and cellular debris showed variable distribution densities within the smears and displayed the typical acid-fast staining characteristics of *Cryptosporidium* spp. (Figure 1).

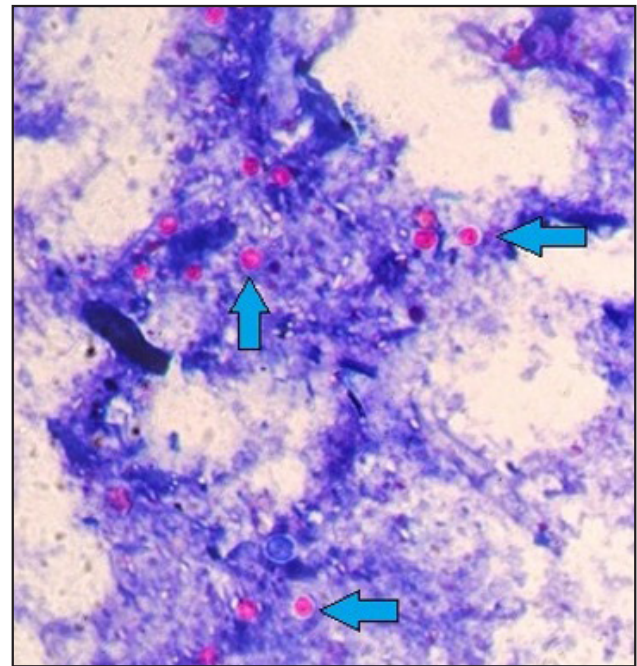


Figure 1. Cryptosporidiosis agents (arrowheads) in feces of calves determined by modified acid fast method.

Table 1. Acute phase proteins and biochemical parameter levels in clinically healthy calves and calves with *Cryptosporidiosis* (Mean $\pm$ SE).

Parameters	Control	Infected	P
HP (g/L)	0.094 $\pm$ 0.004	0.925 $\pm$ 0.035	< 0.001
CP (mg/dL)	9.61 $\pm$ 0.78	14.89 $\pm$ 1.01	< 0.01
Albumin (g/dL)	3.11 $\pm$ 0.09	2.87 $\pm$ 0.04	< 0.05
Total protein (g/L)	7.13 $\pm$ 0.20	7.82 $\pm$ 0.19	< 0.05
Globulin	4.02 $\pm$ 0.22	4.95 $\pm$ 0.18	< 0.01
Albumin/Globulin	0.80 $\pm$ 0.06	0.60 $\pm$ 0.02	< 0.01
AST (U/L)	47.64 $\pm$ 1.81	77.60 $\pm$ 4.49	< 0.001
ALP (U/L)	67.44 $\pm$ 0.77	79.97 $\pm$ 0.71	< 0.01
Urea (mmol/L)	7.54 $\pm$ 0.40	37.10 $\pm$ 1.86	< 0.001
Creatinine ($\mu\text{mol/L}$)	64.61 $\pm$ 4.95	142.03 $\pm$ 7.76	< 0.001
Total bilirubin (mg/dL)	0.15 $\pm$ 0.02	0.21 $\pm$ 0.01	> 0.05

Table 2. Lipid profile and oxidative stress levels in clinically healthy calves and calves with *Cryptosporidiosis* (Mean $\pm$ SE).

Parameters	Control	Infected	P
MDA ($\mu\text{mol/L}$)	0.37 $\pm$ 0.04	0.52 $\pm$ 0.02	< 0.01
GSH (mg/dL)	0.69 $\pm$ 0.07	0.52 $\pm$ 0.04	< 0.05
Triglyceride (mg/dL)	20.12 $\pm$ 0.70	31.43 $\pm$ 1.14	< 0.001
Cholesterol (mg/dL)	77.61 $\pm$ 4.02	50.33 $\pm$ 1.75	< 0.001
HDL-C (mg/dL)	40.47 $\pm$ 3.51	31.35 $\pm$ 1.41	< 0.05
VLDL-C (mg/dL)	4.02 $\pm$ 0.14	6.29 $\pm$ 0.23	< 0.001
LDL-C (mg/dL)	33.12 $\pm$ 4.93	12.69 $\pm$ 1.54	< 0.01

DISCUSSION

In calves infected with *Cryptosporidium*, CD4+ T cells mediate cellular and humoral immune responses against the parasite [17,31]. This immune response is associated with increased production of cytokines such as IFN- γ and IL-12, which stimulate hepatic APP synthesis. Albumin, haptoglobin, and ceruloplasmin have diagnostic significance in ruminants.

Haptoglobin concentrations, which are very low in the serum of healthy cattle, increase in various bacterial [22], viral [24], and parasitic diseases [9,23]. In a study conducted on calves infected with *Cryptosporidium*, although increases were observed in serum HP, C-reactive protein (CRP), amyloid A, CP, and malondialdehyde (MDA) levels, as well as in superoxide dismutase (SOD) and adenosine deaminase (ADA) activities, in blood samples taken before and after treatment, no statistically significant difference was found in SAA, CRP, and MDA levels; however, after treatment, decreases were observed in ADA and SOD activities, as well as in HP and CP levels [11].

In addition, according to Kabu [19], measuring SAA, Hp, IL-1 β , and IL-6 concentrations in *Cryptosporidium*-infected calves may be useful for assessing the severity of the disease, determining the appropriate treatment, monitoring treatment efficacy, and identifying subclinical disease during the treatment period.

This study has demonstrated that haptoglobin and ceruloplasmin levels are elevated in comparison to the control group. This increase may be associated with tissue damage caused by the parasite.

In addition to its role as a negative acute-phase protein (APP), albumin is primarily synthesized in the liver and is subsequently catabolized predominantly through renal pathways. Liver disease, damage to tissues, inflammation and intestinal disease have been reported to lead to a decrease in albumin concentrations [36]. This study revealed that albumin values in diseased animals were lower than those in healthy animals. Tissue damage, infections, and inflammatory conditions in the body lead to the activation of mononuclear phagocytic cells [32]. These cells, which are involved in defense, cause the production of free radicals along with the consumption of large amounts of oxygen.

Research has reported that oxidative stress occurs due to disruption of the oxidant-antioxidant balance in bacterial/viral diseases such as brucellosis

[26], sheep (*Ovis aries*) pox [18], and traumatic reticuloperitonitis [2].

The present study showed that the oxidant-antioxidant balance was disrupted in *Cryptosporidium*-infected calves, as evidenced by increased MDA concentrations and decreased antioxidant activity compared with the control group.

Lipolysis of adipose tissue, together with the increased availability of fatty acids during infection, contributes to enhanced triglyceride synthesis. In parallel with the increase in triglyceride concentration, VLDL synthesis increases. LDL-C is the lipoprotein responsible for transporting fat-soluble vitamins and phospholipids from the liver to tissues. HDL-C is a lipoprotein that facilitates the transport of cholesterol to the liver while also having anti-inflammatory effects. HDL-C concentration has been reported to decrease during inflammatory responses [10].

In studies conducted in calves with neonatal diarrhea, it was reported that cholesterol was decreased in bacterial and parasitic infections and triglyceride, HDL, LDL and VLDL concentrations were decreased in viral and/or mixed infections [5,14]. In the present study, although there was a significant increase in VLDL-C levels compared to the control group, the low levels of cholesterol, HDL-C and LDL-C may possibly be due to enteropathy and/or inflammation.

Parasitic infections have been reported to affect various blood parameters in animals [4]. AST is a marker of liver parenchymal injury, while ALP indicates cholestasis [13]. AST and ALP activities were increased in the study and may be the result of damage to the liver and/or intestinal tissue. Total protein concentration was found to increase in the study, which may be due to an increase in globulin synthesis [37].

In diarrheic calves, elevated serum urea and creatinine concentrations have been reported to result from reduced renal perfusion associated with dehydration [29]. In this study, a significant increase in serum urea and creatinine levels was determined in accordance with other studies [29,37].

CONCLUSION

As a result, it has been determined that an acute phase response develops in calves infected with *Cryptosporidium*, leading to increased synthesis of haptoglobin and ceruloplasmin and significant changes in biochemical parameters.

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Artificial intelligence statement. ChatGPT (OpenAI) was used solely for academic English translation and language editing. All AI-assisted content was reviewed and approved by the authors, who take full responsibility for the final manuscript.

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