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Efficacy of Serological Tests in Comparison with PCR for Diagnosis of Brucellosis

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Abstract: Brucellosis is a complicated disease in terms of epidemiology, diagnosis and control. Although many serological tests and new automated blood culture techniques have been developed to diagnose brucellosis, there are still significant problems in the diagnosis of the disease. The present work aimed to evaluate the diagnostic yield of the two main serological tests used for diagnosis of brucellosis; Rose Bengal test (RBT) and serum tube agglutination test (STAT) in comparison with PCR. Special emphases were given to evaluate the sensitivity, specificity, accuracy, cost and the time consuming of RBT and STAT in comparison with PCR for the diagnosis of this disease. Blood samples were collected from 30 persons and 4 female goats (does) that were serologically positive for brucellosis by STAT and RBT. Conventional PCR was performed for the detection of Brucella DNA. Results for human blood samples showed that specificity and sensitivity values of RBT are 33.3 and 87.5% and are less than STAT (≥1/320; 100 and 91.7%) respectively. Results of examination of the 4 goat blood samples indicated that 4, 3 and 3 does give positive results for STAT, RBT and PCR tests, respectively. In conclusion, the STAT ≥ 1/320 has a greater diagnostic accuracy than that of the RBT (93.3and 76.6% respectively).

Key words: RBT · PCR · STAT · Brucellosis · Human · Goats

INTRODUCTION

Brucellosis is an important zoonosis. It affects a wide variety of mammals causing significant reproductive failure and enormous economic losses. In humans, it is associated with chronic debilitating infection [1]. The epidemiology of brucellosis in goats is more complex as several extrinsic factors such as flock size, mangemental and ecological conditions and socioeconomic factors play important poor defined roles [2].

The global incidence of human brucellosis is estimated at more than 500,000 infections per year [3]. But, the true incidence has been estimated to be 25 times higher than the reported incidence because of the lack of essential statistics, disease reporting and notification systems in many countries [4].

Brucella organisms are usually transmitted between animals by contact with the placenta, fetus, fetal fluids and vaginal discharges from an infected animal. Entry into the body occurs by ingestion and through the mucous membranes, broken skin and possibly intact skin [5].

In Egypt, brucellosis is caused mainly by *B. melitensis* (particularly biovar 3) and *B. abortus* [6]. The pathogenicity of Brucella varies according to its species; *B. melitensis* have the highest pathogenicity; *B. suis* have high pathogenicity; *B. abortus* and *B. canis* have moderate pathogenicity [7].

The clinical picture of brucellosis alone cannot always lead to diagnosis since the symptoms are nonspecific and often atypical; therefore, diagnosis needs to be supported by laboratory tests. Although many serological tests and new automated blood culture techniques have been developed to diagnose brucellosis, there are still significant problems in the diagnosis of the disease [8].

PCR was considered as the golden test for diagnosis of brucellosis as suggested by Maher [9] whereas he stated that this method is more sensitive and specific than culture and serology for diagnosis of brucellosis. Also, Mitka *et al.* [10] stated that PCR is a very useful tool not only for the diagnosis of acute brucellosis but also as a predictive marker for the course of the disease and the post treatment follow-up, which is valuable for the early detection of relapses.

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In our previous study [11], serological investigations (STAT and RBT) revealed that 10 and 6.26 % of examined does and contact persons were positive for brucellosis. Therefore, the present work aimed to evaluate the diagnostic yield of the used serological tests (RBT and STAT) in comparison with PCR for the diagnosis of brucellosis. Moreover, special emphases were given to predict the sensitivity, specificity and cost of RBT and STAT in comparison with PCR for the diagnosis of brucellosis.

MATERIALS AND METHODS

Blood Samples: Blood samples were collected from 30 human subjects and 4 does previously proven to be serologically positive for brucellosis [11]. Samples were centrifuged at 1200 Xg for 5min. at 4°C. Serum samples were harvested and kept at -20°C until used.

PCR

Extraction of Brucella Genomic DNA: The genomic DNA from the investigated human and animal blood samples was extracted with the Biospin Blood Genomic DNA Mini-Prep Kit (BioFlux, Tokyo, Japan) as recommended by manufacturer's instructions. DNA concentration and purity was measured according to Sambrook *et al*. [12].

DNA Amplification by PCR Assay: The following primers: B4: TGGCTCGGTTGCCAATATCAA and B5: CGCGCTTGCCTTTCAGGTCTG [13] were used to amplify a 223 bp fragment from extracted human and animal DNA [14].

Statistical Analysis: The collected data were statistically analyzed using SPSS program (software version 17.0). Student's t-test, Chi-square test, Fisher's Exact test and McNemar test as well as correlation co-efficient test (r-test) were used [15].

RESULTS

This study was conducted on 30 Brucella positive persons living in contact with livestocks raised at Lower Egypt.

Results of STAT and RBT in comparison with PCR for diagnosis of human and animal brucellosis (Table 1) showed that different STAT titers were positive in the 30 studied human cases. RBT was positive in 25 cases and PCR was positive in 24 sample cases. In goats,

Table 1: Percentages of the different Brucella tests used in the studied human and animal cases

	Human		Does	
Variables	No (30)	%	No (4)	%
STAT				
≥ 1/160	8	26.7	4	100
≥ 1/320	9	30.0		
≥ 1/640	4	13.3		
≥ 1/1280	9	30.0		
RBT				
• Positive	25	83.3	3	75
• Negative	5	16.7		
PCR				
• Positive	24	80.0	3	75
• Negative	6	20.0		

Table 2: Distribution of RBT results among different titers of positive STAT in the studied cases.

	RBT		
STAT	Positive (No=25)	Negative (No=5)	p
≥ 1/160	25 (100%)	5 (100%)	1.000
≥ 1/320	19 (76.0%)	3 (60.0%)	0.508
≥ 1/640	11 (44.0%)	2 (40.0%)	0.001*
≥ 1/1280	8 (32.0%)	1 (20.0%)	<0.001*

McNemar test *Significant at p< 0.001

Table 3: Distribution of PCR results among different titers of positive STAT in the studied cases:

	PCR		
SAT	Positive (N=24)	Negative (N=6)	p
≥ 160 (No=30)	24 (100%)	6 (100%)	1.000
≥ 320 (No=22)	22 (91.7%)	0 (0.0%)	0.500
≥ 640 (No=13)	13 (54.2%)	0 (0.0%)	<0.001*
≥ 1280 (No=9)	9 (37.5%)	0 (0.0%)	<0.001*

#McNemat test *Significant at p< 0.001

Table 4: Distribution of RBT results among PCR results in the studied cases

		PCR		
Variables		Positive (N=24)	Negative (N=6)	Total
RBT	Positive	21 (87.5%)	4 (66.7%)	25
	Negative	3 (12.5%)	2 (33.3%)	5
P#		0.221		

#McNemat test

Table 5: Comparison between combined RBT+STAT (= 320) results among PCR results in the studied cases:

		PCR	
Variables		Positive (No=24)	Negative (No=6)
RBT+ STAT ≥320	Positive (No=19)	19 (79.2%)	0 (0.0%)
	Negative (No=11)	5 (20.8%)	6 (100%)
P#		<0.001*	

#McNemat test *Significant at p< 0.001

Table 6: Characteristic of STAT ≥1/160, STAT ≥1/320, STAT ≥ 1/640, STAT ≥ 1/1280 and RBT as a diagnosis of brucellosis (PCR was considered a golden test)

Variables	STAT 1/≥160	STAT ≥ 1/320	STAT 1/≥640	STAT ≥ 1/1280	RBT
Sensitivity	100.0	91.7	54.2	37.5	87.5
Specificity	0.0	100.0	100.0	100.0	33.3
PVP	80.0	100.0	100.0	100.0	84.0
PVN	Na*	75.0	35.3	28.6	40.0
Accuracy	80.0	93.3	63.3	50.0	76.6

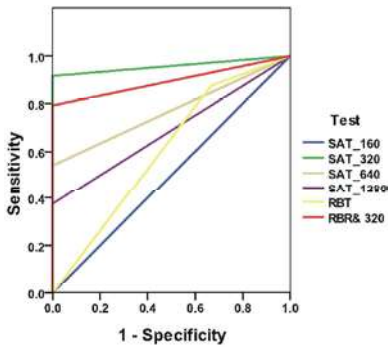
*Na: Non-applicale

Table 7: ROC curve for different tests of diagnosis of Brucellosis

	AUC				
Test	Area	95% CI		P	Test value
STAT≥160	0.50	0.24	0.76	1.000	Bad
STAT≥320	0.96	0.00	1.00	<0.001*	Excellent
STAT≥640	0.77	0.60	0.94	0.043*	Good
STAT≥1280	0.69	0.49	0.88	0.162	Suggestive
RBT	0.60	0.33	0.87	0.437	Bad
RBT+STAT≥320	0.90	0.77	1.00	0.003*	Excellent

Table 8: Comparison between the time and cost of different brucella diagnostic tests

Variables	STAT	RBT	PCR
Time	48 hours	4 minutes	24 hours
Cost	20 pounds	10 pounds	150 pounds



STAT≥320 was the best test to diagnose brucellosis

examination of 4 blood samples indicated that 4, 3 and 3 does give positive results for STAT, RBT and PCR tests, respectively.

The distribution of RBT results among different titers of positive STAT in the studied cases was shown in Table (2). There was no significant difference between diagnosis of Brucella by RBT and both STAT ≥ 1/160 and STAT ≥ 1/320, but there was a significant difference between diagnosis of Brucella by RBT and both STAT ≥ 1/640 and STAT ≥ 1/1280.

The distribution of PCR results among different titers of positive STAT in the studied cases was evaluated in Table (3). There was no significant difference between diagnosis of Brucella by PCR and both STAT ≥ 1/160 and STAT ≥ 1/320, but there was a significant (P ≤ 0.001) difference between diagnosis of Brucella by PCR and both STAT ≥ 1/640 and STAT ≥ 1/1280.

The distribution of RBT results among PCR positive cases was assessed in Table (4). There was no significant difference between diagnosis of brucella by PCR and RBT.

As shown in Table (5), there is a significant difference (P ≤ 0.001) between diagnosis of brucella by PCR and combined RBT+STAT ≥ 320.

Table (6) assessed the characteristics of STAT ≥1/160, STAT ≥1/320, STAT ≥ 1/640, STAT ≥ 1/1280 and RBT as diagnostic tools for brucellosis (PCR was considered as a golden test). This table showed that, STAT ≥1/320 test had better characteristics than STAT at other titers and RBT in diagnosis of brucellosis.

The ROC curve for the different tests used for diagnosis of brucellosis was assessed in Table (7). The STAT ≥ 1/320 was the best test to diagnose brucellosis.

While the comparison between the time and cost of different Brucella diagnostic tests was evaluated in Table (8). The RBT was the cheapest and fastest; STAT was delayed than PCR, but was cheaper.

Figure 1 displays the PCR products (223 bp fragment) obtained by amplification of human DNA using the B4/B5 primer set.

DISCUSSION

The present work aimed to evaluate the commonly used tests for diagnosis of brucellosis in man and animal (goats), whereas brucellosis is a complicated disease, especially in term of its diagnosis. In man, the clinical picture of brucellosis alone can't always lead to actual diagnosis, since the symptoms are nonspecific and often atypical; therefore, diagnosis needs to be supported by laboratory tests. In goats, brucellosis especially in Egypt received little research despite its zoonotic importance and economic drawbacks, so sensitive rapid diagnostic tools for its control must be applied [2].

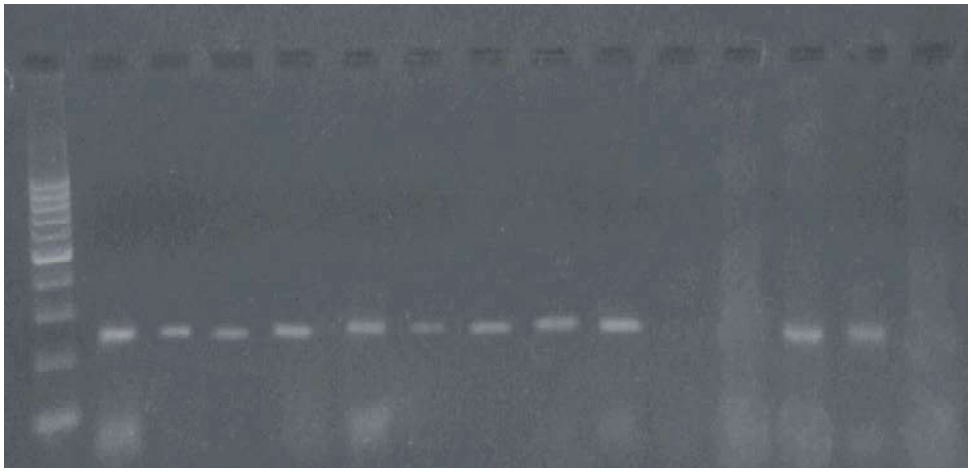


Fig. 1: Agarose gel electrophoresis of PCR products obtained by amplification of human DNA using the B4/B5 primer set. Lane 1, molecular weight DNA ladder; lane 2, positive control (223 bp); lane15, negative control; lanes 3-10, 13 &14are positive samples; lanes-11 & 12 are negative samples.

In the present investigation, comparing to PCR, the sensitivity of STAT ≥1/160 was 100%, but it lacked the specificity, the sensitivity and specificity of STAT ≥ 1/320 were 91.7 and 100%, respectively, while the sensitivity and specificity of RBT were 87.5 and 33.3%, respectively. The accuracy values of STAT ≥ 1/160, STAT ≥ 1/320 and RBT were 80, 93.3 and 76.6%, respectively. So, STAT ≥ 1/320 test had better characteristics than STAT at other titers and RBT in diagnosis of brucellosis. Similar findings were given by Yildiz *et al*. [16] regarding STAT. Also, Sirmatel *et al*. [17] reported that STAT is still the most reliable method for the serological diagnosis of brucellosis. Moreover, Mantur *et al.* [18] reported that STAT remains the most popular and yet used worldwide diagnostic tool for the diagnosis of brucellosis because it is easy to perform, does not need expensive equipments and training. However, in areas of endemic disease, using a titer of 1/320 as cutoff may make the test more specific.

Regarding RBT, Mesa *et al*. [19] revealed that this test is good or very good in patients with no previous exposure to Brucella or history of brucellosis, but poor in patients who are repeatedly exposed to Brucella or had a history of infection. Moreover, Gad El-Rab and Kamba, [20] added that the use of the RBT as the sole diagnostic tool to establish treatment of brucellosis in endemic areas is not a reliable practice with individuals who are repeatedly exposed to the disease, or who have a recent history of the disease.

Regarding goats, as mentioned in our previous study [11], 10% (4/40) of the examined does were serologically positive for brucellosis, these animals suffered from reproductive disorders, mainly low fertility and repeat breeding, included 4 and 3 positive for STAT and RBT, respectively. In the present work, PCR results showed that 3 out of the 4 serologically positive samples were successfully amplified. Special interest must be given to diagnosis of brucellosis in goats whereas this species usually becomes infected with *B. melitensis* and is raised in close contact with farm animals and human, it is preferable to use PCR for actual diagnosis owning to its sensitivity and requiring little labor. AL-Garadia *et al*. [21] reported that the sensitivity of conventional PCR and RBPT are 95.89 and 89.04%, while their specificities are 93.02 and 99.06%, respectively. PCR was higher in sensitivity and had superior ability to detect *Brucella melitensis* in goats' blood comparing with other serological techniques. On the other hand, a number of studies have revealed the effectiveness of the RBPT in the diagnosis of *B. melitensis* infection in sheep and goats although, some researchers reported low efficiency. Several studies have found that the RBPT detects infected animals earlier in the immune response than the CFT and that the sensitivity is good. There is no doubt however that more research is required to standardize the RBPT preparations internationally [22].

AL-Garadia *et al.* [21] stated that a combination of a serological test and confirmation by a molecular technique, especially RT-PCR is the best way to attempt control or eradication of *B. melitensis* infection in goat farms and accurate diagnosis of individuals infected with brucellosis.

In conclusion, diagnosis of brucellosis is still a complicated procedure. The routine classic STAT offers good results for the diagnosis of brucellosis in areas of endemicity when adequate cutoff point ≥1/320 is used as the specificity; sensitivity and accuracy (100, 91.7and 93.3%) were higher than that of RBT (33.3, 87.5 and 76.6%) respectively. However, the RBT is less costly, easier to perform (i.e. simple and rapid), that making it suitable for national serological surveys and laboratories with large diagnostic workloads. Large scale multi-center studies are recommended to evaluate the diagnostic value of RBT and STAT in comparison with PCR.

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