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Efficiency of Serological Tests for Detection of Brucellosis in Ruminant at South Provinces of Egypt

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Abstract: A total of 2138 serum samples(715 from cattle, 1323 from sheep and 100 from goats) from different districts in Assuit governorate, was tested for the detection of antibodies against *Brucella* spp. Results obtained by Buffer acidified plate antigen test (BAPAT) and Rose bengal test (RBT) as screening tests indicated a positive reactors percentage of 4.6-5.3, 4.4-7.6 and 10-15% followed by overall brucellosis incidence of 4.5, 5.2 and 5.0 % in case of cattle, sheep and goats, respectively. The *Brucella* positive reactors were subjected to confirmation by Serum agglutination test (SAT) and Rivanol test (Riv. T) in addition to the previous tests. It confirmed that the BAPAT indicated seroreactors of 91.3 % in sheep and 100% in cattle and goats. *Brucella melitensis* biotype 3 was isolated from 39 seropositive animals (9 Cattle, 25 sheep and 5 goats). In this investigation, the highest rate of sensitivity (97.4%) was detected by BAPAT, while the highest rate and specificity (87.6%) was by RIV.t.

Key words: Brucellosis · Serology · Buffer acidified plate antigen test (BAPAT) · Rivanol test (Riv. T) · Upper Egypt

INTRODUCTION

Brucellosis, a disease caused by various species of the genus *Brucella*, has the most wide-spread zoonosis in the world [1]. Cross-transmission of brucellosis can occur between cattle, sheep, goats, camels and other species. Brucellosis is still endemic in countries of the Mediterranean basin, the Middle East and Central Asia. Human infection due to *Brucella* from camels is known to occur mostly through the consumption of unheated milk [2-4]. Brucellosis in sheep and goats, caused by *B. melitensis*, one of the most virulent species of *Brucella*, is responsible for important economic losses in sheep and goats farming. Ruminant brucellosis can cause abortion, weak offspring, infertility, loss of milk production and has been responsible for major economic losses [5]. The interest in brucellosis has increased since *Brucella* species has been identified as a potential biological weapon [6].

For several decades it has been recognized as a significant public health problem in the Middle East and recent reports suggested that its incidence is increasing in both ruminants and humans [7, 8] and that currently applied control measures may not be capable of reducing the levels of infection in ruminants [9].

In Egypt, *Brucella melitensis* biovar 3 is considered to be the predominant species of *Brucella* isolated from humans and animals [8]. Outbreaks in cattle due to *B. melitensis* have become a worldwide emerging problem particularly difficult to control due to the lack of knowledge on the epidemiology in this host species and of an effective vaccine [10].

Diagnosis of *Brucella* spp. infection is mainly based on the detection of antibodies in serum by serological tests. The Rose Bengal test (RBT) [11] and complement fixation test (CFT) [12] are the most accepted tests worldwide for this purpose [13] and the only approved for certification of sheep and goats flocks due to brucellosis

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status in EU member states [14]. The RBT, due to its low sensitivity on sheep and goats sera, is suggested to be used only for identification of infected flocks (flock screening test) and not for individual animals [15]. Since CFT is regarded as more sensitive and specific, is used for individual testing of animals in infected flocks aswell as a confirmatory test [14, 16, 17].

The Rev.1 vaccine was developed by Elberg and Faunce [18] and has been successfully applied in sheep and goat for the control of ovine and caprine brucellosis. It was recognized that Rev.1 vaccination cause existence of positive reactors in serological tests among vaccinated population which lead to difficulties in distinguishing between infected and vaccinated animals by conventional serological tests [19]. Due to these difficulties, the study of the epidemiological situation of the disease is a key element of a successful control program. The gold standard that confirms the presence of the disease is isolation, identification and biotyping of the bacterial agent [20]. Eradication of brucellosis requires accurate diagnosis of the disease among the infected animal population.

MATERIALS AND METHODS

Animals: All animals tested were Egyptian native breeds from farms with a known history of brucellosis according to the directorate of veterinary medicine, Assiut). The samples were taken from slaughtered animals under strict hygienic conditions, kept on ice and sent to our laboratory as soon as possible. The animals all tested positive to at least one of standard tube agglutination test (SAT) and Rose Bengal plate test (RBPT) [12]. Positive samples to the SAT were those with titres >1/40 (50%) according to the European technique [12].

Sample Collection: Between May 2009 to May 2010, a total number of 2136 blood samples was collected from lymph nodes (retropharyngial, prescapular, prefemoral, internal iliac and supramammary) and spleen tissues from carcases of all serologically positive animals (715 from cattle, 1323 from sheep and 100 from goats) in districts (Al-Badari, Assuit, El-Fath, Abnoub, Manflut, Dyrut and El-Qusia) among Assuit province. Blood samples were allowed to clot and the sera were separated by centrifugation and stored at-20°C until performing serological tests.

Serological Examination: All sera were screened for antibodies against *Brucella* by BAPAT and RBPT as screening tests. All positive serum samples were further retested by SAT and RIV.t as qualitative confirmatory tests described by Alton *et al*. [12].

Bacteriological Examination: All obtained tissues were cultured on *Brucella* agar selective media (Oxoid), *Brucella* spp. were identified and biotyped as the methods of Alton *et al.* [12]. This part of study hasn't been published.

RESULTS AND DISCUSSION

Results obtained by BAPA and RBT as screening tests revealed a positive reactor percentage ranges of 4.6-5.3, 4.4-7.6 and 10-15% followed by overall brucellosis incidence of 4.5, 5.2 and 5.0 % from cattle, sheep and goats at Assuit province, respectively (Table 1).These percentages were similar to that obtained in cattle as 4.8% [21], 4.89% and on sheep as 4.8% [22].

Our percentages were higher than those previously obtained in, cattle as 1.9% [23], sheep as, 1.5% [24-26] and 2.16% [27], 2.31% [28] and in goats as, 4.70 [28], 5.8% [22] and 5.85% [24-26]. Moreover, our percentages were lower than those obtained in, cattle as 6.1% [29], 6.6% [30], 7.1-10 % [31] and 30.6-50 % [32], sheep as, 21.20% [27] and 10.4% [30] and in goats as 14.5% [27].

It is note worthy that no single test can identify all infected animals at all stages of the disease [33, 34] and therefore a combination of serological tests (BAPAT, RBPT, TAT, RIV.T) should be included to reduce the number of both false negative and false positive serological reactions.

It is clearly evident that most of the serological tests used were liable to radical change in their incidence, the great number of false positive detected by BAPA and RBT in the first examination was due to the activity of specific and non-specific antibodies [12].

The results indicated the BAPAT(Table 2), among all tests, gave serorectors of 91.3 in sheep and 100% in cattle and goats as reported [29], followed by 96.9, 82.6 and 100% using RBPT as reported by Shalaby [35] among examined cows and buffaloes.

Interestingly, SAT and Riv.T. indicated seroreactors of 90.6 and 68.75% in cattle, 65.13 and 73.9% in sheep and 100% in goats, respectively (Table 2). Therefore, the above mentioned results indicated the importance of

Table 1: Results of serological diagnosis of brucellosis by BAPAT and RBPT among animals in Assuit province

	Cattle			Sheep			Goats		
Location	No. of animal sampled	Positive	%	No. of animals sampled	Positive	%	No. of animals sampled	Positive	%
Al-Badari	30	-	-	793	60	7.6	15	-	-
Assuit	170	8	4.7	40	-	-	10	-	-
Abnoub	100	5	5.3	90	-	-	15	-	-
El-Fath	200	10	5	140	-	-	10	-	-
El-Qusia	30	-	-	100	5	5	10	-	-
Manflut	110	5	4.6	90	4	4.4	20	3	15
Dyrut	75	4	5.3	70	-	-	20	2	10
Total	715	32	4.5	1323	69	5.2	100	5	5

Table 2: Seroprevalence of brucellosis among reactors animals in Assuit province based on different confirmatory tests

	Serological tests							
	BAPAT		RBPT		SAT		RIV.T	
Animal species/								
Total number	+	%	+	%	+	%	+	%
Cattle/32	32	100	31	96.9	29	90.6	22	68.75
Sheep/69	63	91.3	57	82.6	45	65.1	51	73.9
Goat/5	5	100	5	100	5	100	5	100
Total/106	99	93.39	33	31.13	79	74.52	78	73.58

using several procedures to overcome the problem of escaping of some infected animals in diagnosis of brucellosis as emphasized by Necoletti and Muraschi [36]. Therefore, it is of importance to use more than one diagnostic test for the diagnosis of brucellosis.

In this investigation the highest rate of agreement was between the result of BAPAT and RBT among tested animals which means that should be supported by other confirmatory serological tests. The present results are nearly similar to those previously obtained [37-39] whereas, these authors noticed that BAPAT was similar in its sensitivity to RBT. But it is much higher sensitive than the SAT and Riv. t. in diagnosis of ovine and caprine brucellosis. However, there are some of disagreement obtained between BAPAT and RIV.T among tested animal, thus for the difference in the mode of action of BAPAT detected only IgG_1 and IgG_2 subclasses of immunoglobulin [40] while in RIV.T test case the Rivanol solution (2-ethoxy-6,9 diamino acridine lactate) added to the serum to promotes the reactivity of the IgG_1, the most indicative isotype of infection, reduces the reactivity of IgG_2 and precipitates IgM, the most commonly associated with the non-specific reaction, [41].

The recovery of *Brucella* spp. from culturing of lymph nodes and spleen of serologically positive slaughtered animals, according to bacteriological isolation, the isolation incidence reached to 28.1% in cattle and 40.5% in sheep and goats. Identification and biotyping of all the recovered isolates confirmed *Brucella melitensis* biovar 3 was the sole type detected in this investigation (these results haven't published yet).

The distribution of collective samples were illustrated in fig. 1 and brucellosis percentages were 56.6, 11.3, 9.4, 7.5, 5.7 and 4.7% in Al-Badary, Manflut and El-Fath, Assuit, Dyrut and Abnoub and El-Qusia, districts, respectively.

Brucella strains were isolated from 24 (28%) out of 86 aborted sheep fetus samples. All *Brucella* strains were identified as *B. melitensis* by biochemical tests and PCR. Of the 36 *B. melitensis* isolates, 3, 32 and 1 were identified as biotype 1, biotype 3 and *B. melitensis* Rev-1 vaccine strain, respectively [42].

Using bacteriological isolation as a gold standard by looking for serological profile of bacteriologically positive animals whereas only when the organism could be isolated and identified that give appositive value and 100% infection but negative bacteriological investigation dose not exclude the presence of brucellosis [43].

Table 3: Evaluation of four serological tests in terms of culture results from different infected animal

	Serological tests			
	BAPAT	RBPT	SAT	RIV.T
Sensitivity* (%)	97.4	94.9	84.2	87.6
Specificity** (%)	60	80	81	85.7

*Sensitivity = (True positive/True positive + false negative) X 100

**Specificity = (True negative/True negative+ false positive) X 100

Fig. 1: Assuit administrative map showing the district (O) of the collected samples. Districts with (*) indicate the presence of heavy brucellosis infection of examined animals.

On the other hand, *Brucella* organisms were recovered from serologically negative animals, sensitivity and specificity of different serological tests among different animal species according to bacteriological isolation revealed that the highest rate of sensitivity (97.4%) detected by BAPAT (Table 3) is due to the fact that it detects both IgG and IgM molecules [44]. While the RBPT revealed the high rate of sensitivity (94.9%) more than SAT (84.2%) and Riv.T (87.6%) among tested animal, which was similar to that previously reported [45-48].

SAT appeared to have inferior sensitivity if compared with BAPAT and RBT. This coincided with the results obtained by other investigators [49-52]. While, Riv.T., revealed the highest specificity rate of 85.7% (Table 3) and its sensitivity rate was more than that detected by SAT and lower than by BAPAT or RBT, may be due to the precipitating activity of the Rivanol solution of the IgM, as recorded by Morgan [53, 54] and so the test only detects IgG_1 and IgG_2 immunoglobulins. These results are in agreement to previously reported results [38, 39, 55, 56]. *B. melitensis* strains isolated in Konya region were found to be from different sources by RAPD-PCR. *B. melitensis* biotype 3 was the most common biotype. *B*. [42].

In conclusion, BAPAT and RBPT serological tests revealed the highest rat of sensitivity that guide us to use these tests as screening tests on animals brucellosis. RIV.T showing the highest rate of specificity that bearing in mind the BAPAT and RBT positive samples should be confirmed by this test.

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