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Preparation of buffered acidified plate antigen from *Brucella abortus* strain 19

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A B S T R A C T

Brucellosis is one of the major zoonotic infections worldwide, continues to be a public health problem resulting in significant morbidity and economic losses. Accurate diagnosis must include laboratory tests that allow the direct *Brucella* isolation or indirect detection of antibodies. Practical serological tests are routinely used for the diagnosis of brucellosis by using specific antigens. In the present work, the used buffered acidified plate antigen (BAPA) was prepared from *B. abortus* biovar 1 strain S1119-3 according to the USDA SOPs. For this purpose, a total of 4100 bovine sera from five farms located in different governorates were screened for brucellosis. The effectiveness of prepared BAPA from strain 19 was compared with the standard BAPA and BCT antigens prepared from *B. abortus* strain 99. Evaluation was done by using a panel of known dilutions of the OIEISS (Office International des Epizootie International Standard Serum) as the international reference standard serum. There were no significant differences in results of the BAPA antigen prepared from strain 19 and the conventional antigen prepared from strain 99. The results concluded that, the vaccinal strain 19 can be used instead of strain 99 to prepare the BAPA antigen.

Key word: *Bovine brucellosis, Brucella abortus strain 19, buffered acidified plate agglutination antigen.*

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1. INTRODUCTION

Brucellosis is a worldwide zoonotic infectious disease caused by Gram-negative bacteria from the genus Brucella. 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 with a range between 16.5% to 23% in cattle and 7% to 10% in buffaloes. In 2002, the prevalence of positive serological reactors was 3% in cattle and 2%in buffaloes. Diagnosis of Brucellosis depends on bacteriological isolation from abortion material, udder secretions or tissues collected at post-mortem. Presumptive diagnosis can be made by assessing specific cell-mediated or serological responses to Brucella antigens (OIE, 2012). Among the rapid agglutination assays for brucellosis surveillance are the Buffered Acidified Plate antigen (BAPA) and the Rose -Bengal plate tests. The Rose -Bengal or brucellosis card test (BCT) is rapid qualitative one dilution plate agglutination at acidic pH of 3.65±0.05 attained by lactate buffered phenol saline in which inactivated Rose –Bengal stained *Brucella abortus* cells are suspended and

standardized. The test brings about agglutination of the non-agglutinogenic IgG_1distinctive of the longstanding Brucella infection (Alton *etal.,* 1988). This adds up for more sensitivity and specificity to the test.It was used after the presumptive BAPA test, the Rose –Bengal plate test (RBPT) reduces the number of positive samples demanding confirmation. All of these tests are recommended for international trade in the (OIE Terrestrial Manual 2016). It is prospective that smooth Brucella abortus strain 19 (S-19) as a vaccinal strain, could be used as substitute for S.99 in to preparation of antigen.

The present work aimed to evaluate the specificity and sensitivity of buffered acidified plate agglutination antigen prepared from *B. abortus* S19 against corresponding antigens traditionally prepared from strain 99 used for diagnosis of bovine brucellosis in cows of non-vaccinated history and its relation to the sensitivity of Rose Bengal test.

2. MATERIALS AND METHODS

2.1. Brucella strains:

Smooth *Brucella abortus* biovar 1 strain 99(S-99) (Weybridge, England) and smooth *Brucella abortus* biovar 1 strain 19(S-19(CZ Veterinaria, S.A., Spain) were used.

2.2. Brucella antigens for serologic tests:

2.2.1. Conventional Rose Bengal and Buffer Acidified Plate antigens (BAPA) prepared from *B.abortus* (S-99) were supplied by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (VSVRI). It prepared according to international rules regarding pH and PCV (Alton *et al.,* 1988 and OIE, 2016).

2.2.2. Buffer Acidified Plate antigen (BAPA) prepared from B. abortus

Strain 19 (S-19) was locally prepared according to Alton *et al.,* (1988).

2.3. Serum samples:

A total 4100 serum samples were collected from non-vaccinated cows against Brucellosis from five dairy farms in different governorates in Egypt as list in details in (Table 1). History of the farms reveled of these farms were of no history of vaccination against brucellosis. All examined animals did not show abortion or retained placenta. Animals which have positive results of serological tests were slaughtered immediately, in each farm sera were tested 2-7 times with various intervals as shown in (Table 2 to 6).

2.4. Serologic tests (Alton et al. 1988)

Rose Bengal (RB) test was performed, following the procedure described by Alton *et al.* (1988), through mixing of 25 ul of sera and 25 ul of the antigen. The plates were shaken for 4 min and any agglutination that appeared within this time was recorded as a positive reaction.

BAPA test was carried out, mixing 80 ul of sera and 30 ul of the antigen. The plates were shaken for 8 min and any agglutination that appeared within this time was recorded as a positive reaction

3. RESULTS

Results are summarized in Tables (3) to (11) and Figures (1) to (4).Tables (4) to (8) reveal the overall performance of all acidified plate agglutination test (conventional RBA, conventional BAPA and S.19 BAPA) in each of five farms of cattle. The performance characteristics included the positive and negative result of each agglutination tests, numbers of these tests, numbers of samples in each one and Length of time interval between each test.It respect of rose Bengal test, sensitivity and specificity of the two BAPA antigens preparations were calculated on (http://vassarstats.net/clin1.html) as shown in table (3).

Table (1): Epidemiologic data of dairy farms included in the current study

Farms	Animals		Governorate	Numbers of sera collected	Management Nutration Biosafety level
	Breed	Age			
First	Dairy	36 month	Beni -Seuf	1000	Medium
Second	Dairy	36 month	El-Beheira	600	Good
Third	Holstein Friesian	2 years	Alexandria Desert Road	1800	Very Good
Fourth	Dairy	36 month	Wadi El Natrun	350	Good
Fifth	Dairy	36 month	Hosh Essa El-Beheira	350	Medium
Total				4100	

Table (2): Total number of tested serum samples collected from cows in different farms in Egypt.

Farms	Total Numbers of animals	No.of Serological Tests	No. of Samples in Every Tests	Time Interval Between Every Test
1st farm	1000	6 tests	1st test (1000)samples 2nd test (976) samples 3rd test (967) samples 4th test (961) samples 5th test (952) samples 6thtest (945) samples	Every 21 days
2nd farm	600	6 tests	1st test (600)samples 2nd test (599) samples 3rd test (599) samples 4th test (599) samples 5th test (597) samples 6thtest (597) samples	Most of tests apply every 3months except the period interval between the 3rd and 4th is 6 months.
3rd farm	1800	7 tests	1st test 1800 2nd test 1785 3rd test 1775 4th test 1770 5th test 1767 6th test 1763 7th test1761	21 days Interval Between Every Test
4th farm	350	2 tests	1st test 350samples 2nd test 350samples	3 months interval between 2 tests
5th farm	350	3 tests	1st test 350samples 2nd test 348 samples 3rd test 347 samples	3 months interval between every tests
Total	4100	24 tests		

Table (3): Calculation of sensitivity and specificity with respect of gold standard test.

		Gold standard test (cft) Positive	Negative	Total
Test under evaluation	Positive	A	B	A+b
	Negative	C	D	C+d
Total		A+c	B+d	N (264)

Sensitivity= total positive/ total samples
Specificity= D/D+B
False positive= A/A+B
False negative= C/C+D
Relative Sensitivity= A/A+C
True positive (Positive Predictive Value) = A/A+B
True negative (Negative Predictive Value) = D/C+D

3.1 Sero-diagnostic efficacy of antigens (conventional RBA, conventional BAPA and S.19 BAPA) prepared from *B.abortus* biovar 1 (S.99, S.19) used for agglutination tests.

Table (4): Performance of the conventional Rose Bengal test, (S.99) BAPA, (S.19) BAPA in the first farm.

No. of serological tests	Results of Agglutination Tests							
No. of samples in each one	S.99 BAPA		S.19BAPA		Rose Bengal Antigen		Total	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
First serological test 1000 samples	22	978	22	978	24	976	24	976
21 days interval between first and second test								
Second serological test 976 samples	9	967	9	967	9	967	9	967
21 days interval between second and third test								
Third serological test 967 samples	6	961	6	961	6	961	6	961
21 days interval between third and fourth test								
Fourth serological test 961 samples	9	952	9	952	9	952	9	952
21 days interval between fourth and fifth test								
Fifth serological test 952 samples	7	945	7	945	7	945	7	945
21 days interval between fifth and sixth test								
Sixth serological test 945 samples	5	940	5	940	5	940	5	940
Total	58	942	58	942	60	940	60	940

Table (5): Performance of the conventional Rose Bengal test, (S.99) BAPA, (S.19) BAPA in the second farm.

No. of serological tests	Results of Agglutination Tests							
No. of samples in each one	S.99 BAPA		S.19BAPA		Rose Bengal Antigen		Total	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
First serological test 600 samples	1	599	1	599	1	599	1	599
3months interval between first and second test								
Second serological test 599 samples	0	599	0	599	0	599	0	599
3months interval between second and third test								
Third serological test 599 samples	0	599	0	599	0	599	0	599
6months interval between third and fourth test								
Fourth serological test 599 samples	2	597	2	597	2	597	2	597
3months interval between fourth and fifth test								
Fifth serological test 597 samples	0	597	0	597	0	597	0	597
3months interval between fifth and sixth test								
Sixth serological test 597 samples	0	597	0	597	0	597	0	597
Total	3	597	3	597	3	597	3	597

Table (6): Performance of the conventional Rose Bengal test, (S.99) BAPA, (S.19) BAPA in the third farm.

No. of serological tests No.of samples in each one	Results of Agglutination Tests							
	S.99 BAPA		S.19BAPA		Rose Bengal Antigen		Total	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
First serological test 1800 samples	15	1785	15	1785	15	1785	15	1785
21 days interval between first and second test								
Second serological test 1785 samples	9	1776	9	1776	10	1775	10	1775
21 days interval between second and third test								
Third serological test 1775 samples	5	1770	5	1770	5	1770	5	1770
21 days interval between third and fourth test								
Fourth serological test 1770 samples	3	1767	3	1767	3	1767	3	1767
21 days interval between fourth and fifth test								
Fifth serological test 1767 samples	3	1764	3	1764	4	1763	4	1763
21 days interval between fifth and sixth test								
Sixth serological test 1763 samples	2	1761	2	1761	2	1761	2	1761
Seventh serological test 1761 samples	1	1760	1	1760	1	1760	1	1760
Total	38	1762	38	1762	40	1760	40	1760

Table (7): Performance of the conventional Rose Bengal test, (S.99) BAPA, (S.19) BAPA in the fourth farm.

No. of serological tests No.of samples in each one	Results of Agglutination Tests							
	S.99 BAPA		S.19BAPA		Rose Bengal Antigen		Total	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
First serological test 350 samples	0	350	0	350	0	350	0	350
3months interval between first and second test								
Second serological test 350 samples	0	350	0	350	0	350	0	350
Total	0	350	0	350	0	350	0	350

Table (8): Performance of the conventional Rose Bengal test, (S.99) BAPA, (S.19) BAPA in the fifth farm.

No. of serological tests No.of samples in each one	Results of Agglutination Tests							
	S.99 BAPA		S.19BAPA		Rose Bengal Antigen		Total	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
First serological test 350 samples	2	348	2	348	2	348	2	348
3months interval between first and second test								
Second serological test 348 samples	1	347	1	347	1	347	1	347
3months interval between second and third test								
Third serological test 347 samples	0	347	0	347	0	347	0	347
Total	3	347	3	347	3	347	3	347

Table (9): Comparison between the diagnostic importance of strain 19 BAPA and conventional antigens (Rose Bengal Antigen and strain 99 BAPA) by using 4100 serum samples from naturally infected cattle in different farms.

Diagnostic Tests	Plate Agglutination Tests using antigens prepared from					
Strains	Strain 99				Strain 19	
Agglutination Tests	RBT		BAPA		BAPA	
Samples	Numbers of positive reactor	Numbers of negative reactor	Numbers of positive reactor	Numbers of negative reactor	Numbers of positive reactor	Numbers of negative reactor
First Farm	60	940	58	942	58	942
Second Farm	3	597	3	597	3	597
Third Farm	40	1760	38	1762	38	1762
Fourth Farm	0	350	0	350	0	350
Fifth Farm	3	347	3	347	3	347
Total	106	3994	102	3998	102	3998

Table (10): Total Positivity Percent in all Farms of all plate agglutination tests.

Diagnostic Tests	Plate Agglutination Tests using antigens prepared from					
Strains	Strain 99				Strain 19	
Agglutination Tests	RBT		BAPA		BAPA	
Total percent of Positivity and Negativity	Positivity %	Negativity %	Positivity %	Negativity %	Positivity %	Negativity %
First Farm	6%	94%	5,8%	94,2%	5, 8%	94,2%
Second Farm	0,5%	99,5%	0,5%	99,5%	0,5%	99, 5%
Third Farm	2,22%	97,77%	2,1%	97,88%	2,1%	97,88%
Fourth Farm	0%	100%	0%	100%	0%	100%
Fifth Farm	0,85%	99,14%	0,857%	99,14%	0,857%	99,14%
Total	2,6%	97,41%	2,5%	97,51%%	2,5%	97,51%

Table (11): Results of BABA tests against Rose Bengal test as a Gold Standard test.

Tests	Antigens	Rose Bengal test	
		+ve	-ve
Buffered acidified plate agglutination test	Buffered acidified plate antigens prepared from S	102	0

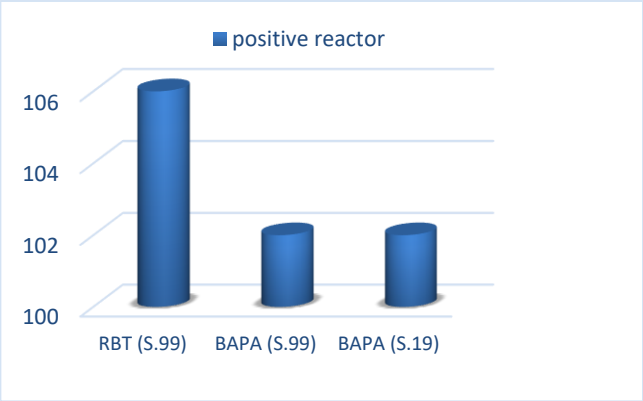

Figure (1): Total positive reactor of Plate Agglutination Tests in all farms.

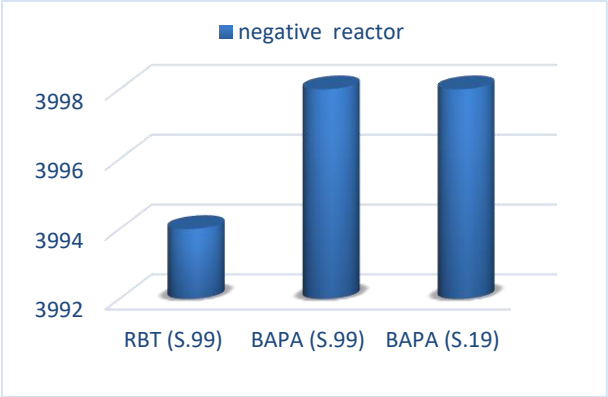

Figure (2): Total negative reactor of Plate Agglutination Tests in all farms.

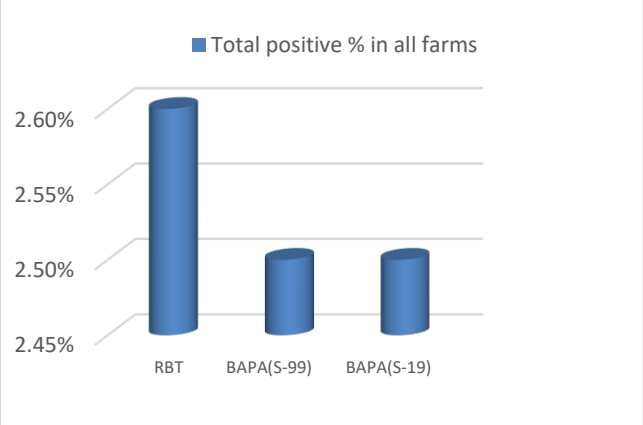

Figure (3): Total positivity percent of Plate Agglutination Tests in all farms

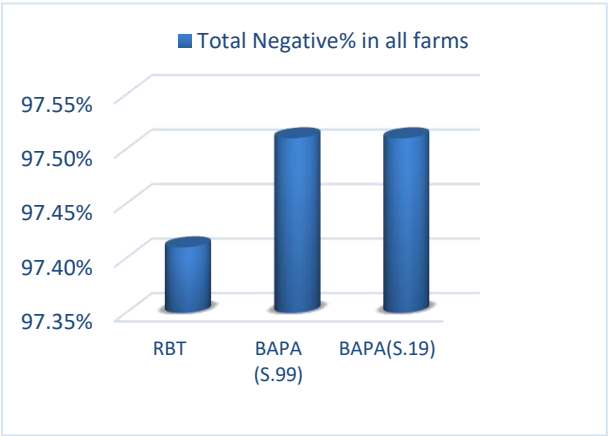

Figure (4): Total Negativity percent of Plate Agglutination Tests in all farms.

4. DISCUSION

The Rose Bengal plate agglutination, Buffered Acidified Plate Agglutination, complement fixation and indirect ELISA tests are usually recommended for screening flocks and individual animals for brucellosis. The complement fixation test is the only test prescribed for confirmation and international trade, but other tests as the Agar Gel Perception test, immunodiffusion and competitive ELISA, are useful for confirmation purposes. All over the world, all agglutination tests use the *B. abortus* strain 99 or 1119 antigens although in some cases different strains were used Erganis *et al.* (2005) used *Brucella melitensis* and *Brucella suis* S2 antigens. The Buffered Antigen Plate Agglutination test (BPAT) has been widely used (Angus and Barton, 1984 and Nielsen and Yu, 2010) and also the Rose Bengal test (RBT) (Nielsen and Yu, 2010 and Morgan *etal.*, 1969).

Among the rapid agglutination assays for brucellosis surveillance and rapid field diagnosis are the Buffered Acidified Plate antigen (BAPA) and the Rose -Bengal plate tests. Both tests are well known as a pilot (screening), cheap, effective and rapid test for the diagnosis of brucellosis. It can be performed with the minimum of facilities, and the end result is read by the naked eye. Because of its apparent simplicity, high level of standardization of antigen and accuracy of reading is needed (Erganis *et al.*, 2005).

After the presumptive diagnosis by BAPA test, using the Rose –Bengal plate test (RBPT) reduced the number of positive samples demanding confirmation. Each of these tests is recommended for international trade (OIE, 2016). It is prospective that smooth *Brucella abortus* (S-19) as a vaccinal strain, could be used as a substitute for S.99 in order to prepare Brucella antigens (Alton *et al.,* 1988).

In this study 4100 bovine serum were tested against all prepared rapid slide agglutination antigens. Rose Bengal test prepared from *B. abortus* S99 was considered as a gold standard test to determine the sensitivity and specificity of tested BAPA antigens in absence of bacteriological isolation. Statistics in this study was considered the 95 % confidence intervals. In this study, No satisfactory differences were observed in specificity and sensitivity of tested BAPA antigens prepared from different brucella reference strains with constant PCV and pH.

With respect to Rose Bengal test and according to results in table(10)and figure(1), sensitivity of different antigens preparations were calculated on (http://vassarstats.net/clin1.html)

with 95% Confidence Intervals (CI) as shown in (table3). Sensitivity of the Rose Bengal test was 2.59% where the sensitivity of the slide agglutination test using the two tested BAPA antigens was equal to each other (2.49%). Relative sensitivity, specificity, true positive and truce negative of both antigens were 96.2%, 50%, 2.49% and 97.51% respectively and prevalence of the diseases in tested farms were 1.31%. The results, in this study revealed that there are no significant differences between conventional antigens and these prepared from brucella abortus biovar 1 strain 19 as recommended by Alton et al., (1988).

5. CONCLUSION

According to the diagnostic performance parameters obtained under conditions of this study, It is concluded that the Buffer Acidified Plate antigen (BAPA) prepared from Brucella abortus biovar 1 strain19(S-19) gave similar results to that antigen prepared from *Brucella abortus* (Weybridge) (S99) on sera collected from the naturally infected farms . So, *Brucella abortus* strain19 (S-19) and *Brucella abortus* strain 99 are indistinguishable for the preparation of Brucella antigens. So, *Brucella abortus* strain19 (S-19) could be used as replacement of *Brucella abortus* strain 99 to prepare the Buffer Acidified Plate antigen (BAPA) for large scale production of such antigens in Egypt.

6. REFERANCES

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