OPEN ACCESS
Journal of Animal Health and Production

Research Article

Clinicopathological Studies on Brucellosis in Sheep and Goat at Matrouh Governorate, Egypt

Asmaa A. Darwish*, Mona A. Mahmoud, Adel M. El-Kattan

Animal and Poultry Health Department, Animal and Poultry Health Division, Desert Research Center. Cairo, Egypt.

Abstract | Brucellosis is a global zoonotic disease. This work aimed to study the clinicopathological changes in brucella-infected sheep and goat at Matrouh governorate and suggest new markers for brucellosis. Sera were obtained randomly from ewes and does then tested serologically by the Rose Bengal Plate test (RBPT) and competitive ELISA test (cELISA). The animals of each specie were divided into 2 groups: the control group CG (results of both tests were negative) and the diseased group DG (results of one or both tests were positive). The clinicopathological parameters were estimated and statistically analyzed. The brucellosis seroprevalence was (17.50%, 22.50%) for sheep, and (30%, 31.25%) for goat by RBPT and cELISA respectively. Both infected species showed a significant ($P<0.05$) macrocytic hypochromic anemia, hyperglobulinemia, hypoalbuminemia, decreased A/G ratio, increased hepatic function tests, hypertriglyceridemia, increased matrix metalloproteinases activity, T/HDL/LDL-hypocholesterolemia, and decreased minerals, electrolytes, and trace elements concentrations and these changes were more prominent in goat than in sheep. While, the diseased ewes suffered from higher degrees of leukocytosis, total hyperproteinemia, hypoglycemia, increased kidney function tests, and oxidative stress than the diseased does. Total antioxidant capacity (TAC) and Zn had the highest likelihood ratios among the suggested markers with acceptable sensitivity, specificity, PPV, NPV, and accuracy rates, and their combination yielded better values. The study concluded that the clinicopathological alterations associated with ovine and caprine brucellosis are a mirror for the immune response against the disease. Most of these changes were more prominent in goat than in sheep. TAC and Zn (separated or combined) are excellent biomarkers for ovine and caprine brucellosis.

Keywords | Brucellosis, Clinicopathological alterations, Matrix metalloproteinases, New biomarkers, Seroprevallance.

Received | November 11, 2022; **Accepted** | December 15, 2022; **Published** | January 15, 2023
***Correspondence** | Asmaa A Darwish, Animal and Poultry Health Department, Animal and Poultry Health Division, Cairo, Desert Research Center. Egypt;
Email: asmaa_vet25@yahoo.com
Citation | Darwish AA, Mahmoud MA, El-Kattan AM (2023). Clinicopathological studies on brucellosis in sheep and goat at matrouh governorate, Egypt. J. Anim. Health Prod. 11(1): 25-33.
DOI | http://dx.doi.org/10.17582/journal.jahp/2023/11.1.25.33
ISSN | 2308-2801

INTRODUCTION

At Matrouh governorate (western gate of Egypt), sheep and goat breeding is a basic part of the socio-economic life, due to the nature of the governorate as a semi-aired area and the ability of these breeds to endure the drought and dry conditions. Matrouh is located near the Libyan border, and there is uncontrolled animal movement between the two countries, and political unrest in Libya facilitated the spreading of transboundary diseases into Matrouh. Among these diseases brucellosis, which is a worldwide problem, not only as a veterinary problem but also as a health issue for humans. It ranked as the second zoonotic disease after rabies. In Egypt, it is an endemic disease. Different species of *Brucella* were isolated from

all Egyptian governorates. The most prevalent strains were *Brucella abortus biovar* 1, next to *B. melitensis biovar* 3. All animal species are susceptible to infection (Hashem et al., 2020; Nayel et al., 2020; Rabah et al., 2022).

Brucella is a Gram-negative, non-spore former, coccobacillus bacteria. It is transmitted through vaginal discharge, semen, and the placenta of aborted animals. Humans are usually infected through contaminated food and milk. Eradication programs are the only method to control it. Abortion, infertility, stillbirth, drop in milk production and cost of vaccination are heavy economic losses associated with brucellosis (Hashem et al., 2020; Nayel et al., 2020; Rabah et al., 2022). Many researchers studied brucellosis at Matrouh governorate, and they focused only on the prevalence and risk factors of the disease. Unfortunately, too little data are available about its effect on the clinicopathological parameters in sheep and goat and its role in disease pathogenesis (Hashem et al., 2020; Nayel et al., 2020; Rabah et al., 2022).

Hence, this work aimed to study the clinicopathological alterations related to brucellosis in sheep and goat and compare between the two species with special reference to the important of the total antioxidant capacity (TAC), Zn, Cu, matrix metalloproteinases (MMP-2 and MMP-9) as biomarkers for brucellosis in both species.

MATERIALS AND METHODS

After the ethical approval of the animal and poultry health department, Desert Research Centre (DRC), Cairo, Egypt, and the owner`s agreement, 160 animals (80 sheep and 80 goat) from different cities of Matrouh governorate, were examined clinically according to Jackson and Cockcroft (2008). The data were collected about abortion history, infertility problems, and their feeding and grazing.

The whole blood samples (in EDTA-containing tubes) and serum were collected from animals. Sera were tested by the Rose Bengal Plate test (RBPT) and competitive ELISA (cELISA) test. RBPT was performed according to the method described by Morgan et al (1969), while cELISA was carried out using commercial kits (COMPELISA 400®, APHA, New Haw, Addlestone, UK). All manual instructions were carefully followed.

Animals of each specie were grouped according to the RBPT and cELISA results into a control group (CG) and a diseased group (DG) as shown in Table (1).

Whole blood samples were used for hematological parameters estimation according to Feldman et al. (2000) method. Sera samples were used for biochemical parameters determination spectrophotometrically, using commercial kits (Biodiagnostic, Cairo, Egypt), and serum Matrix metalloproteinases (MMPs) concentrations using ELISA kits (Cloud-Clone Corp., Huston, USA).

Table 1: Animals grouping.

Sheep		Goat	
Control group (CG)	**Diseased group (DG)**	**Control group (CG)**	**Diseased group (DG)**
Apparently-healthy ewes with negative results for both tests.	Ewes with positive results for one or both tests.	Apparently-healthy does with negative results for both tests.	Does with positive results for one or both tests.

Statistical analysis

Mean values of groups (CG and DG) of the same species were compared by independent-sample T test, using SPSS® program version 23. A difference was considered significant at $P< 0.05$.

Graph Pad Prism version 8 program was used to evaluate the area under the curve (AUC), cut-off points, sensitivity, specificity, and likelihood ratio (LR) for the measured TAC, Zn, Cu, MMPs, and TAC+Zn between groups (DG to CG) of each specie.

The positive predictive value (PPV), negative predictive value (NPV), and accuracy rate for them were calculated according to the next equations:

PPV=True positive÷Total positive×100.
NPV=True negative ÷Total negative ×100.
Accuracy rate=(True positive+True negative)÷Total population ×100.
Percentages of increaese or decrease for all parameters =(The mean value of the marker concentration in DG-The mean value of its concentration in CG)÷The mean value of its concentration in CG ×100.

RESULTS

The screening test for *Brucella* antibodies presented in Table (2) elucidated that out of 80 samples from sheep 14 (17.50%) were positive, and out of 80 samples from goat 24 (30%) were positive, detected by RBPT. Whereas 18 (22.50%) reacted positively in sheep and 25 (31.25%) reacted positively in goat by cELISA test.

The data in Table (3) showed that both species suffered from macrocytic hypochromic anemia (indicated by the significant ($P<0.05$) decrease in red blood cells counts (RBCs), hemoglobin concentration (Hb), packed cell

Table 2: The seroprevalence of *Brucella* antibodies.

	RBPT			cELISA		
	No.	+ve No.	+ve %	No.	+ve No.	+ve %
Sheep	80	14	17.5	80	18	22.5
Goats	80	24	30	80	25	31.25
Total	160	38	23.75	160	43	26.88

No. = No. of samples +ve No. = No. of +ve samples +ve % = % of +ve samples

Table 3: The hematological parameters of the diseased groups and the control groups of both species.

Parameter	Sheep		Goat	
	CG	DG	CG	DG
RBCs (×10⁶/µl)	12.23±0.38	9.27±0.45*	11.28±0.88	8.53±0.33*
Hb (g/dl)	11.25±0.78	8.68±0.29*	10.63±0.57	7.82±0.59*
PCV (%)	37.18±1.21	29.98±1.88*	34.60±0.82	27.80±2.01*
MCV (fl)	30.90±0.50	32.30±1.80*	30.80±1.91	32.66±2.85*
MCH (pg)	9.18±0.34	9.38±0.52	9.45±0.56	9.19±0.85
MCHC (%)	30.24±1.37	29.09±2.08*	30.64±1.64	28.25±2.66*
TLC (×10³/µl)	7.88±0.36	11.90±1.48*	7.52±0.29	10.68±0.73*
Neutrophils (×10³/µl)	5.91±0.47	8.58±2.11*	5.64±0.22	8.01±0.54*
Lymphocytes(×10³/µl)	1.58±0.13	2.28±0.56*	1.50±0.06	2.13±0.15*
Monocytes (×10³/µl)	0.32±0.03	0.46±0.11*	0.30±0.01	0.43±0.03*
Eosinophils (×10³/µl)	0.08±0.01	0.11±0.03*	0.08±0.01	0.11±0.03*
Basophils (×10³/µl)	0.002±0.004	0.002±0.004	0.002±0.004	0.002±0.004

Values are mean ±SD. Significant differences in the values between the diseased and the control groups of the same species, were indicated by (*) at P< 0.05.

RBCs: Red blood cell count, Hb: Hemoglobin concentration, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH; Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, TLC: Total leukocytic counts.

volume (PCV), and mean corpuscular hemoglobin concentration (MCHC) as well as the significant (P<0.05) increase in mean corpuscular volume (MCV) in both diseased groups in relation to both control groups). A significant (P<0.05) leukocytosis accompanied the anemia in both species (represented by the significant increase in total leukocytic counts (TLC), neutrophils, lymphocytes, monocytes, and eosinophils counts in both diseased groups in relation to control groups).

Table (4) illustrated a significant (P<0.05) elevation in total protein (TP), globulin (Glob), hepatic enzymes activity (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)), renal function tests (urea, creatinine (Cr)), triglycerides and MMPs (2, 9). Contrariwise Albumin (Alb), A/G, glucose, T/HDL/LDL-cholesterol, minerals (Ca, P, Mg), electrolytes (Na, Cl, K), trace elements (Cu, Zn) and TAC significantly (P<0.05) declined in both diseased groups when compared to the control groups.

Table (5) displayed that the erythrogram (except MCV), immunological parameters (Alb, Glob, A/G and MMPs), liver enzymes activity (ALT, AST, ALP), lipids profile, minerals, electrolytes, and trace elements were affected in brucella-infected goat with higher percentages than in brucella-infected sheep. In contrast, the degree of MCV increase, total and differential leukocytosis, total hyperproteinemia, hypoglycemia, kidney function tests increase (blood urea, Cr) and oxidative stress were more pronounced in infected sheep than in infected goat.

Concerning the TAC, Zn, Cu, MMP-2, and MMP-9 value as biomarkers for brucellosis, all of them yielded high values of AUC, sensitivity, specificity, PPV, NPV, and AR (except MMP-2 in goat had low values of specificity and PPV), but LR markedly differed. Ordering them according to LR, TAC in both species and Zn in goat (only) scored a high LR as 20, followed by Zn in sheep with a moderate LR as 6.67, then Cu and MMP-9 (in sheep & goat) with low LRs as 4.5 and 4 respectively. While, MMP-2 achieved the lowest values of specificity, LR, PPV, and AR among the estimated markers, especially in goat (Table 6). Interestingly, the combination between TAC and Zn achieved AUC as 1, sensitivity, specificity, PPV, NPV, and AR as 100% in both species.

Table 4: The biochemical parameters of the diseased groups and the control groups of both species.

Parameter	Sheep		Goat	
	CG	DG	CG	DG
Total protein (g/dl)	4.54±0.35	6.64±0.58*	4.22±023	6.04±0.61*
Albumin (g/dl)	3.24±0.22	2.43±0.28*	3.03±0.02	2.16±0.20*
Globulin (g/dl)	1.30±0.27	4.20±0.60*	1.19±0.23	3.88±059*
A\G	2.61±0.64	0.59±0.12*	2.62±0.41	0.57±0.11*
Glucose (mg/dl)	113.60±7.78	83.85±3.06*	107.10±4.70	82.05±3.86*
AST (U/L)	26.40±0.26	32.42±1.87*	23.98±0.54	30.33±1.80*
ALT (U/L)	30.40±0.26	36.42±1.87*	28.98±0.54	35.23±1.80*
ALP (U/L)	27.38±1.22	34.21±1.60*	25.18±1.05	32.71±1.05*
Blood urea (mg/dl)	17.90±1.24	24.25±1.46*	17.37±1.14	22.84±1.63*
Cr (mg/dl)	0.75±0.08	1.77±0.10*	0.65±0.07	1.26±0.04*
Total lipids (mg/dl)	393.57±14.08	389.88±16.01	367.36±7.65	364.89±10.70
Triglycerides (mg/dl)	55.98±2.03	88.45±7.19*	45.98±2.03	76.95±5.12*
Phospholipids (mg/dl)	168.09±13.86	163.53±12.09	162.88±2.02	161.04±8.88
T-cholesterol (mg/dl)	169.50±7.59	137.90±3.91*	158.50±7.59	126.90±3.92*
HDL-cholesterol(mg/dl)	58.31±7.82	50.04±4.66*	51.31±7.82	43.04±4.66*
LDL-cholesterol (mg/dl)	107.20±3.24	83.86±2.51*	102.20±3.24	78.86±2.51*
Ca (mg/dl)	10.36±0.24	8.63±0.25*	10.24±0.31	8.33±0.36*
P (mg/dl)	5.38±0.21	2.66±0.22*	5.24±0.23	2.04±0.03*
Cl (mmol/L)	107.17±2.37	94.24±2.59*	105.07±3.05	91.90±2.91*
Na (mmol/L)	126.00±2.06	96.63±3.12*	123.50±3.57	93.51±4.02*
K (mmol/L)	4.80±0.40	2.71±0.30*	4.70±0.26	2.59±0.33*
Mg (mg/dl)	2.37±0.22	1.53±0.28*	2.27±0.22	1.30±0.17*
Cu (µg/dl)	154.27±4.87	100.11±20.35*	146..27±4.87	92.11±20.35*
Zn (µg/dl)	143.43±4.01	105.59±2.57*	141.78±4.87	103.13±3.66*
TAC (Mm/L)	1.62±0.18	0.50±0.08*	1.19±0.17	0.45±0.04*
MMP-2 (ng/ml)	15.40±0.75	59.96±1.72*	14.19±1.35	56.76±4.82*
MMP-9 (ng/ml)	22.75±0.76	64.74±3.19*	21.98±0.97	63.62±1.86*

Values are mean ±SD. Significant differences in the values between the diseased and the control groups of the same species, were indicated by (*) at P< 0.05.

A/G: Albumin/Globulin ratio, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, Cr: Creatinine, TAC: Total antioxidant capacity, MMP-2: Matrix metalloproteinase-2, MMP-9: Matrix metalloproteinase-9

Table 5: Comparison between the percentages of increase or decrease for the estimated clinicopathological parameters in the diseased groups of both species.

Parameters	Sheep	Goat
RBCs	-24.20%	-24.38%
Hb	-22.84%	-26.43%
PCV	-19.36%	-19.65%
MCV	6.25%	6.04%
MCHC	-3.80%	-7.80%
TLC	51.02%	42.02%
Neutrophils	45.18%	42.02%
Lymphocytes	44.30%	42%

Monocytes	43.75%	43.33%
Eosinophils	37.5%	37.5%
Total protein	46.26%	43.13%
Albumin	-25%	-28.71%
Globulin	223.08%	226.05%
A\G	-77.39%	-78.24%
Glucose	-26.19%	-23.39%
AST	22.80%	26.48%
ALT	19.80%	21.57%
ALP	24.95%	29.90%
Blood urea	35.47%	31.49%
Cr	136%	93.85%
Triglycerides	58.01%	67.36%
Cholesterol	-18.64%	-19.94%
HDL-cholesterol	-14.18%	-16.12%
LDL-cholesterol	-21.77%	-22.84%
Ca	-16.70%	-18.65%
P	-50.56%	-61.07%
Cl	-12.06%	-12.53%
Na	-23.31%	-24.28%
K	-43.54%	-44.89%
Mg	-35.44%	-42.73%
Cu	-35.11%	-37.03%
Zn	-26.38%	-27.26%
TAC	-69.14%	-62.18%
MMP-2	289.35%	300%
MMP-9	184.57%	189.44%

Table 6: Area under the curve (AUC), cut off points, sensitivity, specificity, LR, PPV, NPV and accuracy rate of Cu (µg/dl), Zn (µg/dl), TAC (Mm/L) and MMPs (ng/ml) in DG compared to CG (of the same species).

	Sheep						**Goat**					
	Cu	Zn	TAC	MMP-2	MMP-9	TAC +Zn	Cu	Zn	TAC	MMP -2	MMP -9	TAC +Zn
AUC	0.92	1	1	1	1	1	0.92	1	1	1	1	1
Cut off	149	138.5	1.30	15.90	23.70	-	141	107.8	1.57	15	22.80	-
Sensitivity	90%	100%	100%	100%	100%	100%	90%	100%	100%	100%	100%	100%
Specificity	80%	85%	95%	70%	75%	100%	80%	95%	95%	65%	75%	100%
LR	4.5	6.67	20	3.33	4	-	4.5	20	20	2.86	4	-
PPV	81.82%	86.96%	95.24%	76.72%	80%	100%	81.82%	95.24%	95.24%	74.07%	80%	100%
NPV	88.89%	100%	100%	100%	100%	100%	88.89%	100%	100%	100%	100%	100%
Accuracy rate	85%	92.50%	97.50%	85%	87.50%	100%	85%	97.50%	97.50%	82.50%	87.50%	100%

AUC = 0.5–0.65 (useless marker), AUC = 0.7–0.85 (good marker), AUC = 0.86–1 (with satisfactory sensitivity and specificity: excellent marker). LR= 0.5-5: low; LR=5-10: moderate; LR>10: high.

DISCUSSION

Brucellosis is still remaining a serious problem in farm animals. Previous studies threw the light upon the marked role of sheep and goat especially mature ones, in the epidemiological pattern of brucellosis (Nayel et al., 2020; Rabah et al., 2022). In this study, the seroprevalence of sheep brucellosis was (17.50%, 22.50%) by RBPT and cELISA respectively, while the seroprevalence of goat brucellosis was (30%, 31.25%) by RBPT and cELISA respectively. Lower disease percentages were recorded by different authors in the northern coast area of Egypt (Nayel et al., 2020; Rabah et al., 2022). This differences may be attributed to the time and place of sampling and people habits in reporting cases as well. The high prevalence ratio of brucellosis observed in this study (22.50 % in sheep and 31.25 % in goat by cELISA) may be because of the wrong management practices followed by animals' owners as large-scale animal grazing and mixed breeding which facilitate the spread of infection. Several factors such as lack of research, illegal animal movement, communal clashes, unreported outbreaks, poor vaccination coverage, and absence of brucellosis control programs in this region, may also participate in the increase of brucellosis prevalence, establishment, and maintenance (Nayel et al., 2020; Rabah et al., 2022).

In parallel, the erythrogram of the brucella-infected ewes and does characterized by macrocytic hypochromic anemia. This anemia has an immunological origin as the activated pro-inflammatory cytokines due to the bacterial infection suppress the erythropoiesis process by interfering with erythropoietin formation. It also enhances ferritin and hepcidin synthesis, leading to inadequate intestinal iron absorption and decreased macrophage iron recycling. This action is mainly to prevent iron accessibility to the invading bacteria to prevent its growth and multiplication (Kushwaha et al., 2014; Hashem et al., 2020). By the time, the iron bioavailability to the host bone marrow decreased too, causing the noted reduction of RBCs, Hb, PCV, and MCHC in the infected animals of both species. Similar results were obtained before in brucella-infected camels (El-Boshy et al., 2009), cattle (Kushwaha et al., 2014), and cows (Hashem et al., 2020). On the other hand, the increased MCV values pointed to a regenerative bone marrow response and an increased reticulocytes release in the peripheral blood. These data are compatible with Sikder et al. (2012) findings in cattle infected with *Brucella abortus*. The activated pro-inflammatory cytokines stimulate the formation of different leukocytes, differentiation, maturation, and migration from bone marrow and lymph nodes (lymphocytes) to the circulation for infection fight (Gul et al, 2013; Hashem et al., 2020). This explains the pronounced neutrophilia, lymphocytosis, monocytosis, and eosinophilia and the subsequent leukocytosis which observed in current study in both infected species. The leukocytosis is an evident in *Brucella* infection reported before in brucella-infected cow, ewe and horse (Sikder et al., 2012; Gul et al, 2013; Hashem et al., 2020). This leukocytosis is an important reaction from the host as neutrophils are the first line of defense against bacterial infection and monocytes act as uterine tissue debris scavengers (present due to retained placenta and delayed natural uterine cleaning in case of *Brucella* infection).

Regarding the biochemical parameters, the current data depicted a marked hyperglobulinemia resulted in total hyperproteinemia and decreased A/G ratio in the infected ewes and does. As the presence of the Brucella organism in the host body stimulates its immune system to produce more innate non-specific immune proteins and humoral-specific proteins. Innate proteins such as cytokines, acute phase proteins, and matrix metalloproteinases are responsible for limiting the spread of the pathogen and multiplication till the specific immunity development and they result in the elevation of α and β globulin fractions (Kumar et al., 2015; Mahboub et al., 2015). While, the humoral-specific proteins (immunoglobulins) are responsible for pathogen destruction and removal, resulting in γ globulin elevation (Kumar et al., 2015; Mahboub et al., 2015). Hyperglobulinemia was determined before in brucella-infected ewes (Kumar et al., 2015; Mahboub et al., 2015) and cattle (Kushwaha et al., 2014). Likewise, the hypoalbuminemia in the brucella-infected sheep and goat detected in this study was assigned to the immune response and the strong acute phase response usually observed with *Brucella* infection (Sharifiyazdia et al., 2012; Hamdy et al., 2019). Albumin is a negative acute phase reactant, sensitized in the liver like the above-mentioned α and β globulins. During the infection, the liver gives absolute priority to α and β globulins synthesis at the expense of albumin synthesis causing the significant hypoalbuminemia recorded in both diseased groups. Similar results were obtained by Uluğ et al. (2010), Kumar et al. (2015), Mahboub et al. (2015), and Hashem et al. (2020) in brucella-infected humans, ewes, and cows respectively.

Additionally, the immune response was involved in the hyperthermia and anorexia commonly noted in diseased animals because of prostaglandin E2 secretion and bradykinin formation (de Goeij et al., 2013). The hyperthermia and anorexia had a great contribution to the prior hypoalbuminemia, hypoglycemia and T/HDL/LDL-hypocholesterolemia detected in the infected animals in this research, because of the dietary amino acids, carbohydrates, and fatty acids deficiency. While, hypertriglyceridemia referred to increased adipose tissue lysis and usage of body fat stores to get the energy to overcome the hypoglycemia related to the disease. Similar

findings were recorded in brucella-infected different species by Mahboub et al. (2015), Singh et al. (2016), Merhan et al. (2017), Kumar et al. (2015), and Hashem et al. (2020). Anorexia was also implicated in the spotted hyponatremia, hypokalemia, hypochloremia, hypocalcemia, hypomagnesemia, hypophosphatemia, hypozincemia and hypocupremia in the infected animals (Radostits et al., 2002).

The oxidative stress noticed in brucella-infected ewes and does in the current work (represented by the decreased TAC levels in both diseased groups) is another outcome for the above-described cell-mediated immune response. The immune cells, mainly neutrophils, and macrophages generate free radicals under the effect of the invigorated pro-inflammatory cytokines. Free radicals react with the pathogen cellular components and absorb their electrons to achieve their stability (Merhan et al., 2017; Shalby et al., 2020). Physiologically, free radicals are controlled by the anti-oxidants (enzymes and vitamins) but in this research as well as previous researches free radicals exceed the anti-oxidants neutralizing ability and oxidative stress strongly appeared (Kataria et al. 2010; Bozukluhan et al., 2017; Merhan et al., 2017; Shalby et al., 2020). Anorexia partially participated in this process, whereas Zn and Cu are necessary elements for antioxidants synthesis and their decreased concentrations in the infected animals means lower levels of antioxidants and lead to oxidative stress augmentation (Kataria et al., 2010; Bozukluhan et al., 2017; Merhan et al., 2017; Shalby et al., 2020).

Oxidative stress is a reasonable cause for liver and kidney damage usually determined in brucella-infected species. This was indicated in the current data and previous data by the elevated hepatic and renal function tests in the infected animals (Gul et al., 2013; Mahboub et al., 2013; Kumar et al., 2015; Singh et al., 2016; Bozukluhan et al., 2017; Maruf et al., 2019; Hashem et al., 2020). The liver is the concerned organ with albumin and cholesterol formation and glucose blood levels regulation. Thus, its damage took a part in the hypoalbuminemia, hypoglycemia and T/HDL/LDL-hypocholesterolemia obtained in the infected ewes and goat here (Bozukluhan et al., 2017; Maruf et al., 2019; Hashem et al., 2020). In the same way, the decreased minerals and electrolytes levels in the diseased animals may be attributed to renal insufficiency and its inability to restore minerals and electrolytes from urine due to its damage (Gul et al., 2013; Mahboub et al., 2013; Kumar et al., 2015; Singh et al., 2016; Bozukluhan et al., 2017; Maruf et al., 2019; Hashem et al., 2020). Therefore, oxidative stress had an indirect role in the reported hypoalbuminemia, hypoglycemia, and T/HDL/LDL-hypocholesterolemia and decreased minerals and electrolytes levels in the infected ewes and goat. Interestingly, oxidative stress involved in the hypoalbuminemia noticed in the affected species through another way. As the accumulated free radicals consume the albumin because of its antioxidant characters. These free radicals also attack the circulating erythrocytes causing their lysis and subsequent anemia magnification (Kataria et al., 2010; Bozukluhan et al., 2017; Merhan et al., 2017; Shalby et al., 2020). Consequently, oxidative stress which begins as apart from the host innate immunity, has a major role in the disease pathogenesis exacerbation.

Matrix metalloproteinases are a group of proteolytic zinc-containing Ca-dependent enzymes. They are responsible for extracellular matrix remolding through its degradation. They are upregulated in different physiological and pathological conditions under the effect of the pro-inflammatory cytokines (Scian et al., 2011). Miraglia et al. (2013) and Šiširak and Hukić (2014) referred to their elevated activities in brucella-infected patients especially those with osteoarticular complications and neurobruollsis. They attributed this increase to the elevated pro-inflammatory cytokines activity usually accompanied *Brucella* infection (Demirdag et al., 2003; Scian et al., 2011; Hashem et al., 2020). The current results were compatible with these authors` opinions, as MMP-2 and MMP-9 showed high concentrations in the brucella-infected ewes and does.

The comparison between the two species demonstrated that most of the clinicopathological alterations in goat were more considerable than in sheep. Many researchers found that brucellosis is more prevalent in goat than in sheep (Nayel et al., 2020; Rabah et al., 2022). So, goat has a stronger immune response than sheep against *Brucella* (represented by the higher percentages of hyperglobulinemia, hypoalbuminemia, decreased A/G and increased MMPs activity). This response may cause a higher degree of anemia, hepatic damage, anorexia and hyperthermia and a lower degree of bonne marrow regenerative response and leukocytosis (total and differential) in the affected does than the affected ewes (Tizard, 2013). The hepatic damage and anorexia explained the higher percentages of T/HDL/LDL-hypocholesteremia, hypertriglyceridemia, decreased minerals, and electrolytes levels and trace elements recorded in does with brucellosis than in ewes with brucellosis (Radostits et al., 2002). While, oxidative stress and the subsequent renal damage were more prominent in sheep than in goat because of the higher leukocytosis and neutrophilia degree (are responsible for free radicals production) recorded in sheep than in goat (Malech et al., 2014).

Regarding the importance of the studied markers in the *Brucella* diagnosis, the present work suggested TAC and Zn (especially if combined together) as excellent biomarkers

for caprine and ovine brucellosis and excluded Cu, MMP-2, and MMP-9 as markers for the disease. These results completely disagreed with Demirdag et al. (2003) and Scian et al. (2011) opinions who nominated MMPs as sensitive biomarkers for human brucellosis. Shalby et al. (2020) also disagreed with this result, as they excluded Zn and oxidative stress indicators as markers for ovine brucellosis. This disagreement may be due to the difference in the host species, *Brucella* species, and the disease stage.

CONCLUSION

Brucellosis in sheep and goat is associated with several clinicopathological alterations. These alterations are a reflection of the host immune response against the pathogen and most of them are more prominent in goat than in sheep. Oxidative stress has a major role in disease pathogenesis exacerbation. TAC and Zn (separate or combined) are excellent biomarkers for caprine and ovine brucellosis.

ACKNOWLEDGMENT

The authors thank members of the Animal and Poultry Health Department, Desert Research Center, Cairo, Egypt.

CONFLICT OF INTEREST

Authors have no conflict of interest.

NOVELTY STATEMENT

This research was able to determine the most important clinicopathological alterations related to brucellosis in sheep and goat and compare between them in both species. the research also suggests new biomarkers for the disease in both species

AUTHORS CONTRIBUTION

All authors are equally contributed.

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