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Sero-diagnostic efficacy of antigens prepared from B. abortus and B. melitensis used for agglutination tests and IELISA

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SUMMARY

In this study, 120 bovine and ovine serum samples were collected and tested for brucellosis with Rose Bengal test (RBT), modified Rose Bengal test (mRBT), buffered acidified plate test (BAPT) using conventional Rose Bengal antigen (RBA) and buffered acidified plate antigen (BAPA) and antigens prepared from *B. melitensis* (the main cause of brucllosis in Egypt) strain 16-M. Indirect ELISA was used for testing the same serum samples using 4 different coating antigens which were S-LPS and OMP antigens prepared from 16-M strain and S-LPS and OMP prepared from *S19* strain. There were some differences between the results of conventional Rose Bengal and BAPA antigens and Rose Bengal and BAPA antigens prepared from *B. melitensis* 16-M strain. In the same time, there were no characteristic differences in results of Indirect ELISA

INTRODUCTION

Bovine brucellosis is a disease that causes high economic losses and is hazardous to human health worldwide. Among 176 countries that respond to questionnaires distributed through the FAO, WHO and OIE organizations concerning disease occurrence and control in 1987, 140 countries were still infected (Davidson *et al.,* 1990).

Animal burcellosis 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 (Refai, 2002 and Refai *et al.,* 1990).

Accurate diagnosis must include laboratory tests that allow the direct (isolation) or indirect (serology) demonstration of *Brucella* (OIE 2008). Also PCR test is highly sensitive confirmatory test

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(Vaid *et al.*, 2004) which can be used to differentiate between species and biovar (Bricker and Halling, 1994, Garcia-Yoldi *et al.*, 2006 and OIE, 2008). Classical serological tests are routinely used for the diagnosis of brucellosis. These tests (Rose Bengal and BAPA 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 produced by infection, but these tests till now are a rapid easily applied field tests. As *B. melitensis* is the main cause of brucellosis in Egypt (Refai 2002 and Refai *et al.*, 1990) so. It has long been recognized that the LPS is the major antigen of the surface of smooth brucellae and the relevant molecule in classical diagnostic tests for brucellosis (Alonso-Urmeneta *et al.*, (1998).

The aim of this study was to use antigens prepared from *B. melitensis* as trials to reach the best specific and sensitive antigens for diagnosis of brucellosis in Egypt

MATERIALS AND METHODS

Strains:

B. melitensis biovar 1(16-M) (Weybridge-England)

Serum:

120 Serum samples were used in this study which were collected from Egyptian cattle and sheep and Somalian sheep naturally infected (*B. melitensis* biovar 3 were previously isolated from lymph nodes samples) with *brucella*, cattle vaccinated with *RB51* (1-3.4 X 10^{10} CFU/ dose. S/C) (CZ Veterinaria S.A., Spain), cattle vaccinated with *S19* (6-10 X 10^9 CFU/ dose.S/C)(CZ Veterinaria S.A., Spain), sheep vaccinated with Rev-1vaccine (1-3 X 10^9 CFU/ dose. S/C) (CZ Veterinaria S.A., Spain), sheep experimentally inoculated with killed *E. coli* O: 157 and brucellosis free animals. These samples were tested serologically by modified Rose Bengal test (mRBT) (Blasco *et al.*, 1994a), RBT, slide agglutination test and IELISA (Alton *et al.*, 1988).

Antigens:

Conventional Rose Bengal antigen and buffer acidified plate antigen (BAPA) prepared from *B. abortus S99* were supplied by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (VSVRI) while Rose Bengal antigen and buffer acidified plate antigen (BAPA) prepared from *B. melitensis* 16-M strain were prepared according to Alton *et al.*, 1988 and used for Rose Bengal (RBT), modified Rose Bengal (mRBT) and slide agglutination (BAPT) tests. IELISA was done according to Alton *et al.*, 1988 using OMP (prepared from *B. abortus S19* and *B. melitensis* 16-M according to (Chart 1994) and LPS (prepared from *B. abortus S19* and *B. melitensis* 16-M according to (Alton *et al.*, 1988 and Plackett *et al.*, 1976) as coating antigens.

Preparation of B.abortus S-19 and B. melitensis 16-M Outer membrane proteins (OMP) antigens (Chart 1994):

B.abortus S-19 were grown and harvested from the roux bottles, which contain Brucella agar. Bacterial suspension was sonicated after dispensing in 20 ml volumes of chilled 25 mM Tris-HCl (PH 7.4). Any residual whole bacteria were sedimenated by centrifugation at 5000 g/30 min/4 °C. bacterial envolpe (outer and inner membrane proteins) was sedimenated by centrifugation at 45000/ 4 °C for 1 hour. Outer membrane proteins was separated from inner membrane proteins by solubilization the inner membrane proteins using sarcosyl with incubation 24 hours at 37 °C. Outer membrane proteins were sedimenated by centrifugation at 45000/ 4 °C for 1.

Preparation of B.abortus S-19 and B. melitensis 16-M Lipopolyscchide (HSE) antigens (Plackett *et al.*, 1976):

B.abortus S-19 were grown and harvested from the roux bottles, which contain Brucella agar. To each bottle, 25 ml. of 0.5 % phenol in D.W. at 4°C were added. The growth was suspended by gentle transverse rocking and filtered through several layers of gauze into weighted centrifuge bottles. The cells were washed 3 times in PBS (PH 7.2) at 20.000 rpm for 20 minutes. The pellet was weighted and resuspended in D.W. (400 ml/100 g cells). The suspension was autoclaved at 121°C for 20 minutes, centrifuged at 20.000 rpm for 20 minutes. The supernatant fluid was collected and centrifuged again to be clarified. To the supernatant fluid 1/15 of its volume 4M NaOH was added and heated at 56°C for an hour. The supernatant fluid was cooled and neutralized with glacial acetic acid to PH 7.0 using PH meter. The supernatant fluid was mixed with 4 volumes of ethanol and left for 24 hours. The flocculent formed was collected by centrifugation at 20.000 rpm for 20 minutes. The flocculent was resuspended in a small volume of D.W. and dialyzed against D.W. to remove salt and ethanol.

RESULTS AND DISCUSSION

Brucellosis is a zoonotic disease that has both public health and economic importance. The prevalence of these diseases is related to the management practices of the farm and the ability of a country to finance prevention or control programs. The diagnosis of brucellosis is performed through the isolation of the bacteria and/or serological tests. The Rose Bengal Plate Test (RBPT) and the Complement Fixation Test (CFT) are the most widely used tests for the serological diagnosis of sheep and goats brucellosis. They are also the official tests for international trade (OIE, 2008). To date, the use of the RBPT as a screening test for diagnosis of *Brucella melitensis* infection in sheep and goats was recommended by the joint

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FAO/WHO expert committee on brucellosis (Blasco et al., 1994a, Blasco et al., 1994b, Diaz-Aparicio et al., 1994 and Mikolon *et al.*, 1998). RBPT is a cheap, dependable and giving rapid results method and is frequently used for the diagnosis of brucellosis and detection of its prevalence (Ergans *et al.*, 2005).

The RB test was developed more than 20 years ago for the diagnosis of bovine brucellosis. An important problem affecting the sensitivity of the RB test concerns the standardization of the antigen. The European Union regulations require antigen suspensions in lactate buffer at pH 3.65 ± 0.05 that are able to agglutinate at a dilution of 1:47.5 (21 IU/ml) of the International Standard anti-*B. abortus* serum (ISaBS) but give a negative reaction at a dilution of 1:55 (18.2 IU/ml) of the same serum (Council Directive 64/432/EEC, 1964). These standardization conditions, which seem to be suitable for the diagnosis of *B. abortus* infection in cattle (MacMillan, 1990), limit the sensitivity of the test resulting in reduced performance for the diagnosis of *B. melitensis* infection in sheep (Blasco *et al.*, 1994a, 1994b). This accounts for the relatively low sensitivity of some commercial RB antigens when diagnosing brucellosis in sheep and goats (Falade, 1978, 1983; Blasco *et al.*, 1994a, 1994b) and for the fact that a high proportion of sheep and goats belonging to *B. melitensis*-infected areas give negative results in the RB but positive ones in the CF test (Blasco *et al.*, 1994a). These phenomena have raised serious questions on the efficacy of using the RB as an individual test in small ruminants.

However, if the antigen is standardized differently to give a higher analytical sensitivity, the diagnostic sensitivity is much improved (MacMillan, 1997). Some workers claimed that, at least for sheep, the sensitivity of the RB test can be improved significantly when the antigens are standardized against a panel of sera from several *B. melitensis* culture positive and *Brucella*-free sheep, respectively, or when the volume tested is increased from 25µl to 75µl (Blasco *et al.*, 1994a). RBPT antigens produced from *B. abortus* biovar 1 and 2 strains have A-antigen. However, *B. melitensis* with dominant features causes infections in sheep and goats and has M-antigen (Corbel 1985 and Macmillan 1997). By the fact that brucellosis is mainly caused by *B. melitensis* in sheep and goats (in Egypt mainly *B. melitensis* biovar 3 either in sheep, goats, cattle, baffoles and camels (Refai 2002 and Refai *et al.*, 1990) and since serological test antigens including Rose Bengal antigen are usually prepared from Br. abortus strains so these serological tests may give negative results for brucellosis caused by *B. melitensis* (Ergans *et al.*, 2005).

In this study, the plate agglutinations tests were carried on 120 cattle and sheep sera samples. The BAPT modified RBT (mRBT) and RBT results were expressed as positive or

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negative. The percentage of sera samples reacted positively with RBT using conventional RB antigen and RB antigen prepared from 16-M strain were 53.33% and 51.66% respectively.

mRBT were carried only on serum samples reacted negatively with RBT. The number of serum samples reacted positively with modified RBT using conventional RB antigen and RB antigen prepared from 16-M strain were 16 from 56 negative samples and 20 from 58 negative samples respectively as sensitivity of RB antigens increase when MRBT are carried on although specificity of antigens was decreased.

The percentage of serum samples reacted positively with slide agglutination test using conventional BAPA plate test and BAPA plate test prepared from 16-M strain were 60 % and 60 %, respectively (Table 1,2 and 3). All samples collected from vaccinated cattle and sheep gave same results and differences occurred only in samples collected from naturally infected cattle and sheep. The same antigens were tested against serum of artificially infected sheep with E. coli O: 157 which reacted positively with all antigens (Table (1), (2) and (3) and Chart (1). In this study we cannot evaluate the sensitivity and specificity of the antigens due to the lack of false negative samples and presence of one false positive sample only.

Results of RBT showed that 3 samples of naturally infected animals reacted positively with RB antigen prepared from 16-M strain and negatively with conventional RB antigen either using RBT and mRBT while 5 samples of naturally infected animals were reacted positively with conventional RB antigen and negatively with RB antigen prepared from 16-M strain using RBT but these samples were reacted positively when MRBT was carried on these samples. one sample of naturally infected animals were reacted positively with RB antigen prepared from 16-M strain and negatively with conventional RB antigen using RBT but reacted positively when mRBT was carried on this sample while 1 sample of naturally infected animals were reacted positively with conventional RB antigen and negatively with RB antigen prepared from 16-M strain when RBT and mRBT were carried on.

Results of slide agglutination test using BAPA showed that one of naturally infected samples were reacted positively with BAPA antigen prepared from 16-M strain and negatively with conventional BAPA antigen, while 1 of naturally infected animals were reacted positively with conventional BAPA antigen and negatively with BAPA antigen prepared from 16-M strain.

Conventional antigens and antigens prepared from *16M* strain gave same results in 103 samples with differences only in strength of reaction and speed of appearance of positive reaction which may reach 30-45 seconds (Table 1, 2 and 3).

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From the above results, it was found that there is differences between conventional antigens and those prepared from *16M* strains and this do not agree with (Ergans *et al.*, 2005) who found that Rose Bengal antigen can be prepared from *B. suis* biovar 2 and *B. melitensis* with the same results of conventional Rose Bengal antigen this result were in contrast to (Sahahza *et al.*, 2009) where the conventional antigen were more specific and less sensitive than antigen prepared from 16-M strain.

The large majority of Enzyme Immuno-Assays (EIAs) in use in brucellosis diagnosis are Indirect ELISAs. ELISAs are methods that involve the immobilization of one of the active components on a solid phase, and Indirect ELISAs are those in which the antigen is bound to a solid phase, usually a polystyrene microtitre plate so that antibody, if present in a sample, binds to the immobilized antigen and may be detected by an appropriate anti-globulin-enzyme conjugate which in combination with a chromogenic substrate gives a colored reaction indicative of the presence of antibody in the sample (European commission Scientific committee on Animal health and Animal welfare, 2001).

A wide variety of antigen preparations has been used in the IELISA ranging from whole cells to crude and semi-purified smooth lipopolysaccharide (S-LPS) preparations to polysaccharides and proteins.

Table (1): Results of plate agglutinations tests and IELISA

Samples	Plate agglutination tests using antigens prepared from						IELISA coating antigen			
	S99			*16-M*			LPS		OMP	
	RBT	mRBT	BAPA	RBT	mRBT	BAPA	*S19*	*16-M*	*S19*	*16-M*
1 E. Sheep	++++		++++	++++		++++	+	+	+	+
2 E. Sheep	++++		+++	++++		++++	+	+	+	+
3 E. Sheep	++++		++++	++++		++++	+	+	+	+
4 E. Sheep	++++		++++	++++		++++	+	+	+	+
5 E. Sheep	++++		++++	++++		++++	+	+	+	+
6 E. Sheep	++++		++++	++++		++++	+	+	+	+
7 S. Sheep	++++		++++	++++		++++	+	+	+	+
8 S. Sheep	-	-	-	-	-	-	-	-	-	-
9 S. Sheep	-	-	++++	++		++++	+	+	+	+
10 S. Sheep	-	++	++++	-	++	++++	+	+	+	+
11 S. Sheep	++		++++	+		++++	+	+	+	+
12 S. Sheep	++++		++++	++++		++++	+	+	+	+
13 S. Sheep	+++		++++	+++		++++	+	+	+	+
14 S. Sheep	+++		++++	+++		++++	+	+	+	+
15 S. Sheep	-	-	-	-	-	-	-	-	-	-
16 S. Sheep	++		+	++		++	+	+	+	+
17 S. Sheep	+++		++++	+++		++++	+	+	+	+
18 S. Sheep	++++		++++	++++		++++	+	+	+	+
19 S. Sheep	-	-	-	-	-	-	-	-	-	-
20 S. Sheep	-	-	+	+++		++	+	+	+	+
21 S. Sheep	++++		++++	++++		++++	+	+	+	+
22 S. Sheep	-	-	-	-	-	-	-	-	-	-
23 S. Sheep	+		-	-	-	-	+	+	+	+
24 S. Sheep	-	-	+	-	-	-	+	+	+	+
25 S. Sheep	++++		++++	++++		++	+	+	+	+
26 S. Sheep	+++		++++	+++		++++	+	+	+	+
27 S. Sheep	-	-	-	-	-	-	+	+	+	+
28 S. Sheep	-	-	-	-	-	-	-	-	-	-
29 S. Sheep	+		+++	+		+	+	+	+	+
30 S. Sheep	-	-	-	-	-	-	-	-	-	-
31 S. Sheep	-	-	-	-	-	+++	+	+	+	+
32 S. Sheep	++		++	++		+	+	+	+	+
33 S. Sheep	++++		++	++++		++	+	+	+	+
34 S. Sheep	++++		++	++++		++	+	+	+	+
35 S. Sheep	+		++	+		++	+	+	+	+
36 S. Sheep	+++		++	+++		++	+	+	+	+
37 S. Sheep	-	-	-	-	-	-	-	-	-	-
38 S. Sheep	++		++	++		++	+	+	+	+
39 S. Sheep	-	-	-	-	-	-	-	-	-	-
40 S. Sheep	-	-	-	-	-	-	-	-	-	-
41 S. Sheep	++++		++++	++++		++++	+	+	+	+
42 S. Sheep	+		+++	+		+++	+	+	+	+
43 S. Sheep	-	-	++	-	-	++	+	+	+	+
44 S. Sheep	-	++++	++++	-	++++	++++	+	-	+	+
45 S. Sheep	+		+++	+		+++	+	+	+	+
46 S. Sheep	-	++	+++	-	++	+++	+	+	+	+
47 S. Sheep	-	++	++	-	++	++	+	-	+	+
48 S. Sheep	++++		++++	++++		++++	+	+	+	+
49 S. Sheep	+		+++	++		+++	+	+	+	+
50 S. Sheep	-	-	-	-	-	-	-	-	-	-
51 S. Sheep	-	++	++	-	++	++	+	+	+	+
52 S. Sheep	++		++	++		++	+	+	+	+
53 S. Sheep	++		-	-	++	-	+	-	-	-
54 S. Sheep	-	++	-	-	++	-	+	+	-	-
55 S. Sheep	-	+	+++	-	++	+++	+	+	+	+
56 E. Sheep	-	++	+	-	++	+	+	-	+	+
57 E. Sheep	-	++	-	-	++	-	+	+	+	+
58 E. Sheep	-	-	-	-	-	-	+	+	+	+
59 E. Sheep	+		+	-	++	+	-	-	-	-
60 E. Sheep	++++		++++	++++		++++	+	+	+	+

* Sheep sera vaccinated with Rev-1 vaccine. S. = Somalian. E. = Egyptian

Table (2): Results of plate agglutinations tests and IELISA

Samples	Plate agglutination tests using antigens prepared from						IELISA coating antigen			
	S99			*16-M*			LPS		OMP	
	RBT	mRBT	BAPA	RBT	mRBT	BAPA	*S19*	*16-M*	*S19*	*16-M*
61 E. Sheep sera*	++++		++++	++++		++++	+	+	+	+
62 E. Sheep sera*	++++		++++	++++		++++	+	+	+	+
63 E. Sheep sera*	++++		++++	++++		++++	+	+	+	+
64 E. Sheep sera*	-	-	-	-	-	-	-	-	-	-
65 E. Sheep sera*	-	-	-	-	-	-	-	-	-	-
66 E. Sheep sera*	++++		++++	+		++	+	+	+	+
67 E. Sheep sera#	++		++++	+		++++	+	+	+	+
68 E. Sheep sera#	-	-	-	-	-	-	-	-	-	-
69 E. Sheep sera#	-	-	-	-	-	-	-	-	-	-
70 E. Sheep sera#	-	-	-	-	-	-	-	-	-	-
71 E. Sheep sera#	+++		+++	+++		+++	+	-	-	-
72 E. Sheep sera#	++		++	-	++	++	+	-	+	+
73 E. Sheep sera#	++++		++++	++++		++++	+	+	+	+
74 E. Sheep sera#	++++		++++	++++		++++	+	+	+	+
75 E. Sheep sera#	++++		++++	++++		++++	+	+	+	+
76 E. Sheep sera#	-	-	-	-	-	-	-	-	-	-
77 E. Sheep sera#	-	-	-	-	-	-	-	-	-	-
78 E. Sheep sera#	-	++	-	-	++	-	+	-	-	-
79 E. Sheep sera#	++		-	++		-	+	+	+	+
80 E. Sheep sera#	+++		+++	+++		+++	+	+	+	+
81 E. Sheep sera#	++		-	-	++	-	+	+	+	+
82 E. Sheep sera#	-	-	-	-	-	-	-	-	-	-
83 E. Sheep sera#	-	-	-	-	-	-	-	-	-	-
84 E. Sheep sera#	-	-	-	++		-	+	-	+	+
85 E. Sheep sera@	++++		++++	++++		++++	+	+	+	+
86 E. Cattle sera#	-	-	-	-	-	-	+	+	+	+
87 E. Cattle sera#	-	-	-	-	-	-	-	-	-	-
88 E. Cattle sera#	++++		++++	++++		++++	+	+	+	+
89 E. Cattle sera#	-	++	-	-	++	-	-	+	-	-
90 E. Cattle sera#	++		++	++		++	+	+	+	+
91 E. Cattle sera#	-	++	-	-	++	-	-	+	+	+
92 E. Cattle sera#	-	+++	+	-	+++	+	+	+	+	+
93 E. Cattle sera#	++++		++	++		++	+	+	+	+
94 E. Cattle sera#	++++		+	++++		+	+	+	+	+
95 E. Cattle sera#	-	+++	++	-	+++	++	+	-	-	-
96 E. Cattle sera#	-	-	-	-	-	-	-	-	-	-
97 E. Cattle sera#	-	+++	-	-	+++	-	+	+	+	+
98 E. Cattle sera#	++++		++++	++++		++++	+	+	+	+
99 E. Cattle sera#	+		+	+		+	+	+	+	+
100 E. Cattle sera#	+++		+++	+++		+++	+	+	+	+
101 E. Cattle sera#	+++		+++	+++		+++	+	+	+	+
102 E. Cattle sera#	-	-	-	-	-	-	-	-	-	-
103 E. Cattle sera#	++		++	++		++	+	+	+	+
104 E. Cattle sera#	-	-	-	-	-	-	-	-	-	-
105 E. Cattle sera#	-	++	-	++		-	+	+	+	+
106 E. Cattle sera#	-	-	-	-	-	-	+	+	+	+
107 E. Cattle sera#	-	-	-	-	-	-	+	+	+	+
108 E. Cattle sera#	-	-	-	-	-	-	-	-	-	-
109 E. Cattle sera#	++		-	-	++	-	+	+	+	+
110 E. Cattle sera#	++		++	++		++	+	+	+	+
111 E. Cattle sera#	-	-	-	-	-	-	-	-	-	-
112 E. Cattle sera#	++++		++++	++++		++++	+	+	+	+
113 E. Cattle sera#*	++++		++++	++++		++++	+	+	+	+
114 E. Cattle sera#*	++++		++++	++++		++++	+	+	+	+
115 E. Cattle sera#*	++++		++++	++++		++++	+	+	+	+
116 E. Cattle sera#*	++++		++++	++++		++++	+	+	+	+
117 E. Cattle sera#**	-	-	-	-	-	-	+	+	+	+
118 E. Cattle sera#**	-	-	-	-	-	-	+	+	+	+
119 E. Cattle sera#**	-	-	-	-	-	-	-	-	-	-
120 E. Cattle sera#**	-	-	-	-	-	-	-	-	-	-

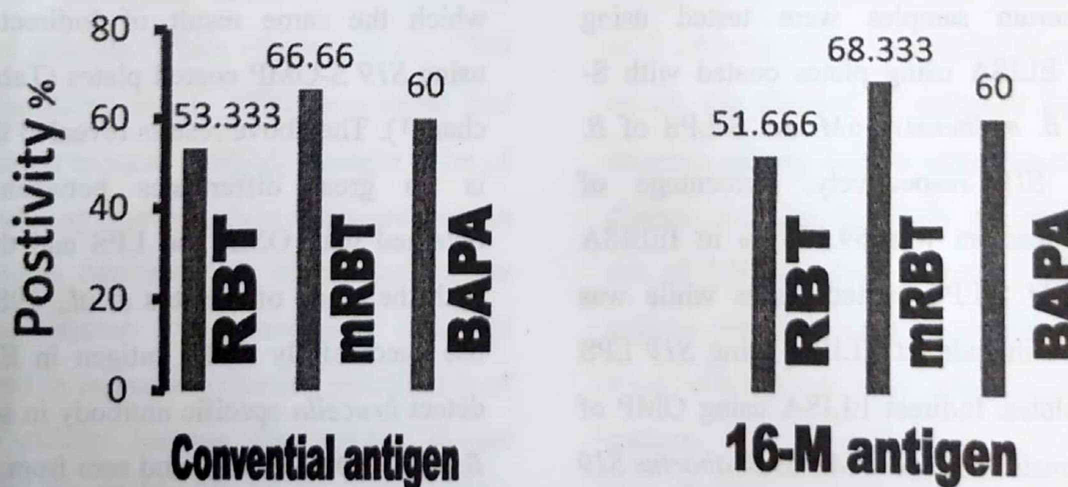
#* Cattle sera vaccinated with *S19* vaccine
#** Cattle sera vaccinated with *RB51* vaccine # naturally infected sheep and cattle
@ Sheep expermintally infected with E. coli O: 157
E. =Egyptian

The antigens of S-LPS are the most immune-dominant and, due to the fact that they are very strongly adsorbed to polystyrene, have become the antigens most widely used for routine diagnosis. Even in relatively crude extracts or when whole cells are used, it is the S-LPS antigens which are most active. In practice, the use of S-LPS antigen derived from either *B. abortus* or *B. melitensis* is adequate for the diagnosis of either, but there is some evidence that it is preferable to use a homologous antigen. There is little evidence for any significant difference between antigens prepared from *B. abortus* or *B. melitensis*, whether LPS, O polysaccharide or native hapten is used for the detection of brucellosis in vaccinated or non-vaccinated cattle (Nielsen *et al.*, 1983a and b, Devi *et al.*, 1987). Although it is recommended that purified S-LPS antigen should be used in the Indirect ELISA, there is little doubt that less purified preparations such as autoclaved or sonicated cell extracts areadequate, at least for screening purposes (Cherwonogrodzky *et al.*, 1986; Nielsen *et al.*, 1988).

Table (3): Results of plate agglutinations tests

Samples	Plate agglutination tests using antigens prepared from					
	S99			*16-M*		
	RBT	mRBT	BAPA	RBT	mRBT	BAPA
Number of positive reactors	64	80	72	62	82	72

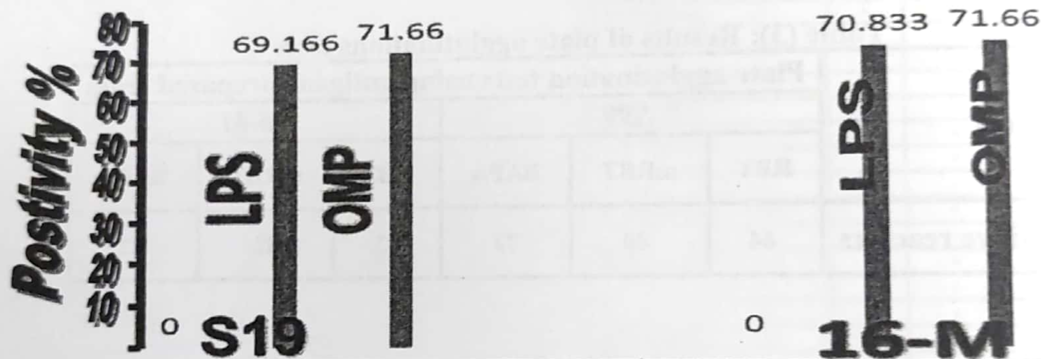
Chart (1): Results of plate agglutination tests



RBT= Rose Bengal Test.
BAPA= buffered Acidified Plate Antigen test.
MRBT= Modified Rose Bengal Test

There is also consensus that the purity of the antigen has little effect on the sensitivity of the assay, but as diagnostic specificity becomes increasingly important with decreasing disease prevalence, it can be expected that less pure antigens give rise to a slightly higher number of false positive reactions. On the other hand, it is suspected that extremely pure S-LPS have the tendency to form micelles in aqueous solution causing it to adsorb irregularly to polystyrene. T[illegible] results in assay variation and high nega[illegible] backgrounds. The hot water/phenol extract[illegible] method appears to be one of the best meth[illegible] currently in use. While the use of ELISA [illegible] the diagnosis of bovine brucellosis is w[illegible] developed, more extensive field trials a[illegible] required to fully validate the test for use [illegible] sheep and goats.

Chart (2): Results of IELISA using OMP and S-LPS coated plates



In the present study, 120 cattle and sheep serum samples were tested using Indirect ELISA using plates coated with S-LPS of *B. melitensis 16M* and S-LPS of *B. abortus S19* respectively. Percentage of positive reactors was 69.166 % in IELISA using *16M* S-LPS coated plates while was 70.833 % in Indirect ELISA using *S19* LPS coated plates. Indirect ELISA using OMP of *B. melitensis 16M* and OMP of *B. abortus S19* coated plates were done on the same cattle and sheep serum samples tested with S-LPS coated plates. Percentage of positive reactors was 71.66 % using *16M* S-OMP coated pla[illegible] which the same result of Indirect ELIS[illegible] using *S19* S-OMP coated plates (Table 4 a[illegible] chart ٢). The above results revealed that the[illegible] is no great differences between resul[illegible] obtained with OMP and LPS and this agr[illegible] with the study of Santos *et al.*, 1984 whi[illegible] use successfully OMP antigen in ELISA [illegible] detect *brucella* specific antibody in sera fro[illegible] *B. canis* infected dogs and sera from *bruce[illegible]* infected human. Araj and Kaufmann (198[illegible] found the same results when use OMP antige[illegible] in ELISA for diagnosis of human brucellosis

ELISA presented with OMPs extracted from *Brucella melitensis* as capture antigen was found to be more sensitive test for detecting antibodies to *Brucella abortus* (Santos *et al.,* 1984 Hunter *et al.,* 1986 and Riezu-Boj *et al.,* 1986) concluded that OMPs antigens have a potential to be antigenically related to all species of *brucella* so ELISA could detect brucellosis caused by all species of *brucella*. Silvia *et al.,* (1995) used the lipopolysaccharide of the cell wall as a coating antigen in IELISA to detect *Brucella melitensis* antibodies in ovine serum. The ELISA also had high sensitivity (94.7%) and a somewhat lower specificity (90.4%).

Anyhow, Gall and Nielsen (2004) concluded that the primary binding assays including IELISA, CELISA and FPA were developed as more sensitive and specific alternatives to conventional tests but IELISA were unable to distinguish between *B. abortus* strain 19 vaccinated animals and naturally infected animals. While (Nielsen *et al.,* 1989 and MacMillan *et al.,* 1990) concluded that vaccination induces antibody thought to be of lower affinity due to a short exposure time to the antigen because it is eliminated by the immune system. Alternatively, antibody produced in response to natural infection is of higher affinity because the antigen is not removed as quickly by the immune system and, therefore, persists for a much longer period.Thus, the CELISA and the FPA were developed to overcome this problem.

In conclusion, these slight difference in the results between the plate antigens is not understood but it may be due difference in animals species or biovars of *brucella* causing brucellosis (although it was known that most *Brucella* infections in Egypt are with *B. melitensis* biovar 3 and nearly there is no infection with *B. abortus* (Refai *et al.,* 1990, Refai 2002 and Refai 2003). Also, these results may be differ by using different samples than those used in this study or other studies but any how to evaluate these antigens more accurately, we need more samples of known species and of known *brucella* biovars causing brucellosis (i.e. serum samples from *brucella* infected animals from them *brucella* organisms were isolated and identified).

From the above results, this study recommended that the combination of the usage of antigens prepared from both *B. abortus* and *B. melitensis* may be useful especially in countries have mixed infection of *B. abortus* and *B. melitensis* or countries in which the disease is endemic and has no accurate survey and no clear situation of the disease and also the study recommended the use of MRBT which increase sensitivity of antigen which is very important in endemic areas although it may affect specificity of antigen.

Further research is needed to develop serological tests of improved sensitivity for the diagnosis of brucellosis in sheep, especially assays which would be able to

discriminate between infected and vaccinated animals.

Table (4): Results of IELISA using OMP and S-LPS coated plates

IELISA	Number of positive reactors	Number of negative reactors
IELISA using *16M* S-LPS	83	37
IELISA using *S19* S-LPS	85	35
IELISA using *16M* OMP	86	34
IELISA using *S19* OMP	86	34

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الكفاءة التشخصية للانتيجينات المحضرة من البروسيلا ابورتس و البروسيلا ميلتينزيس و المستخدمة فى اختبارات التلزن و الاليزا الغير مباشرة

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**المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية ,العباسية ,القاهرة

فى هذه الدراسة تم تجميع و اختبار ١٢٠ عينة سيرم من ابقار و أغنام للكشف عن الاصابة بمرض البروسيلا باختبارات الوزبنجال التقليدى و الروزبنجال المعدل و اختبار الباا باستخدام انتيجين الوزبنجال و انتيجين البابا التقليدية و باستخدام الانتيجينات التقلدية و المحضرة من البروسيلا ميلتينزيس (المسبب الرئيسى لمرض البروسيلوزيس فى مصر) عترة 16ام .تم استخدام اختبار الاليزا غير المباشر لاختبار نفس العينات باستخدام 4انتجينات مبطنة مختلفة و هم ال OMP, S-LPS المحضررين من عترة 16ام و ال OMP, S-LPS المحضرين من عترة اس 19 دلت النتائج انه يوجد اختلافات الى حد ما بين نتائج انتيجينات الوزبنجال و البابا التقليدية و المحضرة من البروسيلا ميلتينزيس ال ١٦ ام فى حين لا يوجد اختلاف جوهرى فى نتائج عند استخدام الانتيجن اختبار الاليزا غير المباشر باستخدام الانتيجينات المختلفة.

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