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Clinicopathological and immunological studies on brucellosis

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ABSTRACT

Our view refers to clarify the seroprevalence of brucellosis infection in dairy cows in Menoufiya Governorate and to evaluate the hematological, biochemical and immunological parameters changes in blood of infected cows compared to healthy group. Blood samples of 100 dairy cows (3-5) years from private farm and Menoufiya abattoir were screened for *Brucella* infection using (BAPAT) test and groups, the first group consistent of (25) samples which serologically positive to brucella and the second group consistent of (75) samples which brucella negative. Hematological analysis revealed normocytic anemia and Lymphopenia. Biochemical analysis of brucella positive serum infected cows when compared with negative control revealed significant elevation in (AST), (ALT), (ALP) and (GGT) activity in addition to non-significant increases in creatinine level, however a significant decrease in serum urea in diseased cows was recorded. Total protein, α1 globulin, β2 globulin revealed significant decrease while non-significant change was observed in α2, β1 and γ globulin in infected group. Immunologically IL-1 β and IL-10 showed significant elevation in infected group when compared with negative control group.

1. INTRODUCTION

Brucellosis is a world re-emerging of zoonotic nature (Godfroid and Kosbohrer, 2005). It is a highly contagious 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 (Hashem *et al*., 2020). It has a great economic and social importance because of the huge losses it can cause in the livestock industry (Quintero *et al.*, 2018). This disease is caused by four to six members of the genus Brucella. Cows take the infection by *Br. abortus*, in swine by *Br. Suis*, in goat and sheep by *Br. Melitensis*, and also in sheep by *Br. Ovis*. Meanwhile, in camels can takes infection by the same organisms according to the animals contact (Howard and Smith, 1999). Africa, the affection has high spread which is characterized primarily by delayed conception, late-term abortions and retention of placenta and temporary or permanent infertility (Kollannur *et al.*, 2007).

Detection of biochemical markers can provide valuable information about the health status of the animal and, therefore, can be used for evaluating the health status of the animal (AbouElazab, 2015).

Changes in blood enzyme levels are good indicators of pathological changes in different tissues because it infects body organs causing damage and change of their function and lead to the release of their enzymes according to the stage of infection (Rita Nath *et al.*, 2014).

for diagnosis of brucellosis at the national or local level, the buffered Brucella antigen tests, i.e., the buffered Acidified plate agglutination test (BAPAT)are sensitive starting test, as well as polymerase chain Reaction (PCR) for confirmation. Our study is to explain the difference in hematological, biochemical metabolites and some immunological parameters of the animals have brucellosis that reflects the adverse effects associated with brucellosis on animals health and performances.

2. MATERIAL AND METHODS

2.1. Animals and sample collection

A total number of 100 mature, non-pregnant, female dairy cows, none vaccinated against brucellosis, 3–5 years age from private farms and slaughtered house in Menoufiya governorate, were applied in this view. Specimens were collected without contamination by vein puncture of the jugular vein. About two milliliters of blood was taken in Vacutainer tube containing EDTA as the anti-coagulant for hemogram evaluation; and another in tubes has no anticoagulant. After clotting, serum was removed from the blood by centrifugation at three thousand RPM for twenty minute Each one was named using codes describing the specific animal.

2.2. Serological testing

Every sample was started screened for antibodies against *B. abortus* using (BAPAT) the buffered Acidified plate agglutination test. carried out according to *Alton et al., (1988).* Then using (PCR) kit for confirmation.in which classified the sample in two group.

Brucella sero-positive group: Consists of 25 animals which proved to be naturally infected with Brucella abortus.

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Control healthy group: Consists of 75 animals which proved to be non-infected with Brucella abortus.

2.3. Haematological examination

Parameters of hemogram were explain by standard techniques described by Jain, (1986). The % and absolute value for each type of leukocytes calculated according to Feldman *et al.*, (2000).

2.4. Serum biochemical analysis

Serum biochemical analysis were assayed spectrophotometrically using commercial diagnostic kits as following (ALT) and (AST) activities according to Bergmeyer *et al.* (1978), (ALP) was determined according to Bowers and McComb (1966); (GGT) was determined according to Szasz *et al.* (1974). Serum urea according to Tietz (1990) and serum creatinine was determined as performed by Fabiny and Ertingshausen (1971).

2.5. Blood protein fraction

Serum protein electrophoresis were done by using a semi-automated agarose gel electrophoresis system according to the method described by Keyser and Watkins (1972).

Albumin /Globulin ratio: (A/G) ratio was calculated by dividing albumin concentration on globulin concentration individually.

2.6. Immunological examination:

IL-1β and IL-10 Level were detected from concentrated serum samples using commercially allowed ELISA Kits (Nori^{R}Bovine IL-1β and IL-10 ELISA Kit Data Sheet from 2009-2016 GENORISE SCIENTIFIC). The plates were read at 450nm and a correction wavelength of five hundred and fifty nm was measured on a computerized automated microplate ELISA reader.

Results expressed in picograms per ml were high plated using linear regression from a standard curve of known level.

2.7. Statistical analysis:

The results obtained were tabulated and statistically analyzed according to Snedecor and Cochran (1967).

Mean values and standard errors were calculated. Significant of changes in the different tested parameters were checked with the student *t*-test.

3. RESULTS

Antibodies were detected by using (BAPA) test and confirmed by PCR of 25 cows of 100 (25%). Type of anemia (have no changes in the shape of the cells) was observed in the *Br. Abortus* infected group and was missing in the control (Table 1); however, there was non-significant changes in platelets count in both brucella infected group and control group, while the DLC indicated lymphopenia only in infected groups and not in the non-infected one (Table 1).

Biochemically: serum ALT, AST, ALP, and GGT activities are presented in table (2) appear a marked increases ($P < 0.05$) in the both Serum leakage enzymes activities (AST and ALT) and serum cholestatic enzymes activities (GGT, ALP) in infected cattle when compared with healthy control group (Table 2), but there were a significant decrease in urea level in sero-positive brucella group comparing with negative group, while serum creatinine level shows a significant increase in brucella infected group (Table 3).

Results of serum protein electrophoresis of brucella positive group and its control are illustrated in table (4) which show a marked lowering in TP, albumin, Alpha 1 globulin and Beta 2 globulin level in sero-positive brucella group comparing with its control While, non-significant changes in Alpha two globulin, β one globulin and γ-globulin results were observed.

Immunologically: Interleukin-1Beta and Interleukin-10level in *Br. abortus* infected cows showed significant increase in contrast with the control group (Table 5).

Table 1 Hematological parameters changes in sero-positive brucella group compared with healthy control group (mean± S.E.)

Parameters	Groups	
	Control	Positive brucella
Haemoglobin (Hb) (g/dl)	10.12±0.36^{b}	9.28±0.25^{a}
RBCs (x10^{6}/µl)	7.42±0.50^{b}	6.11±0.21^{a}
PCV (%)	32.59±1.15^{a}	32.41±1.19^{a}
MCV (fl)	44.78±3.40^{a}	50.37±3.31^{a}
MCH (pg)	13.94±1.22^{a}	16.31±0.73^{b}
MCHC (%)	31.02±0.47^{a}	29.69±1.21^{a}
Platelate (x10^{3}/µl)	194.8±29.1^{a}	187.3±15.79^{a}
Total leucocyte count (TLC) (x10^{3}/µl)	6.98±0.26^{b}	5.28±0.52^{a}
Granulocyte (x103/µl)	3.74±0.39^{b}	2.9±0.28^{a}
Lymphocyte (x103/µl)	2.82±0.26^{b}	1.88±0.17^{a}
Monocyte (x103/µl)	0.42±0.07^{a}	0.50±0.05^{b}

a& b: Superscripts to be compared statistically. Values with different letter superscripts at the same row are significantly different ($P<0.05$).

Table 2 Hepatic enzymes changes in cattle infected with brucella compared with healthy control group (mean± S.E.)

Parameters	Groups	
	Control	Brucella Positive
AST (U/L)	53.8±3.12^{a}	76.72±3.19^{b}
ALT (U/L)	19.60±1.21^{a}	31.00±0.67^{b}
ALP (U/L)	115.20±5.00^{a}	127.32±3.75^{b}
GGT (U/L)	21.30±0.55^{a}	24.72±0.35^{b}

a& b: Superscripts to be compared statistically. Values with different letter superscripts at the same row are significantly different ($P<0.05$).

Table 3 Kidney parameters in brucella infected cattle group compared to healthy control group.

Parameters	Groups	
	Control	Positive brucella
Creatinine (mg/dl)	1.13±0.07^{a}	1.31±0.08^{b}
Urea (mg/dl)	30.24±2.57^{b}	20.5±0.76^{a}

a & b: Superscripts to be compared statistically. Values with different letter superscripts at the same row are significantly different ($P<0.05$).

Table 4 Total protein, Albumin, Globulin and A/G ratio changes and electrophoresis in brucella infected cattle group compared to healthy control group.

Parameters	Groups	
	Control	Positive brucella
Total protein (g/dl)	7.40±0.15^{b}	6.54±0.24^{a}
Albumin (g/dl)	3.14±0.12^{b}	2.56±0.07^{a}
Alph 1 globulin (g/dl)	0.11±0.03^{b}	0.06±0.02^{a}
Alph 2 globulin (g/dl)	0.50±0.06^{a}	0.47±0.09^{a}
Beta 1 globulin (g/dl)	0.93±0.08^{a}	0.87±0.10^{a}
Beta 2 globulin (g/dl)	0.52±0.03^{b}	0.42±0.03^{a}
Gamma globulin (g/dl)	2.20±0.11^{a}	2.20±0.18a
A/G ratio	0.75±0.04^{b}	0.64±0.03^{a}

a & b: Superscripts to be compared statistically. Values with different letter superscripts at the same row are significantly different ($P<0.05$).

Table 5 Serum interleukin 1β and interleukin 10 in examined serum samples.

Parameters	Groups	
	Control	positive brucella
IL-1β(pg/ml)	57.5±2.3[a]	79.6±5.8[b]
IL-10 (pg/ml)	26.1±1.3[a]	34.2±1.5[b]

a & b: Superscripts to be compared statistically. Values with different letter superscripts at the same row are significantly different (P<0.05).

Polymerase chain reaction (PCR)

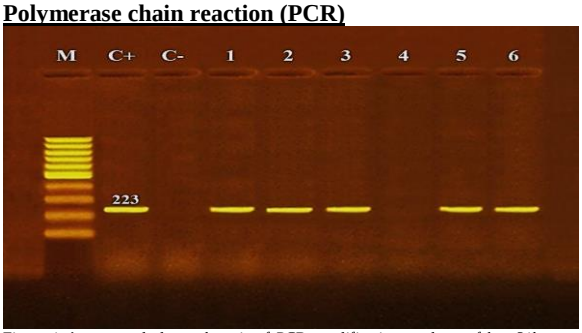

Figure 1 Agarose gel electrophoresis of PCR amplification products of bcsp31k gene specific for identification and characterization of *Brucella abortus*. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *Brucella abortus* forbcsp31kgene. Lane C-: Control negative. Lanes 1, 2, 3, 5 and 6: Positive *Brucella abortus* strains forbcsp31kgene. Lane 4: Negative *Brucella abortus* strain.

4. DISCUSSION

Brucellosis is a sever contagious illness of all domestics, it is classified as one of the most dangerous health problems, especially in non-rich countries (Samaha *et al.*, 2009). It is a chronic bacterial disease with bad effects on livestock production economy (CarvalhoNeta *et al.*, 2010; Poester *et al.*, 2013). Agglutination tests such as BAPAT, RBPT and TAT are commonly used for detection of brucella species antibodies (Jain and Tilak 2008). Large numbers of serological tests and various modifications to enhance accuracy have been developed for diagnosis of brucellosis (Yahaya *et al*., 2019). (PCR)-based tests are applied to be more rapid and higher sensitivity than the traditional tests (Christopher *et al.*, 2018).

Concerning to the blood cellular constituents of *Brucella abortus* antibody positive cows shows normocytic-normochromic anemia as indicated by significant decrease in Hb concentration. Also, there was significant decrease in RBCs count (Hashem *et al*., 2020; Raval *et al.*, 2014); this anemia may be due to the presence of brucella spp. inside every cell which might cause decrease in hemoglobin concentrations (Kushwaha *et al.*, 2014). there was significant decrease in TLC, lymphocyte and this result agreed with EL-boshy *et al.* (2009), and Raval *et al.* (2014). Also, granulocyte count showed a significant decrease and this finding agreed with Kushwaha *et al.* (2014). This lymphopenia condition and the leukocytopenia may be due to lowering the lymphocytes in the thymic cortex in natural and experimental (Enright *et al.*, 1984). The findings of hepatocytosis may be referred to brucella infection as a chronic disease. There are a significant elevation in the ALP Activity and no statistically difference GGT activity between the brucella positive cows and healthy one. high GGT level is mainly considered good diagnostic sensitivity than Alkaline phosphatase to measure the cholestasis or any other disorders in bile duct in cattle and this finding was agreed with that observed with Fernandez (2007) and AbouElazab (2015).

Serum creatinine level in infected cattle and this could be similar to that recorded by (Mohamed *et al*., 2003), who reported elevation in serum creatinine level in brucella infected camel. Meanwhile, urea showed a significant decrease in the infected cows similar to (Kishore *et al.*, 2017) which may be due to damaged liver tissue that cannot form Urea from the ammonia (Hamada *et al.*, 2013). Hypoproteinemia and hypoalbuminemia were observed this could be due to decreased albumin synthesis by reticulo-endothelium in the liver. also, *Brucella* cause sever change and diseased the renal cell of the kidney, which elevate protein out flow in the urine and lead to decrease albumin in blood (AL-Hussary *et al.*, 2010; Kishore *et al*., 2017).

However, no marked changes between mean levels of alpha1-, alpha2-, and beta-globulin amount, the highest globulin concentrations (especially gamma-globulins) are mainly due to chronic antigenic achievement caused by the microorganism. A⁄ G ratio results are in the line with Rita Nath *et al.* (2014).

In our investigation for serum interleukins, IL-1beta and IL-10revealed a marked increase in brucella cows. Our data were in same line of Dzata *et al.* (1991) who reported an elevation in interlukin 1β levels in the blood of cow infected with a *Br. Abortus* antigen. IL-1beta cytokines elevate the expression of adhesion factors on endothelial cells to cable the transferring of WBCS, the cells that attack pathogens, to place of infection (Nicklin *et al.*, 2000). In the other hand, IL-10 displays strong performance to suppress the antigen presentation amount of antigen presenting cells (Moore *et al.*, 2001).

5. CONCLUSIONS

From serological, hematological and biochemical examination in this study we can conclude that Egypt is endemic area Brucellosis. So, periodic sero-prevalence studies in susceptible animal for early diagnosis of brucella infection which is very important way for helping eradication of Brucellosis. brucella infection has degenerative effect on vital organs like liver and kidney.so, biochemical studies would help to identify the extent of hepatic damage and its effect on animal health.

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