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Hyalomma dromedarii TICKS INDUCE A DISTINCT ACUTE PHASE REACTION IN DROMEDARY CAMELS

Mohamed Tharwat[1,2]

[1]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, P.O. Box 6622, Buraidah, 51452, Saudi Arabia

[2]Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

ABSTRACT

The effect of tick infestation in camels on the serum concentrations of the acute phase proteins (APPs) haptoglobin (Hp) and serum amyloid A (SAA) has been described. Twenty-three dromedary camels, naturally infested with *Hyalomma dromedarii* ticks were used. Twelve clinically healthy adult camels were used as controls. From both groups, sera were harvested and analysed for Hp and SAA concentrations. There were statistically significant elevations of Hp and SAA in tick-infested camels compared to uninfested healthy controls. Increases in Hp concentrations in the disease group were estimated to be 4.6 fold (0.52 ± 0.27 mg/L in controls *vs* 2.42 ± 0.51 mg/L in tick infested camels). However, increase in serum SAA were 10.9 folds (0.61 ± 0.25 ng/mL in controls vs 6.67 ± 1.97 ng/mL in tick infested camels). In conclusion, a remarkable acute phase reaction occurs in camels naturally infested with *Hyalomma dromedarii* ticks, and SAA is a more sensitive biomarker for tick infestation in camels than Hp.

Key words: Acute phase reaction, biomarkers, dromedary, *Hyalomma dromedarii*, ticks

Ticks are obligatory haematophagous arthropodes and transmit many tick borne diseases. It may cause severe toxic conditions such as paralysis and toxicosis, irritation and allergy, local skin necrosis resulting in low-quality hides and lowered productivity in terms of weight gain and milk yield (Mehlhorn, 2008).

The acute phase proteins (APPs) are a group of serum proteins that change their concentration in animals following external or internal challenges, and likely play a role in host immunological defense. This response is called the acute-phase response or acute phase reaction (APR), occurring secondary to many types of tissue injury and might be a physiological protective mechanism during inflammatory events (Petersen *et al,* 2004). APPs are synthesised in the liver in response to the systemic presence of high levels of pro-inflammatory cytokines. The principal pathway leading to production of APPs involves initial release of pro-inflammatory cytokines by macrophages at the site of infection or inflammation. The most important inducers of APP are cytokines of the interleukin-1 (IL-1), tumor necrosis factor (TNF) and interleukin-6 (IL-6) families (Eckersall, 2000).

In camel medicine, haptoglobin (Hp) and serum amyloid A (SAA) are the commonly used APPs. The APR can be used in camels for general health assessment. The serum concentrations of APPs has been studied in camels in the non-diseased state during the periparturient period (Tharwat and Al-Sobayil, 2015), following stimulation by electroejaculation (Tharwat and Al-Sobayil, 2018a), and following race (Tharwat and Al-Sobayil, 2018b). These studies suggest that Hp and SAA are sensitive biomarkers and can be used in camels as biomarkers of infection and inflammation (Tharwat, 2020). The aim of the present study was to evaluate the effect of tick infestation in dromedary camels naturally infested with ticks on the serum concentrations of the two major APPs, namely Hp and SAA as markers of APR.

Materials and Methods

Camels, experimental design and blood sampling

The experimental protocol was approved by the Animal Ethical Committee, Deanship for Scientific Research, Qassim University, Saudi Arabia. The experimental design had been reported recently (Tharwat and Al-Sobayil, 2014). Briefly, 23

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Arabian camels (*Camelus dromedarius*), 14 females and 9 males (age: 6.8±2.4 y; weight: 460±115 kg) were admitted to the Veterinary Teaching Hospital, Qassim University for treatment of tick infestation. During the course of the disease, camels showed symptoms of anorexia, incoordination of movement, unsteady gait, recumbency, opisthotonus, anaemia and reduced production. *Hyalomma dromedarii* was identified as the predominant tick species. Twelve clinically healthy adult camels were used as controls. From both groups, blood samples were collected in plain tubes and sera were harvested and stored pending Hp and SAA analysis.

Acute phase protein assays

The serum concentration of Hp was determined in the sera using a colorimetric assay (Haptoglobin kit, second generation, Tridelta Ltd., Ireland) as reported recently (Tharwat and Al-Sobayil, 2015; Tharwat and Al-Sobayil, 2018a; Tharwat and Al-Sobayil, 2018b). The analytical sensitivity of the assay was 0.0005 mg/mL, and intra- and inter-assay CVs were 5-6% and 4-6%, respectively. The SAA was measured in the sera using a commercially available ELISA kit (Multispecies SAA ELISA kit, Tridelta Ltd., Ireland). A monoclonal antibody specific for SAA has been coated onto the wells of the microtitre strips provided. The analytical sensitivity of the assay was 0.15 µg/mL and intra- and inter-assay CVs were 4.5% and 6%, respectively.

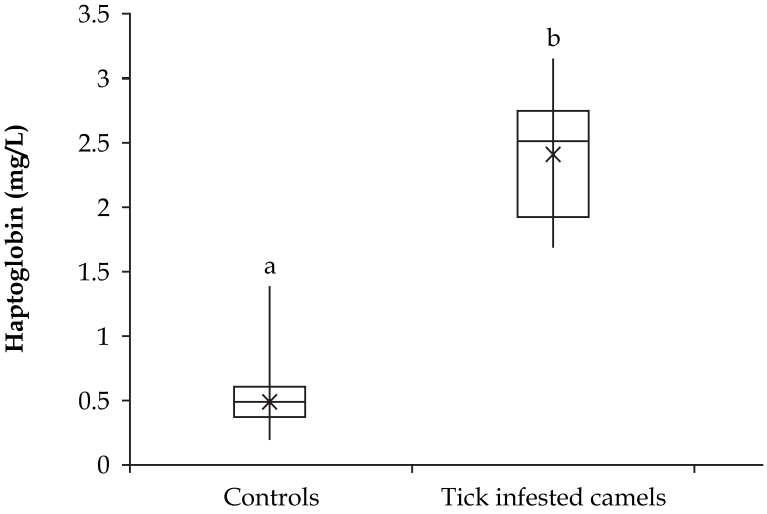

Fig 1. Box and whiskers plots of serum haptoglobin in diseased camels with tick infestation compared to healthy controls. [a,b]Values with different letters differ significantly (P<0.0001).

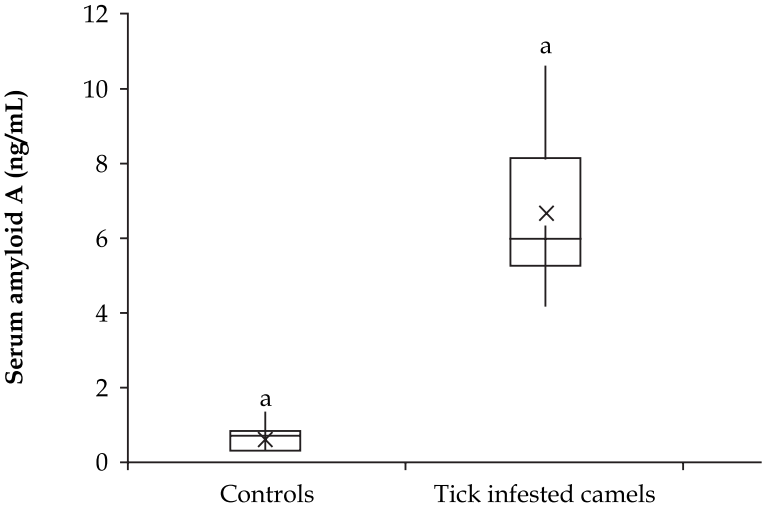

Fig 2. Box and whiskers plots of serum amyloid A in diseased camels with tick infestation compared to healthy controls. [a,b]Values with different letters differ significantly (P<0.0001).

Statistical analysis

Data are presented as means ± standard deviation. A statistical program was used to perform the statistical analyses (SPSS, 2009). The graphical representation of the results was performed using MedCalc Software (Mariakereke). A paired *t-test* for repeated samples was used for comparisons between Hp and SAA values in camels infested with ticks and control values. Significance was set at P<0.05.

Results and Discussion

To the author's knowledge, this is the first report to evaluate the effect on *Hyalomma dromedarii* tick infestation in dromedary camels on the serum concentration of APPs as a marker of APR. The minimum, 25th, 50th and 75th, maximum and mean of serum Hp in camels with tick infestation were 1.7, 1.9, 2.5, 2.7, 3.2 and 2.4 mg/L, respectively compared to 0.2, 0.4, 0.5, 0.6, 1.2 and 0.5 mg/L in controls (Fig 1). Increases in Hp concentrations in the camel with naturally-acquired tick infestation compared to healthy uninfested controls were estimated to be 4.6 fold (0.52 ± 0.27 mg/L in controls *vs* 2.42 ± 0.51 mg/L in tick infested camels) (P<0.0001).

The minimum, 25th, 50th, 75th, maximum and mean of SAA in camels with tick infestation were 4.2, 5.3, 6.0, 8.1, 10.6 and 6.7 ng/mL, respectively compared to 0.3, 0.3, 0.7, 0.8, 1.1 and 0.6 ng/mL, respectively in the controls (Fig 2). Increase in serum

SAA were 10.9 folds (0.61 ± 0.25 ng/mL in controls *vs* 6.67 ± 1.97 ng/mL in tick infested camels) (P<0.0001).

The effect of scab, blood parasites, gastrointestinal and pulmonary nematodes and intestinal protozoa on the APR has been reported in animals. The levels of Hp and SAA has increased in serum following experimental infestation of sheep with *Psoroptes ovis,* becoming statistically significantly elevated from pre-infestation levels at 4 weeks post-infestation. Following successful treatment of infested sheep in the previous study with an endectocide, Hp and SAA serum levels declined rapidly (Wells *et al,* 2013). The serum concentrations of the APPs SAA, Hp, α1-acid glycoprotein (AGP) and ceruloplasmin (Cp) were also higher in Alpine Ibex with clinical signs of *Sarcoptes scabiei* mange when compared to healthy animals (Rahman *et al,* 2010). From the previous study, it was found that SAA and AGP is a major APPs as they increased more than 10 folds, while the increases of Hp and Cp were 2-5 folds so they were classified as minor APPs, a similar finding to our results.

In cattle infected with hydatid cysts, SAA was found to be the major marker in the detection of infection, however Hp was not sensitive marker as it was higher in the control group than diseased one (Sevimli *et al,* 2015). Significant increase were also reported in calves with *Dictyocaulus viviparus* infection (Ganheim *et al,* 2004). Hp concentration was also increased in calves with gastrointestinal and pulmonary nematodes (de Cezaro *et al,* 2016). In calves infected with *Eimeria zuernii* oocysts, serum Hp and SAA levels has increased during the monitoring period of 28 days post-infection (Lassen *et al,* 2015). However, it was reported in the later study that Hp is a more sensitive marker than SAA. In cattle infected with *Theileria annulata*, similar findings were reported (Glass *et al,* 2003). The behaviour of APPs in dairy cattle herd naturally infected with *Trypanosoma vivax* has been investigated (Machado *et al,* 2015). In camels, the serum concentration of the APPs Hp, SAA, Cp and fibrinogen has also been studied in dromedary camels naturally infected with *Trypanosoma evansi* (El-Bahr and El-Deep, 2016).

In the present study, the 4.6-fold and 10.9-fold significant elevations of Hp and SAA, respectively in tick-infested camels reflect the occurrence of severe systemic reaction, probably because the skin inflammation was sufficiently intense to induce a remarkable APR. The local skin necrosis as well as the tick salivary secretions may be the cause of APR induction in this study. The significant elevations of Hp and SAA in tick-infested camels may be attributed to the initial secretion of pro-inflammatory cytokines by macrophages at skin. To secure uninterrupted blood uptake, ticks suppress and evade the complex physiological host immune and homeostatic responses that are raised against them. Haemostasis, which includes coagulation, vasoconstriction, and platelet aggregation, is the first innate host defense mechanism against the mechanical injury caused by intrusion of tick mouthparts into the host skin. This early response further includes complement activation and inflammation, with the host inflammatory response including, among other factors, rapid leukocyte infiltration after skin injury. Pro-inflammatory chemokines and cytokines including IL-8, TNF, and IL 1- β (IL-1β) are released to recruit neutrophils and other inflammatory cells to the area of tick infestation (Kotal *et al,* 2015). Unfortanately, pro-inflammatory cytokines were not determined in this study. In camels, naturally infected with *T. evansi*, there were significant increases in the pool of pro-inflammatory cytokines IL 1- α, IL 1- β, IL-10, IL-6, TNF-α and interferon-γ (El-Bahr *et al,* 2016). It is concluded from this study that a remarkable APR occurs in camels naturally infested with *Hyalomma dromedarii* ticks and SAA appears to be a more sensitive biomarker for tick infestation in camels than Hp.

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