Trypanosoma evansi in naturally infected Dromedary Camels: lipid profile, oxidative stress parameters, acute phase proteins and proinflammatory cytokines

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SUMMARY

Additional biomarkers are essential for control of *Trypanosoma evansi* (*T. evansi*) infection in dromedary camels. Two groups of 30 camels each, one naturally infected with *T. evansi* and other normal healthy were executed. The basis for the infection was the positive findings of clinical examination, blood smears and latex agglutination test. Blood samples of both groups and its harvested serum were used for the estimation of present serobiochemical parameters. The present findings revealed significant decrease ($P \leqslant 0{\cdot}05$) in triacylglycerol, cholesterol, high density lipoprotein cholesterol with significant increase ($P \leqslant 0{\cdot}05$) in low density lipoprotein cholesterol, beta hydroxyl butyric acids, non-esterified fatty acids, haptoglobin, serum amyloid A, ceruloplasmin, fibrinogen, interleukins, tumour necrosis factor-α, interferon gamma, malondialdehyde, superoxide dismutase, reduced glutathione and catalase of infected camels compared with the control. The present study suggests lipid profile, acute phase proteins, proinflammatory cytokines and oxidative stress parameters as biomarkers for *T. evansi* infection in camels.

Key words: Camels, trypanosomosis, acute phase proteins, cytokines, oxidative stress.

INTRODUCTION

Trypanosoma evansi (T. evansi) is the most widely distributed pathogenic trypanosome, affecting domesticated animals (Joshi *et al*. 2005) and the principal host species varies geographically. Although other animals, including wildlife, are susceptible to *T. evansi* infection, buffaloes, cattle, camels and horses are particularly affected (World Organization for Animal Health, 2008). *Trypanosoma evansi* disease (Surra) occurred in acute and chronic forms, however, chronic form is the most common one and characterized by anaemia, emaciation, lachrymation, lymphadenitis and sometimes abortions (Gutierrez *et al*. 2005). Many investigators have shown alterations in haematology of camels infected with trypanosomosis (Chaudhary and Iqbal, 2000; Saleh *et al*. 2009). Recently, chronic camel trypanosomosis was associated with oxidative stress and lipid peroxidation (Saleh *et al*. 2009; El-Deeb and Elmoslemany 2015). Trypanosomosis in camels resulted in significant increase of malondialdehyde, significant reduction of superoxide dismutase and non-significant increase in catalase compared with the control values (Saleh *et al*. 2009). Upon infection, neutrophils responded to a series of reactions (Soethout *et al*. 2002) which involved phagocytosis, elevating antibodies titre, complement fixation and stimulation of reactive oxygen and nitrogen free radicals release (Segal, 2005). In addition, proinflammatory cytokines (tumour necrosis factor-α, interleukins 1β, IL-12 and interferon gamma) produced from monocytes and macrophages mediated the effect of acute phase proteins and favoured T-helper cell differentiation. Acute phase proteins (APP) either positive (upregulated; haptoglobin, serum amyloid A, fibrinogen) or negative (downregulated; albumin, transferring and α-fetoproteins) released in response to the challenge (Eckersall and Bell, 2010). Induction of positive APP in hepatocytes by cytokines was accompanied by changes in lipid profile in the form of higher serum triacylglycerol and lower high density lipoprotein cholesterol levels (Cabana *et al*. 1989). The publications regarding the estimation of the oxidative stress status in *T. evansi* infection in camels are lack (Saleh *et al*. 2009) and additional studies are required. In addition, data

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concerning acute phase proteins, lipid profile and proinflammatory cytokines in dromedary camels infected with trypanosomosis are scarce yet. Therefore, the present study aimed to determine the role of lipid profiles, oxidative stress biomarkers, acute phase proteins and proinflammatory cytokines as biomarkers for *T. evansi* in dromedary camels.

MATERIALS AND METHODS

Animals and sampling protocol

Sixty dromedary camels were assigned equally into two groups. Camels in the first group were healthy and free of trypanosomosis and served as control. Camels in the second group were infected naturally with *T. evansi*. Diagnosis of *T. evansi* infection was based on the presence of the parasite in the fresh heparinized blood using wet-blood film and micro-Haematocrit technique (Woo, 1969). Positive camels were also examined by stained blood smears (Diff-Quick®). Moreover, latex agglutination test was applied on all examined camels according to the method described by (Olaho-Mukani *et al*. 1996). Blood samples were collected from the jugular vein of both groups into plain and ethylene-diaminetetraacetic acid (EDTA) vacutainers. Plasma was used for measurement of fibrinogen. Sera were harvested and stored at −20 °C until assayed for triacylglycerol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, total cholesterol, beta hydroxyl butyric acids, non-esterified fatty acids, superoxide dismutase, catalase, reduced glutathione, malondialdehyde, haptoglobin, serum amyloid A, ceruloplasmin, tumour necrosis factor-α, interleukins-1α, 1β, 6, 10 and interferon gamma.

Analysis of samples

Serum triacylglycerol, low density lipoprotein cholesterol, high density lipoprotein cholesterol and total cholesterol were measured on a Beckman CX-7 auto-analyser using commercial kits (Sigma Chemical Co. Ltd., Poole, Dorset, UK). Serum concentration of beta hydroxyl butyric acids was determined by using a commercial kit (Randox, UK). Serum concentration of non-esterified fatty acids was carried out using commercial kits (Randox, UK). Fibrinogen concentration in plasma was measured with a commercial enzyme-linked immunosorbent assay (ELISA) kit (USCA, Life Science) according to the manufacturer's instructions. Serum haptoglobin was measured with a commercial ELISA kit (Phase SAA kit, Tridelta Ltd., Ireland). Serum amyloid A was measured with a commercial ELISA kit (Phase SAA kit, Tridelta Ltd., Ireland), according to the manufacturer's instructions. Ceruloplasmin activity was measured by using commercial kit (MyBiosource, USA). Levels of malondialdehyde, reduced glutathione, catalase and superoxide dismutase activities were determined using commercial ELISA Kits (Cayman, USA). Interleukins-1α, 1β, 6, 10, tumour necrosis factor-α and interferon gamma levels were determined from undiluted serum samples using commercial ELISA Kits (Biosource, Belgium).

Statistical analysis

All the data were presented as mean ± standard error of mean by using one way analysis of variance (ANOVA). All tests were performed using computer package of the statistical analysis system (SAS, 2002).

Ethics statement

All the animal procedures were performed according to the guidelines of the Animal Ethics Committee of College of Veterinary Medicine and Animal Resources, King Faisal University, Saudi Arabia (committee protocol number: 42/1943).

RESULTS

Clinical examination

The diseased camels showed progressive anaemia, emaciation marked depression, recurrent fever, atrophy of the thigh muscles dullness, corneal opacity, diarrhoea, oedema of the dependent parts, loss of condition and some cases showed nervous signs like trembling and unusual aggressiveness.

Lipid profiles

The data shown in the same table (Table 1) revealed a significant reduction ($P \leqslant 0{\cdot}05$) in the values of triacylglycerol, cholesterol and high density lipoprotein cholesterol with significant elevation ($P \leqslant 0{\cdot}05$) of low density lipoprotein cholesterol, beta hydroxyl butyric acids and non-esterified fatty acids concentrations in serum of infected camels compared with the control.

Oxidative stress, acute phase proteins and cytokines analysis

The results shown in Table 2 indicated that, the activities of superoxide dismutase, catalase and reduced glutathione level were reduced significantly ($P \leqslant 0{\cdot}05$) in infected camels compared with the control whereas, lipid peroxidation was increased significantly ($P \leqslant 0{\cdot}05$) as reflected on higher malondialdehyde value in the serum of infected camels compared with the control. The present study showed also that acute phase proteins namely haptoglobin, serum amyloid A, ceruloplasmin and fibrinogen were significantly ($P \leqslant 0{\cdot}05$) increased in the

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Table 1. Lipids profiles in control ($n = 30$), and *Trypanosoma evansi* infested camels ($n = 30$)

Variables	Units	Control	Diseased
High density lipoprotein cholesterol	mmol L^{-1}	0·52 ± 0·02	0·24 ± 0·01*
Low density lipoprotein cholesterol	mmol L^{-1}	0·15 ± 0·01	0·24 ± 0·02*
Cholesterol	mmol L^{-1}	0·86 ± 0·12	0·33 ± 0·02*
Triacyleglycerol	mmol L^{-1}	0·47 ± 0·13	0·21 ± 0·01*
Beta hydroxyl butyric acids	mmol L^{-1}	0·11 ± 0·01	1·2 ± 0·12*
Non esterified fatty acids	mmol L^{-1}	0·65 ± 0·12	1·6 ± 0·32*

* Means are significantly different at the level ($P \leqslant 0{\cdot}05$) when compared with the control.

Table 2. The levels of oxidative stress biomarkers and lipid peroxidation in control ($n = 30$), and *Trypanosoma evansi* infested camels ($n = 30$)

Variables	Units	Control	Diseased
Superoxide dismutase	U mg^{-1}Hb	5·0 ± 0·4	3·6 ± 0·3*
Reduced glutathione	mmol L^{-1}	6·8 ± 0·3	3·7 ± 0·4*
Catalase	U mg^{-1}Hb	15·7 ± 0·4	11·5 ± 1·8*
Malondialdhyde	nmol g^{-1}Hb	13·2 ± 0·6	27·0 ± 1·9*

* Means are significantly different at the level ($P \leqslant 0{\cdot}05$) when compared with the control.

Table 3. Acute phase proteins in control ($n = 30$), and *Trypanosoma evansi* infested camels ($n = 30$)

Variables	Units	Control	Diseased
Fibrinogen	g L^{-1}	3·3 ± 0·30	5·0 ± 1·20*
Serum amyloid A	mg L^{-1}	9·5 ± 0·40	18·4 ± 2·00*
Haptoglobin	g L^{-1}	0·3 ± 0·03	1·3 ± 0·03*
Ceruloplasmin	g L^{-1}	0·1 ± 0·00	1·7 ± 1·00*

* Means are significantly different at the level ($P \leqslant 0{\cdot}05$) when compared with the control.

Table 4. Proinflamatory cytokines in control ($n = 30$), and *Trypanosoma evansi* infested camels ($n = 30$)

Variables	Units	Control	Diseased
Interleukin-1α	pg mL^{-1}	9·3 ± 0·3	19·1 ± 2·0*
Interleukin-1β	pg mL^{-1}	22·1 ± 0·9	46·0 ± 1·4*
Interleukin-10	pg mL^{-1}	10·2 ± 0·4	13·3 ± 0·9*
Interleukin-6	pg mL^{-1}	13·9 ± 0·5	16·7 ± 1·2*
Tumour necrosis α	ng mL^{-1}	15·3 ± 0·5	19·3 ± 1·2*
Interferon-γ	pg mL^{-1}	19·0 ± 0·5	27·5 ± 3·2*

* Means are significantly different at the level ($P \leqslant 0{\cdot}05$) when compared with the control.

serum of infected camel compared with the control (Table 3). The data shown in Table 4 indicated that, the values of all proinflammatory cytokines namely, interleukins-1α, 1β, 6, 10, tumour necrosis factor-α and interferon gamma were increased significantly ($P \leqslant 0{\cdot}05$) in *T. evansi* infected camels compared with the control.

DISCUSSION

The significant decrease of triacylglycerol, total cholesterol and high density lipoprotein cholesterol in trypanosome infected camels compared with the control agrees with that reported in *Trypanosoma congolense* and *Trypanosoma brucei* infection of goats (Biryomumaisho *et al*. 2003), in *T. congolense* infection of sheep (Adamu *et al*. 2008), in *Trypanosoma rhodesiense* infections of cattle (Wellde *et al*. 1989). As trypanosomes require lipoproteins for multiplication *in vitro* (Black and Vanderweed, 1989), the hypolipidemia and hypocholesterolemia observed in the present study might be due to the utilization of the lipid molecules by trypanosome (Admau *et al*. 2009). Blood-stream forms of trypanosomes require cholesterol, phospholipids and total lipids for membrane biosynthesis

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and maintaining growth (Nok *et al*. 2003). However, the parasites are not able to biosynthesize such molecules and depend upon the host to fulfil this task. During the journey of trypanosomes in the blood stream, they utilize blood glucose as a source of energy (Chaudhuri *et al*. 2006), which could ultimately lead to hypoglycemia in the trypanosome-infected animal. Because of depletion of glucose caused by trypanosome, the body of the infected animal switches on lipid catabolism as an obligation mechanism for energy supply. In the present study, lipid catabolism leads to an increase in the level of free fatty acids that undergoes β-oxidation and gives rise to tremendous amount of ketone bodies (beta hydroxyl butyric acids) as noticed in the serum of infected camels. The significant decrease in serum cholesterol of infected camels may attribute to impaired cholesterol biosynthesis as a result of mechanical liver injury by trypanosome (Logan-Henfrey *et al*. 1992) or insufficient respiration of liver cells due to hypoxia caused by anaemia in infected camels as discussed above. As cholesterol is vital in cell signalling in neuronal synapses formation, its decrease along with lipids could induce the observed neurological disorders.

Parallel to the present study, earlier researches demonstrated higher level of malondialdehyde in naturally infected camel with *T. evansi* (Saleh *et al*. 2009). The decrease in superoxide dismutase and catalase activities may postulate to the consumption of antioxidants as free radical scavengers during the oxidative stress in the naturally chronic *T. evansi* infected camels (Saleh *et al*. 2009). Consistent with the present results, chronic *T. evansi* infection in camels resulted in inhibition of the antioxidants (albumin, ascorbic acid, reduced glutathione and superoxide dismutase) (Saleh *et al*. 2009). The decrease in glutathione level in the serum of infected camels was reflected on the observed reduction in the activities of superoxide dismutase and catalase enzymes as discussed above. The significant increase in haptoglobin, serum amyloid A, ceruloplasmin and fibrinogen levels in *T. evansi* infected dromedary camels is consistent with previous result (Eckersall *et al*. 2001) in mice experimentally infected with *T. brucei brucei*.

The significant elevation of acute phase proteins in infected dromedary camels may attribute to the initial secretion of pro-inflammatory cytokines by macrophages at infection site. The increased levels of selected cytokines may attribute to liver necrosis, spleen necrosis, generalized lymphoid tissue hyperplasia (Enwezor and Sackey, 2005). These lesions induce the release of cytokines such as interleukins-1, interleukins-6 and tumour necrosis factor-α and therefore, acute phase proteins are biosynthesized in liver (Radostits *et al*. 2007). Similar results were observed in mice experimentally infected with *T. brucei brucei* (Eckersall, *et al*. 2001). Recently, it has been suggested that, elevated levels of interleukins-1, tumour necrosis factor-α and interferon gamma in *T. evansi* infected rats participated in the development of anaemia (Paim *et al*. 2011). This increase was associated with the regulation of immune responses against the parasite. In addition, pro-inflammatory cytokines particularly tumour necrosis factor-α have been shared in mediating anaemia associated with tropical theileriosis (Graham *et al*. 2001).

The present study concluded that, *T. evansi* natural infection reflected on the infected camels in the form of several clinical signs are represented by depression, recurrent fever, corneal opacity, diarrhoea and loss of condition. These clinical signs resulted from a series of biochemical reactions inside the body of the infected camels. The parasitaemia induced a state of oxidative stress in infected camels as reflected on significant elevation of measured oxidative stress biomarkers. The body of infected camels responds to this stressful situation by three ways. Firstly, triacylglycerol has been mobilized and the produced fatty acids have been oxidized to provide an extra energy to the body of infected camel as reflected on significant decrease of triacylglycerol, cholesterol and high density lipoprotein cholesterol with significant increase of free fatty acids and ketone bodies. Secondly, the activities of enzymatic (superoxide dismutase and catalase) and non-enzymatic (reduced glutathione) antioxidants levels have been elevated in serum of infected camels. Finally, significant increase in proinflammatory cytokines that mediate the effect of acute phase proteins has been conducted. Therefore, the antioxidant therapy may be useful in the treatment of *T. evansi* infection in camels. In addition, lipid profile, acute phase proteins, proinflammatory cytokines and oxidative stress parameters could be used as biomarkers of *T. evansi* infection in dromedary camel.

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