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Updates of Brucellosis in Egyptian Cattle and Camels with Emphasis on Some Associated Biochemical Values and Genetic Markers

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Abstract: Egypt is endemic for brucella in spite of all efforts done by the government and people in charge.: The aim of this work was to highlight some of the questions related to epidemiology of brucellosis in Egyptian camels and cattle with emphasis on some relevant clinical investigations. Blood samples were obtained from cows (N=102) and camels (N=82) with a history of reproductive disorders and screened for brucella using buffer acidified plate antigen Test (BAPAT), Rose Bengal plate test (RBPT), Tube Agglutination Test (TAT) and complement fixation test (CFT). The electrophoretic patterns and serum chemistry was done. Also, genotyping of blood protein and gene frequency of genetic loci was estimated. The blood protein loci used as genetic markers were Albumin (Al), β globulin presented as transferrin (Tf) and Post-transferrin (Ptf), Fast or alpha globulin ($F_2\alpha$) and slow or gamma-globulin ($S_2\alpha$). Results reveled that : the incidence of brucella screening field test using RBT, TAT, BAPA and CFT are 22.50, 21.60, 23.50 and 22.50 % for cows and 18.30, 15.90, 20.70 and 17.10 for camels, respectively. The prevalence of seropositive cows was higher (21.6-23.5%) than camels (15.9-20.7%). The protein electrophoresis pattern showed significant variation between species where cattle pre-albumin was null compared to camel Also concentration of β and γ globulin fractions was different between species. Infected camels showed very little changes in serum chemistry. Infected cattle showed decrease in β2 globulins and copper While total protein. Total globulin, $\gamma_1$globulin, LDH, AST, MDA were markedly increased. The predominant genetic alleles of sero-positive cows were $S_2\alpha^A$, $S_1\alpha^B$, $F_2\alpha^A$ and Al^A, while in seronegative cows were $S_2\alpha^B$, Tf^A, Ptf^B and $F_2\alpha^B$. In sero-positive camels, the frequency of $S_2\alpha^B$, $S_1\alpha^A$, Tf^B and $F_1\alpha^A$ were high but the frequency of Tf^A, $F_2\alpha^B$, Ptf^B, Al^B and Pr^A increased in sero-negative camels Conclusion: the study revealed that the incidence of brucellosis in camel is less than that in cattle. BAPA was more sensitive than other screening tests. Seropositive camels showed very little changes in serum chemistry compared to seropositive cattle. Analysis of genetic alleles showed significant difference between seropositive and seronegative animals and can be used as genetic markers for susceptible animals.

Key words: Brucellosis · Prevalence · Biochemical Parameters · Genetic Markers · Cows · Camels · Egypt

INTRODUCTION

Brucellosis is worldwide zoonotic disease causes economic losses to animal breeder due to abortions, retained placenta and metritis in females [1], orchitis and epididymitis in males and infertility was reported in both sexes [2].

Brucellosis was first reported in Egypt in 1939 and is now considered endemic in most parts of the country [3] A control program of serological survey and vaccination was stated since 1980s [5]. In Egypt, the prevalence of bovine brucellosis, in 2013, was recorded as 17.8% in Dakahlia, 8.9% in Damietta and, 11.8% in Alexandria governorates [6]. In 2016, Dromedary camels recorded 4.17 and 3.73% sero-prevalence of brucella antibodies in Upper and Lower Egypt, respectively [7]. The sale of brucella infected animals in the Egyptian market is evident and threatened infection of herds and lead to spread of infection and economic losses [8].

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The BAPAT, RBPT, TAT and CFT are common screening serological tests used to monitor the brucella infection during eradication programs [9]. These tests depends mainly on the detection of anti brucella lipopolysccharide (LPS) antibodies. However, these tests cannot define if the antibodies resulting from natural infection or vaccination. It also can give false positive reactions with LPS of other Gram negative bacteria [10]. However these serological tests were to estimate the tendency of disease to increase or decrease and can't be considered as accurate prevalence.

Serological survey of brucellosis in Egypt showed that 22 out of 27 governorate had infected animals. Only Ismailia, Matrouh, north and south saini and Red Sea governorate was free of the disease. The peaks of infection were recorded during 2002, 2003, 2008, 2009 and 2010. The lowest percentage of seropositive animals was recorded in 2011. The number of examined animals was always few compared to total animal population in the location [9].

Brucellosis like other diseases causes imbalance in the oxidants-antioxidant state and induce changes in organ functions and protein electrophoretic pattern of the infected animals [11]. Genetic markers of serum proteins can be used for identification of animals naturally bearing susceptibility and/or resistance to brucellosis in selection programs [12].

This work aimed to throw light on the current prevalence of brucellosis in Egyptian cattle and camels. Also to investigate the effect of infection on some blood biochemical values and genetic markers of serum proteins.

MATERIALS AND METHODS

Animals and Sample Collection: Blood samples were collected from local cows (N=102) and she camels(N=82) belonged to private and governmental farms during 2019 with history of reproductive disorders including abortion, repeat breading and retained fetal membranes. Blood samples were collected from the jugular vein and serum was separated after coagulation by centrifugation (3000 rpm for 15 minutes at 4°C) and kept at -20°C for different biochemical analyses.

Brucellosis Seroprevalence: Serum samples were screened for brucella antibodies using buffer acidified plate antigen (BAPA) and Rose Bengal Test (RBT). Seropositive samples were confirmed using tube agglutination test (TAT) and complement fixation test (CFT). All antigens were obtained from NVSL/DBL, USDA, USA and tests were carried out using standard procedures [13]

Biochemical Parameters: Lipid peroxide product (malondialdehyde, MDA), nitric oxide (NO), Glutathione Perxidase enzyme (GPX), Catalase (CAT), Copper, iron, ALT, AST, Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), total cholesterol, urea and creatinine were calorimetrically determined using commercial kits (Biodiagnostics, Egypt) [13] and Shimadzu UV spectrophotometer.

Genotyping of Blood Protein Loci and Gene Frequency of Their Alleles: Serum total protein (TP) was electrophoretically fractionated on one dimensional polyacrylamide gel electrophoresis (PAGE) [14]. Genotyping of blood protein loci and gene frequency were determined [15]. Blood protein loci of pre-albumin, albumin and Globulin ($\alpha_1, \alpha_2, \beta_1, \beta_2, \gamma_1, \gamma_2$) were analyzed in both species.

Statistical Analysis: Results were computed using SPSS program version. Data were statistically analyzed using independent sample t- test to differentiate between seropositive and seronegative values within species. Simple one way-ANOVA was used to compare between seronegative or seropositive animals within the species. Duncan's Multiple Range test was used to differentiate between significant means. Chi- Square was used to compare the percentages, while genetic equilibrium was determined by X^2.

RESULTS

The present study showed that the sero-prevalence of brucella antibodies averges 22.50, 21.60, 23.50 and 22.50 % in cows and 18.30, 15.90, 20.70 and 17.10 % in camels using RBT, TAT, BAPA and CFT screening tests, respectively (Table 1).

The incidence in cattle (21.60-23.50 %) was higher than that in camels (15.90-20.70%).

Analysis of serum chemistry (Table 2) showed marked variations between species. Results showed clear higher values in pre-albumin, albumin, alb/glb ratio ($p \leq 0.001$), $\beta 2$ globulins ($p \leq 0.01$) and serum iron ($p \leq 0.001$) in camels than cattle. While $\alpha 2$, $\beta 1$ and $\gamma 2$ globulins ($p \leq 0.001$), ALT and plasma copper were significantly higher in cattle than she camels.

Table 1: The seroprevalence of brucellosis in cows and camels

	Serological tests							
	RBPT		TAT		BAPA		CFT	
Animal under examination	+Ve	%	+Ve	%	+Ve	%	+Ve	%
Cows (N= 102)	23	22.50	22	21.60	24	23.50	23	22.50
Camels (N= 82)	15	18.30	13	15.90	17	20.70	14	17.10

Table 2: Serum chemistry and electrophoretic pattern in cows and camels infected with Brucella

	Cattle		Camel		
	Seronegative	Seropositive	Seronegative	Seropositive	P-value
Pre albumin	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.15 ± 0.03^{B}	$0.20\pm0.05^{C**}$	0.001
Albumin	1.56 ± 0.18^{A}	1.77 ± 0.37^{A}	2.27 ± 0.39^{B}	2.09 ± 0.28^{B}	0.001
α_1 globulin	0.56 ± 0.22^{AB}	0.44 ± 0.13^{B}	0.68 ± 0.36^{B}	0.75 ± 0.24^{B}	0.011
α_2 globulin	0.54 ± 0.17^{BC}	0.59 ± 0.15^{C}	0.44 ± 0.23^{AB}	0.32 ± 0.06^{A}	0.001
β_1 globulin	0.79 ± 0.16^{B}	0.83 ± 0.13^{B}	0.46 ± 0.17^{A}	0.40 ± 0.06^{A}	0.001
β_2 globulin	0.79 ± 0.25^{B}	$0.54\pm0.08^{A**}$	1.19 ± 0.36^{C}	1.21 ± 0.11^{C}	0.01
γ_1 globulin	0.72 ± 0.12^{C}	$0.55\pm0.10^{A***}$	0.66 ± 0.09^{BC}	0.62 ± 0.08^{AB}	0.001
γ_2 globulin	2.38 ± 0.94^{B}	2.07 ± 0.43^{B}	$1.57.22\pm0.26^{A}$	1.52 ± 0.08^{A}	0.001
Total protein g/dl	5.66 ± 0.57^{A}	$6.36\pm0.62^{C***}$	5.99 ± 0.81^{B}	6.07 ± 0.61^{B}	0.001
Globulins g/dl	3.27 ± 0.54^{A}	$3.84\pm0.89^{B**}$	3.11 ± 0.95^{A}	3.04 ± 0.70^{A}	0.01
Albumin g/dl	2.38 ± 0.17^{A}	2.52 ± 0.43^{A}	2.89 ± 0.43^{B}	3.04 ± 0.28^{B}	0.001
Albumin/globulin	0.75 ± 0.15^{A}	0.72 ± 0.31^{A}	1.03 ± 0.37^{B}	1.09 ± 0.42^{B}	0.001
Cholesterol mg/dl	38.06 ± 10.74^{B}	$48.23\pm5.48^{C**}$	35.96 ± 4.55^{B}	$32.09\pm2.51^{A**}$	0.01
LDH U/L	9.25 ± 1.49^{A}	$15.51\pm4.07^{B**}$	14.26 ± 1.51^{B}	13.49 ± 2.54^{B}	0.05
Alk. ph U/L	126.36 ± 27.28^{A}	123.37 ± 26.71^{A}	109.95 ± 9.89^{A}	112.07 ± 26.03^{A}	NS
AST U/L	140.39 ± 14.08^{A}	$158.51\pm16.73^{B**}$	139.40 ± 8.59^{A}	136.57 ± 11.56^{A}	0.007
ALT U/L	46.99 ± 1.69^{B}	46.46 ± 3.07^{B}	43.24 ± 2.05^{A}	45.41 ± 2.04^{AB}	0.019
Creatinine mg/dl	0.19 ± 0.12^{A}	0.30 ± 0.0^{A}	0.97 ± 1.89^{A}	1.72 ± 2.72^{A}	NS
Urea mg/dl	54.84 ± 32.17^{A}	39.10 ± 12.46^{A}	44.64 ± 14.99^{A}	56.24 ± 13.01^{A}	NS
Copper µg/dl	369.50 ± 42.67^{B}	$283.63\pm37.69^{A***}$	264.75 ± 28.01^{A}	253.33 ± 27.51^{A}	0.001
Iron µg/dl	161.21 ± 25.03^{A}	157.29 ± 53.81^{A}	230.99 ± 74.60^{B}	219.37 ± 65.73^{B}	0.001

Means with different superscripts (A, B, C) within row are significantly different at P<0.05, NS means Non significant, ** significant at P<0.01, *** significant at P<0.001

Table 3: Oxidative markers in Brucella seropositive and seronegative bows and camels

	Cattle		Camel		
Parameter	Seronegative	Seropositive	Seronegative	Seropositive	P-value
MDA nmol/ml	0.92 ± 0.31^{A}	$1.15\pm0.39^{B**}$	0.71 ± 0.25^{A}	0.82 ± 0.72^{A}	0.01
NO µmol/l	44.82 ± 3.53^{A}	42.46 ± 10.84^{A}	50.48 ± 11.33^{B}	44.87 ± 4.89^{A}	0.002
Catalase U/L	337 ± 125^{A}	320 ± 172^{A}	314 ± 234^{A}	281 ± 127^{A}	NS
GPx U/L	1393 ± 87^{B}	1439 ± 149^{B}	974 ± 229^{A}	1054 ± 371^{A}	0.007

Means with superscripts (A, B, C) within row are significantly different at P<0.05, NS: Non significant, ** means significant at 0.001 within species

Concerning the changes within species due to infection (Table 2). Infected camels showed little changes in serum chemistry. Significant increase in pre albumin ($p\leq0.001$) and decrease in cholesterol level ($p\leq0.001$).

Infected cattle showed significant increase in total globulins and total protein, cholesterol, copper, AST and LDH. While, β_2 ($p\leq0.01$) and γ_1 ($p\leq0.001$) globulins were significantly decreased and γ_2 globulins was slightly decreased.

Analysis of the oxidative markers in cattle and camel (Table 3) showed that the animals have elevated MDA while, NO and the antioxidants did not show significant difference due to brucellosis.

Table 4: Genotyping and gene frequencies of blood protein loci in cows

		Gene frequencies of studied alleles in caws			
		Seronegative		Seropositive	
Blood Protein loci	Genetic alleles	Gene frequency	χ^2	Gene frequency	χ^2
Gamma 2	$S_2\alpha$	$S_2\ \alpha^A$(0.4) $S_2\ \alpha^B$(0.6)	0.13	$S_2\ \alpha^A$(0.7) $S_2\ \alpha^B$(0.3)	1.42
Gamma 1	$S_1\alpha$	$S_1\ \alpha^A$(05) $S_1\ \alpha^B$(0.5)	1.8	$S_1\ \alpha^A$(0.3) $S_1\ \alpha^B$(0.7)	1.42
Beta 2	Tf	Tf^A(0.7) Tf^B(0.3)	1.42	Tf^A(0.5) Tf^B(0.5)	1.8
Beta 1	Ptf	Ptf^A(0.4) Ptf^B(0.6)	0.13	Ptf^A(0.5) Ptf^B(0.5)	1.8
Alpha 2	$F_2\alpha$	$F_2\ \alpha^A$(0.3) $F_2\ \alpha^B$(0.7)	1.42	$F_2\ \alpha^A$(0.7) $F_2\ \alpha^B$(0.3)	1.42
Alpha 1	$F_1\alpha$	$F_1\ \alpha^A$(0.5) $F_1\ \alpha^B$(0.5)	1.8	$F_1\ \alpha^A$(0.5) $F_1\ \alpha^B$(0.5)	1.8
Albumin	Al	Al^A(0.5) Al^B(0.5)	1.8	Al^A(0.7) Al^B(0.3)	1.42

Table 5: Genotyping and gene frequencies of blood protein loci in camels

		Gene frequencies of studied alleles in camels			
		Seronegative		Seropositive	
Blood Protein loci	Genetic alleles	Gene frequency	χ^2	Gene frequency	χ^2
Gamma 2	$S_2\alpha$	$S_2\ \alpha^A$(0.5) $S_2\ \alpha^B$(0.5)	1.80	$S_2\ \alpha^A$(0.3) $S_2\ \alpha^B$(0.7)	1.42
Gamma 1	$S_1\alpha$	$S_1\ \alpha^A$(0.5) $S_1\ \alpha^B$(0.5)	1.80	$S_1\ \alpha^A$(0.8) $S_1\ \alpha^B$(0.2)	0.31
Beta 2	Tf	Tf^A(0.7) Tf^B(0.3)	1.42	Tf^A(0.2) Tf^B(0.8)	0.31
Beta 1	Ptf	Ptf^A(0.3) Ptf^B(0.7)	1.42	Ptf^A(0.5) Ptf^B(0.5)	1.80
Alpha 2	$F_2\alpha$	$F_2\ \alpha^A$(0.3) $F_2\ \alpha^B$(0.7)	1.42	$F_2\ \alpha^A$(0.5) $F_2\ \alpha^B$(0.5)	1.80
Alpha 1	$F_1\alpha$	$F_1\ \alpha^A$(0.5) $F_1\ \alpha^B$(0.5)	1.80	$F_1\ \alpha^A$(0.7) $F_1\ \alpha^B$(0.3)	1.42
Albumin	Al	Al^A(0.3) Al^B(0.7)	1.42	Al^A(0.5) Al^B(0.5)	1.80
Pre – albumin	Pr	Pr^A(0.7) Pr^B(0. 3)	1.42	Pr^A(0.5) Pr^B(0.5)	1.80

$S_2\ \alpha$= slow globulin(gamma) with 2 fractions (1 and 2)

$F_1\ \alpha$= fast globulin(alpha) with 2 fractions (1 and 2)

The current study showed that the predominant genetic alleles of sero-positive cows (Table 4) include $S_2\alpha^A$, $S_1\alpha^B$, $F_2\alpha^A$ and Al^A, while those of seronegative cows ($S_2\alpha^B$, Tf^A, Ptf^B and $F_2\alpha^B$) were more frequent. In sero-positive camels (Table 5) the frequency of $S_2\alpha^B$, $S_1\alpha^A$, Tf^B and $F_1\alpha^A$ were high but the frequency of Tf^A, $F_2\alpha^B$, Ptf^B, Al^B and Pr^A increased in sero-negative camels.

DISCUSSION

The present study showed that the current incidence of brucellosis in the investigated (Table 1) cattle (21.60-23.50%) was higher than in camel (15.9= - 20.70%) (Table 1). In the same time, these figures are higher than that previously recorded in earlier studies in both species

in Egypt. Seroprevalence of camel brucellosis was recorded in earlier studies as 2-5% in nomadic camels and 8-15% in camel kept under intensive or semi intensive breeding system [16]. Also, brucellosis in imported Egyptian camels was reported as 14.70% in females and 17.20% in males [17].

Matrouh governorate was mentioned, in 2002, among the free governorates from brucellosis. however, in 2020, it showed the prevalence of brucellosis in camels as 10.0, 10.0, 9.0 and 9.0% using RBPT, BAPAT, CFT and PCR, respectively [18] . In the past, the camel was thought to be more resistant to diseases commonly affecting other livestock. However, the historic isolation of camel in desert areas apart from other animals may stand behind this finding. Actually camel was found to be more susceptible than other animals to certain diseases including brucellosis. Camels can be infected by either *Brucella abortus* (in Sudan, Egypt and Kuwait) and *Brucella melitensis* (in Iran, Libya and Saudi Arabia) and some authors suggested that camel was affected by *B. melitensis* from the accompanied sheep rearing [19].

In spite of the great efforts of the General Organization of Veterinary Services over the last 30 years to control brucellosis, the case is disappointing and brucellosis is still endemic among ruminants in Egypt [20]. Various authors referred the condition to improper diagnosis of disease. Only 4-5 % of the animal stocks are included in the control program [21]. In the same time, great number of animals of unknown health status came from different governorates intermix weekly in the animal markets leading to spreading of disease [22]. Also, Low compensation for owners for their diseased animals, results in slaughtering of only 0.2% of seropositive animals [23]. The official reports of GOVS about brucellosis from 1999 to 2011 indicated that the total number of infected animals increase steadily by time. According to FAO, 2013 The peak of infection was recorded in 2008-2010 and dropped only with 0.33% in 2011, FAO/WHO [24].

Analysis of serum chemistry (Table 2) showed significant variations between species in protein electrophoretic pattern. Because the proteins are synthesized under genetic control, it would be expected that variations in proteins takes place between species [25].

The current study showed that camel have pre albumin which is not separated in cows electrophoretic bands. The total albumin and A/G ratio in camel was higher than cattle. Previous studies recorded higher albumin than globulin level (A/G) in camels compared to other livestock [26, 27] . Similar higher values of albumin was reported in camel's milk compared to cow's milk [28].

The current study showed significant changes within species due to infection (Table 2). Infected camels showed little changes in serum chemistry. Significant increase in pre albumin and decrease in cholesterol level.

Brucellosis in camels was reported as an insidious disease since it hardly provokes clinical signs [19].

Infected cattle showed significant increase in total globulins, total protein, cholesterol, copper, AST and LDH. while, $\beta 2$ and γ_1 globulins were significantly decreased. Also, infected animals have elevated MDA while, NO and the antioxidants did not show significant difference due to brucellosis (Table 3).

The adaptation of *Brucella* to live inside macrophages is managed by its ability to block receptors for innate immunity [29, 30] Th1 CD4 + T cells exert their protective function against brucella by producing cytokines, such as IFN-γ and IL-2, to activate cytotoxic CD8+Tcells. IFN-γ is necessary to eliminate brucella during acute, active infection [31]. In some cases, brucella has the ability to proceed to a chronic infection, yet the mechanism of IFN-γ inhibition is not completely understood in this setting [32]. Interestingly, brucella can evade the immune system through CD8+Tcell suppression in acute infection and potentially disrupt conversion to effective memory T cells [33]. This may explain the low level of β globulins in the current study as the complement was mentioned as a fraction of β-globulin beside haemopexin, transferrin and C-reactive protein [34].

In the same time, activated macrophages was found to kill brucella by production of reactive oxygen intermediates. Interestingly, nitric oxide is not produced by IFN-γ activated *B. Abortus* infected J774A.1 macrophages, as detected by the presence of the conversion product nitrite [35]. These findings coincide with our findings where NO showed normal level in brucella positive animals.

It is well known that all living organisms need trace elements for survival and replication [36]. As a defense mechanism, the body cells minimize mineral utilization by microbes to control its survival and replication [37]. mammalian cell has homeostasis mechanism by binding minerals in certain proteins keep them non toxic. Iron is bound tightly by transferrin and lactoferrin in the extracellular environment [38]. Transferrin is a negative acute phase protein secreted as β-globulin. It Decreases iron transfer during acute and chronic infections [34]. This may explain the lowered level of β-globulin in infected animals in the current study.

The present study showed slight elevation of α –globulins and marked decrease in γ_1 globulin. The α -globulins are synthesized in the liver as acute and non-acute phase proteins [39]. The γ globulins are two fractions, fast migrating γ_1 globulin such as IgMs and IgEs and γ_2 fraction like IgGs. The IgM antibodies against brucella LPS were the first to appear following infection and rise gradually during the course of acute infection. Thenafter, IgG anti-brucella antibodies appeared later after the onset of infection bound to brucella cytoplasmic proteins and play part in serological tests to differentiate between infected and uninfected hosts [40]. The present study showed down regulation of γ_1 globulin this may be due to the chronic infection.

The current study showed increased activity of AST in seropositive cows and this may be due release of intracellular enzymes from affected cardiac muscle which was previously reported with brucellosis [41]. Elevated LDH and AST were noticed in dairy cows positive for brucellosis. Generally increased LDH activity could be a useful indicator of hemolysis, muscle damage, cardiovascular, hepatocellular injury and uterine and placental pathology [42].

The current study showed elevated serum copper level. It is well known that copper plays a critical function in mammalian cells when bind to protein. It has prooxidant as well as antioxidant properties [43]. The animal body keeps his needs of these elements bound to certain transfer and storage protein to hold them in non toxic form [44]. It has been reported that chronic brucellosis elevated the serum level of copper and lowered the level of zinc while other traces did not show marked changes [45].

Brucellosis can impair liver metabolism and synthesis of ceruloplasmin (CP) in the liver and thereby, the level of copper increased [46, 47]. Also, the body defense mechanism inhibits the synthesis of Cu-Zn superoxide dismutase [48], which inactivates oxygen radicals and promotes the intracellular survival of brucellae inside the Macrophage niches [49].

Results (Table 3) showed elevated MDA and non significant changes in antioxidants levels in diseased animals. Macrophages produced some reactive oxygen intermediates to control the growth of *B. Abortus* [50]. oxidative stress results in lipid oxidation and alter the serum level of trace elements. positive correlation between oxidative stress and copper content was demonstrated [51].

In the present study, 7 blood protein loci in cows ($S_2\alpha$, $S_1\alpha$, Tf, Ptf, $F_2\alpha$, $F_1\alpha$ and Al) and 8 blood protein loci in camel ($S_2\alpha$, $S_1\alpha$, Tf, Ptf, $F_2\alpha$, $F_1\alpha$ Al and Pr) were analyzed. The results showed that all these loci were polymorphic.

The present study revealed that the most predominant genetic alleles in brucella seropositive cows (Table 5) were $S_2\alpha^A$, $S_1\alpha^B$, $F_2\alpha^A$ and Al^A, while seronegative cows are characterized by high frequency of $S_2\alpha^B$, Tf^A Ptf^B and $F_2\alpha^B$.

Also, the current work showed that sero- positive camels (Table 6) showed high frequency of $S_2\alpha^B$, $S_1\alpha^A$, $Tf^B$and $F_1\alpha^A$, while seronegative camels revealed high frequency of Tf^A, Ptf^B, $F^2\alpha^B$, Al^B and Pr^A increased in sero-negative camels.

The present result coincide with the previously reported in brucella sero-positive male camels that showed $S_{2\alpha}{}^B$ gene markers as predominant genotype [52]. Another study reported high frequency of slow transferrin TF^B allel and fast TF^A allel in positive and negative sero-reactors camels for brucellosis, respectively and the genotyping analysis of transferrin locus showed predominance of homozygotic genotypes [12].

Significant correlation among different gene markers gives impression that different genes affect one trait and that may be due to the close connection of these genes on the same chromosome [53]. Also, there is association between the physiological function and genetic constitution [54].

In normal condition transferrin bind to iron to keep it soluble and non oxidant, it transport iron safely in the body to supply the growing body cells [55], Interestingly, brucella, not like other bacteria, can replicate maximally under condition of low iron concentration. Iron act as proxidant and produce free hydroxyl radicals when found with activated macrophages to kill brucellae . when activated macrophages infected with *B.abortus* were supplemented with iron saturated transferrin beside the activation with IFN-γ resulted in fewer brucellae by 48 hours after infection [56]. This may explain the dominant TfA in camels.

The concept of the relation between blood protein loci and susceptibility to brucellosis is based on the theory of protein coding loci [57]. The fractions of transferrin in present study is in line with those reported by Chaudhary *et al.* [58] (2) and Sargent *et al*. [59] but disagree with finding of Ghazy *et al.* [60] who found three fractions and Seichi *et al.* [61] who found only one fraction of Tf. The cause of these variation in transferrin fractions in camel's serum may be attributed to the presence of high abundant proteins in plasma [62].

Interestingly, the finding of present investigation revealed that the immunoglobulin fractions $S_2\alpha^B$, Tf^B, $S_1\alpha^A$ and $F_1\alpha^A$ are the most prominent gene markers in diseased camels, While, in diseased cows the most prominent gene markers are $S_2\alpha^A$, $S_1\alpha^B$, $F_2\alpha^A$ and $Al^{A.}$ The presence of these markers in predominance case could be considered that they are the susceptible functional gene markers for brucellosis in both cattle and camels[63] .

CONCLUSION

The incidence of brucellosis in camel is less than that in cattle. BAPA was more sensitive than other screening tests. Seropositive camels showed very little changes in serum chemistry compared to seropositive cattle. Analysis of genetic alleles showed significant difference between seropositive and seronegative animals and can be used as genetic markers for susceptible animals.

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