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Evaluation of Immunochromatographic Assay for Serodiagnosis of Brucella among Cattle, Sheep and Goats in Egypt

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Abstract: Investigations were carried out on 640 animals (376 cattle, 158 goats and 106 sheep) from different farms and mobile flocks suspected of suffering from brucellosis from different localities in Menoufyia Governorate for evaluation of the sensitivity and specificity of immunochromatographic Assay, ICA or (latex agglutination assay LAT) with other commonly used serological tests. Twenty out of 376 cattle were seropositive for *Brucella* infection using BAPAT (5.32%), RBPT (4.79%), TAT; suspicious and positive (4.52%), ELISA (4.79%) and LAT (4.79%), 14/158 goats were seropositive using BAPAT (8.86%), RBPT (7.59%), TAT {suspect, positive (6.96%), ELISA (8.22%) and ICA (8.22%). 10/106 sheep were seropositive using BAPAT (9.43%), RBPT (8.49%), TAT {suspicious, positive} (7.55%), ELISA (7.55%) and LAT (7.55%). Sensitivity was for BAPAT (100), for RBT (92.9%), for TAT (90.7%), for ELISA (100%) and lastly ICA (100%). While Specificity was for BAPAT(83%), for RBT (83%), for TAT (71.4%), for ELISA(100%) and lastly LAT (100%) which indicated that ICA more specific than BAPAT, TAT and RBT. ICA, Rapid B. *Brucella* Ab Kit is a chromatographic immunoassay proved to be simple, accurate, rapid, does not require specialized training or equipment and economical for the detection of *Brucella* antibody. It can be concluded that this assay could be ideal as a field test for developing countries and rural settings, suitable for large-scale screening or presumptive test.

Key word: *Brucella* · Diagnosis · Serology · ICA · Cattle · Sheep · Goat

INTRODUCTION

Brucellosis remains a major zoonotic worldwide [1-3]. In particular in developing countries the disease may have important economic, veterinarian and public health consequences [4-7]. The eradication of this disease in animals is a necessary step to control the disease in man [8,9]. Also, it is considered as one of the most economically important reproductive diseases of livestock, leading to abortion, sterility and decreased productivity [10]. *B. melitensi*s may also cause abortion in cattle, although it is mainly associated with sheep, goats and wildlife [11]. In man it causes an undulating fever [12]. Brucellosis is widespread with varying prevalence's across Africa, with some areas reportedly having up to 30% seroprevalence, the state of knowledge was recently reviewed by McDermott and Arimi [13].

Currently, diagnosis of brucellosis is based on serological and microbiological tests. Serological methods are not always sensitive or specific [14, 15], these tests are sensitive but many false-positive results have been found [16, 17], mainly due to cross-reactivity with other antigens [18]. Single test is not recommended since this could not detect all positive reactors [19]. Agglutination tests such as: BAPAT, RBT and TAT are commonly used for detection of *Brucella* specific antibody [20-23]. ELISA is a highly sensitive and specific diagnostic assay since it directly detects antibody and has no or minimal false positive reactions of agglutination tests [24]. Although isolation and identification is considered as gold standard as the most reliable methods of diagnosis but *Brucella* culture takes several days to weeks and represents a great risk of infection for technicians [25]. A laboratory that is capable of performing these slow and complicated assays

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may not be available in many places. More recently, the convenience and speed of the test have been achieved by a novel concept of immunochromatographic (ICA) assay which is a simplified version of ELISA [26-28].

Recently (ICA) is a laboratory method to check for certain antibodies in a variety of bodily fluids including blood, saliva, urine, or cerebrospinal fluid [29, 30]. The sensitivity is 89.1% and the specificity 98.2%. The assay is ideal for use as a simple field, rapid screening test to detect the immunoglobulins (IgM and IgG) for the serodiagnosis of brucellosis in livestock species.

Comparing with RBPT in detecting antibodies with brucellosis, the positive or negative results detected by LAT are consistent with those by SAT and ELISA. LAT is stable, specific, sensitive and practicable for the serodiagnosis of brucellosis [31].

ICA is easy to be done and does not require specialized training or equipment and the components are stable and rapid in the management of large numbers of serum samples, these factors make the test ideal for developing countries and rural settings. Many investigators [32-34] stated that ICA is accurate as compared with the standard microscopic agglutination test (MAT) also the sensitivity and specificity of LAT are 88 and 98%, respectively [41]. Watarai *et al*.[36] reported that ICA is more specific than the tube agglutination test. Singer *et al*. [37-40] concluded that the developed ICA is immunodiagnostic assay, simple, rapid, economical and suitable for large-scale screening in endemic areas, also the sensitivity and specificity of ELISA and ICA are 86.84, 93.16 and 95.42 and 98.33%, respectively.

The antigen employed in the *Brucella* LAT is a LPS extract prepared from *B. abortus*. Similar to other serological assays for brucellosis the *Brucella* LAT is based on the detection of IgG antibodies against smooth LPS antigen [41-44]. Other *Brucella* species that do not contain significant amounts of smooth LPS such as *B. ovis and B. canis* may require the use of a different antigen [45, 46].

In order to prevent the further transmission and spread of the infection a rapid test result is desirable. So the objective of this work was to evaluate the clinical and laboratory utility of the ICA device for serodiagnosis of cattle, sheep and goat brucellosis and comparing results with those obtained from other commonly used tests.

MATERIALS AND METHODS

This Study was done in corporation with the Egyptian General Organization for Veterinary Services (EGOVS) at Menoufyia governorate during the period from March 2011 to August 2011.

Animals: A total number of 640 animals (376 cattle, 158 goat and 106 sheep) from different farms having history of brucellosis were examined. Animals free of clinical signs of brucellosis for at least 6 months and subjected to two rounds of negative serological results were included as control.

Samples Collection: Blood samples were collected and serum samples were prepared and kept frozen (-20°C), till analysis [47].

Serological Examination: Seroprevalence of brucellosis was investigated using commonly used serological tests as: Buffered acidified plate antigen test (BAPAT) [47], Rose Bengal plate test (RBPT) [48], as well as tube agglutination test (TAT) [47] and ELISA [49]. Lastly immunochromatographic assay (Rapid B.Brucella Ab. Test), imported by the authors from Quicking Biotech Co. Ltd. No. 1998, China was applied.

Immunochromatographic Assay (ICA) (Brucella Ab Rapid Test) for Vet. Use Only: (Brucella test device for detection of Brucella antibodies in (whole blood, serum/plasma). ICA, latex agglutination test (LAT) or lateral flow assay LFA) is an immunoassay system for the qualitative detection of Brucella antibody [50] in whole blood, serum (Cat No.: W81085), rapidly visualize an antigen-antibody reaction through the easily observed clumping that occurs between polystyrene latex beads coated with a specific antibody and the target antigen for the antibody. The sensitivity and specificity of the assay were determined [47].

Statistical Analysis: Data were collected and statistically analyzed [51].

RESULTS

In the present investigation, comparisons were performed between immunochromatographic assay (ICA) and commonly used serological tests for laboratory diagnosis of *Brucella* among cattle (Table 1), goats (Table 2) and sheep (Table 3) as shown in tables.

Table 4 shows comparison between sensitivity and specificity of different serological tests compared with ELISA among cattle, sheep and goats.

Table 1: Comparison between immunochromatographic assay (ICA) and commonly used serological tests in laboratory diagnosis of *Brucella* among cattle

			BAPAT		RBPT		TAT			ELISA Results Mean (OD)		Latex agglutination test	
	Total	Number	----------		----------		----------			----------		----------	
Animal	examined	Of positive	--	+	--	+	--	±	+	--	+	--	+
Control	2	0	2	0	2	0	2	0	0	2	0	2	0
No. of +ve		20	0	20	2	18	3	6	11	2	18	2	18
% of +ve according to total examined	376		5.32 %		4.79		4.52%			4.79%		4.79%	
% of +ve according to total +ve		20	100 %		90%		85%			90 %		90 %	

Cut-off point of ELISA (OD =0.143) TAT: 1/10 Negative (--) 1/20: Suspect >1/40: Positive(+)

Table 2: Comparison between immunochromatographic assay (ICA) and commonly used serological tests in laboratory diagnosis of *Brucella* among goats.

			BAPAT		RBPT		TAT			ELISA Results Mean (OD)		Latex agglutination test	
	Total	Number	----------		----------		----------			----------		----------	
Animal	examined	Of positive	--	+	--	+	--	±	+	--	+	--	+
Control	2		2	0	2	0	2	0	0	2	0	2	0
No. of +ve		14	0	14	2	12	3	5	6	1	13	1	13
% of +ve according to total examined	158		8.86%		7.59 %		6.96 %			8.22%		8.22%	
% of +ve according to total +ve		14	100 %		85.7%		78.6%			92.9 %		92.9 %	

Table 3: Comparison between immunochromatographic assay (ICA) and commonly used serological tests in laboratory diagnosis of *Brucella* among sheep

			BAPAT		RBPT		TAT			ELISA Results Mean (OD)		Latex agglutination test	
	Total	Number	----------		----------		----------			----------		----------	
Animal	examined	Of positive	--	+	--	+	--	±	+	--	+	--	+
Control	2		2	0	2	0	2	0	0	2	0	2	0
No. of +ve		10	0	10	1	9	2	1	7	2	8	2	8
% of +ve according to total examined	106		9.43%		8.49 %		7.55 %			7.55%		7.55%	
% of +ve according to total +ve		106	100 %		90%		80%			80%		80%	

Table 4: Comparison between sensitivity and specificity of different serological tests compared with ELISA among cattle, sheep and goats

Test	NO. of +ve by each test	Sensitivity (%)	Specificity (%)
BAPAT	44	100 %	83%
RBPT	39	92.9 %	83%
TAT	36	92%	71.4 %
ELISA	39	100 %	100%
LAT	39	100 %	100%

Table 5: Percentage of seropositive samples among cattle, sheep and goats in correlation with the immunochromatographic assay (ICA)

		BAPAT		RBPT		TAT			ELISA		LAT	
	Total	----------		----------		----------			----------		----------	
Species	examined	+	%	+	%	±	+	%	+	%	+	%
Cattle	376	20	5.32	18	4.79	6	11	4.52	18	4.79	18	4.79
Goat	158	14	8.86	12	7.59	5	6	6.96	13	8.22	13	8.22
Sheep	106	10	9.43	9	8.49	1	7	7.55	8	7.55	8	7.55
% of +ve according to total No. examined	640	44	6.87	39	6.09	12	24	5.63	39	6.09	39	6.09
% of +ve according to total +ve		44	100%	39	88.64%	12	24	81.82%	39	88.64%	39	88.64%

The Gold Standard used in this study for True positive was ELISA.

Table 5 shows the pPercentage of seropositive samples among cattle, sheep and goats in correlation with the immunochromatographic assay (ICA)

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 endemic areas. Isolation and identification of the causal agent is considered as gold standard but takes several days to weeks. Diagnosis of brucellosis by serological study largely depends on the use of two or more tests and then use more specific test to confirm any positive animals. Single test is not recommended since this could not detect all positive reactors [2, 3, 19, 21,23]. The best estimates using all the information available suggest that the LFA is a sensitive and highly specific test, this means that positive test result for the LFA (assuming a sensitivity of~87% and a specificity of~97%). Its ease of use makes it a very attractive screening tool without need for laboratory facilities [50].

As regards to the presented results in table (1) which showed that the percentage of reactors among cattle 20/376 were seropositive for *Brucella* infection using BAPAT(5.32%), RBPT (4.79%), TAT; suspicious and positive (4.52%) N.B: suspicious treated as positive in Egypt, ELISA(4.79%) and ICA (4.79%). Looking to table (2); the percentage of reactors among goats 14/158 goats were seropositive using BAPAT (8.86%), RBPT(7.59%), TAT; suspect, positive (6.96%) and ELISA(8.22%) and ICA (8.22%). Looking to table (3) the percentage of reactors among sheep 10/106 were seropositive using BAPAT (9.43%), RBPT (8.49%), TAT; suspect and positive (7.55%) and ELISA (7.55%) and ICA (7.55%).

So, it is concluded from tables 1, 2 and 3 that conventional agglutination tests have good sensitivity but their lack of specificity and the occurrence of false positive serological results make a specific test necessary which agree with Bronsvoort *et al.* [50] who stated that although some diagnostic or screening tests are referred to as the "gold standard" but it need the use of a more specific test to confirm any positive animals. In the present study, ELISA successfully detected the actual number of positive and negative reactors. Similar results of ELISA were also reported by Lucero *et al*. [52]. Lower sensitivity of TAT was attributed to the high incidence of suspicious cases as well as to the prozone phenomena [47]. ICA was more sensitive and specific than the TAT which agrees with the results of Lu *et al*. [31] who reported that LAT is more specific than the TAT using whole bacterial cell antigens. Also agree with Dey *et al* [34] who reported that ICA is found to be sensitive, specific and accurate as compared to the standard agglutination test. Results of table 4 showed that and RBT was more sensitive than ICA but ICA was more specific than RBT as ICA detect both IgG and IgM antibodies to Brucella in animal. ICA has advantage of higher specificity than RBPT, like ELISA which was considered the gold standard test. Our results agree with many investigators [50, 31, 38-40, 53], who stated that the sensitivity and specificity of the ICA were 89.70 and 90.45%.

Results of table 1, 2 and 3 showed some samples were negative by RBT, BAPAT and TAT reacted in the ICA. These negative samples collected from recently infected cattle, goats and sheep that reacted in the ICA and ELISA, the result is fairly similar to those obtained by Abdoel and Smits [29] and Nielsen *et al*. [24] who found that RBT showed the highest false positive reactions as compared to the ELISA and ICA also speed of ICA make it available for the rapid presumptive test which can replace RBPT in brucellosis control programs.

The absence of reactivity in ICA for all-samples from animals from herds free of brucellosis indicates a high specificity of close to "100% because of know false-positive reactivity in conventional serological tests which agree with results of Abdoel and Smits [29]. Also for most groups the percentage of animals that reacted in the ICA was similar to the number of animals that reacted in the ELISA and almost of the RBT which agrees with that of Abdoel and Smits [29].

In this study it was noticed the presence of some samples which reacted positively to the BAPAT, RBPT and TAT and proved negative by ELISA as a specific test may be attributed to cross reaction by some bacteria as *Escherichia coli, Salmonella dublin, Yersinia enterocolitica* O:9 and others in the body fluids and secretions in diagnosis of brucellosis [54-56] or background antibody levels due to earlier exposure or vaccination thus causing faults or error in the interpretation of the results. Also, it was noticed the presence of some samples which reacted negatively to the RBPT and TAT and reacted positively by ELISA and ICA, our results agree with Abdoel and Smits [29], Nielsen *et al.* [24], Jacques *et al.* [57] and Abd El-Razik *et al.* [14] who stated that ELISA is a highly specific diagnostic assay as it has no minimal false positive reactions as compared to agglutination tests.

Reading table (5) which indicates that the percentage of seropositive reactors 44/640 among examined animals using BAPAT (6.87%), RBPT(6.09%), TAT; suspicious and positive (5.63%), ELISA(6.09%)and finally ICA (6.09%). While the percentage of seropositive reactors detected were BAPAT (100%), RBPT (88.64%), TAT {suspicious, positive (81.82%), ELISA(88.64%) and finally ICA (88.64%) and the percentages of animals from infected flocks that reacted in the *Brucella* LFA and

ELISA ranged from 90% for cattle, 92.9% for goats and 80 % for sheep which agree with results of Abdoel and Smits [29].

Sensitivity remarked to either BAPAT (100%) RBT (92.9%), TAT (90.7%), ELISA (100%) and lastly ICA (100%). While Specificity was BAPAT (83%),RBT (83%), TAT (71.4%), ELISA (100%) and lastly ICA (100%) and the sensitivity of the LFA were calculated to be 100% for the bovine Brucella LFA. 100% for the caprine, 100% for the ovine and none of the samples from animals from herds free of brucellosis reacted in the new assays (LATsimilar to ELISA) indicating a high specificity (100%), our results nearly similar with the that of Abdoel and Smits [29], Birnbaum, *et al.* [26], Lou *et al.* [27], Zuk, *et al.* [28] and Kim *et al*. [16] who stated that complicated assays may not be available in many places and 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.

Sensitivity was ELISA (100%) and ICA (100%) while Specificity was ELISA (100%) and ICA (100%) which nearly similar with that of Abdoel and Smits [29] who reported that the sensitivity and specificity of ELISA and ICA were 86.84% and 93.16% and 95.42% and 98.33% respectively. Both the tests are found to be specific.

On the other hand, the ICA has several practical advantages that make it the method of choice when testing animals in remote areas or other migratory populations. Practical advantages include that the use of the ICA does neither 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.

In conclusion, both ELISA and ICA are sensitive and specific tests but the procedure of ICA is more simple than ELISA.

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