Animal Health Research Journal Vol. 7, No. 3, September 2019 pp. 42-53

ISSN: 2356-7767

Acute phase biomarkers of sheep flocks naturally infected with brucellosis

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Received in 18/8/2019
Accepted in 18/9/2019

Abstract

One hundred and sixty selected positive and negative serum samples were collected during the period ranged from January 2018 to July 2018 from the blood of 730 native ewes belonged to different mobile flocks located at Giza Governorates with a previous history of late term abortion and stillbirth. Positive serum samples to BAPAT, RBPT, and CFT were divided into two groups namely CFT low and high titer groups. This was aimed to evaluate the performance of the serological tests used for the diagnosis of brucellosis in mobile sheep flocks, and to investigate the acute phase proteins, oxidant and antioxidants biomarkers on both brucella positive and negative serum groups. Considering cELISA as a gold standard, sensitivities and specificities of serological tests under this study was estimated. BAPAT and RBPT recorded the highest relative sensitivities of (93.5%) and (91.3) on the expense of their specificities. While the confirmatory CFT was superior in specificity (92.8%) than both screening tests. The overall performance of serological tests in small ruminants based on both ROCs and AUCs is very good being ≥ 0.9. Haptoglobin, fibrinogen, superoxide dismutase (SOD), glutathione peroxidase (GPx), ascorbic acid, nitric oxide (NO), iron, zinc and copper were measured in Brucella positive and negative sera. One-way ANOVA and independent sample t-test were performed and the ROC curves were plotted. the fibrinogen (P=0.0001), copper (P=0.05), NO (P=0.001), and GPx (P=0.029) concentrations decreased in the sera of infected ewes. While, ascorbic acid (P=0.0001) and zinc levels (P=0.0002) increased in serologically positive animals. In conclusion, *Brucella* does not appear to induce antioxidant enzyme activities and inflammation during long lasting infection as shown by the significant lower figures of oxidative stress biomarkers (SOD, NO, and GPx) and non-significant results of positive acute phase proteins (haptoglobin and fibrinogen) and that reflecting the stealthy strategy of brucella.

Key words: *Acute phase reactants, Brucella, Serological tests, sheep, trace minerals*

Introduction

Brucellosis is the common name used for the animal and human infections triggered by several species of the genus Brucella (OIE, 2016). Brucellae show a wide range of host preference. Currently, 11 Brucella species exist **(Whatmore *et al.*, 2014)** including three that have been reported in Egypt **(Menshawy *et al.*, 2014)**, viz. *B. abortus, B. melitensis,* and *B. suis.* The main symptoms of brucellosis in ruminants are reproductive disorders in form of abortion or birth of weak off-springs that do not survive, low milk yield (20-25% reduction), orchitis, epididymitis and less commonly arthritis. Assessment of antibody response employing serological test plays a major role in the routine diagnosis of brucellosis **(Alton *et al.*, 1988).**

Bacterial infection is the major initiators of acute phase response in animals. The macrophage is the main responsible cell for the release of several mediators, reactive oxygen

species and regulatory proteins during infection. The acute phase proteins (APP) are a group of blood proteins mainly produced by the liver that relate to defense to pathological damage, to restoring homeostasis and limiting microbial growth in animals subjected to infection **(Ceciliani *et al.,* 2012).**

Functionally, haptoglobin is an acute phase protein that binds to heme preventing it from serving as a nutrient for pathogens and initiating deleterious oxidation reactions resulting in a rapid inflammatory response **(Matson *et al.,* 2006, 2012).**

Fibrinogen is a plasma glycoprotein originates from the liver and is not only involved in the blood clotting **(Jayachandran *et al.,* 2016),** but its levels are elevated during any form of inflammation; (Page and Schroeder 1976). The changes in the concentrations of blood biochemical parameters and enzyme profiles shows the impact of brucellosis on the vital organs' functioning in the body and thereby helps to understand the health status of individual **(Radostits *et.al.,* 2007).**

In keeping view of the above facts, the present work was undertaken to evaluate the diagnostic performance of some immunoassays used in the diagnosis of ovine brucellosis and to assess and compare the serum zinc, copper and iron in association with the acute phase response (haptoglobin and fibrinogen), and SOD (Superoxide dismutase), glutathione peroxidase (GPx) ascorbic acid and nitric oxide (NO) of native ewes infected with Brucellosis with different CFT titers

Materials and Methods

Ethical approval

Prior to the study, an approval from the Institutional Animal Ethical Committee of the National Research Centre was obtained for blood collection of the animals underwent this study.

Animals and sample collection

Selective positive and negative 160 serum samples were collected during the period ranged from January 2018 to July 2018 from the blood of 730 native ewes belonged to different mobile flocks located at Giza Governorates with previous history of late term abortion. serum samples were separated and divided into aliquots preserved at - 20ºC until being examined for brucellosis.

Brucella serological tests

Sera were tested using buffered acidified plate antigen test (BAPAT) rose Bengal plate test (RBPT) as screening tests and complement fixation test (CFT) as a confirmatory test for detection of brucella antibodies **(Alton *et al.,* 1988; OIE, 2016).**

Multispecies competitive ELISA kit (**SVANOVIR®** Brucella-Ab C-ELISA), was produced by Svanova Biotech AB, Uppsala, Sweden. This kit uses Brucella abortus smooth lipopolysaccharide antigen, horseradish peroxidase conjugated goat anti-mouse IgG monoclonal antibodies and tetramethylbenzidine in substrate buffer containing H2O2. Validation of the kit was done according to the kit instructions, the validation guidelines of the (ISO/IEC 17025, 2005). The test was performed according to the kit instructions.

The biochemical analyses

Haptoglobin (**HP3222)** quantitative immunoturbidimetric assay was. The sensitivity limit was 2.9 mg/dL. Within –run precision and Run to Run precisions were 2.1%. Fibrinogen was measured using immuno-turbidimetry commercial diagnostic kit (Salucea, The Netherlands). The sensitivity of the test was 4.5mg/dL. The test depends on the antigen –antibody (reaction polyclonal anti-fibrinogen) prepared in goat.

Suproxide disutase, glutathione peroxidase, ascorbic acid, nitric oxide, copper, iron and zinc were estimated using commercially available kits (Biodiagnostics, Egypt).

Superoxide dismutase (Cayman chemical company, USA) assay relied on the ability of the enzyme to inhibit the phenazine methosulphate -mediated reduction of nitroblue tetrazolium dye. The GPx assay is a reduction of an organic peroxide by c-GPx, is recycled to its reduced state by the enzyme glutathione reductase (GR): c-GPx R-O-O-H + 2 GSH R-O-H +GSSG +H2O GR GSSG + NADPH + H+ 2GSH + NADP+ The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm providing a spectrophoto-

metric means for monitoring GPx enzyme activity. Redox reaction of Ascorbate with 2,6-dichlorophenol indophenol in acid solution involved reduction of this dye to a colourless leucobase The resulting azo dye has a bright reddish – purple color which can be measured at 540 nm

For measuring zinc (Biodiagnostics, Egypt) present in the sample is chelated by zincon (2carboxy-2'-hydroxy-5-sulphoformazyl-benzene) in the reagent at alkaline pH. The formation of this complex is measured at a wavelength of 610nm. For measuring iron, the serum is deproteinized by trichloroacetic acid and the iron is dissociated from the protein transferrin by hydrochloric acid then reduced to ferrous by thioglycolic acid. The colored complex which the iron form with bathophenanthroline is measured calorimetrically. Copper is released from protein by hydrochloric acid then protein was precipitated by trichloroacetic acid, after that copper in the supernatant reacted with Diethylthiocarbamate forming a golden yellow colored complex with copper which was extracted by n-butanol.

Statistical analyses (All analyses were done using **SPSS 2016)**

One-way analysis of variance (ANOVA):
In order to compare the levels of acute phase response in case of sero-negative *vs*. sero-positive animals and their interaction, generalized mixed linear models were designed separately for each parameter. Each individual was included as a random factor with a normal distribution and an identity link. Then one-way ANOVA was processed. ANOVA post hoc test (Independent sample t-test) was performed using Duncan's Multiple Range test to differentiate between significant means of negative and infected animals at $P<0.05$.

Receiver operating characteristics (ROC) curves: ROC curves were plotted for all agglutination tests as well as haptoglobin, fibrinogen, superoxide dismutase (SOD), glutathione peroxidase (GPx), ascorbic acid, nitric oxide (NO), iron, zinc and copper. Values of AUC between 0.5 and one are interpreted as low (0:5 >: AUC ≤ 0:7), moderate (0:7 >: AUC ≤ 0:9), or high (0:9 >: AUC < 1), accuracy **(Swets, 1986, 1988).**

Estimation of Sensitivities and specificities:
Sensitivity and specificity of the RBPT, BAPAT, and CFT were estimated considering cELISA as a gold standard.

Results and Discussion

Table (1). Relative sensitivities and specificities of serological tests used in the diagnosis of ovine brucellosis

Serological tests	TP	TN	FP	FN	Relative sensitivities	Relative specificities
RBPT	84	46	22	8	91.3%	67.6%
BAPAT	86	43	25	6	93.5%	63.2%
CFT	75	65	5	15	83.3%	92.8%

TP= True positive, TN= True negative, FP= False positive, FN= False negative, Complement fixation test (CFT), Rose Bengal plate test (RBPT), Buffer acidified plate agglutination test (BAPAT)

Table (2). Mean± SD (Standard Deviation) acute phase proteins in ewes infected with Brucella

	Parameters	Positive according to CFT			Negative (group 3)	P-value
		Low titer <10 (group 1)	high titer >10-320 (group 2)	Total		
Immunoassays	BAPAT	2.4±0.1[b]	3.1±0.1[c]	2.8±0.08	1.0±0.1[a**]	0.0001
	RBPT	1.9±0.1[b]	2.8±0.1[c]	2.5±0.08	0.76±0.07[a**]	0.0001
	CFT	6.9±0.4[a]	148±18.5[b]	97.9±13.5	0.00±0.00[a**]	0.0001
Oxidative stress biomarkers	NOμmol/L	27.7±1.4[a]	28.2±2.2[a]	27.9±1.39[a]	52.3±9.3[b**]	0.001
	GPx mU/mL	183±63[ab]	49±7[a]	88.7±20	258±123[b*]	0.029
	SOD U/ml	316±61	273±19	290±27	369±72[*]	NS
Positive acute phase proteins	Fibrinogen mg/dL	273±17[a]	345±16[b]	318±12	300±15[ab]	0.0001
	Haptoglobin mg/dL	28.6±6.9	33.9±7.3	32.0±5.2	29.5±4.2	NS
Blood parameters	Ascorbic mg/ L	39.1±5.1[b]	48.7±4.3[b]	45.±3.3	17.4±1.9[a**]	0.0001
	Zinc µg/dL	167.3±8.9[b]	178.2±7.7[b]	174.3±5.9	141±3.3[**a]	0.002
	Iron µg/dL	803±65	825±4	817±37	776±64	NS
	Copper µg/ dL	488±59[ab]	653±77[b]	591±35	438±26[a#]	0.04

Means with different superscripts (a, b, c) are significantly different at P<0.05, #P>0.05, *P<0.05, **<0.001, non-significant (NS), Complement fixation test (CFT), Rose Bengal plate test (RBPT), Buffer acidified plate agglutination test (BAPAT), Nitric oxide (NO), Glutathione peroxidase (**GPx)**, Superoxide dismutase **(SOD).**

Table (3). Area under the curve (AUC) of the ROC cure for each parameter

	Test Result Variable (s)	AUC	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
Immunoassays	BAPA	.972	.012	.000	.949	.996
	RBPAT	.964	.013	.000	.938	.990
	CFT	.996	.008	.000	1.000	1.000
Blood parameters	Iron µg/dl	.538	.087	.652	.366	.709
	Copper µg/dl	.501	.085	.993	.335	.667
	Zinc µg/dl	.720	.070	.008	.583	.857
	Ascorbic mg/L	.896	.041	.000	.815	.977
Oxidative stress biomarkers	NO	.203	.071	.000	.063	.343
	SOD	.521	.090	.804	.344	.698
	GPX	.599	.098	.239	.407	.791
Positive acute phase proteins	Fibrinogen mg/dL	.537	.065	.555	.410	.665
	Haptoglobin mg/Dl	.415	.060	.179	.297	.532

The test result variable (s): copper ug/dl, Zinc ug/dl has at least one tie between the positive actual state group and the negative actual state group. Complement fixation test (CFT), Rose Bengal plate test (RBPT), Buffer acidified plate agglutination test (BAPAT), Nitric oxide (NO), Glutathione peroxidase (**GPx)**, Superoxide dismutase **(SOD).**
Statistics may be biased a. Under the nonparametric assumption. b. Null hypothesis: true area = 0.5

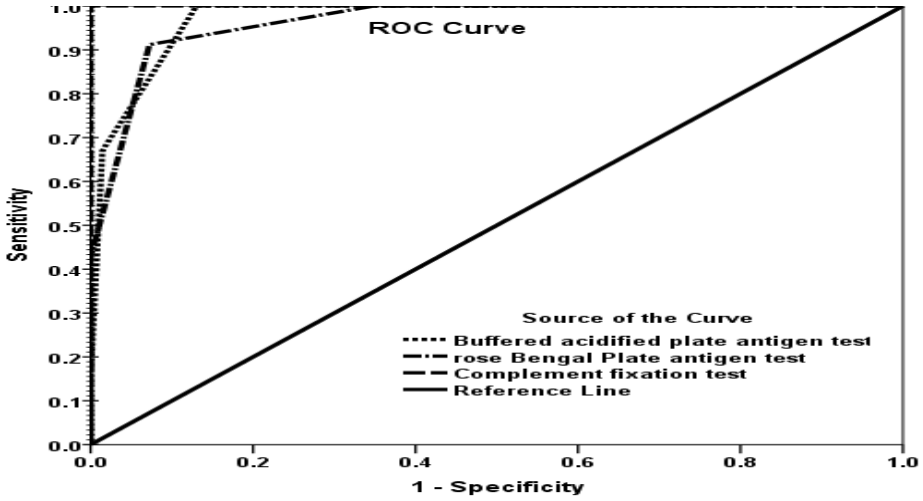

Figure (1). ROC curve of Buffered acidified plate antigen test, Rose Bengal plate antigen test and complement fixation test.

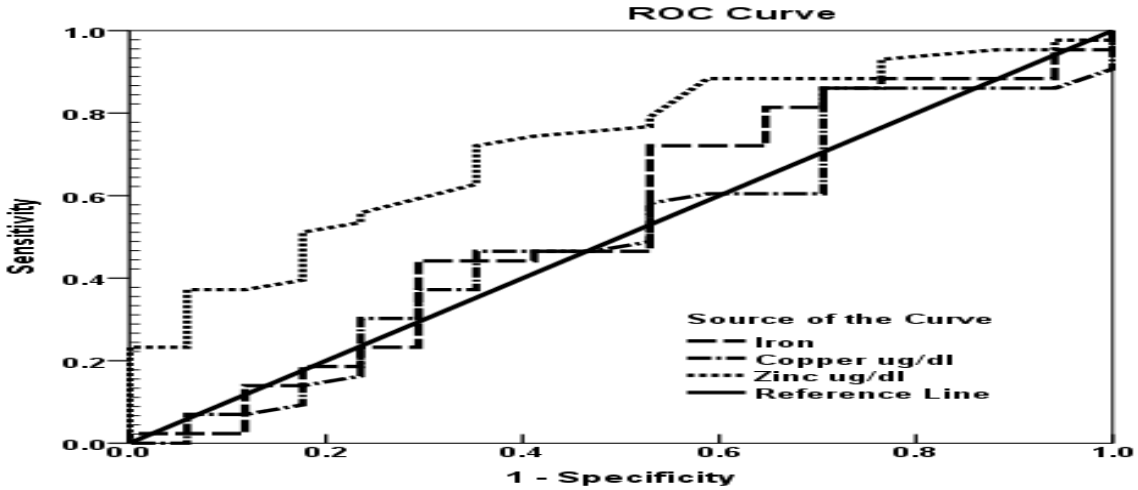

Figure (2). ROC curve of iron, copper and zinc in animals infected and free from Brucellosis.

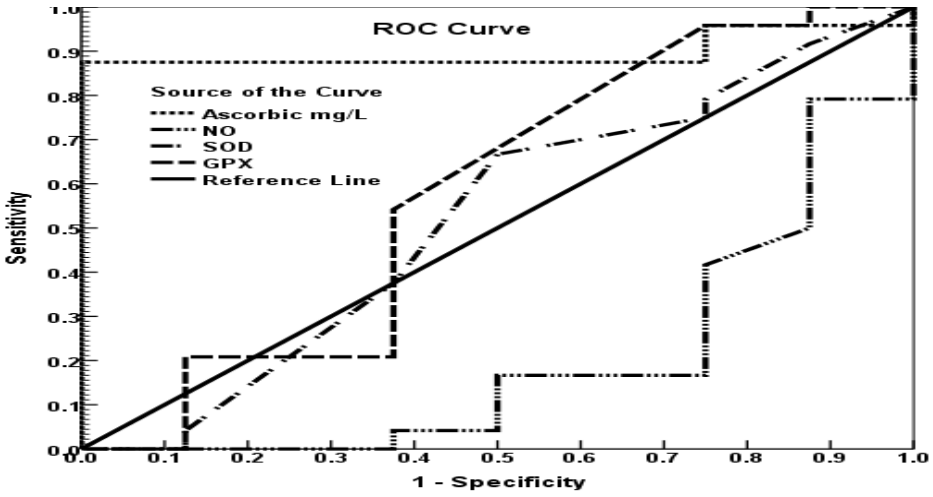

Figure (3). ROC curve of ascorbic acid, NO, SOD, GPx of animals with or without Brucellosis

Table (1), reveals the estimated relative sensitivities of different serological tests used in the diagnosis of brucellosis in ewes and they were as follows: BAPAT, mRBT, CFT of 93.5%, 91.3%, and 83.3%, respectively. While the estimated specificities were as follows: 63.2, 67.6, 92.8 of BAPAT, mRBT, CFT respectively.

The Brucella sero-negative animals (group 1) had lower (P=0.0001) ascorbic acid than brucellosis positive animals (Group 2 and 3) with low and high CFT titers (Table 2). In contrast, NO (P<0.001) and GPx (P=0.029) concentrations of infected sheep were low compared to the non-infected animals. Though SOD concentrations of sero-positive animals were lower (P<0.05) than sero-negative ones but SOD levels of low CFT titer infected animals were not significantly low compared to high CFT titer infected ones and the sero-negative ones. Concentrations of both copper (P=0.04) and zinc (P=0.002) of diseased animals were significantly higher than non-diseased animals. The levels of fibrinogen of infected animals are significantly higher (P<0.0001) than those non-infected animals. Haptoglobin showed a non significant increase of acute infected animals compared to non-diseased and chronic form of the disease (Table 2).

When ROC curve was plotted for all studied parameters (Table 3), the test serological complement fixation test (CFT) had the largest area under the curve (AUC) of 0.996 proving that the CFT is the most accurate test to differentiate between diseased and non-diseased animals (Figure 1). By comparing between the two serological screening tests, the Buffered acidified plate antigen test (BAPAT) had larger AUC of 0.97 than Rose Bengal plate antigen test which had AUC of 0.96. Only the Ascorbic acid (Figure 3) and Zinc (Figure 2) of diseased animals had moderate accuracy where their AUC were 0.89 and 0.72, respectively. Whereas the other parameters had low accuracy and their area AUC ranged from ≤0.5 to <0.7.
The RBPAT correlated positively with iron (r=0.20; P=0.03), zinc (r=0.25; P=0.004), copper (r=0.26; P=0.04), fibrinogen (r=0.20; P=0.05), and ascorbic acid (r=0.49; P=0.0001, but has negative correlations with NO (r=-0.26; P=0.009 and GPx (r=-0.20; P=0.05). BAPAT showed a positive correlation with ascorbic acid (r=0.31; P=0.001) but a negative one with nitric oxide (r=-0.31; P=0.002). The CFT tended to correlate with ascorbic acid (r =-0.16; P=0.079)

Discussion

Sheep and goats get infected not only by the very virulent biotype B. *melitensis* but can contract other types that have major impacts on human health **(Wang *et al.*, 2017)** with causing reproductive disorders with significant economic losses in animal husbandry **(Antunes *et al.*, 2013; Costa *et al.*, 2016;).** A number of serological tests are widely used for the diagnosis of brucellosis because infected animal may or may not produce all antibody types in detectable levels. The highest relative sensitivities of the of BAPAT (93.5%) and RBPT (91.3) in ewes as shown by **Table (1),** can be attributed to the acidic pH of lactate buffer at which the antigens were preserved. The acidic pH alters the isoelectric point of IgM, thus reducing its agglutinability usually responsible for nonspecific serological reactions **(Corbel, 1972).**

The acidic pH also enhances the agglutinability of IgG1 which is non agglutinogenic at neutral pH. Likewise, the final pH after addition of serum in BAPAT is (4.02) and 3.65 in RBPT and the final packed cell volume in case of BAPA is (3%) while that of RBPT is 4% **(Alton *et al.*, 1988).**

The low final packed cell volume of BAPA compared with RBPT in addition to slightly low final acidic pH of BAPA relative to RBPT are the key reasons why the BAPAT is to somewhat sensitive than the RBPT. The highest sensitivities even on the expense of their specificities (63.2%, and 67.6%) are matching with their usage as screening tests.

When it comes to the confirmatory test (CFT), it gives less false positive results (5) compared to screening tests, 25, and 22 for BAPA, and RBPT respectively, reflecting the highest specificity (92.8%) over the screening tests and this finding may be attributed to the high affinity of the CFT test to detect principally IgG1 charac-

teristic to the long lasting infection (**Alton *et al.,* 1988; Mikolon *et al.,* 1998**).

The overall performance of serological tests in ewes (**Table 3 and figure 1**) based on both ROCs and AUCs is very good being ≥ 0.9 and is a reflection of how good the tests are distinguishing between Brucella infected and healthy animals. The main reason stands behind the better performance of screening tests as well as the confirmatory test (CFT) in ewes are attributed in part to the better sensitivities and/or specificities estimated under the umbrella of the current investigation.

In diseased ewes of this study, the increase of ascorbic acid, fibrinogen, zinc and copper especially in high CFT titer group were associated with a decrease of NO, SOD and GPx. In agreement with our results, the levels of acute phase proteins are greatly enhanced after the exposure to the pathogenic factors during the acute phase of inflammation, **(Ceciliani *et al.,* 2002),** whereas, the chronic inflammation is considered as a series of individual inflammatory stimuli and is characterized by longer and slight increase in the serum concentration of APPs as compared to acute inflammation **(Murtaugh, 1994; Jain *et al.,* 2011).** As well as, the *Brucella* infection in ewes was characterized by a significant increase of globulin, cholesterol, Aspartate transaminase and Alanine transaminase with a decrease of albumin and glucose compared to healthy ewes **(Kumar *et al.,* 2015).**

In agreement with the decrease of NO in Brucella infected animals of this study, healthy mares showed similar patterns of their total proteins, globulins and NO **(AboEl-Maaty *et al.,* 2012).** In the serum of Brucella infected animals, a significant decrease of albumin levels and the insignificant decrease of total protein and blood urea nitrogen (BUN) concentrations were recorded ewes **(Kumar *et al.,* 2015),** cattle **(Nath *et.al.* 2014),** ewes, goats, and cattle, **(Hamada *et.al.,* 2013, El-Boshy *et. al.,* 2009)**. This reduced serum albumin and urea in cattle infected with Brucella was referred to damaged liver tissue as expressed by disturbance in liver enzymes **(Kumar *et al.,* 2015; Al- Hussary *et.al.*, 2010, Nath *et.al.* 2014).** The decreasing pattern of nitric oxide in ewes of this study may follow the decreasing pattern of albumin. Albumin as one of the negative acute phase proteins, its concentrations decrease in response to inflammation or infection **(Tothova *et al.,* 2014).**

This decline of serum albumin concentration in the *Brucella* affected ewes was attributed to the loss of albumin through urine due to kidney damage, decreased feed intake by the affected ewes and reduced production of albumin by the liver due to hepatic damage **(Al-Hussary *et.al.*, 2010).**

Nitric oxide as a gas and a free radical is synthesized enzymatically from the amino acid L-arginine in a number of tissues using the three isoforms of nitric oxide synthase and the endothelium one is responsible for the regulation of blood flow and the activation of blood platelets **(Bruckdorfer, 2005).** In the present study, the decrease of NO can be attributed to the lowered feed intake due to *Brucella* which results on the lowered its synthesis of proteins or that endothelial NO as a potent vasorelaxant is used to cause vasodilatations during inflammation, a consequence of either damaged/dysfunctional endothelium and the down-regulation of IL-1 β which induce nitric oxide synthase (NOS) and NO synthesis **(Rosselli *et al.,* 1998).**

All the ruminants, can synthesize ascorbic acid from glucose in their liver **(Comb, 2008).** Vitamin C is also an important water-soluble antioxidant that prevents the *oxidation of protein, DNA and nitric oxide* **(*Frei et al.,* 1989).** Ascorbate increases neutrophils protection against oxidative stress induced by free radicals during the oxidative burst **(Wolf, 1993),** and stimulates interferon production **(Goetzl *et al.,* 1974).** In contrast to the decrease of plasma ascorbic acid concentrations in sheep infected with Fasciola hepatica till week10 after infection then subsequently increased **(Gameel 1982),** ewes naturally infected with brucella in this study obtained high ascorbic acid concentrations. The comparable increase of ascorbic acid may refer to the destructive effects of Fasciola on the animal hepatic cells which synthesize ascorbic acid.

Haptoglobin (Hp) is an acute phase protein that binds to heme preventing it from serving as a nutrient for pathogens and initiating deleterious oxidation reactions resulting in a rapid inflammatory response **(Matson *et al.,* 2006, 2012),** and was used as a predictive marker after an endotoxin challenge **(Matson *et al.,* 2012).**

A haptoglobin (Hp) concentration above 1 mg/ mL was considered the approximate cut-of of severe inflammation **(Skinner and Roberts, 1994; Wells *et al.,* 2013).** In sheep, Hp increases locally through its release by neutrophils in response to early production of TNF-alpha in inflammatory reactions **(Bastos *et al.,* 2011; Ulutas and Ozpinar, 2006; Lepherd *et al.,* 2009).** The insignificant increase of haptoglobin in sheep infected with Brucella may refer to the chronic infection with Brucella infection. Moreover, the sheep breed played an important role in modulating immune response that was observed when Suffolk ewes and Dorset ewes received the same lipopolysaccride, the Suffolk ewes showed an enhanced acute-phase response demonstrated by increased concentrations of plasma haptoglobin **(Hadfield *et al.,* 2018).**

Moreover, age and physiological status of the sheep influenced their immune response **(Miglio *et al.,* 2018).** Our concentrations of haptoglobin and fibrinogen in ewe's infected and non-infected with brucella lied within the normal values in healthy ewes **(Simplício *et al.,* 2017)** which explain the inability of the two major acute phase proteins to be used as predictive values of the disease.

The insignificant decrease of both haptoglobin and fibrinogen concentrations in the serum of ewes with brucella infection was also noted 2 months post-treatment in sheep during a field outbreak of sheep scab **(Wells *et al.,* 2013).**

However, among biochemical parameters, total proteins fibrinogen, and aspartate aminotransferase did not differ, whereas higher (P=0.0062) alanine aminotransferase and lower (P=0.030) alkaline phosphatase concentrations were noted in brucellosis positive horses **(Gul *et al*., 2013).**

The significant increase of haptoglobin between infected and non-infected ewes with brucellosis was also observed in rams experimentally infected with rough virulent strain of B. ovis (R-B. ovis) where the pro-inflammatory cytokine expression was up-regulated for only 30 days after infection then no changes were observed in the expression of of epididymal IL-1α and IL-1β, and testicular IL-12 and INF-γ from Day 30 till Day 240 after infection indicating that with the development of infection, cytokine gene expression levels decreased, providing evidence of immuno-suppression and evidence of immune evasion that favored persistence of chronic R-B. ovis infection **(Antunes *et al,* 2013).**

The mouse model experimentally infected with whole cell of B. melitensis or its lipopolysaccharide via subcutaneous route of exposure demonstrated significant clinical signs and histopathological evidence of B. melitensis infection than LPS. However, the infected groups showed elevated levels of interleukins (IL-1β & IL6), antibody levels (Ig^M and Ig^G) as early as 3 days post-infection with predominance in LPS infected group **(Osman *et al.,* 2018).** The fibrinogen of mobile sheep naturally infected with brucella of this study had higher area under the curve than haptoglobin **(El-Deeb and Elmoslemany, 2016).**

The disadvantage of a natural infection with regard to the determination of Hp responses is that timing cannot be controlled, and the host can have other entero-pathogens which alter the expression of cytokines at different stages of the infection causing different profile of APP responses. Previous studies on the changes of serum Hp and SAA concentrations have focused mostly in natural infectious diarrhea in calves.

Similar to the response of mobile sheep to natural infection with Brucella, Haptoglobin and fibrinogen concentrations and white blood cell counts were not significantly different for seropositive and seronegative sheep to caseous lymphadenitis and their values did not differ significantly among seronegative, acute, and chronic of infection **(Bastos *et al.,* 2011).** As well as, haptoglobin and fibrinogen levels re-

mained within normal limits in lambs infected with ovine lentivirus **(de la Concha-Bermejillo *et al.,* 2000).** Moreover, various infections and inflammatory processes induced different APPs response **(Gruys *et al.,* 2005; Tothova *et al.,* 2014).**

The decrease of enzymatic Superoxide dismutase (SOD), glutathione peroxidase (GPx) and non enzymatic antioxidants (NO) in naturally infected ewes with Brucella species compared to the non-infected ones could be related to prevent the activation of some cytokines **(Ceciliani *et al.,* 2012),** or the inactivation of others or the presence of type IV secretion system gene which is responsible for the virulence of Brucella by increasing its ability to invade and replicate within the macrophages and the over-secretion of effecter proteins causing the death of infected macrophages via activating IRE1α pathway of endoplasmic reticulum stress **(Li *et al.,* 2017).**

SOD and GPx activities in treated ewes after challenge with LPS were significantly lower than positive control one three hours after induction of treatment **(Chalmeh *et al.,* 2016).** SOD promotes the conversion of an anion superoxide to H2O2 **(Al-Gubory *et al.,* 2010)** which is eliminated by catalase **(Klotz *et al.,* 1997; Lim *et al.,* 1998).** In contrast to the seropositive sheep in the current study, cows infected with brucella had higher nitric oxide concentrations and they referred their increase to the stimulation of NO synthesis in macrophages by bacterial lipopolysaccharides **(Nisbet *et al.,* 2007).**

The insignificant increase of iron in the blood serum of ewes infected with brucella of the current study could be attributed to the effect of ascorbic in increasing iron bioavailability through the reduction of ferric ions to ferrous ions **(Wollenberg and Rummel, 1987).** The different values of zinc and iron in lactating sheep **(Yokus *et al.,* 2004)** was attributed to differences in the breed, sex, age, illness, seasonal and physiologic variations, nutritional content of the diet, feeding, environment and geography affect **(García *et al.,* 2000; Kaneko, 1997; Yokus *et al.,* 2004).**

Hypoferremic response of sheep was observed as early as 6 h after lipopolysaccharide (LPS) challenge and iron reached its lowest level at 16 h after LPS infusion and this hypoferremi was attributed to the up-regulation of hepcidin in sheep liver, spleen and kidney in response to the systemic inflammation **(Ridler, and West, 2011).** Moreover, the administration of LPS reduced serum iron levels via modulating hepcidine **(Wallace and Subramaniam, 2015)**. Similar to the increased copper concentrations in sheep seropositive to Brucella, both men and women infected with Brucella had higher copper concentrations in their serum. In contrast to the increased zinc concentrations in but in agreement sheep seropositive to Brucella, women infected with Brucella had significantly low zinc concentrations in their serum **(Mobaien *et al.,* 2010*).***

Conclusions

Authors concluded that *Brucella* does not appear to induce antioxidant enzyme activities and inflammation during long lasting infection as shown by the significant lower figures of oxidative stress biomarkers (SOD, NO, and GPx) and non-significant results of positive acute phase proteins (haptoglobin and fibrinogen) and that reflecting the stealthy strategy of brucella.

The overall performance of serological tests in ewes based on both ROCs and AUCs is very good being ≥ 0.9 and is a reflection of how good the tests are distinguishing between Brucella infected and healthy animals.

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