Global Veterinaria 4 (5): 495-503, 2010
ISSN 1992-6197

Pathophysiological Investigations on Brucellosis in She-Camels

[1]Aziza Kh. Omar, [2]Abd El-Aziz S. Bahbil, [1,3]Nibal A. Hassan and [2]Amany M. Abd EL-Wahab

[1]Department of Science, Faculty of Sciences in Kurma, Taif University, KSA
[2] Department of Science, Faculty of Sciences, Taif University, KSA
[3]Animal Reproduction Research Institute, Giza, Egypt

Abstract: The present study was carried out on two groups of she-camels in different localities in El-khurma and Taraba governorates, KSA. Serum samples were obtained from 165 she-camels kept in close contact with small ruminant (1st group) and 95 she-camels kept in closed farm (2nd group). These samples were subjected to different serological tests for detection of antibody against Brucella. Also, samples were subjected to biochemical analysis. Lymph nodes, spleen, mammary gland and uterus were collected from serologically positive animal for bacteriological, histopathological and immunopathological examinations. The sero-prevalence in 1st group (camel kept in close contact with small ruminant) were 7.88, 8.48, 6.67, 6.67 and 6.67% by using Rose Bengal Test (RBT), Buffered Acidified Plate Antigen Test (BAPA), Tube Agglutination Test (TAT), Rivanol Test (Riv. T) and Complement Fixation Test (CFT), respectively. Regarding to 2nd group (kept in closed farm) only one sample (1.05%) was positive to RBT, BAPA and TAT as well as one sample was suspicious to TAT. Biochemical analysis of serum samples taken from Brucella positive she camels showed elevation in liver enzymes (AST and ALT) and blood glucose, urea, uric acid and creatinine levels. Bacteriological examination showed that all Brucella strains detected in tissue specimens of reactor animals were typed as *Brucella melitensis* biovar 3. The pathological study revealed individual variation with respect to organ affected, severity and extension of lesion. Histopathological studies of lymph node revealed granuloma like structure infiltrated by inflammatory cells. Spleen showed depletion in lymphoid follicle with proliferation of fibrous tissue. Mammary gland, showed granuloma from mononuclear cells mainly macrophages and plasma cells. While uterus showed partial destruction and fibrosis in mucosal epithelial lining as well as atrophy in underlying uterine glands. Immunopathological examination showed intense brown granules inside inflammatory cells in lymph node, spleen and uterus. It was concluded that Immunohistochemistery can be applied in case of biopsy samples from mammary lymph node tissue of she-camel for detection of brucellosis.

Key words: Brucellosis · Pathophysiological · Diagnosis

INTRODUCTION

Brucellosis is considered as an important disease in both human and domesticated animals in which the main symptom is reproductive failure. A number of characteristics make Brucella species attractive targets for weaponization and the organism included in the list of CDC category B potential biological warfare [1].

Brucellosis is a serious zoonotic disease affecting man and all domestic animals including camels. It is considered as one of the great public health problem allover the world [2].

Brucellosis can be acquired through ingestion and through breaks in skin, aerosol transmission, also occurs [3]. Organisms first localize in regional lymph nodes, subsequent entry into lymphatic system and development of bacteremia allows localization of bacteria in a variety of tissues and they proliferate within reticuloendothelial cells. Organisms in lymphoreticular system attract macrophages and proliferate within them leading to small granulomas growth. These granulomas may be grossly or microscopically visible according to its size. It is a classic lesion of Brucellosis [4]. Recently immunoenzymatic techniques have been introduced to detect the location of brucella organisms in formalin fixed tissues of goats and in cows [5].

The camel has been considered as a symbol of stability for pastoralists in the arid zone of the world [6].

Corresponding Author: Nibal A. Hassan, Animal Reproduction Research Institute, Giza, Egypt
E-mail: nibalamin@yahoo.com.

In the past, the camel was thought to be resistant to disease. However, the camel was found to be more susceptible than other animals to certain disease such as paratuberculosis (Johnes's disease), Clostridia enterotoxaemia and brucellosis [7].

Camel could play an important role in the epidemiology of brucellosis and act as important source of infection to other domestic animals [8].

Kiel and Khan [9] suggested that the epidemiology of brucellosis in camels in a country or region was complicated by importation of living animals with higher prevalence of brucellosis than in the local animals and human across national border.

The present study was carried out to throw light on some pathophysiological aspects of brucellosis in she-camels at Saudi Arabia with emphasis on comparing different serological and bacteriological tests used for its diagnosis and using of immunoperoxidase technique as a confirmative diagnostic tool.

MATERIALS AND METHODS

Samples:

a- Blood samples were collected to separate sera from two animal groups.

1st group comprised of 165 she camels kept in close contact with small ruminant.

2nd group comprised of 95 she camel kept in closed farm. Both groups belonged to different areas in El-Khurma and Taraba governorates, KSA during January to November 2009.

Each serum sample was divided into 2 parts, the first part was used for serological examination of brucellosis, the second part was used for analysis of some relevant biochemical parameters.

Control Group: From fifty Brucella negative samples only fifteen samples were selected to be control negative to give normal values for kidney and liver function tests.

These animals get slaughtered due to owner complain from repeated abortion which lead to great economical loss.

b- Tissues Samples: for bacteriological examination, supra-mammary, retropharyngeal, pre-scapular lymph nodes, spleen, uterus and mammary glands were collected from each slaughtered serologically positive animals in sterile bags directly after slaughtering and transferred on ice packs to laboratory as soon as possible. They were kept frozen at-20°C until cultured.

c- Tissue Samples for Histo-pathological Examination: lymph nodes, spleen, uterus and mammary glands were collected from serologically and bacteriologically positive animals for brucellosis. Samples were kept in 10% neutral buffered formalin until processed.

Serological Tests for Brucellosis: Serum samples intended for serological examination were subjected to adsorption method against killed antigen of *Pasteurella multocida* and *Yersenia enterocolitica* O.9 strain according to modification described by Turcott [10] to eliminate non-specific reaction of brucellosis.

- Rose Bengal test (RBT) was done as described by Morgan *et al.* [11].
- Buffered acidified plate antigen test (BAPA) was done as described by Alton *et al.* [12].
- Tube agglutination test (TAT) was done as described by Alton *et al.*[12].
- Rivanol test (RivT) was done according to the technique described by Alton *et al.* [12] using antigen and Rivanol solution.
- Complement fixation test (CFT) was done according to Alton *et al.* [12].

Bacteriological Examination: Tissue samples were exposed to Brucella isolation whereas direct culture of the samples were performed under complete aseptic condition into Brucella agar plates and incubated in special incubator with 10% carbon dioxide; the plates were examined at 4th day and daily for 10 days till 14 days. Identification of Brucella isolates was applied according to morphological characters, microscopical examination and reaction with positive sera. The typing of Brucella isolates was done according to CO_2 requirement, H_2S production and growth in the presence of dyes (Thionin and fuchsin), reaction with nonspecific sera (A and M) and bacteriophage typing. All these procedures were done according to Alton *et al.* [12].

Serum Biochemical Analysis: Some relevant serum constituents were spectrophotometrically assayed using commercial diagnostic chemical kits as follows:-

Serum Alanine aminotransferase (ALT) and serum Aspartate aminotransferase (AST) was determined according to Reitman and Frankel [13]. Glucose was determined according to Trinder [14] urea was measured according to Patton and Crouch [15], uric acid was measured according to Caraway [16] and creatinine was measured according to Bartels *et al.* [17].

Histopathological and Immuno-histopathological Examination: Tissue specimens collected for histopathological examinations were fixed in 10% neutral buffered formalin solution, processed by the paraffin embedding technique, stained with hematoxylin and Eosin according to Bancroft *et al.* [18]. Detection of Brucella antigen was done by immunoperoxidase test according to Liellile and Harlod [19]. The conjugate working solution 1:200 slides were examined using light microscope for detection of specific positive reaction.

Data were computed and statistically analyzed

RESULTS

The number of positive to each serological test in camel kept in close contact with small ruminant (1st group) are shown in Table 1. The seroprevalence in this group revealed that out of 165 serum samples 13 (7.88%) were positive using RBT, 14 (8.48%) using BAPA, 11 (6.67%) using TAT, 11 (6.67%) using RivT and 11 (6.67%) using CFT while 3 (1.8%) were suspicious using TAT.

Regarding to 2nd group (kept in closed farm) out of 95 serum samples, only one sample (1.05%) was positive to RBT, BAPT and TAT as well as one sample was suspicious to TAT.

The Brucella positive serum showed elevation of AST, ALT, Glucose, Urea, Uric acid and Creatinine as compared to the control group.

As regarded to the results of bacteriological examination (Table, 4), tissue culturing give 81.81% in camel kept in close contact with small ruminant (1st group) and 0% in camel kept in close farm (2nd group) in correlation with serological test.

Histopathological Findings

Lymph Nodes: Gross pathological examination of lymph nodes of Brucella reactor she camels,,especially supra-mammary node recealed hat it appear firm and its capsule was tout with slight pink coloration, slightly enlarged and edematous.Histopathological examination showed varying degrees of lymphoid hyperplasia with wide germinal centers of lymphoid follicles. Granulomatous

Table 1: Results of sSerological tests of she camels kept in close contact with small ruminants (first group)

	Total reactor		Positive		Suspicious		Negative	
Serological test	No.	%	No.	%	No.	%	No.	%
RBT	13	7.88	13	7.88	-	-	152	97.12
BAPA	14	8.48	14	8.48	-	-	151	91.5
TAT	14	8.48	11	6.67	3	1.8	151	91.5
RivT	11	6.67	11	6.67	-	-	154	93.3
CFT	11	6.67	11	6.67	-	-	154	93.3

RBT: Rose Bengal plate test
BAPA: Buffered Acidified Plate Antigen Test
TAT : Tube Agglutination test.
RivT: Rivanol Test
CFT : Complement fixation test.
TAT titre= 1/20 consider suspicious and = 1/40 consider positive.
CFT titre= 1/4 consider suspicious and = 1/8 consider positive.

Table 2: Results for serological tests of she camels kept in close farm (Second group)

	Total reactor		Positive		Suspicious		Negative	
Serological test	No.	%	No.	%	No.	%	No.	%
RBT	1	1.05	1	1.05	0	0	94	98.95
BAPA	1	1.05	1	1.05	0	0	94	98.95
TAT	2	2.1	1	1.05	1	1.0 5	93	97.89
RivT	-	-	-	-	-	-	-	-
CFT	-	-	-	-	-	-	-	-

RBT: Rose Bengal plate test
BAPA: Buffered Acidified Plate Antigen Test
TAT : Tube Agglutination test.
RivT: Rivanol Test
CFT : Complement fixation test.
TAT titre= 1/20 consider suspicious and = 1/40 consider positive.
CFT titre= 1/4 consider suspicious and = 1/8 consider positive.

Table 3: Some serum biochemical parameters in Brucella positive she camel

Biochemical parameters.	Control	Brucella positive
AST	12.6 ±1.42	25.61 ±2.19*
ALT	82.00 ± 2.86	163.0 ± 6.72*
Glucose	59.99 ± 2.16	78.92 ± 6.21*
Urea	27.00 ± 0.42	36.97 ± 2.96***
Creatinine	1.12 ± 0.06	2.53 ± 0.03***
Uric acid	2.99 ± 0.12	3.45 ± 0.16**

Mean ± Standard error.

*, **, *** significant difference at probability at P<0.05, 0.01, 0.001.

ALT: Serum Alanine aminotransferase

AST: and serum Aspartate aminotransferase.

Table 4: Results of bacteriological examination of reactor animals

Examined animal group	Total slaughtered reactor	No. of +ve	% of positive
1st group	11	9*	81.81
2nd group	1	0	0

All isolated strains belonged to *Brucella melitensis* biovar 3.

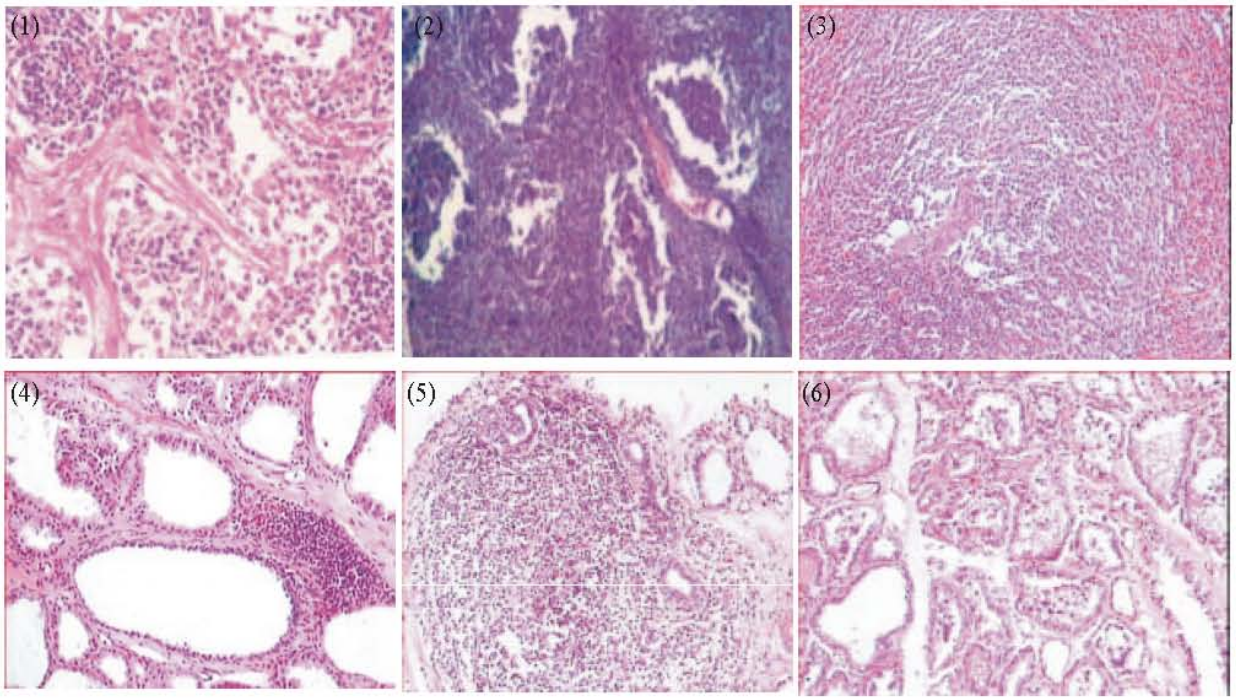

Fig. 1: Lymph node of she-camel infected with Brucella melitensis showing granuloma (H&E- X400)

Fig. 2: Spleen of she-camel showing depletion of lymphoid follicle (H&E- X200)

Fig. 3: Spleen of infected she-camel showing haemosedrin granule scattered through out splenic parenchyma (H&E- X100)

Fig. 4: Mammary gland of infected she-camel showing granulomatous mastitis (H&E- X200)

Fig. 5: Mammary gland of infected she-camel showing granulomatous mastitis (H&E- X400)

Fig. 6: Mammary gland of infected she-camel showing connective tissue proliferation and atrophied secretory acini (H&E- X100)

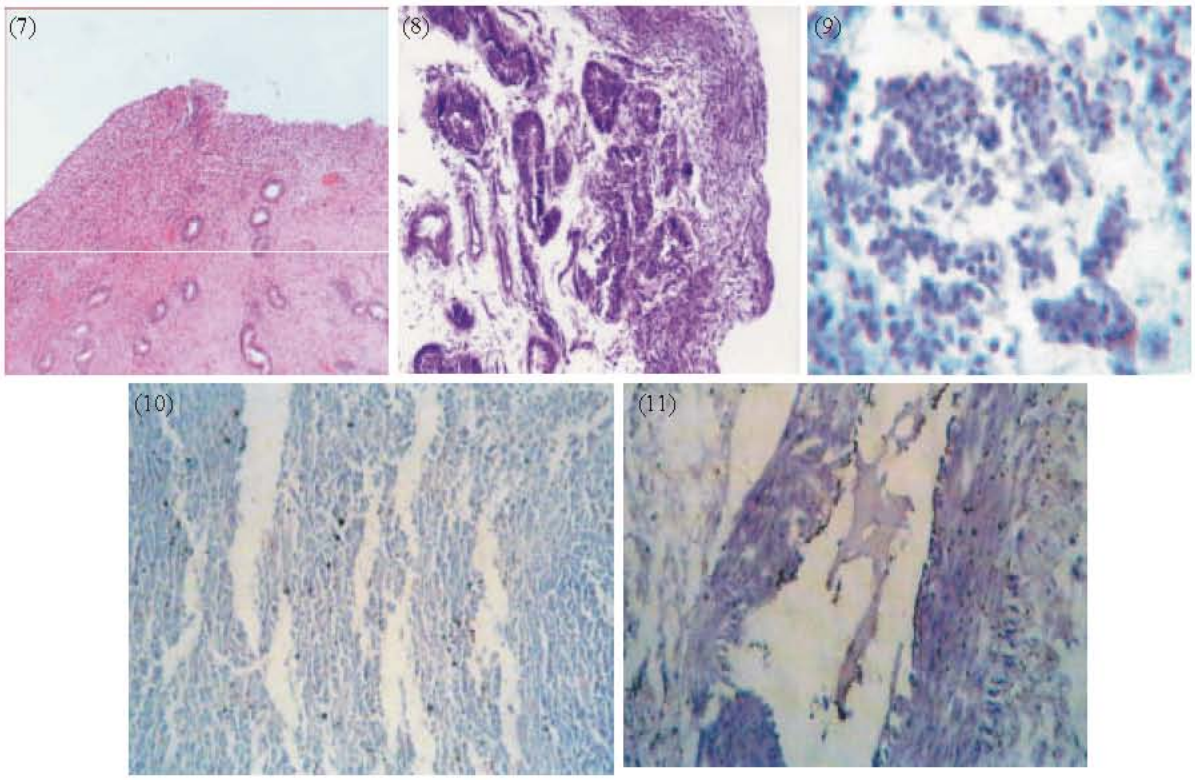

Fig. 7: Uterus of infected she-camel showing ulcerative endometritis with destructive gland (H&E- X100)
Fig. 8: Uterus of infected she-camel showing endometrial oedema (H&E- X100)
Fig. 9: Supra-mammary lymph node of infected she-camel revealed deposition of golden brown chromogen pigment at the site of antigen - antibody complex in cytoplasm of macrophage and lymphocyte (X200)
Fig. 10: Spleen of infected she-camel revealed immunoperoxidase brown granule in macrophage of lymphoid follicle (X100)
Fig. 11: Uterus of infected she-camel showed deposition of golden brown chromogen pigment at the site of antigen - antibody complex in cytoplasm of cytoplasm of endothelial cell lining blood vessels (X200)

reaction of different patterns was observed in cortical area of lymphoid follicle. This granuloma consisted of aggregation of macrophages, lymphocytes, plasma cells and sometimes multinucleated giant cells (Figure 1).

Spleen: Grossly the spleen appeared increased in size with granular surface in some cases. Histopathological examination revealed marked hyperplastic activation of the white pulp with the presence of abundant histocytes and plasma cells around the medullary cord of red pulp, some lymphoid follicles showed depletion (Figure 2) and proliferation of fibrous tissue, haemosidrine granules scattered through out the spleenic parenchyma (Figure 3) and spleenic blood vessels showed thickening of their walls.

Mammary Gland: Grossly the mammary glands were hard in texture on cut section the milk secretion was scanty and slight turbid with yellowish coloration in some cases. Histopathological examination revealed that granulomatous mastitis in few cases. There was granulomatous structure found in glandular parenchyma consisted of abundant of mononuclear inflammatory cells mainly macrophages and plasma cells (Figures 4 and 5). In most cases, the mammary glands exhibited intralober and interlobular fibrous connective tissue proliferation the secretory acini appeared small and atrophid with narrowing lumen and infiltrated with inflammatory cells, also the epithelium lining of secretory acini showed vacuolar degradation (Figure 6). Cystic dilatation of some acini were noticed and the lumen become highly dilated and its lining epithelium become flattened and desquamated into the lumen.

Uterus: Grossly erosion and ulceration of endometrial mucosa with the presence of moderate amount of mucous exudates, some cases showed mild degree of congestion and oedema.Histopathologically multifocal desquamates of the surface epithelium and its basement membrane was seen in the lamina propria subepithelialis showed diffuse and heavy infiltration of mononuclear inflammatory cells mainly macrophages and lymphocytes (Figure 7). endometrial stroma revealed edema dispersing the uterine element from each other (Figure 8) some glands appear degeneration and their epithelium revealed vacuolar degeneration, few gland revealed inflammatory cell in the lumen mainly lymphocyte, blood vessels appeared markedly dilated and congested.

The Immunoperoxidase Technique: Specific immunoperoxidase brown granules against Brucella organism within cytoplasm of macrophages and lymphocytes of lymph nodes was seen (Figure 9). In spleen of infected she-camel, the brown specific immunoperoxidase specific immunoperoxidase granules were observed within macrophages in lymphoid follicle (Figure 10).While, in uterus of infected camel, the brown specific immunoperoxidase granules were observed within epithelial lining endometrium, within endothelial blood vessels and within mononuclear cells infiltration around blood vessels (Figure 11).

DISCUSSION

Brucellosis is a contagious, zoonotic bacterial disease that affects several species of domestic animals cause abortion and it, manifests it self in human as a systemic febrile illness [20].

The resistance of animals to Brucella infection is influenced by sex, age and reproductive status. Brucella organisms first localized in regional lymph node, they proliferate within reticuloendothelial cells with subsequent entry into lymphatics and localized in different tissues like spleen and reproductive organs [4]

The magnitude of brucellosis sero-prevalence in camels is based on serological surveys by a variety of procedures.

In this study different serological tests (RBT, BAPA, TAT, RivT and CFT) were carried out to show a prevalence of the disease among examined she camels.

The sero-prevalence revealed that out of 165 samples collected from camels kept in close contact with small ruminants 13 (7.88%) were positive using RBT, 14 (8.48%) using BAPA, 11 (6.67%) using TAT, 11 (6.67%) using RivT and 11 (6.67%) using CFT while 3 (1.8%) were suspicious using TAT. The higher percentage of RBT and BAPA suggested the efficiency of these tests as screening tests for detection of recent and chronic infections of camel's brucellosis [21]., The higher percentage of RBT and BAPA as compared to TAT explained the basis that TAT may miss some infected animals, especially in chronic stage of the disease [22]., RivT in this study detected 11 (6.67%) positive reactors this may be attributed to that RivT is useful in detection of chronic sera that mainly contain IgG [23]., As shown from the collective data, CFT gave negative results in many serum samples that were identified as reactor in other tests, such reaction may be regarded as false positive. EL-Gibaly *et al.* [24] concluded that TAT must be confirmed by CFT to prove that all animal are Brucella free. This test has been recommended as a confirmatory test.

It seems advisable to use CFT as a confirmatory, quantitative, more sensitive, specific test and easy to be done for detection of Brucella antibodies, which gave 100% correlation ship with direct culturing on selective media as compared with other serological tests [25].

There are some problems of the specificity of serological tests for brucellosis since antibodies against Brucella species epitopes may be present due to animal vaccination and or of contacts with other Gram negative bacteria (mainly Yersinia enterocolitica 0:9) sharing cross-reactive epitopes with Brucella [26]., Also *Mitk et al.* [27] reported that conventional serological methods have important limitation such methods display poor sensitivity in the early stage of the disease during which the level of antibodies may be low. Furthermore, there can be cross reacted with certain other negative bacteria.

Camels of 2nd group showed lowest percent of positive reactors, this group was kept in closed farm under good hygienic condition, this might explains the lower incidence of positive reactor among this group.

Our results showed that highest percent of positive reactors was observed in camels in contact with other animals (1st group). Therefore, we can conclude the possibility of spread of brucellosis between different animal species and this might explain the higher incidence of the disease in the 1st group. This result, agree with Hisham *et al.* [28] who reported that contact between camels and small ruminants was incriminated in transmission of brucellosis to camels., Radwan *et al.* [29] suggested that lateral transmission of the disease between different animal species can occur and play a serious role in spread of the disease. Also, it was demonstrated that camel pastoralists invariably keep relatively large flock of sheep and goats along side the camels [6]. arger herds provide more chances of contact between animals leading to more chance of infection.

The sera of infected camels with Brucella showed elevation of AST and ALT levels. These enzymes are liver specific enzymes; this indicates that in camels, liver may be affected as consequence of brucellosis [30].

In this study, significant increase in glucose level was found in Brucella infected camels and the condition may be attributed to the increase of several enzymatic activities in brucellosis involved glucose metabolism like glucosyltransferase [31].

Urea, uric acid and creatinine levels were significantly increased herein in brucella infected camels. These alterations in liver protein metabolism affect kidney functions manifested by elevation of urea, uric acid and creatinine [32].

Bacteriological examination in this study showed that all Brucella strains detected from tissue specimens collected from reactor animals were typed as *Brucella melitensis* biovar 3. In Saudia Arabia since there are mixed populations of sheep, goats and camels so *Brucella meltensis* biovar 3 is easily to be introduced into these populations [28,33]

Brucella melitensis persists in Mediterranean and Middle East countries and sporadically allover the world. It is associated with nomadic animal husbandry which it self is related to developing countries [34,35]. For this reason a test and slaughter policy is not realistic in the majority of places whereas *B. melitensis* is endemic due to lake of financial resources needed for compensation. International agencies have, therefore, proposed that whole flock vaccination should precede any test and slaughter programs until disease prevalence is significantly reduced. Only then should test and slaughter be implemented as a part of national eradication scheme [36].

Primary isolation of Brucella species in the diagnostic laboratory presents problem, because all species are potentially pathogenic to man and are relatively slow growing since colonial development may take as along as 5 days [25].

Pathological examination of our cases revealed the presence of individual variation with respect to organ affected extension and severity. The histopathological finding of supramammary lymph node revealed granulomatous reaction characterized by aggregation of macrophages, lymphocytes and plasma cell. This result is in agreement with Montaser and Nashwa [37] and El-Nser and Mahdy [38]. This may be attributed to localization and multiplication of organism in macrophages and lymphocytes of lymph node followed by rupture of phagocytic cells.

The histopathological changes in spleen due to brucellosis in the present study revealed depletion of lymphoid follicle and proliferation of fibrous tissue, the alteration of this tissue attributed to multiplication and responsive to immune reaction [5].

In infected animals, different pathological patterns were recorded in mammary gland as granulomatous structure, intra and inter lobular fibrous connective tissue proliferation in addition of cyctic dilation of some acini. This results could be explained as Brucella is invades blood stream and will result in localization of the organism in mammary duct and alveoli [39].

It can be concluded that the most common type of endometritis association with brucellosis in she-camel was ulcerative endometritis which characterized by destruction of epithelial lining and atrophied underlining uterine gland. These result agree with the previously reported by Sohir *et al.* [40].

Regarding to immunopathological studies, our results revealed brown specific immunoperoxidase granules within macrophage in lymph nodes, spleen and uterus as well as within endothelial lining uterine blood vessels and epithelium lining mucosa and uterine glands. These results were in agreement with Perez *et al.* [41] as they recorded that the immuno-reactivity pattern is located mainly in the cytoplasm of macrophage. The immunohistochemical technique in our study was sufficiently sensitive for detecting Brucella antigen in formalin fixed tissue. This agrees with Vincent *et al.* [42] who recorded that the immunohistochemical test may be useful for performing retrospective studies because antigen detected by this method have been shown to be stable in paraffin section for 10 years.

From serological, biochemical, bacteriological and pathological examination in this study we can conclude that camels play an important role in epidemiology of brucellosis and as important source of infection so camel should be included in surveying programs in Saudi Arabia for complete eradication of the disease.

REFERENCES

1. Rotz, L.O., A.S. Khan, S.R. Lillibridge, S.M. Ostroff and J.M. Hughes, 2002. Public health assessment of potential biological terrorism agents. Emerg. Infect. Dis., 8: 225-230.
2. Radositits, O.M., D.C. Blood and C.C. Cary, 1994. "Veterinary Medicin" 8th Ed. Baillier Trindall. England. London.

3. Mina, J.I., Y. Polosky, M.G. Mense, A.K. Bhattacharjee, C.M. Paranavitana, T.L. Halfied and D.L. Hoova, 2000. Impaired control of Brucella infection in Rag1. Infect. Immun., 9(68): 5314-5320.
4. Jones, T.C., R.D. Hunt and N.W. King, 1999. Efficacy of Veterinary pathology. 6th ed. Philadelphia, New York, London and Tokyo.
5. Meador, V.P., W.A. Hagemeser and B.L. Deyoe, 1988. Histopathological findings in Brucella abortus pregnant goats. A.M. J.Vet. Res., 49(2): 274-280.
6. Abbas, B.Q., A.A. Qarawi and S.A. Aihawa, 2000. Survey of camel husbandry in Qessium region Saudi Arabia. Rev. Elv. Vet. Med. Trop., 53: 285-293.
7. Wernery, U.I. and O.R. Kaaden, 1995. Infectious disease of camel lids Blackwell Wissen scafts Verby. Berlin.
8. EL-Sawalhy, A.A., A.M. Montaser and L.G. April, 1996. Diagnostic and biochemical evaluation of camel brucellosis. 4th Sci. Proc. April3-6 1996 Vet. Med. J. Giza., 2(44): 332-335.
9. Kiel, F.W. and M. Khan, 1989. Brucellosis in Saudi Arabia. Soc. Sci. Med., 29: 999-1001.
10. Turcotte, R., 1975. The participation of common and species specific antigen of Mycobacterium in tuberculin skin reaction. C. J. Microbial., 21: 774-783.
11. Morgan, W.J., D.J. Mechinnon, K.P. Gill S.G. Gower and P.I.B. Norris, 1987. Brucellosis diagnosis standard laboratory techniques 2nd ed.,Central vet. Laboratory New How, Weybridge, Surrey Kt 153 NB, England.
12. Alton, G.G., L.M. Jones, R.D. Angus and J.M. Verger, 1988. Techniques For The Brucellosis Laboratory. INRA, Paris, France.
13. Reitman, S. and S. Frankel, 1957. Colormetric determination of GOT and GPT activity according to the Reitman and Frankel method. Am. J. Clinc. Path., 28(2): 56-64.
14. Trinder, P., 1969. Enzymatic determination of glucose. Ann. Clin.
15. Patton, C.J. and S.R. Crouch, 1977. Enzymatic determination of urea. Anal. Chem., 49: 464-469.
16. Caraway, W.G., M.M. Jon and J.M. Meryer, 1966. Techniques for uric acid determinations in alkaline medium. Clin. Chem, 12: 16-18.
17. Bartels, G., C. Marin, M. Belas, M. Diano and K. Moriy, 1972. Kinetic determinations of creatinine without deproltainzations. Clinc. Acta, 37: 193-197.
18. Bancroft, D.J., C.H. Cook, R.W. Strilling and D.R. Turner, 1994. Manual of histological technique and their diagnostic application. Churchill Livingstone, New York.
19. Liellile, R.D. and M.F. Harold, 1976. Histological technique and particle histochemistry. 4th ed. Mc-Graw-Hill Inc., New York.
20. Kreeger, T.J., W.E. Cook, W.H. Edwards and T. Cornish, 2004. Brucellosis in captive Rocky mountain sheep caused by Brucella abortus biovar 4. J. Wild Life Dis., 40(2): 311-315.
21. Yagoub, L.A., A.A.L. Mohammed and M.O. Salim, 1990. Serological survey in the one humped camel (Camelus dromedaries) from Easter Sudan. Rev. Elv. Med. Trop., 43: 167:171.
22. Morgan, W.J., 1967. The serological diagnosis of bovine brucellosis. Vet. Rec., 80(21): 612-623.
23. Amir, M.A., 1988. Occupational brucellosis in human contact. Mvsc thesis (Zoonosis) Fac. Vet. Med. Cairo Univ.
24. EL-Gibaly, S.M., F.F. Gada, S.M. Nada and E.M. Sayour, 1975. Trials for control and eradication of Brucella infection in a farm. J. assuit Vet. Med., 11(34): 219-223.
25. Ghanem, F.M., 1998. Specificity and sensitivity of some serological tests used for diagnosis of bovine brucellosis. Beni-Suef Vet. Med. Res., 8(2): 181-189.
26. Grain Bustuji, B., J.M. Balsco, C. Marin and D. Albert, 2006. The diagnosis of brucellosis in sheep and goats old and tools. Small Ruminant Research, 62: 63-70.
27. Mitk, S., C. Anetukis, E. Soulion, E. Diza and A. Kcensouzidou, 2007. Evaluation of different PCR assays for early detection of acute and relapsing brucellosis in human in comparison with conventional methods. J. Clin. Microbiol., 45(4): 1211-1218.
28. Hisham, N.H., G.A. Galil, M.A. Hulaibi and E.M. AL-Sleim, 1987. The incidence of brucellois and species of Brucella organisms isolated from animal AL Has, Saudi Arabia. World Anim. Rev., 61: 32-53.
29. Radwan, A.I., S.I. Bekairi, A.A. Mykayel, A.M. AL-Bokmy, P.V. Prasaxd, F.N. Azar and E.R. Coloyan, 1995. Control of Brucella melitensis infection in large camel herd in Saudi Arabia using antibiotherapy and vaccination with Rev.1 vaccine. Rev. Sci. Technol., 14: 719-732.

30. Sisteron, O.A., I. Soucia, P. Chevalliera, E. Cuab and J.N. Brunetona, 2002. Hepatic abscess caused by Brucella US, CT and MRI findings-case report and review of the literature. A service de Radiologie, Hospital l`archet II, 151 Route de Saint-Antine de GinestiEre, 06202.
31. Corbel, M.J., 1997. Brucellosis an overview. Emerg. Infect. Dis., 3(2): 213-221.
32. Cazevieille, C., F. Porte, S. Bettache, J.P. Liantard and J. Sirwidada, 2000. Early events and implication of actin and annexin I associated structure in the phagocytes uptake of Brucella suis by the J-74A.1 murine cell line and human monocytes. Microb. Patholo., 28(6): 343-325.
33. Kadry, M.K., 1996. Some studies on brucellosis in cattle and buffaloes. MVSc, Thesis, Fac. Vet. Med., Cairo University.
34. Jacques, I., V. Olivier-Bernardin and G. Dubray, 1998. ELISA compared to conventional tests (RBPT and CFT) for diagnosis of Brucella melitensis in infection sheep. Vet. Microbiol., 64: 61-73.
35. Cardoso, P.G., G.C. Macedo, V. Azevedo and S.C. Oliveiro, 2006. Brucella SPP non canonical LPS: Structure, biosynthesis and interaction with host immune system. Microbiol. Cell Factories, 5: 13.
36. WHO (World Health Organization), 1998. The development of New/Improved Brucellosis Vaccines: Report of WHO meeting. Geneva, Switzerland, December. 11-12 1997. WHO/EMC/ ZDI/ 98. 14 WHO Geneva.
37. Montaser, A.M. and Nashwa M. Helmy, 2004. Application of insitu hybridization in Diagnosis of Brucellosis in tissue of naturally infected sheep and goats. Assuit Vet. Med. J., 50(101).
38. EL-Nser, K.A. and E.A. Mahdy, 2006. Lymphoadenopathy characterized by fatty infiltration in cattle and sheep. Egypt J. Comp. Pathol. Clinic. Pathol., The 15th Sci. Congress 25-26 January.
39. Hegazy, A.A., M. El-Shazly, M.A. Wahdan and H.A. Amer, 1998. Pathological and serological studies on brucellosis in she camel. Egypt J. Comp. Path and Clinc. Path., 11(2): 23-32.
40. Sohir, I. Badr, Hoda, M. Zaki and Zeinab R. Mohamed, 2007. Mycoplasmal and Bacteriological studies on genital affections of She-Camel and the Pathological features associated. 2nd. sci. conf. fac. vet. Med., Benha Univ. Biochem., 6: 24-34.
41. Perez, J., M. Quezada, J. Lopez, O. Casquet, M.A. Sieries and J. Martien, 1998. Immunohistochemical detection of Brucella Abortus antigens in tissues from aborted bovine fetuses using a commercially available polyclonal antibody. J. Vet. Diag. Invest., 10(1): 17-24.
42. Vincent, L.L., B.H. Jake, P.S. Paul and P.G. Halbur, 1997. A Monoclonal-antibody-based immunohistochemical method for the detection of swine influenza virus in formalin fixed, paraffin embedded tissues. J. vet. Diagn. Invest., 9: 191-195.