



Decreased Serum Adenosine Deaminase Activity Correlated with Clinical Score and Serum Proteins in Calves with Cryptosporidiosis

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ABSTRACT

This study was aimed to investigate serum adenosine deaminase (ADA) activity in calves with cryptosporidiosis. Significantly higher serum concentrations of globulin ($P<0.01$), blood urea nitrogen ($P<0.01$) and creatinine ($P<0.01$) were found and significantly lower albumin concentration ($P<0.01$) and albumin to globulin (A/G) ratio ($P<0.001$) in calves with cryptosporidiosis compared to healthy calves. Serum adenosine deaminase (ADA) activity was determined as 4.12 ± 0.91 U/l in calves with cryptosporidiosis compared to 10.27 ± 1.71 U/l in healthy calves, which was significant at $P<0.01$ level. Serum ADA was negatively correlated with clinical score ($P<0.05$), which serum total protein concentration ($P<0.05$), serum globulin concentration ($P<0.01$) and serum A/G ratio ($P<0.05$) were positively correlated in calves with cryptosporidiosis. From these results we concluded that cryptosporidiosis infection is closely related to low serum ADA activity which correlated with clinical score and serum total protein, globulin and A/G ratio in calves. The results of the present study indicate possible role of ADA in development and progression of cryptosporidiosis.

Article Information

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Authors' Contribution

GFY conceived and designed the study. BBY performed clinical diagnosis and collected the samples. GFY and FK performed biochemical analyses and statistically analyzed the result. GFY, BBY and AE wrote the article.

Key words

Adenosine deaminase, calf, cryptosporidiosis.

INTRODUCTION

Cryptosporidiosis is a zoonotic disease caused by *Cryptosporidium* spp. of farm animals and human beings (Prichard and Fleetwood, 1995; de Graaf *et al.*, 1999; Chalmers and Davies, 2010). Disease most often is seen in calves 1-4 weeks old and characterized by watery and loose diarrhea (Tzipori *et al.*, 1982; Olson *et al.*, 2004). Calves less than 2 months of age are especially at much greater risk for cryptosporidiosis. It has been reported that the prevalence of infection is higher in pre-weaned calves (1-8 weeks of age) than post-weaned calves (3-12 months of age) (Santín *et al.*, 2008). Other symptoms of disease include apathy, anorexia, malabsorption, dehydration and low-grade fever. Poor condition, high morbidity and low mortality in calves from cryptosporidiosis continue to plague the livestock industry (de Graaf *et al.*, 1999). Villous atrophy and inhibition of mucosal enzyme activity are frequently accompanied by inflammation in the intestines (O'Donoghue, 1995). Cryptosporidiosis causes destruction of the functional mucosal barrier system and increase in paracellular permeability of the small intestine in neonatal calves (Klein *et al.*, 2008). In severe infection, chronic life-threatening diarrhea ensues in immunosuppressed cases (Booth, 1985; Mead *et al.*,

1991b; Navin *et al.*, 1999; Hunter and Nichols, 2002; Leav *et al.*, 2003).

Adenosine deaminase (ADA) is an enzyme that is found exclusively in lymphocytes and monocytes that catalyzes the deamination of adenosine to inosine and 2'-deoxyadenosine to 2'-deoxyinosine (Gakis, 1996). It has been considered essential for the function and maturation of blood lymphocytes and monocytes (Ungerer *et al.*, 1992). It is well known that a decrease in ADA activity is associated with number of lymphocytes and suppression of lymphocyte proliferation and differentiation (Bremer *et al.*, 1981; Inigo *et al.*, 1992; Tonin *et al.*, 2012). Genetic deficiency of ADA causes severe combined immunodeficiency disease characterized by inability of cellular and humoral immune responses (Giblett *et al.*, 1972; Hirschhorn *et al.*, 1979; Apasov *et al.*, 2001). Decreased (Tripathi *et al.*, 2008; Da Silva *et al.*, 2013) and increased (Kontas and Salmanoglu, 2006; Altug *et al.*, 2008; Rai *et al.*, 2011) serum activities of ADA have been reported in parasitic infections. These reports supported that the serum ADA activity might prove useful in the evaluation of immune status of various parasitic disorders in animals. Ungar *et al.* (1991) have demonstrated that the immune system, particularly T-lymphocytes are required to resolve *Cryptosporidium* infection in mouse model. However, our knowledge about the role of serum ADA activity in bovine cryptosporidiosis is still limited. Therefore, the purpose of this study was to evaluate serum ADA activity in calves with cryptosporidiosis and its relevance to disease.

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Table I.- Scoring system with clinical findings for diarrheic calves.

Parameters								
Temperament	Active	0	Sedate	1	Aphatic	2	Coma	3
Body condition	Good	0	Normal	1	Weak	2	Cachexia	3
Appetite	Good	0	Slow sucking	1	Few sucking	2	No sucking	3
Body temperature °C	38-39.5	0	39.6-40	1	40.1-41	2	38>-41<	3
Bronchopneumonia	No sign	0	Mild	1	Moderate	2	Several	3
Hair coat	Normal	0	Confused	1	Dirty	2	Very dirty	3
Heart rate	90-130/min	0	111-130/min	1	131-150/min	2	>151/min	3
Lack of skin elasticity	No sign	0	Mild	1	Several	2	Moderate	3
Mucosa	Normal	0	Pale	1	Anemic			2
Capillaries	Normal	0	Filling	1	Over filling			2
Eye position in the orbita	Normal	0	Moderately sunken	1	Severe sunken			3
Fossa paralumbalis	Normal	0	Moderately sunken	1	Severe sunken			2
Joints	Normal	0	Moderately swollen	1	Severely swollen			3
Jugular veins filling	Immediately	0	Short time	1	Long time/Collapsed			3
Stool Consistence	Solid	0	Soft	1	Liquid			3
Stool Color	Normal	0	Yellowish- greenish	1	Bloody			5
Smell of stool	Normal	0	Bad	1	Very bad			2

MATERIALS AND METHODS

Study design, animals and samples

This study was conducted in dairy herds in Kirikkale between January to March 2014. Eight calves with healthy and 10 calves with cryptosporidiosis aged between 10-30 day old, male and female and of different breeds were used. A complete clinical examination was performed on all animals to detect any abnormal findings. As shown in Table I, clinical score (CS) of diarrheic calves was defined by a numerical scoring system (Sahal *et al.*, 1994). Fresh faecal samples were tested for *Cryptosporidium* antigen using the rapid stool test kit (*Cryptosporidium* strip kit, BIO K 155, Bio-X Diagnostics, Belgium). For oocysts detection, faecal smear staining technique was performed in faecal samples collected from calves (Heine, 1982). Briefly, twenty grams of fresh faecal specimens were collected from calves and homogenized. Fifty µl of faecal specimens were mixed a drop of concentrated carbol-fuchsin solution on glass microscope slides and faecal smears were prepared. The slides were dried and under the microscope (Leica Microsystems Inc., Illinois, USA) using 40x magnification (immersion).

Blood samples were collected from the jugular vein from each calf into vacutainer tubes without anticoagulant. Serum was removed by centrifugation at 1550 g for 10 min and stored at -80°C until analyses.

Serum biochemistry analyses

Total protein, albumin, glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST) and

alanine aminotransferase (ALT) were determined by an automatic chemistry analyser (Autolab PM4000, AMS - Analyzer Medical System S.r.l., Rome, Italy) using commercial test kits (Audit Diagnostics, Ireland). The concentration of globulin was calculated by subtracting albumin concentration from total protein concentration.

ADA activity assay

Measurement of ADA activity was performed by spectrophotometric method according to the procedure described by Giusti and Galanti (1984). All experiments were done in triplicate. Principle of this method based on the detection of ammonia by enzymatic deamination of adenosine to inosine. The release 1 µmol ammonia from adenosine is reflect the 1 unit of ADA. The ADA activity is expressed in U/l.

Statistical analysis

Assessment of the results was performed using Mann-Whitney *U* test by the SPSS statistical program version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Data are given as mean±SD and P-values lower than 0.05 were evaluated as significant.

RESULTS

Clinical findings

The clinical score of 23.70±4.67 (n=10) was found in calves with cryptosporidiosis. Infected calves were markedly dehydrated and mildly febrile, depressed and anorectic and watery diarrhea containing mucus were observed in these animals.

Table II shows concentration of serum biochemical components for calves with cryptosporidiosis and the control group. Significantly higher serum concentrations of globulin (P<0.01), BUN (P<0.01) and creatinine (P<0.01) and significantly lower albumin concentration (P<0.01) and A/G ratio (P<0.001) was found in calves with cryptosporidiosis compared to healthy calves. The serum values of glucose, AST and ALT of two groups were not significantly different.

Table II.- Serum biochemistry of calves with cryptosporidiosis and healthy.

	Control group (n=8)	Affected group (n=10)
Tp (g/dl)	6.09±0.42	6.27±0.41
Alb (g/dl)	3.24±0.18	2.71±0.33*
Glob (g/dl)	2.86±0.40	3.57±0.49*
A/G ratio	1.16±0.19	0.77±0.16**
Glu (mg/dl)	64.13±2.53	61.60±4.37
BUN (mg/dl)	19.63±2.50	30.26±5.84**
Cre (mg/dl)	0.94±0.23	1.41±0.32*
AST (U/l)	17.25±2.37	19.32±2.90
ALT (U/l)	9.88±1.88	10.80±2.52

A/G, albumin/globulin; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen, Cre, creatinine; Glob, globulin; Glu, glucose; Tp, total protein.

* P<0.01, ** P<0.001 compared with the control group (Mann-Whitney U-test).

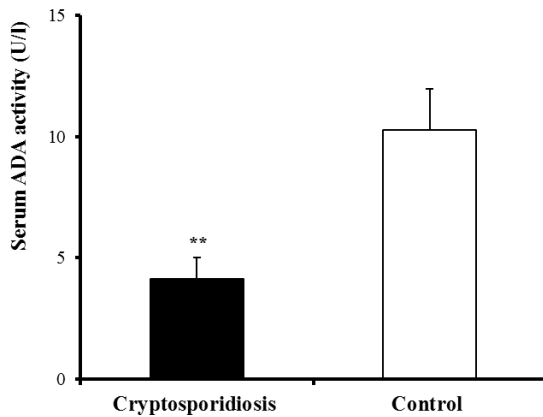


Fig. 1. Serum adenosine deaminase activity of calves with cryptosporidiosis and healthy, Mann Whitney-U test, * P<0.01.

Calves with cryptosporidiosis showed 4.12±0.91 U/l of ADA activity in serum, which was significantly lower than those found in healthy control calves (10.27±1.71 U/l) (P<0.01) (Fig. 1).

The serum ADA activities (r= -0.758, P<0.05), serum total protein concentration (r= -0.826, P<0.01) and serum globulin concentration (r= -0.929, P<0.01) were negatively correlated with clinical score and were positively correlated with serum A/G ratio (r= 0.844, P<0.05) in calves with cryptosporidiosis.

DISCUSSION

Cryptosporidiosis continues to pose threats to health of human and animal (Mason *et al.*, 2010; Meireles, 2010; Wang *et al.*, 2011; Jiang *et al.*, 2014; Mahfouz *et al.*, 2014; Raja *et al.*, 2014; Sirisena *et al.*, 2014; Shafiq *et al.*, 2015). Infected calves spreads cryptosporidiosis to each other or to humans. Study of Dixon *et al.* (2011) indicate that dairy calves pose a greater risk of infection humans than beef cattle. Inpankaew *et al.* (2009) have reported that cattle could play a role in zoonotic cryptosporidiosis in Thailand. In the cattle farming, disease lead to significant economic losses due to slower growth rate and death of calves (Tzipori *et al.*, 1982). Neonatal calves are quite susceptible to cryptosporidiosis and they carry maximum risk for the disease at the age of 9-12 days (Castro-Hermida *et al.*, 2002). Cryptosporidiosis is more likely to cause severe symptoms including prolonged diarrhea, dehydration, and possibly death in immunosuppressive individuals (Tzipori, 1988; Mead *et al.*, 1991a; Hunter and Nichols, 2002). Several studies have shown that cell-mediated immunity plays a major role in recovery from *Cryptosporidium* infection and T-lymphocytes appear to be necessary for protection against this infection (McDonald *et al.*, 1994; O'Donoghue, 1995; Riggs, 2002). Innate immune responses also have a significant protective role for infection (McDonald *et al.*, 2013). Chronic *Cryptosporidium parvum* infection has been reported in congenitally severe combined immune deficient mice (Mead *et al.*, 1991b).

ADA is believed to play a crucial role in lymphocyte maturation which eliminate substances foreign to the body such as parasitic agents (Ungerer *et al.*, 1992). The deficiency of the ADA results lymphopenia and severe combined immunodeficiency disease (Giblett *et al.*, 1972; Hirschhorn and Candotti, 2006). Low lymphocyte ADA activity has been reported in human immunodeficiency virus infection characterized by suppression of the immune system (Renouf *et al.*, 1989). Kameoka *et al.* (1993) reported that direct relationship between the CD26 and ADA asserted to these interactions may provide a clue for pathophysiology of severe combined immune deficiency related to ADA deficiency. Lymphocyte ADA activity was found to be correlated with the number of lymphocytes in rats

infected with leptospirosis (Tonin *et al.*, 2012). The serum ADA activity consist of ADA-1 and ADA-2 isoenzymes. ADA-1 is widely distributed in lymphoid tissues, which may contribute to the immune response. ADA-2 is produced mainly by monocyte-macrophage cell system and plays a role in the defense against pathogenic organisms (Ungerer *et al.*, 1992). It has been reported that activated monocytes secretes ADA-2 isoenzyme (Kurata, 1995). Macrophages have been reported as extracellular source of ADA-2 in rat model of septic peritonitis (Conlon and Law, 2004).

ADA activity is used for differential diagnosis and evaluation of prognosis of many disease causing cellular immune response (Baganha *et al.*, 1990; Blackburn and Kellems, 2005). ADA has been demonstrated as potential marker of tuberculous pleurisy (Ungerer *et al.*, 1994; Baba *et al.*, 2008) and rheumatoid arthritis (Nalesnik *et al.*, 2011). The result of present study showed the decreased ADA activity in calves with cryptosporidiosis compared with healthy calves. In addition, the serum ADA activity correlated well with clinical score, serum total protein concentration, serum globulin concentration and A/G ratio. The strong correlation of serum ADA activity with clinical score may be explained by the major role of ADA in the host responses to cryptosporidium infection. The measurement of plasma protein fractions provides valuable information reflecting prognosis of various diseases (Bishop *et al.*, 2000). In this study, the increase of serum total proteins and globulin and the decrease of serum albumin correlated with serum ADA activity in calves with cryptosporidiosis may have been result of immune response to cryptosporidium and malnutrition causing by diarrhea.

A limited number of clinical studies have evaluated the ADA activity in parasitic infections. It has been reported that low serum ADA activity due to increased adenosine level in extracellular fluid of rats experimentally infected with *Trypanosoma evansi* (Da Silva *et al.*, 2011). Karaman and co-workers (2009) reported that decreased serum ADA activity in *Toxoplasma gondii* seropositive and *Giardia intestinalis* positive patients compared with controls and they postulated that this finding associated with increased oxidative stress resulted from parasitic infection. High serum ADA activity have been determined in patients with visceral leishmaniasis indicating the activation of T-lymphocytes (Khambu *et al.*, 2007). Decreased serum and erythrocyte ADA activity has been demonstrated in lambs experimentally infected with *Haemonchus contortus* (Da Silva *et al.*, 2013). Researchers have asserted that low ADA activity may be due to inflammatory response to this infection and clinical signs of the disease. We found that low ADA activity is

associated with clinical score and serum total protein and globulin concentrations and A/G ratio in calves suffering from cryptosporidiosis. The results of the present study indicate possible role of ADA in development and progression of cryptosporidiosis, but further research needs to be done in order to fully elucidate the cause and the role of observed alterations in ADA activity.

Statement of conflict of interest

Authors have declared no conflict of interest.

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