

Application of Three Diagnostic Serologic Techniques to Detect of Dromedary Camel's Brucellosis

Mithal Kareem Abass Al-Hassani¹ Hasanain A.J. Al-Gharban²

Luma foad Manher³

Dept. of Biological Science, Coll. of Education, Al-Qadisiyah University¹
Dept. of Internal & Preventive Medicine / Coll. of Veterinary Medicine/ Wasit University²

College of Enviromental Science AL-Qassim Green University³

Email: Mithal.al-hassani@qu.edu.iq¹ hghirban@uowasit.edu.iq²

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Abstract

This study was aimed to evaluate the prevalence of brucellosis in camel by using of Rose-Bengal test (RBT), Standard-tube agglutination test (STAT) and competitive Enzyme-Linked immunosorbent assay (c-ELISA) for first time in Iraq. From some regions of Al-Qadisiyah governorate, a totally of 148 camels from both sexes were submitted for collection of blood samples, and the overall results showed that 8.78%, 4.1%, and 12.84% of camels were seropositives by RBT, STAT and c-ELISA, respectively. In addition, the cross-classification results have been discussed as follow: by both c-ELISA and RBT, the seropositive result was 7.43%, while, 5.41% by c-ELISA and 1.35% by RBT, only. Whereas, the positive result was 4.1% by both c-ELISA and STAT, while, 8.78% positive camels by c-ELISA, only. In regarding to sex factor, the positive results in female camels were 9.92%, 4.96% and 13.22%; while in males, they were 3.7%, 0% and 11.11% by RBT, STAT and c-ELISA. In association to age factor, ≥ 4 years group were reported 13.85%, 7.69% and 18.46%, while in 1-4 years group, they were 4.82%, 1.2% and 8.43% seropositive camels by RBT, STAT and c-ELISA, respectively. Statistically, the significant difference ($P \leq 0.05$) was reported among the total positive results and within the cross-classification results for applied serological tests. Also, the female camels were showed an infection rate more than males by RBT and STAT, whereas, both sexes were at a similar level of infection by c-ELISA. As well as, > 4 years group were revealed on a high prevalence rate than 1-4 years group by all assays.

Keywords: Brucellosis, Dromedary Camel, Serologic techniques, Diagnostic, Application
Physiology Classification QR1-502-75-9905

Introduction

Brucellosis is a greatly zoonotic worldwide disease resulted by a Gram-negative bacterium, *Brucella* that classified at a risk group III by the WHO (1). The disease can cause substantial economical losses in livestock and severe or chronic debilitating illness in humans which obliged for a long period of treatment with a combination of antibiotics to get the cure (2). Although, the genus of *Brucella* has ten pathogenic species that infecting many primary and secondary hosts of domestic and wildlife animals as well as humans; but in camels, two species (*B. abortus* and *B. melitensis*) are implicated to be more frequent isolates (3). As reported by many previous studies, camels are very susceptible to brucellosis like other animals and their infection depends on the *Brucella* species that persist, abundantly, in their habitats (4). From public health view point, *Brucella* is considered to be as an occupational pathogen that affecting directly on slaughter house workers, butchers, as well as veterinarians (5). Camels can be infected, mostly, by ingestion during the grazing upon infected pasture or consuming of feedstuff or water supplies that contaminated by discharges, fatal membranes, and aborted fetuses of diseased animals; as well as to venereal and congenital transmission (6, 7).

There is neither a single diagnostic test available by which a bacterium can be identified as *Brucella* nor any signs and

symptoms are specific for brucellosis. Therefore, the combination of more one diagnostic method is necessary for accurate diagnosis (8). Although, the reliable diagnosis can only made by direct isolation and detection of causative pathogen by culture, this procedure is complicated and constitute a large infection risk (9). Also, this method is impractical for regular screening of large population and cannot be used as criteria for control and eradication of disease (10, 11). Many serological techniques were applied for the evaluation of specific *Brucella*-antibodies (IgG and IgM) in humans and animals, among which they have a key role in the rapid and proper diagnosis (12). Rose-Bengal test and Standard-tube agglutination test are used, largely, for long period as the tests of field with reasonable sensitivity and an acceptable, to some extent, specificity for monitoring of infection because of their easily, safety to use and quit inexpensively (13). ELISA is, relatively, simple to perform and provided a practical advantage in detecting a prevalence of infection. In addition, it's posses a very high sensitivity and specificity that can reach to 100%, ready to be given an automated results, rapidly and accurately (14).

The aims of this study were carried out to evaluate the prevalence of camel brucellosis by application of three serological assays including the Rose-Bengal test (RBR), Standard-Tube agglutination test (STAT) and ELISA on serum samples of camels.

and from some areas related to Al-Qadisiyah governorate, a totally of 148 camels with more than one year of age and from both sexes were submitted for this study. From each one, 10 ml of blood samples were drained from jugular vein by using a

II. Serological assays

1. Rose-Bengal test (RBT)

According to manufacturer's instruction (Bioveta/Czech), the serum samples and antigen bottle were left at 37°C for 30 minutes. The antigen bottle was shaken, gently, to ensure that suspension was homogenous. Then, 30µL of antigen with 30µL of serum samples were placed into each square of a plate, mixing with a spreader, rotated manually for four minutes, and the results was considered as positive for any degree of agglutination. With each set of test sera, the positive and negative controls were applied.

2. Standard-Tube Agglutination Test (STAT)

Materials and Methods

I. Samples collection

During the period from May to July/2016

vacationer tube without anticoagulant, which centrifuged at 3000 rpm for 15 minutes for serum separation. The serum samples were packaged in a numbered 1 ml eppendorfs and kept at -20°C until be used.

According to manufacturer's instruction (Bioveta/Czech), the serum samples and an antigen bottle were left at 37°C for 30 minutes. The doubling dilution of tested serum had been done, starting from 1:5, with 0.5% of phenol saline in ten tubes. The antigen bottle was shaken, well, and 50µL of antigen was added to each tube and mixed by rolling in between the palms. At the same time, five tubes were prepared positive and negative controls by using of an antigen in 0.5% phenol saline. All test and controls tubes were incubated in a water bath at 37°C for 24 hours, and the received results of serum samples were compared with control tubes as detailed in (Table 1).

Table (1): Interpretation of STAT results

Control Tubes	0.5% Phenol Saline	Antigen	Agglutination Degree	Interpretation
Tube 1	-	2 ml	No - Agglutination	Negative
Tube 2	1.25 ml	0.75 ml	25% - Agglutination	Positive
Tube 3	1.50 ml	0.50 ml	50% - Agglutination	
Tube 4	1.75 ml	0.25 ml	75% - Agglutination	
Tube 5	2 ml	-	100% - Agglutination	

3. Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA)

The qualitative camel *Brucella* antibody IgG (B-IgG) ELISA kit (Cat. No: MBS059216 / MyBioSource / Canada) was used in this study. The serum samples and ELISA kit were left at 37°C for 30 minutes, and

according to manufacturer's instructions, the assay was performed and read at a wave length of 450 nm optical density (OD) by using of a microplate photometer ELISA-reader (BioTek-USA). The test validity and results interpretation were discussed as in (Table 2).

Table (2): Test validation and interpretation of ELISA results

1	Test Validity	Positive Control	OD \geq 1
		Negative Control	OD \leq 0.15
2	Critical CUT OFF	Criteria	Average of Negative Controls + 0.15
3	Results	Positive Samples	OD Samples \geq Critical CUT OFF
		Negative Samples	OD Samples < Critical CUT OFF

III. Statistical analysis

All data were tabled and analyzed by a computerized Microsoft Excel Word (2013) and IBM/SPSS programs (v.23). Descriptive statistics and Chi-square (X^2) test were used

Results

In (Table 3) that dealt with an application of three serological tests on a totally of 148

to detect the significant differences ($P \leq 0.05$) between the results of serological assays that applied in this study (15).

serum camel's samples, and revealed on 13 (8.78%), 6 (4.1%), and 19 (12.84%) positive

camels by RBT, STAT and c-ELISA, respectively.

Table (3): Total results of serological assays on 148 camels' serum samples

Serological Assays		Seropositives	Seronegatives
1	RBT	13 (8.78 %) ^b	135
2	STAT	6 (4.1%) ^c	142
3	c-ELISA	19 (12.84%) ^a	99

Variations in small letters, vertically, referred to significant differences at $P \leq 0.05$

The cross-classification of positive and negative results by the adopted serological tests was discussed as follow: by both c-ELISA and RBT, there were a totally of 11 (7.43%) positive samples and 127 (85.81%)

negative samples; while, 2 (1.35%) samples were positive by RBT and negative by c-ELISA, and 8 (5.41%) samples were positives by c-ELISA and negatives by RBT (Table 4).

Table (4): Cross-classification results of c-ELISA and RBT in total 148 camels

Results of c-ELISA	Results of RBT		Total
	Positive	Negative	
Positive	11 (7.43%) ^{Ba}	8 (5.41%) ^{Bb}	19
Negative	2 (1.35%) ^{Bb}	127 (85.81%) ^{Aa}	129
Total	13	135	148

Variations in large and small letters, horizontally and vertically, referred to significant differences at $P \leq 0.05$

Also, by both c-ELISA and STAT, there were 6 (4.1%) and 129 (87.16%) positive and negative samples, respectively; while,

13 (8.78%) samples were positive by c-ELISA and negative by STAT (Table 5).

Table (5): Cross-classification results of c-ELISA and STAT in 148 camels

Results of c-ELISA	Results of STAT		Total
	Positive	Positive	
Positive	6 (4.1%) ^{Ba}	13 (8.78%) ^{Ab}	49
Negative	0 (0%) ^{Bb}	129 (87.16%) ^{Aa}	99
Total	6	142	148

Variations in large and small letters, horizontally and vertically, referred to significant differences at $P \leq 0.05$

In (Table 6) that dealt with an association of obtained risk factors (sex and age) with the seropositive results by the applied

serological assays (RBT, STAT, and c-ELISA).

Table (6): An association of risk factors to seropositive results by the applied assays

Risk Factor	No.	RBT (13)	STAT (6)	c-ELISA (19)
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1	Sex	Male	27	1 (3.7%) ^{Bb}	0 (0%) ^{Cb}	3 (11.11%) ^{Ab}
		Female	121	12 (9.92%) ^{Ba}	6 (4.96%) ^{Ca}	16 (13.22%) ^{Ab}
2	Age	1- 4 Years	83	4 (4.82%) ^{Bb}	1 (1.2%) ^{Cb}	7 (8.43%) ^{Ab}
		≥ 4 Years	65	9 (13.85%) ^{Ba}	5 (7.69%) ^{Ca}	12 (18.46%) ^{Aa}

Variation in large and small letters, horizontally and vertically, between groups of each factor referred to significant differences at a level of ($P \leq 0.05$)

In (Figure 1): In regarding to sex factor, the seropositive results in 27 males were 1 (3.7%), 0 (0%), and 3 (11.11%), while in 121 females, 12 (9.92%), 6 (4.96%), and 16 (13.22%) by RBT, STAT, and c-ELISA

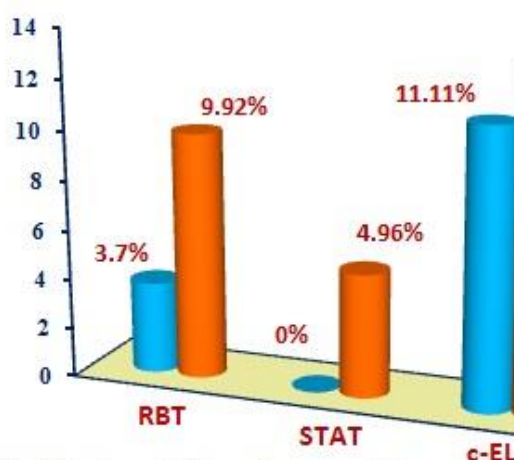


Fig. (1): Association of seropositive results

In (Figure 2): In regarding to age factor, the positive results in 83 camels with (1-4) years age old were 4 (4.82%), 1 (1.2%), and 7 (8.43%), whereas in 65 camels with ≥ 4 years age old, the positive results were 9 (13.85%), 5 (7.69%), and 12 (18.46%) by RBT, STAT, and c-ELISA, respectively.

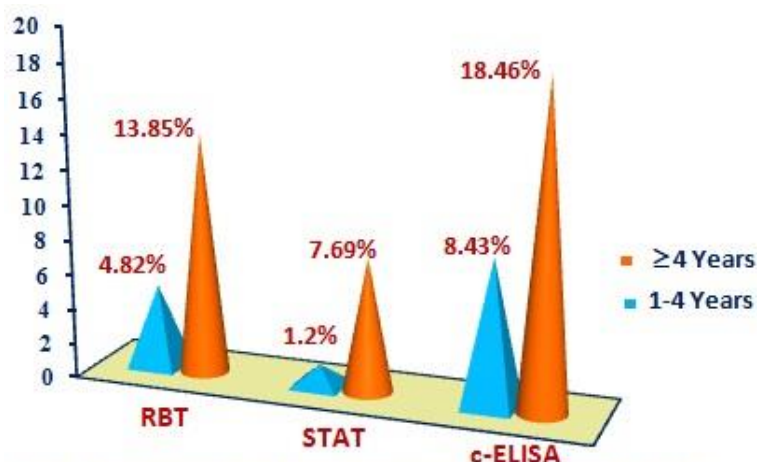


Fig. (2): Association of positive results with Age factor

Discussion

Although, most countries have been attempted to provide the sources for eradicating brucellosis from the domestic animals populations, it's still widespread as an agriculture disease, and several cases of human brucellosis have been reported every year in areas where the disease has not eliminated in livestock (16). The economic and public health impact of *Brucella* remain of particular concern as the disease poses a barrier for trading of animals and animal's products that represented a public health hazard causing an impediment to free-animals movement (17). In this study, the seroprevalence of antibodies to camel brucellosis was estimated by using three serological techniques, for first time in Iraq, which revealed on 8.78% by RBT, 4.1% by STAT, and 12.84% by ELISA (Table 3). Also, the cross-classification results of ELISA with RBT (Table 4) and ELISA with STAT (Table 5) were showed a significant difference between their seropositive and seronegative camels. In Iraq, by using of RBT only, the prevalence of *Brucella* antibodies was evaluated to be 3.03% (18) and 6.73% (19), while globally, the seroprevalence of camel brucellosis reported a greatly different data between regions,

countries and the diagnostic methodologies, which had been ranged from 0% to 51% (19, 20, 21). Several studies have been done worldwide by using of many serological techniques to evaluate the prevalence of camel brucellosis and to explain the caused that lead to the variable results between these techniques (23, 24). Therefore, the selection of a gold standard test was represented as one of the most important and difficult steps in studies that dealt with diagnostic tests. In brucellosis, a gold standard test is a bacterial isolation of *Brucella* spp. from body fluids or tissue specimens, which required a long cultivation period and, in often, resulted on an unsuccessful of culture (25, 26). Some studies believed that the findings of a true gold standard have not achievable, and for this cause, the finding of diagnostic tests that able to completely differentiate between the infected and healthy individuals is impossible. It's quite obvious that the different criteria in detection of infected animals could affect the study results (27).

In regarding to RBT that developed as a diagnostic method having the rapidity, simplicity in its performance, and low cost with absence of infection's risk to laborers; though, the disadvantages of this test involved a high possibility of false negative results due to presence of blocking antibodies, in addition to the low specificity particularly in endemic areas due to presence of high antibody prevalence in the healthy population (28, 29). In respecting to STAT that represented as the first developed serological test applied in diagnosis of brucellosis in dependently on bacterial antigen agglutination under neutral pH, the test is slow in an antibody-antigen reaction, required high skills and has a very low specificity (30). In countries where there was a strategy for using of vaccination, a considerable lack of specificity in results of RBT and STAT that might be occurred due les with a high sensitivity and specificity varies between (92-100%) and (90-99%), respectively (34). In addition, the assay was characterized by its capability for differentiating of a vaccine antibody response from actual infections, and the false-negative or false-positive results might be resulted due to inequalities between the commercial ELISA kits or presence of rheumatoid factors that occurred in some

to a cross-reaction (31). Hence, in endemic areas, the diagnosis of brucellosis must be supported with a high sensitivity and specificity technique to avoid or reduce the faults. However, as reported by several previous studies, ELISA demonstrated a high specificity where compared to other diagnostic techniques, especially, in endemic regions or countries (32, 33). Also, the technique is excellent in screening of large populations for *Brucella* antibodies ((IgM and IgG) and for differentiation between acute and chronic phases of the disease. Also, it's the test of choice in complicated cases under a high clinical suspicion, particularly, when other tests displayed on negative results (23). The kit of ELISA that used in this study, has a smooth *Brucella* lipopolysaccharide (LPS) as antigen for detection of anti-*Brucella* in serum samp chronic cases of suppurative brucellosis (35).

In relation to sex factor, RBT and STAT were reported that the female camels were having a high seropositive prevalence in their *Brucella* antibodies than males, while, c-ELISA had been showed that the seroprevalence were without significant differences between both sexes (Table 6, Figure 1). Several studies have been reported

that the female camels were more susceptible to brucellosis than males and this could be attributed to the fact that the females have more physiological stresses than males or due to presence of erythritol in females, only (36, 37). Other studies were reported an equal distribution of *Brucella* antibody between both sexes (38, 39). Even though, the number of breeding males kept by the pastoralists in the camel herds of the present study was small on which random sampling method was applied and this predictably bias the statistical analysis.

Although, all age groups were susceptible to brucellosis, young animals tend to be more resistant to brucellosis than adult that have an increasing in their susceptibility with increasing of age and this could be interpreted to the facts that the sex hormone and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase with age and sexual maturity (40, 41). With advancing of age, immunity against various infections could be depressed due to different causes such as stress factors and frequent exposures to diseased pathogens (42).

In conclusion, c-ELISA was demonstrated a high effectiveness in detection of *Brucella* antibodies than RBT and STATA, and can be used as a test of gold standard for

diagnosis of camel brucellosis. Also, the study was reported that age and sex might play an important role in an incidence of infection among camels.

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استعمال ثلاث تقنيات مصلية

تشخيصية لأكتشاف داء بروسيلال جمال العربية

مثال كريم عباس الحسني¹ حسنين عبد الحسين جعفر الغريان² لمى فؤاد منحر³

قسم علوم الحياة / كلية التربية / جامعة القادسية¹

فرع الطب الباطني والوقائي البيطري / كلية الطب البيطري / جامعة واسط²

جامعة القاسم الخضراء / كلية علوم البيئة³

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الخلاصة

هدفت الدراسة الحالية الى تقييم الانتشار المصلي لداء البروسيلال جمال باستخدام اختبار وريدية البنغال (RBT) ، اختبار تلازن الانبوب المعياري (STAT) واختبار الفحص المناعي المرتبط بالانزيم التنافسي (c-ELISA) لأول مرة في العراق . من بعض المناطق في محافظة القادسية ، خضع اجماليا 148 جمل من كلا الجنسين الى جمع عينات الدم وظهرت النتائج الكلية بان 8.78% ، 4.1% ، و 12.84% من الجمال كانت موجبة مصليا بواسطة RBT ، STAT ، و c-ELISA ، على التوالي . اضافة الى ذلك ، تم مناقشة نتائج التصنيف المتقاطعة كالتالي : بواسطة كلا من c-ELISA و RBT ، كانت النتيجة الموجبة مصليا 7.43% ، بينما كانت 5.41% بواسطة c-ELISA و 1.35% بواسطة RBT ، فقط . في حين ، كانت النتيجة الموجبة 4.1% بواسطة كلا من c-ELISA و STAT ، و 8.78% بواسطة c-ELISA فقط . فيما يتعلق بعامل الجنس ، كانت النتائج الموجبة في اناث الجمال 9.92% و 4.96% ، و 13.22% ؛ بينما في الذكور ، كانت 3.7% و 0% ، و 11.11% بواسطة RBT و STAT و c-ELISA ، على التوالي . فيما يتعلق بعامل العمر ، سجلت مجموعة ≤ 4 سنوات 13.85% و 7.69% و 18.46% جمال موجبة مصليا ، اما في مجموعة 4-1 سنوات ، كانت 4.82% و 1.2% و 8.43% جمال موجبة مصليا بواسطة RBT و STAT و c-ELISA ، على التوالي . احصائيا ، سجلت الاختلافات الاحصائية ($P \leq 0.05$) بين النتائج الموجبة الكلية وفيما بين نتائج التصنيف المتقاطعة للاختبارات المصلية المستعملة . كذلك ، اظهرت الاناث معدل اصابة اعلى من الذكور في RBT و STAT ، في حين كان كلا الجنسين عند مستوى اصابة متشابه بواسطة c-ELISA . اضافة الى ذلك ، اظهرت مجموعة ≤ 4 سنوات معدل انتشار اعلى من مجموعة 4-1 سنوات بواسطة كل الاختبارات .

الكلمات المفتاحية : داء البروسيلال جمال عربية ، تقنيات مصلية ، تشخيصية ، استعمال