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RESEARCH ARTICLE

Hematological, Biochemical and Immunological Studies on Brucellosis in Cows and Ewes in Dakahlia and Damietta Governorates, Egypt

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Abstract

Brucellosis is an important disease of public health significance that leads to severe losses. The current study was carried out to identify the recovered *Brucella species* , and its prevalence in cows and ewes in Dakahlia and Damietta Governorates during May 2018 to March 2019, with the hemato-biochemical analyses in the positive cases. In the current study, *B. melitensis biovar 3* was identified with a total prevalence of 8.75% and 6.98% in cows and ewes, respectively. Hematological results denoted a normocytic normochromic anemia in infected cows and ewes at the 2nd and 4th weeks after abortion group (GP) 2 and GP.3, respectively, with leucocytosis especially in GP.3. Significant hypoproteinemia and hypoalbuminemia were detected in infected cows at the 2nd week after abortion, with non-significant changes at 4th week after abortion. While Brucellosis-infected ewes showed a significant ($P < 0.05$) hypoproteinemia and hypoalbuminemia at both 2nd and 4th weeks post abortion. The serum AST, ALT, ALP and LDH activities and levels of IL-6 and TNF-α were markedly elevated ($P < 0.05$) in both infected cow and ewes with markedly lowered serum urea level. Whereas serum glucose level was significantly decreased ($P < 0.05$) in infected cows (GPs 2 and 3), as well as, infected ewes of GP. 2, with non-significant changes in infected ewes of GP3. The serum creatinine level showed non-significant changes in all infected cows and ewes. Serum immunoglobulin M was significantly increased in both infected cows and ewes of GPs 2 and 3, while immunoglobulin G was significantly elevated in both infected cow and ewes of GP3. Moreover, phagocytic percent and index showed a significant decrease in both infected cows and ewes (GPs2 and 3). Thus, evaluations of hematological, biochemical and immunological parameters together with specific diagnostic tests are necessary to obtain more reliable results for the diagnosis of Brucellosis in cows and ewes.

Keywords: Brucellosis, IgM, IgG, TNF- α, Interleukin 6.

Introduction

Brucellosis is a highly contagious zoonotic bacterial disease, which is considered the second most important zoonotic disease after rabies and has gained prominence over years since its discovery in island of Malta [1], moreover it results in high reproductive losses in animals, owing to increasing rates of abortion and infertility [2]. Brucellosis in Egypt, considered as an endemic and national wide disease in livestock and humans as it has been reported in cows, buffaloes, ewes, goats and camels [3]. Despite its economic and public health importance, in recent years, the Official Egyptian Brucellosis Control Program does not appear to have been fully implemented [4, 5]**.** El-Diasty [6] recorded the disease in Damietta (10.4%) and in Dakahlia (7.18%) Governorates . However, Shalaby *et al.* [7] reported a prevalence rate of 17.8%, 8.9% and 11.8% in Dakahlia, Damietta and Alexandria Governorates, respectively. Based on Rose Bengal Plate test (RBPT), the survey study published between 1948 and 2009 in Egypt the prevalence of Brucellosis in cows

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and buffaloes were 5.4 % [8]. Isolation of *Brucella species* is still the golden key for an accurate diagnosis to avoid the false positive reaction of serological diagnosis but long incubation period and samples handling act as barrier for this method in diagnosis [9].The most prevalent serotypes in Egyptian veterinary field are *Brucella abortus biovar* 1, next to *B. melitensis biovar* 3 [10]. Regarding the biochemical changes with B125rucellosis the available literature is little. The liver is the most important organ of the reticuloendothelial system (RES) that occupies a central position in the metabolism of the animal .The disturbance in hepatic function may be accompanied with brucellosis in cows which has serious consequences for the productivity and reproductively of the diseased animals [11]. Brucellosis plays a role in the remarkable disturbance of the biochemical parameters in the blood of the diseased cows and man; this reflects a disturbance in the general health condition of the host [7].]. In our opinion more attention should be paid for careful, early diagnosis of this zoonotic disease followed by eradication of the sero-reactor animals, vaccination of non-reactors and a therapeutic plan for the diseased farm workers. Veterinary authorities must encourage the cooperation between the Veterinary Services and Public Health Authorities to put a plan for the control of Brucellosis in Egypt. The objective of this work was to investigate the occurrence as well as to study the serological, hematological, biochemical and immunological changes as a result of brucella infection in the diseased cows and ewes at the high risk when compared with healthy ones.

Materials and Methods

Animals

During the period from May 2018 to March 2019, a total of 217 adult cows of 3-5 years old (98 from Dakahlia Governorate and 119 from Damietta Governorate) and 415 ewes aged 1.5-3 years (220 from Dakahlia Governorate and 195 from Damietta Governorate) were surveyed. The examined animals were 198 apparently healthy cows and 386 apparently healthy ewes, as well as, 19 cows and 29 ewes were suffered from abortion at the last third of pregnancy with retained placenta and endometritis.

Sampling

Blood samples

Blood samples were obtained from 217 adult cows and 415 adult ewes. After Oneblood sample was taken without anticoagulant, and left at room temprature till cloting then was centrifuged at 3000 rpm for 10 min. and the collected sera were stored at -20 °C until assayed for serological, biochemical and immunological studies. The second one was collected on EDTA from 7 cows and 7 ewes that were positive to Brucellosis for hematological examination.

Milk and tissue samples

Six milk samples, lymph nodes, liver, spleen and abomasal content from two aborted foeti were obtained for bacteriological examination (Table 1).

Table (1): Number and percent of positive samples collected from seropositive slaughtered cows and ewes for *Brucella melitensis biovar* in Dakahlia and Damietta Governorates.

Species	No. of positive samples/ total samples (%)											
	Tissue samples		Lymph nodes		Spleen		Liver		Aborted feoti		Milk	
cows	3/21	(14.3)	2/8	(25)	1/5	(20)	0/2	0	**0**	**0**	2/6	(33.3)
ewes	9/43	(20.9)	**0**	**0**	**0**	**0**	**0**	**0**	2/2	(100)	1/2	(50)

Serological tests

Different serological tests including Rose Bengal plate tests (RBPT) [12], Buffered acidified plate antigen test (BAPAT) [13] and Complement fixation test using warm fixation (CFT) [13] were performed on the 632 collected sera samples. The antigens for BAPAT and RBPT were kindly supplied from Veterinary Sera and Vaccine Research Institute, Abbassiya, Cairo, Egypt, while the antigen used in CFT was obtained from National Veterinary Services Laboratories, Ames, IA 50010, USA.

Bacteriological examination

Isolation, identification and typing of *Brucella species* was performed according to the methods recommended by Alton *et al*. [14] from milk samples, lymph nodes, liver, spleen and aborted foeti abomasal content from positively reacted cows and ewes to one or more of previously mentioned serological tests. Trypticase soya agar medium with antibiotics (BBL, Becton Dickinson Company, USA, catalogue no. BB11043) and trypticase soya agar medium with thionin ((Sigma Chemical Company, U.S.A.) were used for isolation,then the suspected colonies were identified and typed according to morphological and biochemical characters of colonies [13].

***Animals groupi*ng**

Cows

Group (GP1) was considered as control group contain 198 apparently healthy cows which were sero-negative for all previous serological tests, were in the same farms, received same food and rearing condition as other animals in (GPs2,3). GP2: *Brucella melitensis biovera* 3 infected cows (9 seropositive cows) at the 2nd week post abortion. GP3: *B. melitensis biovera* 3 infected cows (10 seropositive cows) at the 4th weeks post abortion.

Ewes

Three hundred eighty six apparently healthy ewes which are sero-negative for all previous serological tests and was considered as a control group GP1. GP2 included the infected ewes (10 seropositive ewes) with *Brucella melitensis biovera 3* at the 2nd week post abortion. GP3: contained the infected ewes (19 seropositive ewes) with *Brucella melitensis biovera 3*at the 4th week post abortion.

Hematological studies

Red blood cells (RBCs) count, Hemoglobin (Hb) concentration, Hematocrit value (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total and differential leukocytic count were determined using automatic cell counter (Abcus 380)

Biochemical studies

All biochemical kits were obtained from Diamond-Diagnostic Co., Cairo, Egypt. Cow serum IL 6 was measure by ELISA Kit Catalog number MBS703712, while ELISA Kit Catalog number MB S2500510 was used for ewes IL 6 . Bovine TNF-α was measured by ELISA kit Catalog number: MBS702888, while ewes TNF-α was measured by ELISA kit Catalog Number: MBS702888. Serum was used to determine aspartate aminotransferase (AST), alanine aminotransferase (ALT) [15], alkaline phosphatase (ALP) [16] and Lactate dehydrogenase (LDH)[17] activities, Total protein [18]and albumin[19] levels. Serum globulins were calculated by subtracting the obtained albumin level from the obtained total proteins level [20].Serum was used to estimate urea [21], creatinine [18] andglucose [22] levels. Interleukin 6 [23] and TNF-α [24].

Immunological studies

The serum immunoglobulin (Ig) G and M levels were measured as previously described [25]. In addition, phagocytic activity [26] and phagocytic assay [27] were determined.

Data analysis

All data were expressed as mean ±standard error, analyzed by the computer program SPSS/PC (2001) using one way ANOVA test, followed by Duncan's multiple range test [28]. The minimum level of significance was set at $P < 0.05$.

Result and Discussion

The Nile Delta regions in Egypt not only have the highest human and animal densities in the world [5, 9], also more endemic in Brucellosis in domestic animals, dogs and cats [10].

As presented in Table 2, BAPAT, RBPT and CFT were employed for detection of seroprevalence of brucellosis in serum samples of 98 dairy cows and 220 ewes from Dakahlia Governorate and 119 dairy cows and 195 ewes from Damietta Governorate. An animal was classified as positive when two or more tests were positive and confirmed by CFT. Prevalence of brucellosis in Dakahlia Governorate was 10.7% and 7.27% in cows and ewes, respectively. While, in Damietta Governorate was 7.56% and 6.66% in cows and ewes, respectively. So the total prevalence was 8.75% in cows and 6.98% in ewes.

Table (2): Seroprevalence of *Brucellosis* in cows and ewes by using BAPAT, RBPT and CFT in Dakahlia and Damietta Governorates

Animal species	Governorate	Total No.	No. of positive samples (%)					
			BAPAT		RBPT		CFT	
Cows	Dakahlia	98	9	(9.18)	9	(9.18)	7	(7.14)
	Damietta	119	8	(6.72)	7	(5.88)	7	(5.88)
Ewes	Dakahlia	220	15	(6.8)	13	(5.90)	13	(5.90)
	Damietta	195	11	(5.64)	11	(5.64)	11	(5.64)

BAPAT: Buffered acidified plate antigen test, RBPT: Rose Bengal plate tests
CFT: Complement fixation test using warm fixation

From the previous results, there is a high prevalence of brucellosis among the investigated cows and ewes in these Governorates indicating that brucellosis is one of the important persistent and endemic diseases that have been reported at variable prevalence in Egypt. Previous literature declared that the prevalence of brucellosis was 4.95-5.8% among cows in Dakahlia and Damietta Governorates **[**29], 17.8% in Dakahlia Governorate, 8.9 in Damietta Governorate, while 11.8% was in Alexandria Governorate [7]. A total prevalence among buffaloes populations was 2.10% in Behaira, Menofia, Sharkia, Beni-Suef and Assiut Governorates [30], 7.18% in Dakahlia Governorate and 10.49% in Damietta Governorate [31] Such variation in the prevalence of brucellosis among the different localities in Egypt could be attributed to variation in the sensitivity and specificity of the employed tests in each study and the rate of exposure to infection in certain years.

As revealed in Table 3, *Brucella melitensis biovera 3* was the identified species from the samples that were obtained from the seropositive animals that were presented in Table 1. Of cows's tissue samples, 14.3%, 25% of lymph nodes, and 20% of spleen and, 33.3% milk samples were positive for *Brucella melitensis biovera 3.* While, 20.9% of ewe's tissue samples(two aborted foeti and one milk sample) were positive. This biovar of *B. melitinsis* was previously identified as the predominant type of Brucella in Egypt in different animals [32, 33]. Originally *B. melitensis* affects mainly ewes and goat. Such inter-species transmission situation may be the outcome of close contact between ewes, goats and cows [34]. This may illustrate the occurrence of this biotype in cows and ewes in the current study which consider the most dominant Brucella biotype in Egypt [35- 38].

Table (3): Morphological and biochemical characters of *Brucella melitensis biovar 3* in Dakahlia and Damietta Governorates.

A. Identification of *Brucella species*

Colonial morphology	Microscopic appearance	Biochemical characters		
		Catalase	Oxidase	Urease
Smooth, convex, honey colored	Gram negative coccobacilli, weak acid fast	+	+	+

b. Identification of *Brucella species* at the biovar level

CO_2requirment	H_2S Production	Growth on dyes					Reaction to monospecific sera		
		Thionin			Basic Fuchsin				
		1:25000	1:500000	1:100000	1:25000	1:50000			
-	-	-	+	+	+	+	+	+	-

Concerning the erythrogram in the present study, both cows and ewes infected with *B. melitensis* at the 2nd week post abortion (GP2) showed a significantly reduction in the RBCs count, Hb concentration and HCT value with normocytic normochromic anemia (Table 4). But both infected cow and sheep at the 4th week post abortion (GP3) showed significant decrease in RBCs and Hb concentration, with non -significant changes in HCT, MCV, MCH and MCHC. These results completely agreed with El-Olemy *et al.* [39] and Ghazi *et al.* [40], that attributed the decrease in RBCs and Hb concentration to the reduction of red cell formation as a result of in adequate production of erythropoietin hormone. Moreover, normocytic normochromic anemia is an anemia of chronic disease associated with increased inflammatory cytokines such as TNF-α and IL-6, that directly affect erythroid cell and also inhibit the production and effectiveness of erythropoietin [41]. Non-regenerative normocytic normochromic anemia could be attributed to the inflammatory chemical mediators as IL-1β [42].

Table (4): Erythrogram and Leukogram of cows and ewes in different groups under investigation of brucellosis in Dakahlia and Damietta Governorates.

Parameters	Cows			Ewes		
	GP1	Gp2	GP3	Gp1	GP2	Gp3
RBCs ($\times10^6$/µl)	6.66^a ±0.13	4.61^c ±0.17	5.92b ±0.24	10NN .37^a ±0.31	7.88^c ±0.31	9.19^b ±0.27
Hb (g/dl)	11.81^a ±0.54	7.73^c ±0.12	10.56b±0.35	12.22^a ±0.37	8.80^c ±0.43	10.50^b ±0.27
HCT (%)	30.5^a ±2.10	19.1^b ±0.64	27.1^a ±1.78	38.95^a ±0.81	30.55^b ±1.34	37.21^a ±1.14
MCV (fl)	45.35 ±2.74	41.64 ±1.36	45.91 ±3.13	37.65 ±0.84	38.74±0.64	40.57 ±1.23
MCH (pg)	17.76 ±0.96	16.81 ±0.36	17.91 ±0.86	11.79 ±0.19	11.18 ±0.45	11.44 ±0.25
MCHC (%)	39.44±1.89	40.45±0.91	39.37 ±1.80	31.37 ±0.78	28.91 ±1.44	28.30 ±0.98
WBCs $\times10^3$/ µl	7.90^b ±0.19	7.86^b ±0.23	9.40^a ±0.31	12.77^b±0.41	12.88^b±0.27	16.08^a± 0.42
neutrophils$\times10^3$/ µl	2.01^b ±0.20	2.00^b ±0.26	3.17^a ±0.38	4.59^b±0.19	4.79^b±0.11	6.70^a±0.21
Lymphocytes$\times10^3$/ µl	4.54^a ±0.08	4.40^a ±0.004	4.46^a ±0.51	7.54^a± 0.20	7.53^a±0.29	7.64^a±0.32
Monocytes$\times10^3$/ µl	0.75^b ±0.03	0.83^b ±0.01	1.16^a ±0.08	0.36^b±0.03	0.33^b±0.04	1.51^a±0.20
Eosinophils$\times10^3$/ µl	0.51^a ±0.05	0.54^a ±0.04	0.55^a ±0.02	0.21^a±0.02	0.17^a±0.01	0.19^a±0.03
Basophils$\times10^3$/ µl	0.08^a ±0.004	0.08^a ±0.005	0.07^a ±0.007	0.06^a±0.02	0.04^a±0.01	0.05^a±0.01

GP.(1: apparently healthy cows as control group. GP2: infected cows with *Brucella melitensis biovera 3* at 2nd week post abortion. GP 3: infected cows with *B. melitensis biovera 3* at the 4th weeks post abortion.

Regarding the leukogram (Table 4), both of cows and ewes in GP2 showed non-significant ($P > 0.05$) changes when compared with the normal control (GP1). On contrary, the total leukocytic count (TLC) in GP.3 in cows showed highly significant increase ($P < 0.01$) in leucocytes, neutrophils and monocytes with non-significance changes ($P > 0.05$) in basophils and eosinophils when compared with the control group(GP.1). This result is in agreement with Ahmed and Nada [43] who attributed the leukocytosis to activation of the lymphoreticular system for production and transportation of the antibodies in trial to fight the infection. Also the leukocytosis might be due to stimulation of cell mediated immunity [40, 44]. The neutrophils and monocytes remains always higher in non-specific bacterial infection [45].The infected ewes of GP3 revealed highly significant increase ($P < 0.01$) in leucocytes, neutrophils and monocytes with non-significance changes ($P > 0.05$) in basophils, eosinophils and lymphocytes. Some studies had reported that brucellosis infected animals suffer from severe monocytosis [46, 47]. Monocytosis in brucellosis could be attributed to the presence of tissue debris in the uterus, as natural uterine cleaning is hampered owing to retention of placenta in brucellosis and in this cases the monocytes acting as scavengers [48].

Regarding the biochemical results, the activity of aminotransferases (AST and ALT) was measured to detect the hepatocellular injury (Table 5). ALT is an important liver-specific enzyme in small animals but offers no specificity for detection of liver injury in large animals [49]. In accordance with the previous researches [50- 52], the serum activities of ALT, AST, ALP and LDH showed significant increase ($P < 0.05$) in GP.2 of both cows and ewes in comparison with the normal control (GP1). The significant increase ($P < 0.05$) in serum ALT and AST activities were attributed to liver damage caused by *Brucella species* which leads to an increase in the release of liver enzymes into the plasma [46].The elevation in the enzymatic activities of the liver may be related to hepatic malfunction [53] or due to increased breakdown of hepatocytes as in case of granulomatous hepatitis [54, 55].

Table (5): Biochemical and immunological parameters of cows and ewes in different groups under investigation of brucellosis in Dakahlia and Damietta Governorates.

	Cows			Ewes		
Parameters	**GP1**	**Gp2**	**GP3**	**Gp1**	**GP2**	**Gp3**
ALT(U/L)	$27.92^{c} \pm 1.27$	$58.76^{a} \pm 2.17$	$43.96^{b} \pm 1.36$	$24.34^{c} \pm 0.62$	$53.88^{a} \pm 2.84$	$34.00^{b} \pm 2.55$
AST (U/L)	$38.76^{c} \pm 0.50$	$61.40^{a} \pm 2.35$	$44.56^{b} \pm 1.61$	$42.16^{b} \pm 1.67$	$76.20^{a} \pm 17.25$	$66.52^{b} \pm 8.92$
ALP (U/L)	$60.04^{b} \pm 1.77$	$70.78^{a} \pm 2.03$	$63.50^{b} \pm 1.13$	$69.64^{b} \pm 3.77$	$102.20^{a} \pm 2.13$	$67.24^{b} \pm 2.10$
LDH (U/L)	$256.20^{c} \pm 12.27$	$893.80^{a} \pm 5.33$	$679.40^{b} \pm 36.15$	$221.00^{b} \pm 9.15$	$591.00^{a} \pm 34.02$	$226.40^{b} \pm 18.80$
TP (g/dl)	$6.97^{a} \pm 0.25$	$5.67^{b} \pm 0.16$	$6.79^{a} \pm 0.14$	$8.58^{a} \pm 0.12$	$6.75^{c} \pm 0.07$	$7.14^{b} \pm 0.10$
Albumin (g/dl)	$4.75^{a} \pm 0.16$	$2.93^{b} \pm 0.24$	$4.48^{a} \pm 0.17$	$4.43^{a} \pm 0.07$	$3.04^{b} \pm 0.16$	$3.44^{b} \pm 0.20$
Globulins (g/dl)	$2.22^{a} \pm 0.18$	$2.74^{a} \pm 0.32$	$2.31^{a} \pm 0.14$	$4.15^{a} \pm 0.06$	$3.72^{ab} \pm 0.18$	$3.70^{ab} \pm 0.20$
A/G ratio	$2.18^{a} \pm 0.16$	$1.17^{b} \pm 0.21$	$1.97^{a} \pm 0.16$	$1.07^{a} \pm 0.012$	$0.88^{a} \pm 0.09$	$0.95^{a} \pm 0.10$
Urea (mg/dl)	$39.54^{a} \pm 1.69$	$20.50^{c} \pm 0.86$	$30.14^{b} \pm 1.52$	$34.94^{a} \pm 1.29$	$25.61^{c} \pm 1.23$	$30.80^{b} \pm 0.73$
Creatinine (mg/dl)	$0.87^{a} \pm 0.09$	$0.83^{a} \pm 0.06$	$0.85^{a} \pm 0.07$	$0.72^{a} \pm 0.05$	$0.71^{a} \pm 0.04$	$0.68^{a} \pm 0.06$
Glucose (mg/dl)	$88.76^{a} \pm 0.94$	$53.20^{c} \pm 2.59$	$68.02^{b} \pm 1.61$	$77.67^{a} \pm 2.82$	$52.20^{b} \pm 1.11$	$73.82^{a} \pm 1.18$
IL-6 (pg/ml)	$17.67^{c} \pm 2.07$	$59.65^{a} \pm 2.39$	$49.06^{b} \pm 1.06$	$290.84^{c} \pm 12.4$	$700.06^{a} \pm 6.81$	$477.48^{b} \pm 36.58$
TNF-α	$2.90^{c} \pm 0.32$	$9.12^{a} \pm 0.33$	$7.25^{b} \pm 0.33$	$35.64^{c} \pm 1.48$	$77.13^{a} \pm 2.54$	$59.83^{b} \pm 2.52$
IgM (mg/dl)	$1.79^{c} \pm 0.09$	$12.86^{a} \pm 0.46$	$3.48^{b} \pm 0.21$	$1.96^{c} \pm 0.02$	$7.68^{a} \pm 0.83$	$3.56^{b} \pm 0.17$
IgG (mg/dl)	$8.16^{b} \pm 0.38$	$8.36^{b} \pm 0.35$	$21.54^{a} \pm 2.93$	$8.88^{b} \pm 0.51$	$9.11^{b} \pm 0.65$	$20.37^{a} \pm 2.50$
Phagocytic %	$82.60^{a} \pm 1.29$	$41.80^{c} \pm 2.86$	$76.20^{b} \pm 1.79$	$80.40^{a} \pm 0.93$	$39.80^{c} \pm 1.28$	$75.60^{b} \pm 1.29$
Phagocytic index	$6.26^{a} \pm 0.18$	$2.66^{c} \pm 0.34$	$5.61^{b} \pm 0.17$	$6.10^{a} \pm 0.07$	$2.48^{c} \pm 0.13$	$5.56^{b} \pm 0.13$

Values are mean ± SE, GP1: apparently healthy cows as control group. GP2: infected cows with *B. melitensis biovera 3* at 2nd week post abortion. GP3: infected cows with *B. melitensis biovera 3* at 4th weeks post abortion.

ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, LDH :Lactate dehydrogenase, TP :Total protein, IL-6: Interleukin 6, TNF-α: Tumor Necrosis Factor –alpha, IgM: immunoglobulin M and IgG: immunoglobulin G.

ALP is a membrane-bound glycoprotein mainly found in various animal tissues and used as a biochemical marker to diagnose osteoporosis and hepatobiliary disorders, as well as, fatty liver disease [56, 57].The significant increase in serum activity of alkaline phosphatase was attributed to pregnancy, that additional alkaline phosphatase produced by the placenta [58]. LDH, similar to AST, is found in many types of cells and should not be considered liver-specific [46]. LDH activities in the present study were significantly increased ($P < 0.05$) in infected cows in GP. (3). Increased LDH activity may result from hemolysis, muscle damage, or hepatocellular injury [59]. Moreover, the increase in serum LDH activity could be a useful indicator of the presence of uterine and placental pathology [60].

Concerning the proteinogram in the present study, a significant decrease ($P < 0.05$) in the serum levels of total protein and albumin in Brucella infected cows in GP2 and ewes GPs (2, 3) compared with the healthy control. But there were non-significant change ($P > 0.05$) in serum total protein, albumin and globulin levels in cows in GP3. These results were in line with previous findings that hypoprotenemia and hypoalbuminemia was due to liver damage caused by Brucella infection which leads to decrease in albumin synthesis in the liver [43]. The decrease in the serum total protein concentration observed in Brucella infected animals might be attributed to the restricted food intake in infected animals [61]. The hypoalbuminemia correlates with the results of Arslan *et al.* [50]. The hypoalbuminemia in the Brucella infected ewes may be due to less feed intake and diminished production of albumin by the liver owing to hepatic damage [62].

Concerning with the serum creatinine level, the infected cows and ewes showed non-significant change as compared with normal control (Gp1). While serum urea concentration was significantly decreased in cows and ewes of both groups (2, 3). Our results were in agreement with those obtained by Kishore *et al.* [63], who recorded a significant decrease in serum urea level. The reduced serum urea levels in the Brucella infected cows and ewes may be due to the damage of hepatic tissue, which cannot form urea from ammonia [61].

A significant decrease of glucose concentration was detected in Brucella infected cows and ewes in both groups (2, 3).Similar findings were recorded by Kushwaha *et al.* [51] and Nath *et al.*[64], and they explained hypoglycemia by the decreased feed intake due to Brucella infection or to liver functions impairment [43]. However, different result was reported in ewes and native goats [50].

TNF-α is the key mediators of cellular immunity, forming a loop that ultimately results in IFN-γ production based on general studies of macrophages. In addition, TNF-α is necessary for the optimal killing of *brucella species* by macrophages [65]. In agreement with Demirdag *et al.* [66] who reported that TNF-α level were significantly higher in the acute phase of the disease in comparison to the post-treatment and the control group values, also they observed that in addition to the high levels in the acute phase of the disease, TNF-α was also in correlation with the increase in IFN-γ levels. Moreover the increased level of TNF-α as pro inflammatory cytokines are involved in pathophysiology of brucellosis and have a close relationship with the inflammatory activation of the disease. In our work, the significant increase in serum IL-6 level in *B. melitensis* infected animals indicates the role of these cytokines in the inflammatory response. Since IL-6 plays a major role in inflammation [67].

Specific IgM antibody usually develops early in infection and remains for several weeks to months [68]. While IgG antibodies develop somewhat later and remain for several months to years, as well as, after recovery [69]. There was a significant increase of IgM in *B. melitensis* infected cows and ewes at the 2^{nd} and 4^{th} weeks post abortion (GP2,.3). Meanwhile, a non-significant change of IgG was found in both cows and ewes at the 2^{nd} week post abortion (GP2), but significant increase at the 4^{th} week post abortion (GP3) was observed. These results are in accordance

with Gad El-Rab and Kambal [70] and Memish *et al.* [71] who reported that the IgG antibody has a delayed appearance that found after four weeks from the initial antigenic stimulus and the IgM antibody level always exceeds the IgG antibody level in the acute stage of the disease.

The significant decrease in phagocytic percent and phagocytic index (Table 5) in GP2 and GP3 explained by the ability of *B. abortus* to invade phagocytic and non phagocytic host cells, resist the acidified intraphagosomal environment and inhibit phagosome-lysosome fusion [72].

Conclusion

B. melitensis biovar 3 is the main cause of Brucellosis in cows and ewes in Dakahlia and Damietta Governorates. Hence, it is a very dangerous alarm and gives spotlight for the application of preventive hygienic measures and control program of *B. melitensis*. Brucellosis leads to great disturbance in hematological, biochemical and immunological parameters in the diseased cows and ewes, suggesting its potential for the early diagnosis of Brucellosis for application of preventive hygienic measure and control program.

Conflict of interest

The authors have no conflict of interest to declare.

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الملخص العربي

دراسات الدم،الكيمياءالحيوية و المناعةعلى مرض البروسيلا في الأبقار و الأغنام في محافظتي الدقهلية و دمياط - مصر

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مرض البروسيلا ذو أهمية للصحة العامة ويؤدي إلى خسائر فادحة. أجريت الدراسة الحالية لتحديد أنواع البروسيلا المعزولة ، وانتشارها في الأبقار والنعاج في محافظتي الدقهلية ودمياط خلال الفترة من مايو ٢٠١٨ إلى مارس ٢٠١٩ ، مع دراسة تحليلات الكيمياء الحيوية للدماء في الحالات الإيجابية. تم التعرف على .B melitensis biovar 3 مع معدل انتشار ٨.٧٥ ٪ و ٦.٩٨ ٪ في الأبقار والنعاج ، على التوالى. وبالنظر الي صورة الدم وجد ارتفاع في خلايا الدم البيضاء مع وجود أنيميا. اما بالنسبه الي الدراسات الكيميائية فقد تبين وجود نقص في البروتين الكلي والزلال في المجموعة الثانية من الابقار (المصابة بالبروسيلا بعد اسبوعين من الاجهاض) فقط بينما حدوث نقص في كلا المجموعتين الثانية المصابة بالبروسيلا بعد اسبوعين من الاجهاض والثالثة(المصابة بالبروسيلا بعد أربع أسابيع من الاجهاض) بالنسبه للنعاج. كماحدث ارتفاع في نسبة الالاناين تراسفريز والاسبرتيت امينوتراسفريز واللاكتات ديهادروجيناز والالكالين فوسفاتيز والانترليوكين ٦ وكذلك عامل نخر الورم ($P < 0.05$)بينما نقص في نسبة اليوريا في كل من الابقار والنعاج. كما وجد نقص في نسبة الجلوكوز في كلي المجموعتين في الابقار ($P < 0.05$) بينما في الاغنام حدث نقص فقط في المجموعة الثانية بينما لم يحدث تغيير في المجوعه الثالثة(المصابة بالبروسيلا بعد أربع أسابيع من الاجهاض) مع عدم تغير في نسبه الكرياتين في الابقار والنعاج ، اما بالنسبه الغلوبولين المناعي م فقد اظهرت النتائج زياده ملحوظة في المجموعة الثانية وعودتها الي المعدل الطبيعي في المجموعه الثالثة ,بينما شوهد زيادة في نسبة الغلوبولين المناعي ج في المجموعه الثالثة وعدم تغيرها في المجموعة الثانية وبالإشاره الي نتائج الدراسات المناعة الخلوية نجد ان كل المجموعات أظهرت نقص معنوي في قياس النشاط التبلعمي والليزوزيم. وبالتالي ، فإن تقييم القياسات الدموية والكيمياء الحيوية والمناعية مع الاختبارات التشخيصية المحددة، ضرورية للحصول على نتائج أكثر واقعية لتشخيص داء البروسيلا في الأبقار والنعاج.