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Research Article

Investigation of Acute Phase Reactants and Antioxidant Capacity in Calves Infected with *Cryptosporidium parvum* [1]

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Abstract

Cryptosporidiosis is a zoonotic infection contaminating via fecal-oral route. *Cryptosporidium parvum* has a wide host prevalance, but is more epidemic in calves. This disease courses with high morbidity and mortality resulting considerable economic losses. In this study, halofuginon (100 µg /kg/day for 7 days) was applied to calves infected with *C. parvum* and the effect of this treatment on acute phase proteins and antioxidant capacity were investigated. Study group was comprised of sera of 10 Holstein calves aged 1-3 weeks, infected with *C. parvum*. Blood samples were obtained from the animals before and after treatment of 7 days and serum amyloid A (SAA), haptoglobin (Hp), C-reactive protein (CRP), ceruloplasmin (CP), malondialdehyde (MDA) levels and superoxide dismutase (SOD) and adenosine deaminase (ADA) activities were measured in sera. Obtained data showed that there was no statistical difference between pre and post treatment SAA, CRP and MDA levels, but a decrease was determined in post treatment Hp ($P<0.001$) and CP ($P<0.05$) levels, with ADA ($P<0.05$) and SOD ($P<0.001$) activities. Eventually, it was determined that ADA and SOD activities and Hp and CP levels decreases by treatment in calves infected with *C. parvum*.

Keywords: *Acute phase reactants, Antioxidant capacity, Cryptosporidium parvum*

Cryptosporidium parvum ile Enfekte Buzağılarda Akut Faz Reaktanları ve Antioksidant Kapasitenin Araştırılması

Özet

Kriptosporidiozis, fekal-oral yolla bulaşan bir zoonoz enfeksiyondur. *Cryptosporidium parvum* yaygın prevalans göstermekle birlikte, buzağılarda daha epidemik olarak seyretmektedir. Hastalık yüksek morbidite ve mortalitesine bağlı olarak, ciddi ekonomik kayıplara neden olur. Bu çalışmada, *C. parvum* ile enfekte buzağılara halofuginon (100 µg /kg/gün-7 gün) tedavisi uygulanmış ve bu tedavinin akut faz proteinleri ile antioksidant kapasite üzerindeki etkileri araştırılmıştır. Çalışma grubu, *C. parvum* ile enfekte, 1-3 haftalık 10 Holstein buzağıdan oluşturulmuştur. Tedavi öncesi ve sonrası alınan kan numunelerinde, serum amiloid A (SAA), haptoglobulin (Hp), C-reaktif protein (CRP), seruloplazmin (CP), malondialdehid (MDA) seviyeleri ile süperoksid dismutaz (SOD) ve adenozin deaminaz (ADA) aktiviteleri tespit edilmiştir. Elde edilen veriler, tedavi öncesi ve sonrası SAA, CRP ve MDA seviyelerinde istatistiksel bir farklılık olmadığını, fakat tedavi sonrası Hp ($P<0.001$) ve CP ($P<0.05$) seviyeleri ile ADA ($P<0.05$) ve SOD ($P<0.001$) aktivitelerinde istatistiksel olarak anlamlı bir düşüş meydana geldiğini ortaya koymuştur. Sonuç olarak, *C. parvum* ile enfekte buzağılarda tedavi ile ADA ve SOD aktiviteleri ile Hp ve CP seviyelerinde düşüş sağlandığı tespit edilmiştir.

Anahtar sözcükler: *Akut faz reaktanları, Antioksidant kapasite, Cryptosporidium parvum*

INTRODUCTION

C. parvum is the most common enteral pathogen of neonatal calves and mortal cause of neonatal calf diarrhoea worldwide [1,2]. Nearby leading major economic losses in breeding, the agent is zoonotic and has a potential of public health concern. Humans gain the infection by direct contact with infected individuals or animals and ingestion

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of *Cryptosporidium* oocysts via contaminated food or water [3].

C*ryptosporidium* spp. directly affects the intestines by multiplying at the microvillus borders of the enteric epithelium, giving serious damage to the villi thereby reduction of the absorptive surface and maldigestion and malabsorption followed by diarrhoea [4]. Occasionally, the parasite may affect other tissues such as the respiratory and renal epithelia in all the species of its spectrum [5,6].

Majority of cattle infections are due to *C. parvum, C. bovis,* and *C. Andersoni*, but in pre-weaned calves *C. parvum* is the dominant species [2,7].

The sporulated oocysts spread by the faeces which has long survival capacity with high resistence to environmental conditions and this is the form frequently used in the diagnosis in practice [6]. Studies comparing the diagnostic methods for *Cryptosporidium parvum* in calf faeces revealed high sensitivity and specificity for the rapid tests [8].

There is no definite effective treatment assigned currently for the therapy and prevention in bovine cryptosporidiosis, but conventional therapy includes halofuginone lactate together with providing good husbandry. Studies revealed that halofuginone lactate is satisfactory both in the clinical and prophylactic aspects reducing the environmental contamination with Cryptosporidium oocysts [9].

Acute phase proteins are involved in the restoration of homeostasis and preventing bacterial growth before the acquired immunity development [10]. Haptoglobin, ceruloplasmin and α-1 acidglycoprotein are some of the acute phase proteins triggered by infection. It is concluded that, oxidative stress and reactive oxygen species (ROS) due to tissue damage has a crucial role in the enteric damage pathogenesis of farm animals [11]. Acute phase proteins are an alternative path for monitoring clinical course and may be useful for providing information in the severity and prognosis [12].

The aim of this study is to investigate the effects of conventional halofuginon (100 µg/kg/day for 7 days) treatment on acute phase proteins and antioxidant capacity by determining serum amyloid A (SAA), haptoglobin (Hp), C-reactive protein (CRP), ceruloplasmin (CP), malondi-aldehyde (MDA) levels and superoxide dismutase (SOD) and adenosine deaminase (ADA) activities in calves infected with *C. parvum*.

MATERIAL and METHODS

Animals

The study group was comprised of 10 Holstein calves aged 1-3 weeks, infected with *C. parvum*. The calves were housed in a 200 cow farm in which yellow, nasty smelling and watery diarrhoea was observed in the neonates. Stool specimen obtained directly from the rectum were analysed for cryptosporidium oocyts with rapid kit, BİO K 155 (1x10 strips- Bio X Diagnostics) according to the manufacturers instructions. All specimen were also analysed with carbol fuchsin method. Blood specimen were obtained from the calves before and after the treatment. Conventionel treatment included Halocur® (MSD), Baytril® (Bayer) nearby fluid therapy and supplemental vitamins.

Carbol Fuchsin Staining Method

Stool specimen obtained directly from rectum were transferred to the laboratory in sterile plastic containers in cold chain. All specimen were analysed with Heine's carbol fuchsin staining method. For this purpose, 50 µl of homogenised stool specimen were placed on slides cleaned with ether-alcohol mixture. Same amount of carbol fuchsin was added and a thin specimen smear was prepared. After drying, a drop of immersion oil was added and the slide cover was placed. Smears were examined at X40 aggrandizement at microscope for *Cryptosporidium* oocysts *(Fig. 1)*.

SAA, CRP, Hp Analyses

CRP, SAA and haptoglobuline values were analysed with ELISA, using phase bovine (Tridelta Development Limited, Ireland) kits. Tests were performed according to the standards and guidelines provided by the manufacturer. All samples were calculated with a spectrophotometer (Digital and Analogue Systems S.R.L.) at 450 nm.

Ceruloplasmin Analyses

Serum ceruloplasmin analyses were performed with spectrophotometrically revised modified Ravin method based on the oxidation of colorless phenilen diamine to a blue-purple color product [13].

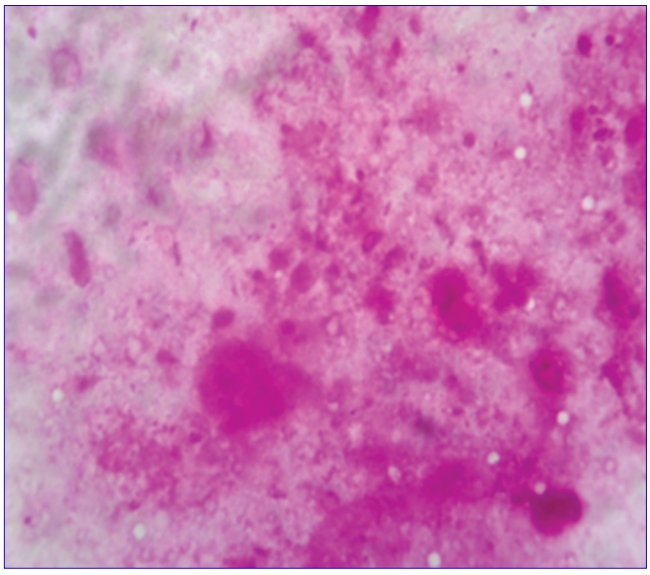

Fig 1. Cryptosporidium oocyts with carbol fuschin staining

MDA Analyses

MDA levels were determined according to the method of Yoshioko and Kawada [14], based on thiobarbuturic acid (TBA) reactivity. According to this method, a stabile red matter giving absorbans at 535 nm was formed by warming lipid content in low pH and thiobarbituric acid (TBA) containing medium resulting in unition of MDA and two TBA molecules was spectophotometrically determined. 1.1.3.3-tetraetoxypropan dissolved in 2.5-5-10 and 20 µmol/L concentration ethyl alcohol was used for calibration. MDA concentration was measured as an indirect marker of oxidative stress in terms of TBARS (thiobarbituric acid reactive substances), spectrophotometrically.

ADA Analyses

ADA in sera was determined at 37°C according to the method of Giusti and Galanti [15], based on the Bertholet reaction, formation of coloured indophenol complex from ammonia liberated from adenosine, and quantified colorimetrically with spectrophotometer (Thermo Scientific, Genesys 10S UV-Vis, USA). One unit of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia/min from adenosine at standard assay condition. Results were expressed as international unit of enzyme activity.

SOD Analyses

SOD analyses were performed according to the method of Podczasy and Wei [16]. The method is based on the principle of reduction of nitroblue tetrazolium (NBT) by xantine-xantinoxidase system which is a superoxide producer. Reducted NBT transforms to blue colored fomazon and measured spectrophotometrically at 560 nm (Thermo Scientific, Genesys 10S UV-Vis, USA). SOD activity was expressed as unit/g protein (U/g).

RESULTS

Obtained data revealed that there was no statistical difference between pre and post treatment SAA, CRP and MDA levels, but a decrease was determined in post treatment Hp ($P<0.001$) and CP ($P<0.05$) levels, with ADA ($P<0.05$) and SOD ($P<0.001$) activities *(Table. 1, Fig. 2)*. Eventually, it was determined that ADA and SOD activities and Hp and CP levels decreases by treatment in calves infected with *C. parvum*

Table 1. *Pre and posttreatment acute phase proteins and antioxidant capacity parameters*

Parameter	Pretreatment (n=10)	Posttreatment (n=10)
SOD	51.8±16.4	138.6±14.4
MDA	0.34±0.06	0.44±0.08
ADA	42.33±5.21	16.84±4.22
CP	20.65±1.25	18.41±0.68
SAA	426.3±52.7	339.4±26.6
HAPTO	1.26±0.13	0.54±0.07
CRP	0.29±0.03	0.22±0.04

DISCUSSION

Enteritis and diarrhoea are major causes of neonatal calf mortality and *C. parvum* is the most common enteral pathogen of neonatal calves [2] also having a zoonotic potential [17]. The disease is spread by the oocyts shed with stool and once ingested by the host, the endogenous phase starts with the invasion of target cells. Following biological steps are schizogony, gametogony, fertilization, and sporogony [6].

$CD4^+$ T cells has a crucial defense action in the immune response against *C. parvum* infection [18]. One other component of protective immunity is interferon (IFN)-γ action and Th2 cytokines [18]. Interleukin (IL)-12 is another part of defens against *C.parvum* [19]. The involvement of IL-12 and (IFN)-γ in host defense shows that a Th1 cell-mediated response is important [20]. IFN-c is also a significant element in the immune response against *C. Parvum* [21].

The acute phase response is a nonspecific reaction to tissue damage as the result of infection, inflammation, neoplasia and immunological disease [10]. It triggers the production of acute-phase proteins (such as a-1 acidglycoprotein, haptoglobin and ceruloplasmin) and involves local and systemic effects and acute phase proteins are also the components of innate immunity mediated by cytokines [10]. In the present study, elevation of Hp and ceruloplasmin in clinically ill calves infected with *C. parvum* and statistically significant decrease following recovery by the therapy is concordant with literature.

Oxidative stress is created as the result of insufficient antioxidant enzyme asset or overproduction of free radicals in the body. Free radicals and lipid peroxidation has detrimental effects to the cell [22]. MDA a product of lipid peroxidation is an important indicator of oxidative damage of cell membrane as it is the most abundant aldehyde formed [23]. Our findings was surprising in this aspect because MDA was the only parameter elevated following therapy. This may be attributed to the very abundant nature of the enzyme as the posttreatment specimen obtaining was just after the therapy and the animals were newly recovered from a devastating disease condition.

Superoxide dismutase (SOD) is a component of the compensatory reflex of the metabolism to oxidative damage targeting to neutralize the free radicals [24]. Our findings supported the literature as the high levels of SOD regressed by the treatment.

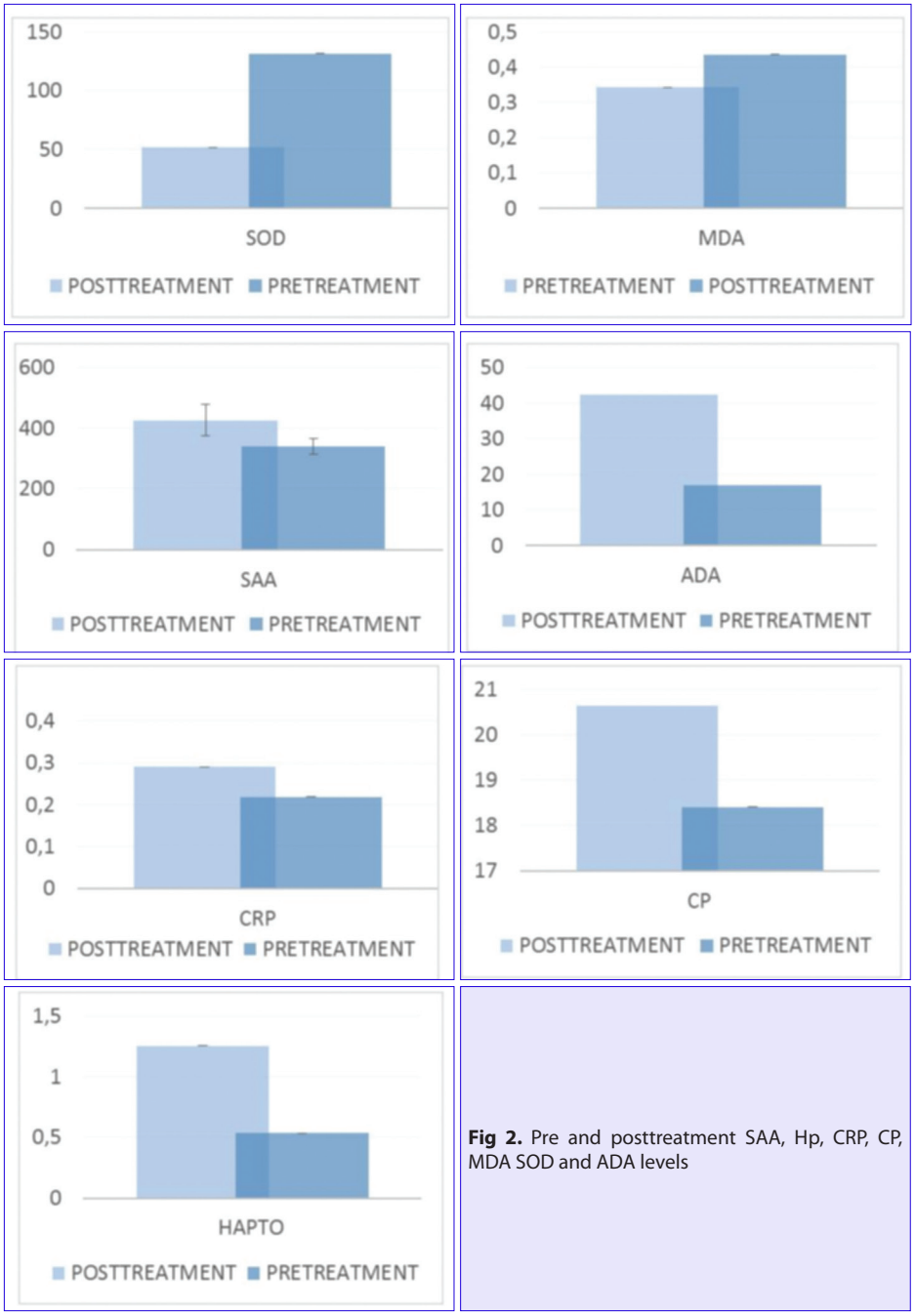

Fig 2. Pre and posttreatment SAA, Hp, CRP, CP, MDA SOD and ADA levels

ADA is important in cellular immunity and its major site of action is in the formation and differentiation of lymphocytes in lymphoid cells [25]. ADA binds cell surface receptors and promps T cells and ADA activity is directly related to the immune resonse [26], therefore the high ADA activity observed pretreatment in the present study reveals the potent immune response against *C. Parvum* infection and though statistically insignificant, a slight decrease in ADA level following therapy shows the regression of the infection.

The acute phase response SAA was characterised by a large individual variation [27], SAA is reported as one of the major acute phase proteins that increases significantly in diarrhoeic calves up to four weeks of age and could be used as a reliable indicator of clinical severity [28]. Concordantly, high SAA levels were determined in clinically infected neonatal calves in the present study, but decrease in SAA level after therapy was not statistically significant.

CRP increase during acute phase response like other acute phase proteins [29]. Our data showed high CRP levels in clinically ill calves whereas not a significant decrease was determined following therapy. This may be related to the regression period.

As the existence of oxidant activity is proven in *C. parvum* infection of calves, some authors reports that antioxidant

supplementation with standard treatment will promise a better therapeutic response [29,30], where on the other side some other researchers administration of antioxidants will exacerbate *C. parvum* infection [31,32]. Although a therapy attempt with antioxidants and evaluation of the effects was not aforementioned in the present study, obtained data on the acute phase response and antioxidant capacity in *C. parvum* infection of calves makes us think that the effects of antioxidant supplementation additional to the conventional therapy must be enlightened with the further studies.

In conclusion, obtained data of the present study showed that serum Hp and CP levels with ADA and SOD activities significantly decreased after treatment in calves clinically infected with *C. parvum* and screening for these values, though not sufficient for establishing a specific diagnosis, may be alternative indicators of the severity and the prognosis of the disease.

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