Comparative evaluation of standard serological tests for diagnosis of ovine brucellosis

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ABSTRACT

One hundred blood samples (55 sheep and45 goats) (include 79 females and 21 males)were selected from farms suffer from brucellosis and examined serologically by Buffered Acidified plate Antigen Test (BAPAT), Rose Bengal plate Antigen test (RBPAT) , Tube Agglutination test (TAT), Complement Fixation Test (CFT) and Immunochromatography assay (ICA). The results of BAPAT were (61.81%) and (73.33%), RBPAT& TAT were (61.81%) and (66.66%) and CFT &ICA were (60%) and (66.66%) in sheep and goats respectively for all tests. Moreover, the positive reactors in females among sheep and goats were (79.1%), (76.56%), (81.25%), (80.95%) and (79.36%) using BAPAT, RBPAT, TAT, CFT and ICA respectively. The sensitivity, specificity and agreement of BAPAT with CFT in sheep and goats were (92.06%, 75.67%, 86%) respectively. The sensitivity, specificity and agreement of RBPT with CFT in sheep and goats were (88.88%, 78.37%, 85%) respectively. The sensitivity, specificity and agreement of TAT with CFT in sheep and goats were (84.12%, 70.27%, 79%) respectively. The sensitivity, specificity and agreement of ICA with CFT in sheep and goats were (98.41%, 97.29%, 98%) respectively. The sensitivity, specificity of CFT was 100%. In conclusion the ICA proved to be the most accurate, cheapest, rapid and simplest test for diagnosis of ovine brucellosis.

Keywords: Brucella, serological tests, sheep and goats.

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1. INTRODUCTION

Brucellae are facultative gram negative intracellular bacteria of genus Brucella which are survivors in both extracellular and intracellular environments. The main domestic animals that are affected are cattle, sheep, goats and pigs, (Nicoletti and Tanya, 1993). Although isolation and identification is considered as gold standard as the most reliable methods of diagnosis but brucella culture takes several days and weeks and represents a great risk of infection for technicians, so a variety of serological tests can be used for detection of brucella specific antibodies as Rose Bengal plate Antigen test (RBPAT), Buffered Acidified plate Antigen test (BAPAT), Tube Agglutination Test (TAT) and Complement Fixation Test (CFT) (Blasco et al., 1994). Recently, Immuno chromatographic Assay (ICA) is a rapid and simplified test for the qualitative detection of specific antibodies in a variety of body fluids (Abdoel and smits 2007, Mizanbayeve et al., 2009 and Abdoel et al., 2008). So (ICA) which considered a simple version of Enzyme Linked Immunosorbant Assay (ELISA) can be used as a substitution of complicated confirmatory tests such as Complement Fixation Test (CFT) and ELISA (Montasser et al. 2012). The study was planned to evaluate the different serological tests for diagnosis of brucellosis among sheep and

goats to determine the most reliable methods for detection of the disease.

2. MATERIALS AND METHODS

2.1. Animal Samples:

A total of one hundred blood samples were collected from (55 sheep and 45 goats). These animals were randomly selected from veterinary clinics, farms and /or from small holder farms located in some village in Qalyoubia, El-Behera, El-Sharkia, El-Garbia and El-Fayoum governorates. All the examined animals were mature and had history of brucellosis. Animals were subjected to clinical and field investigation to collect history on their fertility status.

2.2.Serological Examination:

Blood samples collected from animals were centrifuged at 3000 r.p.m for 10 min to separate sera. Each serum sample was, labeled and stored at – 20°C until used. All sera were sent to the Animal Health Research Institute, (AHRI) " Brucella Department", Dokki, Giza, Egypt to be examined by Buffered Acidified plate Antigen Test (BAPAT), Rose Bengal Plate Antigen Test (RBPAT), Tube Agglutination test (TAT), Complement Fixation Test (CFT) as described by Alton et al., (1988)and by Lateral Flow Assay (LFA) : Immunochromatography assay (ICA) according to the manufacturer's instructions. The test kits were obtained from Quiking Biotech Co. Ltd. No. 1998, China.

3. RESULTS

3.1. Infection rate of brucellosis among sheep and goats:-

The results of BAPAT were 34/55 (61.81%) and 33/45 (73.33%) in sheep and goats respectively. The results of RBPAT& TAT were 34/55 (61.81%) and 30/45 (66.66%) in sheep and goats respectively. The results of CFT &ICA were 33/55 (60%) and 30/45 (66.66%) in sheep and goats respectively table (1).

3.2. Infection rate of brucellosis in examined males & females among sheep and goats:-

The positive reactors in females among sheep and goats were (79.1%), (76.56%), (81.25%), (80.95%) and (79.36%) using BAPAT, RBPAT, TAT, CFT and ICA respectively. While in males among sheep and goats the positive reactors were (20.9%), (23.5%), (18.7%), (19.1%) and (20.6%) using BAPAT, RBPAT, TAT, CFT and ICA respectively table (2).

3.3. Determination of true positive and true negative samples:-

By comparing results of BAPAT, RBPT, TAT and ICA with CFT as control standard test the true positive samples were 58, 56, 53 and 62/63. The true negative samples were 28, 29, 26 and 36/ 37. The false positive samples were 9, 8, 11 and 1/0. The false negative samples were 5, 7, 10, and 1/0 in BAPAT, RBPAT, TAT and ICA / CFT respectively. Table (3)

3.4. Sensitivity, specificity and agreement of all serological tests used for diagnosis of brucellosis among sheep and goats:-

The sensitivity, specificity and agreement of BAPAT with CFT in sheep and goats were (92.06%, 75.67%, 86%) respectively. The sensitivity, specificity and agreement of RBPT with CFT in sheep and goats were (88.88%, 78.37%, 85%) respectively. The sensitivity, specificity and agreement of TAT with CFT in sheep and goats were (84.12%, 70.27%, 79%) respectively. The sensitivity, specificity and agreement of ICA with CFT in sheep and goats were (98.41%, 97.29%, 98%) respectively. (4).

4. DISCUSSION

Serological evaluation of five serological

Table (1): Infection rate of brucellosis among sheep & goats

Species	Examined No	BAPAT		RBPAT		TAT		CFT		ICA	
		Positive	%	positive	%	positive	%	positive	%	positive	%
Sheep	55	34	61.81%	34	61.81%	34	61.81%	33	60.0%	33	60.0%
Goats	45	33	73.33%	30	66.66%	30	66.66%	30	66.66%	30	66.6%
Total	100	67	67%	64	64%	64	64%	63	63%	63	63%

Table (2): Infection rate of brucellosis in examined males & females among sheep and goats

Species	Examined Number			BAPAT		RBPAT		TAT		CFT		ICA	
				% of positive		% of positive		% of positive		% of positive		% of positive	
	males	females	total	males	females	males	females	males	females	Males	females	males	females
Sheep	11	44	55	23.6%	76.4%	23.6%	76.4%	17.6%	82.3%	24.3%	75.7%	24.3%	75.7%
Goats	10	35	45	18.2%	81.8%	23.4%	76.6%	20%	80%	13.4%	86.6%	16.7%	83.3%
Total	21	79	100	20.9%	79.1%	23.5%	76.5%	18.7%	81.3%	19.1%	80.9%	20.6%	79.4%

Table (3) Determination of true positive and true negative samples

	BAPAT	RBPT	TAT	ICA	CFT
Examined samples	100	100	100	100	100
True positive	58	56	53	62	63
True negative	28	29	26	36	37
False positive	9	8	11	1	0
False negative	5	7	10	1	0

Table (4): Sensitivity, specificity and agreement of all serological tests used for *Brucella* diagnosis among sheep & goats.

Test	Sensitivity			Specificity			Agreement		
	sheep	goats	total	Sheep	goats	total	sheep	goats	total
BAPAT	90.9%	93.33%	*92.06%*	81.81%	66.66%	75.67%	87.27%	84.44%	86%
RBPT	90.9%	86.66%	88.88%	81.81%	73.33%	78.37%	87.27%	82.22%	85%
TAT	84.84%	83.33%	84.12%	72.72%	66.66%	70.27%	80%	77.77%	79%
ICA	100%	96.66%	98.41%	100%	93.33%	97.29%	100%	95.55%	98%
CFT	100%	100%	100%	100%	100%	100%	100%	100%	100%

Determination of sensitivity, specificity and agreement: according to Alton *et al*. (1988)

$$\text{Sensitivity \%} = \frac{\text{True positive samples}}{\text{True positive samples + false negative samples}} \times 100$$

$$\text{Specificity \%} = \frac{\text{True negative samples}}{\text{True negative samples + false positive samples}} \times 100$$

$$\text{Agreement \%} = \frac{\text{True positive samples + True negative samples}}{\text{Number of tested samples}} \times 100$$

tests revealed that the infection rate was higher in goats than in sheep as shown in table (1).This agreed with the results of (Abeer 2013) who found that positive reactors among sheep were (19.44%) and among goats (26.6%). Also similar to results of (Lobna *et al.,* 2014) who found that the occurrence of brucellosis was more in goats (7.5%) tan sheep (6%) using BAPAT. Also agreed with results of (Ammar 2000) revealed that, the rate of *Brucella* infection was markedly higher among goats (3.49%) using BAPAT than among sheep (2.58%) and (Aggad 2003) who found that seroprevalence of brucella among goats by BAPAT was (3.05%) and among sheep was (1.42%). But the results disagreed with results of (Montasser *et al.,* 2012) who found that the incidence of positive reactors among goats using BAPAT was (8.86%), which is lower than that of sheep (9.43%). And (Safaa 2011) who reported that total percentage of positive reactors among sheep reached 32.5 % and among goats reached 30 %. On the other hand the results showed that the infection rate among females was higher than among males this come in accordance with results of (Rahman *et al.,* 2011) who found that the positive reactors were relatively higher in females (4.04%) than in males (0.0%) in goats and (2.6%) in females, (0.0%) of males in sheep. Also (Pandeya *et al.,* 2013) found that the incidence of infected females was (14.6%) higher than males (10.6%). The positive reactors in BAPAT in this study were higher than total positive reactors in RBPT. This could be attributed to the fact that the amount of serum used in BAPAT is greater than the amount of serum in RBPAT. Moreover the PH (3.65) of Rose Bengal antigen allowed less amount of IgM to share in the reaction but final PH of BAPAT (4.2 ±0.04) permitted the test to detect most classes of immunoglobulins (IgM, IgG1, IgG2& IgA) in serum of infected animals. Although IgM was the first class of immunoglobulins appearing after infection, yet it was proved to be of nonspecific nature, besides, most Gram negative bacteria as *Escherichia coli, Salmonella Dublin, Yersinia enterocolitica*: 9 share *Brucella* in its antigenicity and produce IgM similar to those produced by *Brucella*e (Corbel 1985 and Alton *et al.,* 1988). RBPT provided positive reactors more than TAT, more over due to its ability for earlier detection of recently infected animals as well as the longer persistence of its reaction in those chronically infected as mentioned by (Awad *et al.,* 1977). CFT is considered as gold standard serological test used for detection of

brucellosis as it detect only IgG specific for *brucella* infection so it overcome cross reaction with other similar gram negative bacteria and so no false results detected. The test has relative specificity about 100% (Abernethy *et al.,* 2012). From mentioned results of (BAPAT, RBPT and TAT) (table (3&4)) the CFT proved to be the most accurate, sensitive and specific this results agreed with the results of (El-Kholi 2007)who applied BAPAT and RBPT and the results were 6.4%, 5.8% reactors in sheep and goats respectively. The positive reactors were confirmed with TAT and CFT. The results were 94.6%, 89.2 % in sheep and 95.4%, 89.4% in goats respectively. In comparing results of BAPAT, RBPT and TAT with CFT the agreement were 89.64%, 92.22%, 91.44% in sheep and 88.72%, 91.12% and 88.61% in goats., (Abernethy *et al.,* 2012) applied complement fixation test as a confirmatory test and found that its relative specificity was about 100%.

In this study CFT was used as a control test for detection of false results and comparative test for detection of sensitivity, specificity and agreement of other tests with results of CFT. The results of (BAPAT, RBPT, TAT, CFT and ICA) proved that the ICA have similar results of CFT this indicates that ICA is the most accurate, sensitive and specific test among other serological tests due to this test detects only IgG specific to *brucella* and is considered as simple version of ELISA and so avoid false results (Montasser et *al.,* 2012).These results was similar to that reported previously by (Kaltungo *et al.,* 2013, and Tharwat *et al.,* 2014).

Determination of sensitivity, specificity and agreement of BAPAT, RBPT, TAT and ICA with CFT the results showed that CFT has highest sensitivity, specificity due to its avoidance of false results and cross reaction with other gram negative bacteria which has smooth antigen similar to *brucella*. As it detects only IgG1 specific to *brucella*. The BAPAT, RBPT, TAT have lower sensitivity and specificity rate than ICA, CFT. This may be due to the presence of samples reacted positively to the RBPT and TAT which proved negative by CFT as a specific test for diagnosis of brucellosis. The false results may be due to cross reaction with other gram negative bacteria which share brucella in its antigenicity. (Montasser *et al.,* 2012 and Morgan *et al.,* 1978).

The last seroprevalence rate recorded with the LFA (ICA) was indicative of its very high specificity, since it only detects antibodies due to Br.abortus, and due to the higher sensitivity, specificity and simplicity of the test and especially that the test not need any expertise nor refrigeration. It is recommended that ICA should be used for serological survey of brucellosis, particularly in the rural and nomadic areas. (Kaltungo *et al.,* 2013 and Montasser et *al.,* 2012). Practical advantages of ICA include that the assay is very simple to perform without the need for specific equipment, training, or electricity. Basically, the assay gives a very clear result and is very easy to read by visual inspection for staining of a line in the test zone of the assay device. Furthermore, the assay components are highly stable and well standardized and the devices can be stored without need for refrigeration (Smits *et al.,* 2003 and Abdoel *et al.,* 2008).

In conclusion it is approved that ICA is simple, rapid, highly sensitive and specific test can be used as confirmatory test giving results similar to CFT and could be ideal as a field rapid screening test for developing countries nomadic and rural settings, suitable for large - scale screening or presumptive test not require specific technicians or specific laboratories and. Moreover, the high sensitivity and specificity of LFA allows its use as a confirmatory test in combination with BAPAT, RBPT as screening tests.

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تقييم مقارن للطرق السيرولوجية المستخدمة فى تشخيص البروسيلا فى الأغنام والماعز

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الملخص العربي

صممت هذه الدراسة من أجل تقييم بعض الاختبارات المستخدمة فى تشخيص مرض البروسيلا فى الأغنام والماعز والتوصل لأدق وأرخص وأسهل اختبار يمكن الاعتماد عليه فى التشخيص .أجريت الدراسة على 100 حيوان (55 اغنام و 45 ماعز):(79 اناث و 21 ذكور) تم تجميعها من مزارع بها اشتباه بالإصابه بالمرض بمحافظات القليوبية و المنوفية و الشرقية والغربية والفيوم.تم عمل اختبارات المحمض المخمد، الروزبنجال ، التلزن الأنبوبى البطيء ، المثبت المكمل واختبار الكروماتوجرافى المناعى. وأظهرت النتائج فى المحمض المخمد أن نسبة العينات الايجابية فى الأغنام والماعز كانت على التوالي (61.81%) و (73.33%) وبينما كانت (61.81%) و (66.66 %) فى اختبار الروزبنجال والتلزن الأنبوبى البطيء على التوالي بينما كانت فى المثبت المكمل واختبار الكروماتوجرافى المناعى (60%) و (66.66 %) فى الأغنام والماعز على التوالي. كما بلغت نسبة العينات الإيجابية فى الإناث فى الأغنام والماعز باستخدام اختبار المحمض المخمد واختبار الروزبنجال والتلزن الأنبوبى البطيء و المثبت المكمل و الكروماتوجرافى المناعى كالآتى : 79.1 % و 76.56 % و 81.25 % و 80.95 % و 79.36 % على التوالي . وعند عمل اختبارات مدى الحساسية والدقة والتوافق لاختبار المحمض مع المثبت المكمل كانت النتائج فى الأغنام والماعز كالآتي:- 92.06% و 75.67% و 86% على التوالي وفى اختبار الروزبنجال كانت النتائج 88.88% و 78.37% و 85% على التوالي . وفى اختبار التلزن الانبوبى البطيءكانت النتائج 84.12 % و 70.27% و 79 % على التوالي وكانت نتائج اختبار الكروماتوجرافى المناعي 98.41% و 97.29 % و 98% على التوالى بالمقارنة بنتائج اختبار المثبت المكمل كانت النتيجة 100% من حيث الحساسية والدقة. من النتائج السابقة اتضح أن اختبار الكروماتوجرافى المناعي يمكن استخدامه كبديل لاختبار المثبت المكمل نظرا لسهولته ودقته ورخص تطبيقه.

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