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OXIDATIVE STATUS AND ORGAN DAMAGE MARKER PARAMETERS IN CALF CRYPTOSPORIDIOSIS

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ABSTRACT

Aim: Neonatal calf diarrhoea is the major cause of calf deaths in the world. Although some pathogens are including calf diarrhoea, Cryptosporidium spp. is the most determined microbial agent as protozoa. The main aim of this research was to determine the blood oxidative status parameters of the calf with cryptosporidiosis. Also, heart, liver and renal damage markers were also determined.
Materials and Methods: Totally healthy 15 calves and cryptosporidiosis-caused diarrheic 15 calves were used, in this research. Serum oxidative status parameters (Malondialdehyde, total antioxidant status, superoxide dismutase, catalase) were measured with ELISA. Liver (Aspartate aminotransferase, alkaline phosphatase, gamma glutamyltransferase) and kidney (Creatinine, blood urea nitrogen) damage markers were measured by auto-analyzer, whereas troponin I level, cardiac damage marker, was determined with chemiluminescence immunoassay technique.
Results: Increased total antioxidant status was determined ($P<0.05$) in the diarrheic calves compared to control group, while there was no any statistically significance changes determined in the oxidative status parameters. Also, increased troponin I and blood urea nitrogen levels were measured ($P<0.05$) in the diarrheic calves.
Conclusion: It may be stated that major changes are not observed in the diarrheic calves with cryptosporidiosis, but it may cause slightly cardiac and renal damage. However, histo-pathological exams should be done.

Keywords: Cryptosporidiosis; oxidative status; organ damage markers; calf.

INTRODUCTION

Cryptosporidiosis is an intestinal protozoan parasite caused by Cryptosporidium species in many species of mammals. This protozoon is a zoonotic character, and it is usually located in the digestive system epithelial cells of humans and mammals (Fayer, 2010). Cryptosporidiosis has been reported to cause severe economic losses in a variety of animal species (such as calves, lambs and goats) in the neonatal period and it has been reported that calves are most infected with Cryptosporidium species in studies (Castro-Hermida et al. 2002; Ramirez et al. 2004; Xiao, 2010; Ekici et al. 2011; Aydogdu et al. 2018). The species that cause

cryptosporidiosis in the cattle are *Cryptosporidium parvum* and *C. anderson*. *C. parvum*, and they are located in the small intestine, and *C. anderson* is located in the abomasum (Inci, 2013). Infection is usually caused by oral intake of contaminated foods and drinking water with sporulated oocysts that are excreted in the stool of the host. The most important clinical symptom of cryptosporidium infections is diarrhoea with yellowish colour (Dubey et al. 1990). In particular, 1-3 week old calves are very sensitive to the disease (Inci, 2013). Routine stool examination methods such as native examination and flotation can be used in the diagnosis of the disease, but it is difficult to distinguish Cryptosporidium oocysts from other small particles in the stool. For this reason, dyeing methods such as Modifiye Ziehl-Neelsen, Kinyoun acid-fast, Carbol fucksin, Tristram, Acridine Orange, Modifiye Köster are used in acid-fast staining methods for definite diagnosis (Sakarya et al. 2010; Inci, 2013).

The determination of malondialdehyde (MDA) levels from biological samples are the most useful biological parameter for detecting lipid peroxidation in oxidative stress studies (Sezer and Keskin 2014; Ilgin et al. 2015; Elbe et al. 2016). In the living cells, reactive oxygen species (ROS) continuously are produced especially after various metabolic events including infection, cancer, etc. The created ROS are detoxified by some antioxidants [catalase (CAT), superoxide dismutase (SOD)] in the body. SOD enzyme converts the superoxide radical into hydrogen peroxide, which is subsequently inactivated by direct conversion into the water by the CAT enzyme. When produced ROS cannot be inactivated because of deficiently antioxidant capacity and excessive produced ROS, ROS may cause many pathological events. This situation is called as oxidative stress derived from lipid peroxidation in cells (Yazar and Tras 2002; Ogut and Atay 2012). In last decade, the totals antioxidant status (TAS) parameter was used in the oxidative stress research, and measured TAS level is accepted the total activity of antioxidant substances against free radicals in vivo (Guler et al. 2004; Sirmatel et al. 2009).

Cardiac troponins, heart-specific proteins, have three subtypes. Troponins regulate the calcium-dependent interaction of actin and myosin in the heart. Just after the cardiac damage, troponin I (TnI) is released simultaneously from the heart, and TnI level is accepted as primer diagnostic marker of myocardial damage (Larue et al.1993; Fromm, 2007; Corum et al. 2015). Liver and bile duct damage may be determined with some values measured from blood samples. Increased serum alkaline phosphatase (ALP) activity reflects the bile duct damage while increases in the gamma glutamyltransferase (GGT) and aspartate aminotransferase (AST) activities are accepted the determination of liver damage. On the other hand, increased blood urea nitrogen (BUN) and creatinine levels are indicative of renal failure and decreased renal perfusion (Turgut, 2000; Kerr, 2002).

In this study, it was aimed to determine the blood oxidative status parameters of the calf with cryptosporidiosis. Also, heart, liver and renal damage markers were also determined.

MATERIALS AND METHODS

In the present study, serum samples of different sexes 15 healthy and 15 diarrheic calves with cryptosporidiosis were used. Research samples were selected from routinely coming samples to parasitology laboratory, which were diarrhoea with

cryptosporidium. Cryptosporidium oocysts were diagnosed by microscopic examination of faecal smears with a modified acid-fast technique (Ok et al. 1997). Healthy calves' serum samples were obtained from our laboratory stock samples. Blood MDA (Bioxytech MDA-586 Kit, OxisResearch, OR, USA), TAS (Total Antioxidant Status Kit, Rel Assay Diagnostics, Gaziantep, Turkey), SOD (Cayman Superoxidase Dismutase Assay Kit, MI, USA) and CAT (Cayman Catalase Assay Kit, MI, USA)) levels were measured by an ELISA reader (MWGt Lambda Scan 200, Bio-Tec Instruments, Winooski, VT, ABD). Hepatic (AST, ALP, GGT) and renal (BUN, creatinine) damage markers were measured by auto-analyser (ILab-300 plus, Instrumentation Laboratory, Milano, Italy). TnI, specific cardiac damage markers, were measured with the chemiluminescence immunoassay technique (Siemens Advia Centaur XP, Erlangen, Germany).

Research data were presented as mean±SEM. Data were evaluated by 2-sample t-test (SPSS 22.0). $P<0.05$ level was accepted as statistically significant.

RESULTS AND DISCUSSION

Serum oxidative status values (MDA, SOD, CAT), liver-bile duct (AST, ALP, GGT) and renal (Creatinine) damage markers of healthy calves and calves with cryptosporidiosis are presented Table 1, and there was no any statistical significance determined these values.

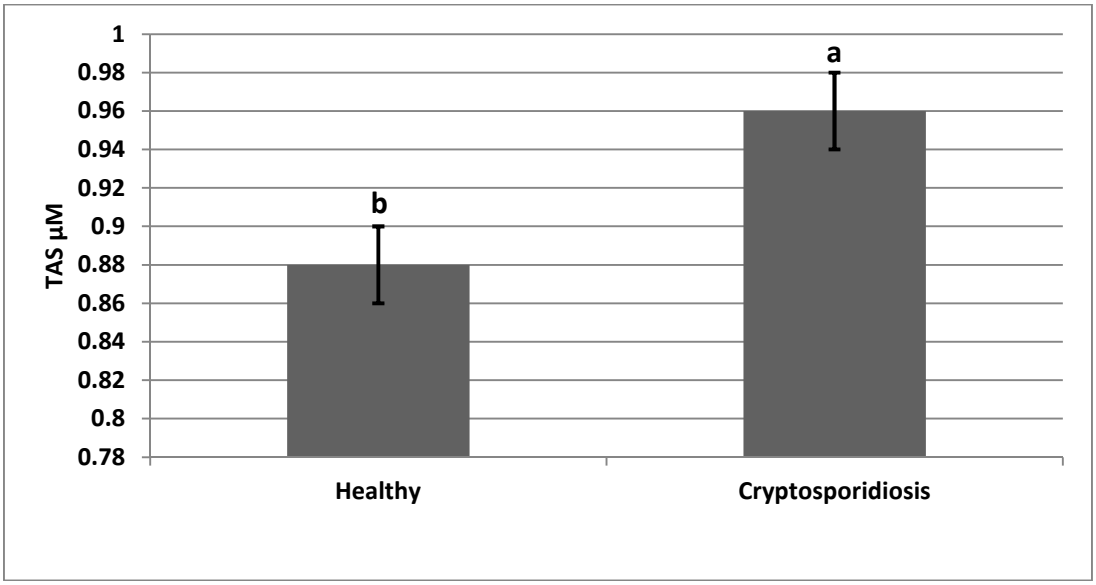
TAS, TnI and BUN levels are shown in the Graphics 1, 2, and 3, respectively. Statistically significant increases in the levels of TAS, TnI and BUN of the calves with cryptosporidiosis were measured ($P<0.05$) when compared Healthy group.

It is well known that neonatal diarrhoea is the main cause of calf death. Although bacterial (*E. coli*, Salmonella spp., Campylobacter spp. etc.) and viral (Calicivirus, astrovirus, parvovirus, etc.) agent are playing the central role in the neonatal calf diarrhoea, Eimeria spp. and Cryptosporidium spp. may always be determined as protozoal agents. It is reported that Cryptosporidium spp. may cause diarrhoea in calves less than four months (Birdane, 2017).

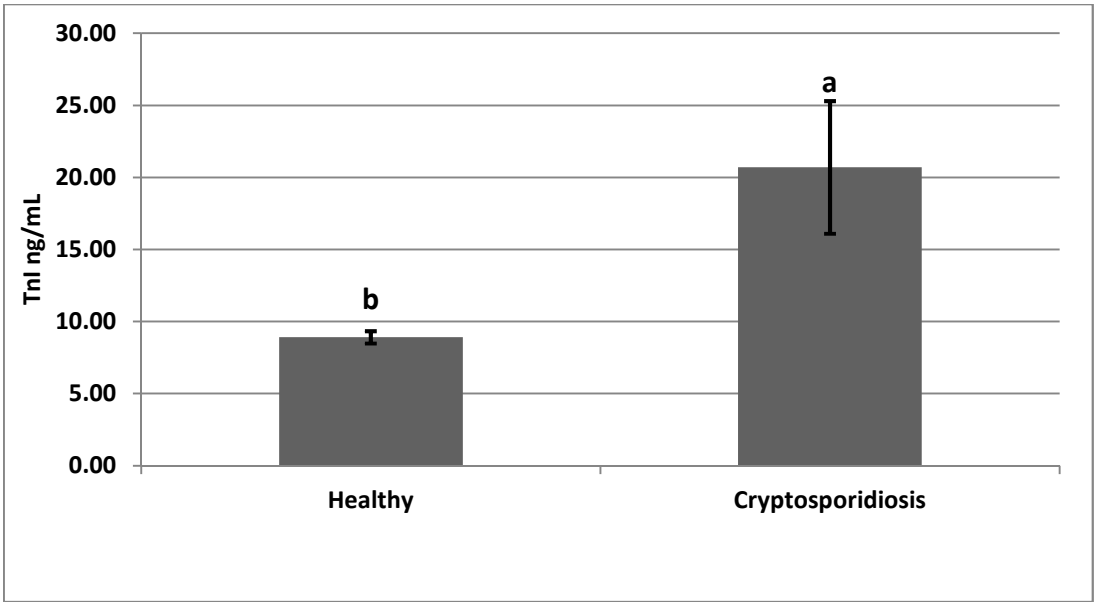
Especially MDA is a very important value within oxidative status parameters in the living cells. The level of MDA has been used for the determination of lipid peroxidation caused oxidative stress for many years with commercially available kits. SOD enzyme dismutase superoxide radical to hydrogen peroxide, and CAT enzyme converts the hydrogen peroxide to molecular

Table 1. Serum oxidative status and organ damage markers of healthy calves and calves with cryptosporidiosis

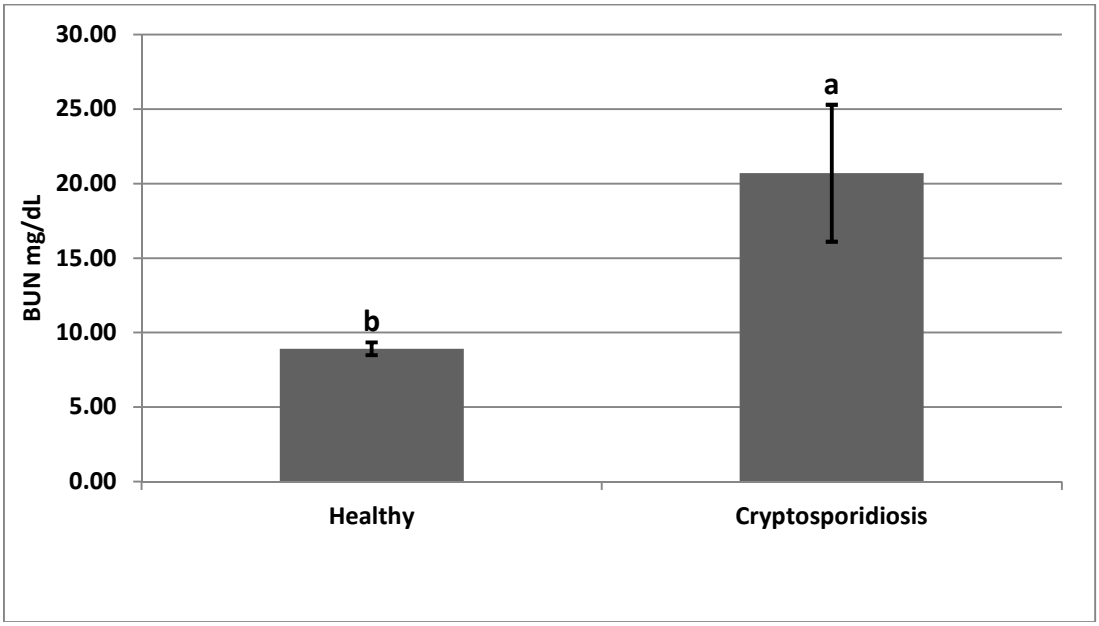
Parameters	Healthy (Control)	Cryptosporidiosis	P value
MDA µM	0.88±0.15	0.82±0.32	P>0.05
SOD U/L	0.08±0.001	0.07±0.003	P>0.05
CAT nmol/min/mL	94.3±21.0	105.6±24.0	P>0.05
AST U/L	38.1±4.8	51.4±13.0	P>0.05
ALP U/L	120.6±14.0	93.0±19.0	P>0.05
GGT U/L	25.0±9.8	69.3±23.0	P>0.05
Creatinine mg/dL	0.58±0.04	0.71±0.07	P>0.05



Graphic 1. Total antioxidant status (TAS) levels of healthy calves and calves with cryptosporidiosis (mean±SE)



Graphic 2. Troponin I (TnI) levels of healthy calves and calves with cryptosporidiosis (mean±SE)



Graphic 3. Blood urea nitrogen (BUN) levels of healthy calves and calves with cryptosporidiosis (mean±SE)

oxygen and water (Yazar and Tras, 2002; Ayala et al. 2004). In the current research, no statistically significance changes were determined in the MDA, SOD and CAT levels of calves infected with cryptosporidium compared to Healthy calves (Table 1). In the literature, unchanged, decreased or increased MDA levels in calves, cows, piglets and mice which were infected with cryptosporidium have been reported (Yassien et al. 2001; Gookin et al. 2002; Zhou et al. 2012; Cenesiz et al. 2017; Asadpour et al. 2018). In this study, statistically significantly increased TAS level in the diarrheic calves with cryptosporidiosis was measured when compared to Healthy group (Graphic 1). TAS value is commonly accepted as the total antioxidant activity of the body against to free radicals (Guler et al. 2004; Sirmatel et al. 2009; Coskun et al. 2018). Increased total antioxidant capacity has been reported in the mice infected with cryptosporidium by Zhou et al. (2012). The different result of these researches may be derived from especially severity of infection stage, the antioxidant capacity of infected animals and animal kinds.

In the present research, increased TnI levels were measured in the calves infected with cryptosporidium when compared to Healthy group (Graphic 2). TnI is very specific cardiac damage marker, and it increases within 2-6 hour after cardiac damage (O'Brien, 2008). We can no find directly related article that mentioned cryptosporidium associated with TnI level. However, it has been reported that cryptosporidial gastroenteritis may change heart function and neonatal cryptosporidiosis may be a potential cardiovascular diseases risk factor (Anatskaia et al. 2011; Anatskaia et al. 2012; Anatskaia et al. 2013). It may be

stated that Cryptosporidium spp. cause heart damage, and heart function may be considered by the clinician in the cryptosporidiosis caused diarrhoea.

In the current research, levels of ALP, AST and GGT which are liver-bile duct damage markers and levels of creatinine which is renal damage marker has no changed (P>0.05, Table 1). However, BUN level statistically significantly increased in the calves infected with cryptosporidium compared to Healthy group (Graphic 3). Similarly, Zhou et al. (2012) reported that ALP and ALT levels did no change in cows infected with Cryptosporidium. However, Krause et al. (2012) reported that Cryptosporidium might cause acute renal failure in the immune depressant patients. Only increased BUN levels, a marker of renal damage (Turgut, 2000) in this research, but not creatinine which is another renal damage marker, is considered, it may be stated that cryptosporidium induced infection in calf causes slightly renal damage due to diarrhoea by inducing the fluid-electrolyte balance disorder.

CONCLUSION

As a result, it may be stated that Cryptosporidium-caused diarrhoea has no impressive effect on the blood oxidative status parameters (MDA, SOD, CAT) in calves, but increased TAS levels may be observed in the sick animals. Interestingly, it may also be stated that cardiac and renal damage may be observed in the calves with cryptosporidium-caused diarrhoea. However, histo-pathological exams should be done in the sick animals.

ETHICAL APPROVAL

No need ethical approval because of using routine laboratory samples.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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