Serological Diagnosis Of Camel Brucellosis At Sharkia Governorate, Egypt

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ABSTRACT

The present study was carried out on 210 camels serum samples collected from a various abattoirs at Sharkia Governorate to clarify the prevalence of the brucellosis among camels during the period from June 2012 to August 2013 in addition to find the most reliable diagnostic method. In this investigation, serological tests on 210 camel sera using Buffered Acidified Plate Antigen Test (BAPAT), Rose Bengal Plate Test (RBPT), Rivanol Test (Riv.T), Complement Fixation Test (CFT) and Immunochromatographic Assay (ICA) to evaluate traditional and recent tests which used for the diagnosis.

The obtained results were 7.6%, 6.7%, 6.2%, 6.2% and 6.7% using BAPAT, RBPT, Riv.T, CFT and ICA respectively .The sensitivity percent of different serological tests were 95.45%, 87.75%, 90.90%, 8885% and 92.15% for BAPAT, RBPT, Riv. T., CFT and ICA respectively. While their specificity percent were 99.27%, 99.61%, 99.80%, 99.80% and 99.75% for the same test respectively.

ICA could be recommended as a rapid screening test, easily performed, sensitive and high specific and as confirmatory test for diagnosis of brucellosis in camels. So, ICA the method of choice when testing animals in remote areas, nomadic and other migratory population. Thus study throw a spot light to include camels in the national program for control and eradication of brucellosis in Egypt on the base that where brucellosis exists in stock animals.

INTRODUCTION

Animal brucellosis has been recorded in Egypt since 1939 and the prevalence of serological reactors on limited surveys has varied from one survey to another *(1)*.The prevalence of the disease is related to the management practices of the farm and the ability of a country to finance prevention or control. Early detection, control and elimination of reactors are important consideration for the control of brucellosis *(2)*.The prevalence of serological reactors among camels was 9%,9% and 8.4% by RBPT, BAPAT and CFT respectively*(3)*while, it was 8.74%, 9.53% and 9.26%,by using RBPT, BAPAT and Riv.T respectively *(4)* but, it was 5%by using RBPT*(5)*.

Classical serological tests are routinely used for the diagnosis of brucellosis. These tests (RBPT and BAPAT tests) deactivate IgM, which is responsible for non specific reactions. These tests are highly sensitive, but have low specificity and are ineffective in discriminating vaccinal antibodies from those by infection, but these tests still now are a rapid easily applied field tests *(1)*.

As complicated assays may not available in many places, more recently, the convenience and speed of the test have been achieved by a novel concept of immunochromatographic assay(ICA) which is a simplified version of ELISA *(6)*.

ICA assay does not require specific expertise, equipment or electricity, and test kits may be kept in stock without the need for refrigeration, thus, making the assay a very useful one for poor resource countries including most African Countries and migratory herds/flock *(7)*. ICA test used for detection of antibodies against *Br. abortus* in sera samples, the ICA had 94.44% sensitivity and 100% specificity versus RBPT as a gold standard *(8)*.

This study was formulated to study the prevalence of brucellosis in camels.and diagnosis of brucellosis by using traditional and recent serological tests and evaluate these tests which used for the diagnosis.

MATERIAL AND METHODS

Animals: A total of 210 adult mature and apparently healthy camels in different localities at Sharkia governorate during the period from June 2012 to August 2013.These camels were admitted for slaughter in different abattoirs.

Serum samples: Blood serum samples were collected from210 investigated camels.

The serum stored at -20ºC in the deep freezer till used for different serological tests *(9)*.

Antigens for serological tests: *Brucella abortus* antigen for BAPAT,RBPT and Riv.T were obtained kindly from the Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt.

Brucella abortus concentrate for CFT was kindly offered by the National Veterinary Service Laboratories (NVSL), Ames, USA.

Immunochromatographic assay (ICA): Brucella ICA manufactured according to *(10)*. Product Feature (Kit component) obtained kindly from Brucellosis Research Department, Animal Health Research Institute, Dokki, Giza, Egypt.

Serological tests: Serum samples were tested by different serological tests. Buffered acidified plate antigen test (BAPAT). Rivanol test (Riv.T.) and Complement fixation tests were carried out according to *(9)*, while, Rose Bengal plate test (RBPT) according to *(11)* and ICA procedures according to *(12)*.

Interpretation of Immunochromatographic Assay

Negative: The presence of only one purple color band within the result window indicates a negative result .

Fig. 1. Negative ICA

Positive: The presence of two color bands ("T" band and "C" band) within the result window, no matter which band appears first, indicates a positive result.

Fig. 2. Positive ICA

Invalid: If the purple color band is not visible within the result window after performing the test, the result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be re-tested.

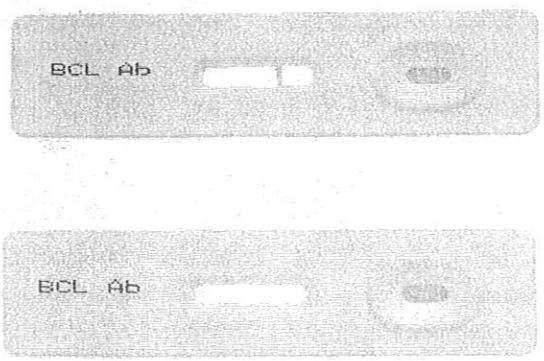

Fig. 3. Invalid ICA

Determination of sensitivity% and specificity% of serological tests: Sensitivity and specificity% of different serological tests were determined according to ***(9)***.

RESULTS

Results of serological tests: The prevalence of brucellosis among camels was 7.6%, 6.7%, 6.2%, 6.2% and 6.7% by BAPAT, RBPT, Riv. T, CFT and ICA respectively as shown in Table (1) and Figure (4)

Table 1. Prevalence of brucella reactors camels by different serological tests

Test / Total No.of examined camels	BAPAT Positive		RBPT Positive		Riv. T Positive		CFT Positive		ICA Positive	
	No.	%	No.	%	No.	%	No.	%	No.	%
210	16	7.6	14	6.7	13	6.2	13	6.2	14	6.7

CFT titer :-1/16 -1/256.

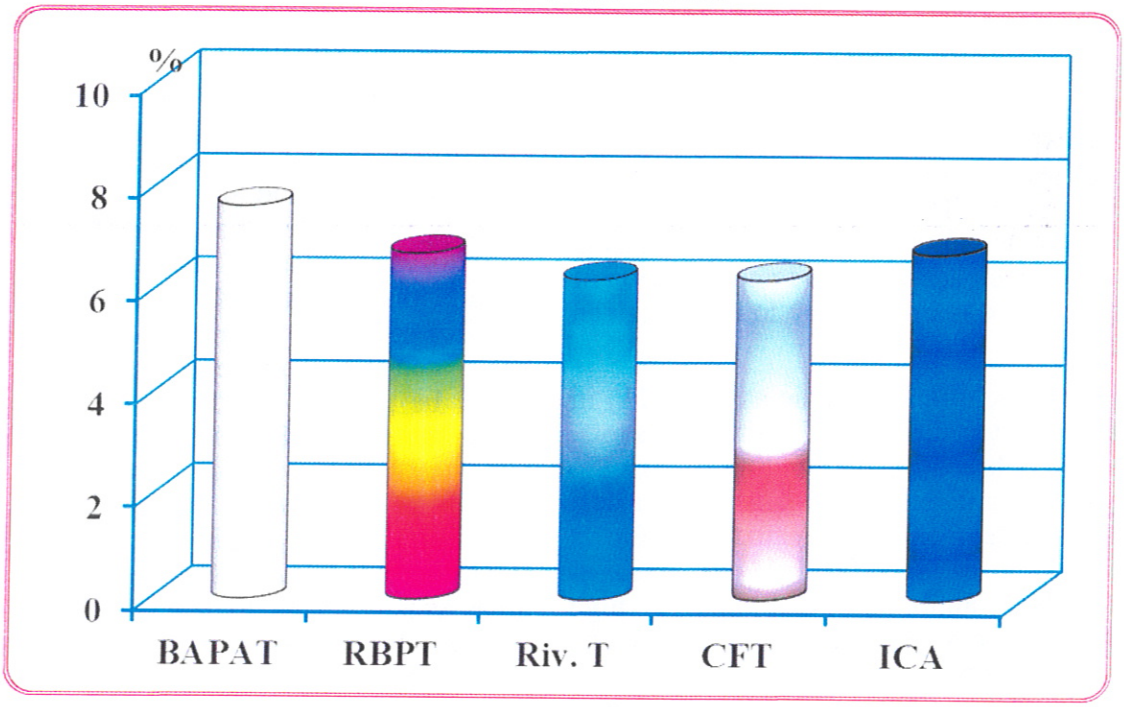

Fig. 4. Prevalence of brucella reactors among camels by different serological tests

Results of sensitivity and specificity of different serological tests

The sensitivity of different serological tests was 95.45%, 87.75%, 90.90%, 88.85% and 92.15% for BAPAT, RBPT, Riv. T,CFT and ICA respectively. While the specificity of the same tests was 99.27%, 99.61%, 99.80%, 99.80% and 99.75%). It was found as shown in Table (2).

Table 2. Results of sensitivity %and specificity % of different serological tests

Test	Sensitivity %	Specificity %
BAPAT	95.45	99.27
RBPT	87.75	99.61
Riv. T	90.90	99.80
CFT	88.85	99.80
ICA	92.15	99.75

DISCUSSION

In order to control and eradicate brucellosis from livestock animals it is very important to establish an appropriate serological method for diagnosis of brucellosis in the enzootic areas ***(13)***. In the present study, the prevalence of brucella reactors among camels by different serological tests as shown in Tables (1) and Figures (4) reveled that camels showed the highest percentage of brucella infection (7.6%) using BAPAT, while 6.7% by RBPT. In camels, the prevalence of Brucella recorded cases reached 7.9% ***(14)***, 11.37% ***(15)***, 10.7% ***(16)*** and 9.53% ***(4)***.

The results of CFT (6.2 %) revealed that the majority of positive CFT titers were 1/16 or higher (up to 1/256) indicating very active infection status where the rate of transmission of the disease among animals was high. This reflected some shortage in the disease control process as well as poor hygienic measures. Rivanol test in this study detected (6.2%) positive reactors. The results of the test showed detection of large number of positive reactors this due to precipitation of IgM and detection of IgG only this was in harmony to that obtained by ***(17,18)***.

Immunochromatographic assay (ICA) in this study detected (6.7%) positive reactors. The assay is based on the binding of specific antibodies to antigen imminobilised on a test strip (cellulose membrane matrix). It allows the detection of specific IgM as well as specific IgG antibodies and that a high sensitivity is assured for all stages of the disease as reported by ***(19)***. The results also agree with ***(6,20)*** who recorded that the speed of ICA make it available for the rapid presumptive test which can replace RBPT in brucellosis control programs.

In this study, the sensitivity % and specificity % of different serological tests have been calculated. Table (2) illustrated that the sensitivity was 95.45%, 87.75%, 90.90%, 88.85% and 92.25% for BAPAT, RBPT, Riv. T., ICA and CFT respectively while the specificity was 99.27%, 99.61%, 99.80%, 99.80% and 99.75% for the same tests respectively. The highest sensitivity in this study was that of BAPAT (95.45%). Complement fixation test showed the highest specificity (99.80%) followed by RBPT (99.61%). Such finding indicate that the suitability of BAPAT and RBPT for screening purposes. The obtained results agreed with those reported by ***(12)*** who reported that conventional agglutination tests have good sensitivity but their lack of specificity and occurrence of false positive serological results make a specific test necessary. The same results were obtained by ***(22)*** who stated that infected animals with brucellosis could be screened with a test such as RBPT or BAPAT and confirmed with ICA as a more specific test.

The sensitivity % of ICA (92.15%) and specificity% (99.75%) were higher than RBPT. Such findings indicate the suitability of the test to be used to replace RBPT in brucellosis control program advantages that make it the method of choice when testing animals in remote areas, nomadic and other migratory population. Practical advantages include that the use of the ICA does either requires specific training, expertise, electricity nor expensive equipment, that assay devices may be stored without the need for refrigeration and that test results are obtained almost instantaneously and by visual inspection with naked eye. Immunochromatographic assays may have important advantages when testing in remote areas where access to laboratory facilities is problematic and when testing animals from rural, nomadic and other mobile migratory farmers. Moreover ICA is a sensitive and highly specific test.

Comparing the results obtained by the different serological tests employed in this study, it is clear that no single test is cable to identify all positive cases of brucella infected animals due to variation in sensitivity and specificity of different serological tests as a result of difference in sensitivity of these tests to different antibody classes and variation in antibody classes in the examined sera. The obtained results assured that different

serological tests could detect infected animals with brucella with a different sensitivity and specificity. CFT is still superior one among the employed tests as it gave the higher balance of sensitivity and specificity than other tests used. Whereas BAPAT was found to be an effective test for initial screening of brucellosis in farm animals as it was more sensitive for detection of brucella infected animals than RBPT beside that, it is simple, easy and inexpensive test.

The prevalence of brucella infection in camels in this study may be attributed to lacking of a national program for camel brucellosis eradication including periodical testing and slaughtering of reactors, absence of vaccination program for camels according to Egyptian Field Strain and which proved with imported camels and several exposure of this species to brucella infection than other animals.

CONCLUSION

From the results of the present study, it is concluded that:

1. The buffered acidified plate antigen test is an effective test for initial screening of brucellosis in farm animals
2. No single serological test could be identify all brucellosis infected camels.
3. Complement Fixation test is still the superior one among the employed tests as it gave the highest balance of sensitivity and specificity.
4. Immunochromatographic assay (ICA) could be recommended as a rapid screening or presumptive test in brucellosis control programs as it easily performed ,high sensitive and specific. Also as confirmatory test so it can replaces the RBPT .
5. Thus study throw a spot light to include camels in the national program for control and eradication of brucellosis in Egypt as the base that where brucellosis exists in stock animals.

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الملخص العربى

التشخيص السيرولوجى لمرض البروسيلا فى الجمال بمحافظة الشرقية ، مصر

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تم تجميع ٢١٠ عينة سيرم من الجمال من عدة مجازر مختلفة بمحافظة الشرقية وذلك للتعرف على معدل الاصابة بمرض البروسيلا فى الجمال. وتم فحصها سيرولوجيا بواسطة اختبار الانتيجين الحمضى المتوازن والروزبنجال الأحمر الحمضى السريع وتحليل المناعة الكروماتوجرافى بالإضافة إلى إجراء الإختباراتالتأكيدية الأخرى مثل اختبار التبثيث المكمل واختبارالريفانول ، ومقارنتهم باختبار التحليل المناعى الكروماتوجرافى وذلك الوصول إلى أنسب طريقة لتشخيص المرض ودراسة التحليل الإحصائى لحساسية وخصوصية هذه الاختبارات التشخيص.

وقد أظهرت النتائج بالاختبارات السيرولوجية المختلفة نسبة أصابة بمعدل ٧,٦٪ ، ٦,٧٪ ، ٦,٢٪ ، ٦,٢٪ و ٦,٧٪ وذلك باستخدام إختبارالأنتيجين الحمضى المتوازن وإختبار الروز بنجال وإختبارالريفانولوإختبار التثبيت المكمل وتحليل المناعة الكروماتوجرافى على التوالى. بينما كانت نسبة الحساسية لكل من إختبارالأنتيجين الحمضى المتوازن وإختبار الروزبنجال وإختبارالريفانول وإختبار التثبيت المكمل وتحليل المناعة الكروماتوجرافى٩٥,٤٥٪ ، ٨٧,٧٥٪ ، ٩٠,٩٠٪ ، ٨٨,٨٥٪ و ٩٢,١٥٪على التوالى أما بالنسبة للخصوصية فكانت ٩٩,٢٧٪ ، ٩٩,٦١٪ ، ٩٩,٨٠٪ ، ٩٩,٨٠٪ و٩٩,٧٥٪ لنفس الاختبارات السابقة على التوالى.

ومن نتائج هذه الدراسة نوصى بالآتى:

١- استعمال إختبار الانتيجين الشريحى الحمضى كإختبار مسح أولى لتشخيص المرض لشدة حساسيته فى الكشف عن الحيوانات المصابة بالإضافة إلى أنه اختبار بسيط وسريع وغير مكلف. مع تأكيد نتائجه بنتائج اختبارتثبيت المكمل التى أكدت الدراسة كفاءته العالية فى معرفة الحيوانات المصابة.

٢- استعمال التحليل الكروموتوجرافى كاختبار مسح لتشخيص المرض وأيضاً كاختبارتأكيدى فمن الممكن استخدامه ليحل محل اختبار الروزبنجال الأقل فى الحساسية والخصوصية كما أنه يمكن استخدامه كبديل لكل من الاختبارات المعقدة عالية التكلفة وعالية الجهد وذلك لشدة حساسيته وسرعته وسهولة إجرائه وكفاءته وانخفاض تكلفته كما أنه من الممكن استخدامه فى المناطق الريفية والنائية دون الاحتياج إلى الامكانيات الخاصة بالمعامل لإجرائه.