Cytokine response and clinicopathological findings in *Brucella* infected camels (*Camelus dromedarius*)

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ABSTRACT: The present study had the aim of assessing the cytokine response and selected clinicopathological findings associated with brucellosis in camels (*Camelus dromedarius*). 340 dromedary camels were examined for brucellosis using agglutination and Complement Fixation tests (CFT). Twenty-five camels (7.35%) were positive by both tests; 14 (4.12%) for *B. abortus* and 11 (3.23%) for *B. melitensis*. IL-1β and IL-10 interleukin levels in both *B. abortus* and *B. melitensis* infected camels showed significant elevations ($P < 0.05$) compared with controls. Moreover, there was significantly larger increase in IL-1β interleukins in camels infected with *B. abortus* compared with *B. melitensis*. TNF-α, IFN-γ and IL-1α levels showed significant decreases ($P < 0.05$) in *Brucella* infected camels compared with non-infected ones; however, there was non-significant changes in IL-6 levels in *Brucella* infected camels compared with controls. Lymphopenia was recorded in infected camels but not in controls. However, normocytic normochromic anemia, hypoproteinemia, hypoalbuminemia and hypoglycemia were recorded in the *B. abortus* group only. Sorbitol dehydrogenase (SD), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed significant increases ($P < 0.05$) in infected camels compared with controls, and in *B. abortus* infected camels compared with *B. melitensis* infected animals. This is the first report that describes changes in selected cytokines and various haematological and biochemical parameters associated with brucellosios in dromedary camels. Emphasis should be placed on multidisciplinary research to elucidate the immunomodulatory features of camel brucellosis.

Keywords: camel; Brucellosis; cytokines; hematology; biochemical parameters; Egypt

Brucellosis is an infectious disease of animals caused by a number of host-adapted species of Gram negative intracellular bacteria of the genus *Brucella* (Mantur et al., 2007). The disease in female animals is characterized by abortion and retained placenta and in male animals by orchitis and epididymitis (Adams, 2002). It is a worldwide zoonotic disease that is recognized as a major cause of heavy economic losses to the livestock industry, and also poses serious human health hazards (WHO, 1986).

Camels are not known to be primary hosts of *Brucella* organisms, but they are susceptible to both *B. abortus* and *B. melitensis* (Cooper, 1991). Consequently, the infection rate in camels depends upon the infection rate in primary host animals in contact with them. *Brucella melitensis*, Biovar types 1 and/or 2 have been isolated from the supramammary lymph nodes of female-camels without remarkable gross lesions, and from serum-positive Saudi Arabian, Libyan and Egyptian one-humped camels (*Camelus dromedarius*) (Gameel et al., 1993; Hegazy et al., 2004).

The epidemiology of brucellosis in camels from different geographical locations of African and Arabian countries has been investigated extensively (Omer et al., 2000; Hamdy and Amin, 2002; Teshome et al., 2003; Schelling et al., 2004; Al-Majali et al., 2008; Musa et al., 2008). The prevalence of brucellosis may vary with time even in the same region (Yagoub et al., 1990).

Several studies on the cytokine levels associated with brucellosis have been conducted in both human and animals (Demirdag et al., 2003; Fernandez-Lago et al., 2005; Wyckoff et al., 2005). In humans, several genes encoding different cytokines may play crucial roles in host susceptibility to brucellosis, since cytokine production capacity varies among individuals and depends on cytokine gene polymorphisms (Budak et al., 2007). To the authors' knowledge, research on the effect of infection with *Brucella* spp. on immunological, biochemical and haematological parameters in camels is very scarce. Only one report has described the effect of experimental vaccination with *B. abortus* strain 19 vaccine on cytokine expression in camels (*Camelus bactrianus*) (Odbileg et al., 2008). Consequently, the aim of the present study was to delineate cytokine responses as well as clinicopathological changes in *Brucella* infected camels (*Camelus dromedarius*).

MATERIAL AND METHODS

Animals

Three hundreds and forty male camels aged 30–42 months were used in this study. Camels were raised in an open yard in a government quarantine station (Nobaria City) with free access to food and water *ad libitum* for three weeks.

Serological testing

Each serum sample was initially screened for the presence of antibodies against both *B. abortus* and *B. melitensis* using specific commercial antigens (Tulip Diagnostics LTD, India; Batch number: 10562 and 561005, respectively). In such tests, 50 µl of reagent were added to 10 µl of serum. Complement Fixation was carried out as a confirmatory test for each serum sample (Alton et al., 1988). A serum sample with an antibody titre equal to or exceeding 1:4 was classified as a CFT-positive. *Brucella* seropositive camels were designated as those with both a positive screening test and CFT results.

Based on the approach of Odbileg et al. (2005) and Odbileg, et al. (2006) who recorded cross reactivity between camel and cow, horse, human, and mouse cytokines, IL-1β and IL-10, TNF-α, IFN-γ, IL-6 and IL-1α levels were determined from undiluted serum samples using commercially available ELISA Kits (Biosurce, Diagnostic Corporation, Belgium). The plates were read at 450 nm and a correction wavelength of 550 nm was measured on a computerized automated microplate ELISA reader (Bio TEC, ELX800G, USA). Values expressed in picograms per millilitre were extrapolated using linear regression from a standard curve of known amounts of human cytokines.

Haematological examination

Haematological investigation with haematological indices was performed on the positive samples as well as for 20 seronegative control camels.

Biochemical analysis

Glucose, bilirubin, uric acid, creatinine, total protein, albumin, AST and ALT were estimated following standard methods using commercial test kits (Spinreact, Spain). Sorbitol dehydrogenase (SD) was also assayed following commercial test kits (Diagnostic Chemicals Limited Co. Oxford, Connecticut, USA)

Statistical analysis

Data was statistically analyzed by ANOVA with *post-hock* Duncan multiple comparison test using statistical software program (SPSS for Windows version 15, USA). Differences were considered significant at $P < 0.05$.

RESULTS

A total of 25 camels (7.35%) were found to be positive for either *B. abortus* or *B. melitensis* by both agglutination and CFT tests. 14 camels (4.12 %) were infected with *B. abortus*, whereas 11 (3.23%) were found to be infected with *B. melitensis*.

Interleukin IL-1β and IL-10 levels in both *B. abortus* and *B. melitensis* infected camels showed significant elevations ($P < 0.05$) compared with controls. Moreover, there was a significant increase of IL-1β interleukins in camels infected with *B. abortus* compared with *B. melitensis* (Table 1). TNF-α, IFN-γ and IL-1α levels showed significant decreas-

es ($P < 0.05$) in *Brucella* infected camels compared with non-infected animals; however, IL-6 levels did not change significantly in *Brucella* infected camels compared with controls (Table 1).

Non-regenerative, normocytic, normochromic anemia was observed in the *B. abortus* infected group and was missing in both the control and *B. melitensis* infected groups (Table 2). Moreover, the differential leukocytic count indicated lymphopenia only in infected groups and not in the non-infected one (Table 3).

Biochemically, there were significant increases ($P < 0.05$) in serum SD, AST and ALT levels not only in infected camels when compared with controls, but also in *B. abortus* infected camels compared with *B. melitensis* infected ones (Table 4). Moreover, there was significant decrease in total protein and albumin levels in the *B. abortus*

Table 1. IL-1β, IL-10 , TNF-α, IL-6, IFN-γ and IL-1α levels (mean ± SD) of camels with brucellosis

Group	IL-1β (pg/ml)	IL-10 (pg/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)	IFN-γ (pg/ml)	IL-1α (pg/ml)
Control (n = 20)	$21.93^{a} \pm 2.10$	$10.12^{a} \pm 0.41$	$15.85^{b} \pm 0.45$	$14.8^{a} \pm 0.78$	$19.1^{a} \pm 1.85$	$9.45^{a} \pm 0.21$
B. abortus (n = 14)	$45.1^{b} \pm 3.65$	$13.4^{b} \pm 0.69$	$7.15^{a} \pm 1.85$	$15.94^{a} \pm 0.89$	$11.18^{b} \pm 0.91$	$4.36^{b} \pm 0.33$
B. melitensis (n = 11)	$35.2^{c} \pm 3.45$	$12.1^{b} \pm 0.54$	$8.84^{a} \pm 1.64$	$16.92^{a} \pm 0.94$	$14.27^{c} \pm 0.98$	$5.87^{b} \pm 0.42$

Means in the same column with different superscript letters are significantly different at $P < 0.05$

Table 2. Erythrogram (mean ± SD) of camels with brucellosis

Group	RBC (10^6/μl	Heamoglobin (mg/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Control (n = 20)	$7.15^{a} \pm 0.35$	$11.12^{a} \pm 0.51$	$30.85^{a} \pm 0.12$	43.14 ± 1.95	15.55 ± 1.65	36.05 ± 0.21
B. abortus (n = 14)	$6.38^{b} \pm 0.24$	$9.85^{b} \pm 0.41$	$27.61^{b} \pm 1.01$	43.8 ± 1.15	15.44 ± 1.39	35.68 ± 0.24
B. melitensis (n = 11)	$6.74^{ab} \pm 0.29$	$10.54^{ab} \pm 0.45$	$28.99^{ab} \pm 0.24$	43.01 ± 1.36	15.64 ± 1.29	36.36 ± 0.28

Means in the same column with different superscript letters are significantly different at $P < 0.05$

Table 3. Leukogram (mean ± SD) of camels with brucellosis

Group	TCL (10^3/μl)	Neutrophils (10^3/μl)	Esinophils (10^3/μl)	Basophils (10^3/μl)	Lymphocytes (10^3/μl)	Monocytes (10^3/μl)
Control (n = 20)	8.95 ± 0.41	3.51 ± 0.15	0.36 ± 0.06	–	$4.62^{a} \pm 0.31$	0.46 ± 0.08
B. abortus (n = 14)	8.04 ± 0.51	3.29 ± 0.18	0.41 ± 0.05	0.08 ± 0.08	$3.75^{b} \pm 0.24$	0.51 ± 0.09
B. melitensis (n = 11)	8.18 ± 0.44	3.35 ± 0.21	0.44 ± 0.04	–	$3.86^{b} \pm 0.21$	0.53 ± 0.07

Means in the same column with different superscript letters are significantly different at $P < 0.05$

Table 4. Biochemical findings (mean ± SD) in camels with brucellosiss

Group	CD (IU/l)	ALT (IU/l)	AST (IU/l)	Glucose (mg/dl)	Total protein (g/dl)	Albumin (mg/dl)
Control (n = 20)	$8.1^{a} \pm 0.31$	$12.46^{a} \pm 0.35$	$81.5^{a} \pm 6.15$	$95.6^{a} \pm 8.45$	$7.81^{b} \pm 0.55$	$4.12^{b} \pm 0.31$
B. abortus (n = 14)	$13.25^{b} \pm 0.85$	$21.8^{b} \pm 0.85$	$116.4^{b} \pm 0.12$	$86.7^{b} \pm 9.18$	$6.15^{a} \pm 0.58$	$3.14^{a} \pm 0.25$
B. melitensis (n = 11)	$10.12^{c} \pm 0.72$	$16.25^{c} \pm 0.64$	$98.4^{c} \pm 5.74$	$91.3^{a} \pm 7.85$	$6.54^{ab} \pm 0.61$	$3.42^{b} \pm 0.29$

Means in the same column with different superscript letters are significantly different at $P < 0.05$

infected group compared with the *B. melitensis* and control groups (Table 4). However, levels of bilirubin, uric acid, and creatinine showed no significant variations.

DISCUSSION

The aim of the present study was to study the effect of brucellosis on the levels of selected cytokines as well as hematological and biochemical parameters. The overall prevalence of brucellosis in examined camels was 7.35%. In all 3.23% were infected with *B. melitensis* and 4.12% with *B. abortus*. A lower prevalence was previously recorded in the Sudan (Damir et al., 1984), Somalia (Baumann and Zessin, 1992), The United Arab Emirates (Afzal and Sakkir, 1994), Yemen (Al-Shamahy, 1999), Chad (Schelling et al., 2004), Ethiopia (Teshome et al., 2003), Egypt (Hamdy and Amin, 2002), and Eritrea (Omer et al., 2000); however, a higher prevalence was recorded in Saudi Arabia (Radwan et al., 1995), Nigeria (Kudi et al., 1997) and Jordan (Al-Majali et al., 2008). In the present study, the higher prevalence may be due to the presence of infection by more than one species. Our findings thus suggest that infection still poses a threat to the health of camels and that the risk of transmission is increasing. The prevalence of *B. abortus* is quite close to that of *B.melitensis*, suggesting that the risk of infection is equal.

In the present study, IL-1β, IL-10, TNF-α, IL-6, IFN-γ and IL-1α levels were assayed for the first time in dromedary camels (*Camelus dromedarius*) infected with brucellosis. IL-1β and IL-10 interleukins showed a significant increase in both *B. abortus* and *B. melitensis* infected camels ($P < 0.05$) compared with controls. However, TNFα, IFN-γ and IL-1α levels displayed significant decreases ($P < 0.05$) in infected camels compared with non-infected. Moreover, IL-6 levels did not vary significantly. These findings are in accordance with other studies that have estimated IL-10 production following *Brucella* infection (Hoover et al., 1999; Pasquali et al., 2001). Similar findings were also recorded in camels (*Camelus bactrianus*) experimentally vaccinated with *B. abortus* strain 19 (Odbileg et al., 2008).

IL-10 displays potent abilities to suppress the antigen presentation capacity of antigen presenting cells (Moore et al., 2001). Erdogan et al. (2007) outlined the mechanisms by which *Brucella* species evade clearance by the immune response to chronically infect their host. They reported that the experimental infection of *B. melitensis* induced lipid peroxidation and stimulated IL-10 transcription in the spleen but not in the liver of rats. Fernandes and Baldwin (1995) reported that IL-10 can down-regulate protective immunity to *B. abortus*. *In vivo*, neutralization of IL-10 with an anti-IL-10 monoclonal antibody resulted in up to 10-fold fewer bacteria in the spleens of BALB/c mice infected with a virulent *B. abortus* strain.

Cytokine IL-1β was first defined as a polypeptide derived from mononuclear phagocytes that enhanced T-cell response to antigens or polyclonal activators. It is now clear that a principle function of IL-1β is as a mediator of the host inflammatory response in natural immunity (Dinarello, 1996). IL-1β cytokines increase the expression of adhesion factors on endothelial cells to enable the transmigration of leukocytes, the cells that fight pathogens, to sites of infection (Nicklin et al., 2000). Stevens and Olsen (1994) recorded an elevation in IL-1β levels from bovine peripheral blood mononuclear cells incubated with live or killed *B. abortus* cells. Moreover, Dzata et al. (1991) recorded an elevation in IL-1β levels in the blood of cattle injected with a *B. abortus* soluble antigen.

TNF-α levels showed a significant fall ($P < 0.05$) in infected camels compared with controls. IL-10 has been demonstrated to inhibit synthesis of pro-inflammatory cytokines like IFN-γ, IL-2, IL-3 and TNF-α made by cells such as macrophages and the Type 1 T helper cells (Moore et al., 2001; Trinchieri, 2007). However, the results of Demirdag et al. (2003) and Odbileg et al. (2008) showed low levels of expression. The present results suggest that IL-10 may be down-regulating the immune response to *B. abortus* by affecting both the function of macrophage effectors and the production of the protective Th1 cytokine gamma interferon. Moreover, diminished production of Th1 cytokines may contribute to T-cell unresponsiveness in chronic human brucellosis (Giambartolomei et al., 2002).

It has been suggested that IFN-γ and TNF-α are involved in the pathophysiology of brucellosis and that they have a close relationship with the inflammatory activation of the disease (Zhan et al., 1996; Dornand et al., 2002). Among the various cytokines, IFN-γ plays the predominant role in generating macrophages with strong intracellular *Brucella*-killing activity (Seder and Hill, 2000; Murphy et al., 2001). It was found that IFN-γ-induced T-cell

responses have important roles in the immunity to intracellular *B. abortus* (Saunders et al., 2000). In both human and animals, IFN-γ has been shown to have a role in the control of brucellosis (Zhan and Cheers, 1993; Murphy et al., 2001).

IL-6 levels were not found to vary significantly between infected and non-infected camels. This finding coincided with that of Saunders et al. (1993) who found that IL-6 titres decreased after the peak of infection, falling to baseline levels before these chronic infections were eradicated. On the other hand, Odbileg et al. (2008) recorded a significant increase in IL-6 levels in camels vaccinated with *B. abortus* strain 19 compared with non-vaccinated camels. This result suggests that the state of infection could affect the cytokine level.

The cytokines chosen for the present investigation were selected based on their significance in human and animal brucellosis (Pasquali et al., 2001; Akbulut et al., 2007). Their levels in dromedary camels indicate similarities between camels and other animals in the response to brucellosis. Although it has been documented that cytokines may provide protective immunity to brucellosis, it is suggested that optimal development and maintenance of a protective response against infection depends on a finely regulated balance of cytokines, rather than upon the level of a single cytokine.

Non-regenerative, normocytic, normochromic anemia was observed in the *B. abortus* group only and this could be attributed to the inflammatory chemical mediators as IL-1β. It is now clear that elevated blood levels of IL-1β contribute to non-regenerative anemia associated with chronic disease (Dinarello, 2005).

Brucella is a facultative intracellular pathogen and the etiological agent of brucellosis. In some cases, animal brucellosis results in a persistent infection that may reactivate years after the initial exposure (Sarafidis et al., 2006).

Differential leukocytic counts indicated lymphopenia in both infected groups compared with the control ones. However, there were no changes in the other parameters. Abu Damir et al. (1989) observed generalized lymphadenitis in camels experimentally infected with *Brucella abortus*. Similarly, Palmer et al. (1996) and Stevens et al. (1994) recorded lymphoid depletion of white pulp in mice, which were inoculated intra-peritoneally with suspensions of *Brucella abortus* strains. Also Enright et al. (1984) reported lymphoid depletion in the thymic cortex in bovine fetuses naturally and experimentally infected with *Brucella abortus*.

Serum SD, AST and ALT were significantly ($P < 0.05$) elevated in *B. abortus* camels compared with *B. melitensis* and controls. Moreover, their levels were significantly ($P < 0.05$) higher in *B. melitensis* infected camels in comparison with controls. This finding may be attributed to the liver damage caused by brucellosis. Abu Damir et al. (1989) recorded focal granulomata in the liver of camels experimentally infected with *B. abortus*. This is the first report to describe the levels of SD in camels; however, its elevation has been attributed to hepatocellular damage (Radostits et al., 2007).

Hypoproteinemia and hypoalbuminemia were observed only in the *B. abortus* infected group. This finding may be ascribed to the hepatic damage caused by brucellosis (Abu Damir et al., 1989).

Hypoglycemia was observed only in *B. abortus* infected camels and not in the healthy controls. It is suggested that liver function impairment in *B. abortus* infected camels could explain the presence of hypoglycemia.

Total serum bilirubin in ruminants is slight elevated in hepatocellular jaundice (Braun et al., 1995). Total bilrubin has been shown to possess sufficient discriminatory power to differentiate reliably between mild and severe fatty liver in dairy cows (Kalaitzakis et al., 2007). Our data revealed a non-significant increase in total serum bilirubin in both infected groups in comparison with the clinically healthy one.

Uric acid is a major end product of nitrogen metabolism in animals. It is synthesized in the liver and 90% of it is excreted via tubular secretion largely independent of urine flow rate (Lee et al., 2006). The rate of secretion is mainly dependent on the hydration state of animals and renal function disorders can eventually lead to increased plasma uric acid concentrations (Dash and Joshi, 1989). Our results showed a non-significant increase in the levels of serum uric acid and creatinine in both infected groups. This could be attributed to the absence of renal impairment in *Brucella* infected camels. This conclusion was supported by Abu Damir et al. (1989), who recorded no clinical signs in four camels inoculated with a field bovine strain of *B. abortus*.

The results of the present study indicate that both *B. abortus* and *B. melitensis* can infect male camels but we found that *B. abortus* showed more clinicopathological changes than *B. melitensis*. The estimation of cytokine levels in camels is essential for an understanding of camel disease development

and protective immune responses. This is the first report of *in vivo* camel cytokine quantification under field conditions. Although serological testing is not a routine examination carried out on imported camels, it may be necessary for the early diagnosis and prevention of such disease. Moreover, the assaying of cytokines and biochemical parameters in camels may be helpful in elucidating the etiopathogenesis of the adverse effects associated with brucellosis in camels.

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