Some studies on *Brucella* among camels with reference to isolated strains

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ABSTRACT

A number of 271 apparently healthy camels of both sexes at different ages were used in this study during a period from April 2016 to May 2017. Samples (271 serum samples, 30 milk samples , 21 tissue specimen and lymph nodes) were obtained from slaughtered camels in different localities (Cairo, Giza, El Sharkyia, El Behira, Matroh). Three serological tests were applied including Buffered Acidified Plate Test (BAPT), modified Rose Bengal Plate Test (mRBPT), and Tube Agglutination Test (TAT). The prevalence of reactors for brucellosis was 9.5%, 8.8% and 7.7% respectively. All samples confirmed by using Rivanol Test (Riv.T) and Immunochromatographic assay (ICA) with 8.5% and 9.2% prevalence respectively. All collected milk samples were Negative by MRT (0/30). ICA or (LFA) is highly sensitive, accurate and specific diagnostic assay since it directly detects antibodies of *Brucella* organism and is considered as rapid confirmatory test.

Culturing from 21 tissue samples and L.N revealed positive isolates as 5/21 (23.8%). The isolated strain was identified biochemically and by Polymerase Chain Reaction (PCR) with 498 bp and revealed predominance of *Brucella abortus* in all isolates as 5 (100%). This work aims at providing an overview on diagnostic investigations, as brucellosis has an economic impact on the production and reproduction in camels.

Key words: *Brucella*, camels, BAPT, mRBPT, TAT, Riv.T, ICA, MRT.

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1. INTRODUCTION

Camel brucellosis has been reported in Egypt for the first time by Ahmed (1939) since that time the disease continued to appear with fluctuation in its prevalence. A large number of camels are continuously introduced to Egypt from many African countries; these camels are not tested for brucellosis in quarantines. Egypt imports camels from east Africa to compensate for the gap in meat production. The sero-prevalence of brucellosis among camels in the source countries is an indicator for

potential transboundary brucellosis being introduced to Egypt through untested camels (Sayour, 2015). Camels are affected by any of the three major *Brucella* species, *Brucella abortus*, *Brucella melitensis* and *Brucella suis* (Rutter and Mack, 1963 and Higgins, 1986). The clinical picture of brucellosis in camels can vary from asymptomatic to abortion (Musa *et al*, 2008). Dams can develop ovario-bursal adhesions, hydrobursitis, and granulomatous endometritis. Placental retention, infertility, and delayed sexual maturity have also been reported (Musa and Shigidi 2001). Males may suffer from orchitis and arthritis also was recorded accompanied by acute lameness (Musa *et al*, 2008 and Abbas and Agab, 2002). *Brucella* organisms were isolated from 20 (87%) out of 23 serologically positive females camels and typed as *B.abortus* sero type 7 and *B.melitensis* sero type 3 (El-Seedy *et al.*, 2000). *B. abortus biovar 7* was isolated from camels (Refai, 2003). ICA, Rapid *Brucella* Ab Kit is a chromatographic immunoassay proved to be simple, accurate, rapid, does not require specialized training or equipment and economical for the detection of *Brucella* antibody. A novel concept of Immunochromatographic (ICA) assay which is a simplified version of ELISA make it used as confirmatory test (Montasser *et al.*, 2012).

The present work designed for isolation and identification of *Brucella* strain by using PCR, elucidate the Prevalence of brucellosis among camels from some localities in Egypt. Moreover, evaluation of the diagnostic efficiency of the commonly used serological tests which including BAPA, Modified RBT, TAT and MRT as screening test and confirmation of positive sera using Riv. test & ICA.

2. MATERIAL AND METHODS

2.1-samples:

During a period from April 2016 to May 2017 a number of 271 blood samples were collected from camels of both sexes (Table1) different ages (Table 2) were used in this study from different localities in Egypt for serum separation to be used for serological diagnosis, 30 milk samples were collected from milking she-camels for Milk Ring Test (MRT) and 21 Tissue specimen and lymph nodes from slaughtered camels in Cairo and Giza abattoirs for bacteriological examination (Table 3).

2.2-Antigens:

Brucella abortus antigen for BAPAT, mRBPT, TAT, MRT and Riv.T were obtained kindly from the Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt.

2.3- Methods:

a. *Serological tests:* Serum samples were tested by different serological tests: modified Rose Bengal plate test (mRBPT) according to Blasco, (1994).Buffered acidified plate antigen test (BAPAT), Tube Agglutination Test (TAT), Milk Ring Test (MRT), Riv. T and ICA acc. to Alton, *et al.*, (1988).
b. *Immunochromatographic assay (ICA)* (Rapid *Brucella* Ab Test), from Quicking Biotech Co Ltd. No. 1998, China was applied for Vet. Use Only (Cat No.: W81085), As *Brucella*-specific antigen a

LPS extract from a solid culture of *Brucella abortus* strain 1119-3 was used.

c. *Bacteriological examination and Isolation* according to FAO/WHO (1986).

d. *Polymerase Chain Reaction (PCR)* according to QIAamp DNA mini kit instructions

They have specific sequence and amplify a specific product Table (4).

3. RESULTS

The obtained results revealed that the prevalence of *Brucella* reactors among 271 camels by screening serological tests were 26 (9.5%), 24 (8.8%) and 21 (7.7) using (BAPAT), (mRBPT), and (TAT) respectively as Table (5) which showed the highest percentage of *brucella* infection by BAPAT than that mRBPT and 23 (8.5%), 25(9.2%) by using Riv. Test &ICA as confirmatory tests as Table (6). All milk samples were Negative by MRT 0/30(0%).

1. ICA test results were read by visual inspection for staining of C band and T band

Positive: The presence of both C band and T band, no matter T band is clear or vague.

Negative: Only clear C band appears.
Invalid: No colored band appears in C zone, no matter whether T band appears.

2. Bacteriological examination revealed 5/21 isolates (23.8%) as Table (7) all 5 isolates were identified biochemically as *Brucella abortus*.

3. Results of Polymerase Chain Reaction (PCR): revealed 5 isolates with molecular size 498 bp which identified as Br.abortus, DNA as Table (8),

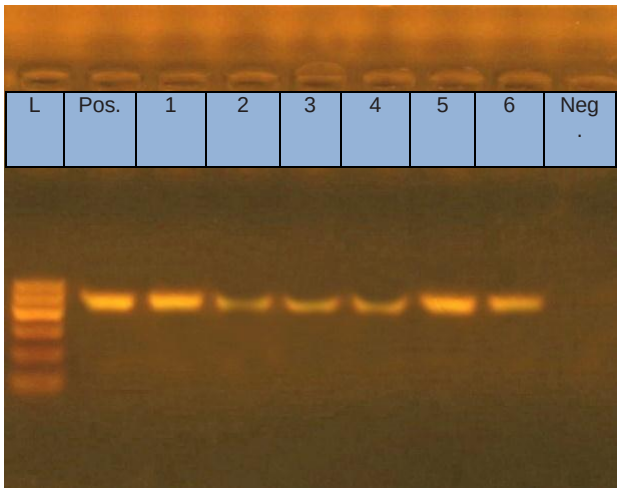

Fig. (1): B. abortus (498 p): Agarose gel electrophoresis of PCR amplification of Br.abortus extracted DNA, Lane L: 100-600bpDNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 1; positive control .Lane 2:6 *Br. abortus* (positive).

Table (1) Number of blood and milk samples collected from different governorate as follows:

locality	No. of examined camel		
	Total no. examined	females	males
Cairo(Al Basatin abattoir)	100	30	70
EL Sharkyia	65	50	15
El Behira	50	25	25
Matroh	50	48	2
Giza(Al Warrak abattoir)	6	1	5
Total	271	154	117

Table (2) Number of examined camels from different localities according to age:

locality	No. of examined camel		
		≤4-5 years	>4-5 years
Cairo (Al Basatin abattoir)	100	50	50
EL Sharkyia	65		65
El Behira	50		50
Matroh	50		50
Giza(Al Warrak abattoir)	6		6
Total	271	50	221

Table (3) Number of collected tissue and L.N from slaughtered camels:

Governorate	L.N				Tissue				
	Supra mammary	Retro-pharyngeal	inguinal	mesenteric	uterine	testes	Umbilical cord	spleen	liver
Cairo abattoir	2	1	4		1	2	1	2	
Warrak abattoir	1		3	1		1		1	1
	3	1	7	1	1	3	1	3	1
Total	13				8				

Table (4): Oligonucleotide primers sequences

Target gene	Target	Primers sequences	Amplified segment (bp)	Reference
1S711 Brucella generic specific gene	*B. abortus*	1S711-specificPrimer TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT *B. abortus*-specific Primer GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC	498	Bricker and Halling, 1994
	B. melitensis	1S711-specificPrimer TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT *B. melitensis*-specific Primer AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA	731	

Table (5): Seroprevalence of camel brucellosis from different localities using screening tests:

Test	BAPAT		mRBPT		TAT	
Total No. of examined samples	Positive		Positive		Positive	
	No.	%	No.	%	No.	%
271	26	9.5	24	8.8	21	7.7

*the percentages were calculated according to the total number of examined samples

Table (6): Percentage of camel brucellosis from different localities using confirmatory test (Rivanol test and ICA):

Test	Riv. T		ICA	
Total No. of examined samples	Positive		Positive	
	No.	%	No.	%
271	23	8.5	25	9.2

*the percentages were calculated according to the total number of examined samples

Table (7): Trial for isolation and identification of *Brucella* isolated from sero positive slaughtered camels:

Examined tissue	No.	+ve	*% of +ve
Inguinal L.N	7	2	9.5%
Supra-mammary L.N	3	2	9.5%
retropharyngeal L.N	1	0	0
Mesenteric L.N	1	0	0
Uterine L.N	1	0	0
Umbilical cord	1	0	0
testes	3	1	4.8%
Liver	1	0	0
Spleen	3	0	0
Total No. of isolates	21	5	23.8%

*the percentages were calculated according to the total number of isolates/ 21

Table (8): Results of Polymerase Chain Reaction (PCR):

Sample code	sample	Results	
		B. abortus	*B. melitensis*
1	Positive control	+	+
2	Testicles	+	-
3	Superficial inguinal L.N 2	+	-
4	Superficial inguinal L.N 1	+	-
5	Supra mammary L.N 1	+	-
6	Supra mammary L.N 2	+	-

4. DISCUSSION

Camel brucellosis can be encountered in all camel rearing countries with exception of Australia. High animal and herd prevalences have been reported from numerous countries, which not only pose a continuous risk for human infection, but also increase the spread of infection through uncontrolled trade of clinically inconspicuous animals (Sprague *et al*, 2012). Brucellosis in Egypt is still one of the most serious problems facing animal production (Sayed, *et al.*,2010). The economic impact of brucellosis on camels can be estimated on the basis of losses due to morbidity and mortality and by estimating

treatment costs (McDermott and Arimi, 2002) . Moreover, camels are not known to be primary host of *Brucella* organisms, but they are susceptible to both *B. abortus* and *B. melitensis* (Cooper,1991). In Butana area, Eastern Sudan, where camels are reared together with cattle, sheep and goats, (Agab, *et al.,*1994) isolated many strains of *B. abortus* from lymph nodes of camels serologically positive for brucellosis.

In the present study, the prevalence of *Brucella* among camels by different serological tests revealed the percentage of positive reactors camels were 26 (9.5%), 24 (8.8%), 21 (7.7%), 23 (8.5 %) and 25(9.2%). using BAPAT, mRBPT, TAT, Riv.T, and ICA respectively as Tables (5) and (6) which agree with (Hegazy, *et al.,* 1998) in Egypt who examined 500 slaughtered female camels for brucellosis. A total of 45 (9%) camels were positive by RBPT and disagree with (El-Sawalhy, *et al.,* 1996) who examined 500 camels at different abattoirs of Sharkia and Kaluobia governorates and the sero prevalence were 14%, 11.6%, 7%, 4.4%, 2.93% and 2.29% using BAPAT , TAT, RBPT , Riv.T, MET and cELISA respectively. While (Horton, *et al.,*2014) who stated that blood samples were collected from domestic and imported livestock slaughtered at the Muneeb abattoir in central Egypt Antibodies against *Brucella spp*. in 12 (8%) cattle, one (1%) buffalo, seven (4%) sheep, and one (10%) camel.

Lateral flow assay (LFA) could be ideal as a field rapid screening test for developing countries and rural settings, suitable for large- scale screening or presumptive test. Moreover, the high sensitivity and specificity of LFA allows its use as a confirmatory test in combination with RBPAT as a screening assay (Lobna *et al.,*2014) also Tharwat El-Shemey (2014) stated that the immuno-chromatographic brucellosis test (ICT) is rapid, card-based test for detection of antibodies directed against *Brucella abortus* antigen. ICT has 94.44% sensitivity and 100% specificity in cattle.

Culturing from 21 tissue samples and L.N collected from slaughter house revealed 5 positive isolates 5/21 (23.8%), all isolated strain identified biochemically and by Polymerase Chain Reaction (PCR) with 498 bp and revealed predominance of *Brucella abortus* in all isolates (5)100% which agree with (Musa *et al*., 2008) In a subsequent abattoir survey of apparently healthy camels, six animals were seropositive, of the six seropositive slaughtered camels, five were shown to have lymph nodes (prescapular and supramammary) infected with *brucella* (*Brucella melitensis* biovar 3, two camels; *Brucella abortus* biovar 6, three camels). and disagree with (El-Sayed *et al.,*2017) who isolate *Brucella melitensis* biovar 3 from stomach content of aborted camel fetus.

The obtained *Brucella* isolates from positive reactors slaughtered camels were examined with PCR for detection and identification of *Br.abortus* and *Br.melitensis*. The obtained results revealed 5 isolates with molecular size 498 bp which identified as *Br.abortus,* DNA. Which agree with Alshaikh *et al*., (2007) who use Polymerase chain reaction (PCR) to

diagnose brucellosis in camels using primers that amplified the IS711 locus, 8 samples revealed DNA amplification at around 500 bp, indicating that the organism involved was *Brucella abortus*.

5. Conclusion& Recommendations

In times of increasing human population, more resources are needed and camels are an ideal asset. The economic impact of brucellosis on camels can be estimated on the basis of losses due to morbidity and mortality and by estimating treatment costs. In turn, we have to improve the laboratory diagnostic methods against brucellosis in camels, ICA or (LFA) is highly sensitive, accurate and specific diagnostic assay since it directly detects antibodies of *Brucella* organism so is considered as rapid confirmatory test. Camels must be tested or vaccinated against *Brucella* before introduced to Egypt and also before slaughter and the positive camels must be executed. More hygienic measurers must be taken to protect veterinarians, butchers and workers from infection. National program and surveillance plane recommended to be done in cooperation with neighboring countries and also support of international organization to control zoonotic diseases for public health.

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