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Comparative Study on Serodiagnostic Techniques of *Brucella* Infection in Egypt

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Abstract

Background: Brucellosis is a zoonotic disease that affects wild and domestic animals causing a decrease in reproductive efficiency and abortion and can be transmitted to human. The incidence of human disease is closely tied to the prevalence of infection in animals and considered as an important health problem in Egypt.

Methods and Findings: In this study, blood specimens from 68 patients that showed clinical signs and/or history of brucellosis and from different investigated animals (76 buffalo, 145 cattle and 191 sheep) were collected and serodiagnosed for *Brucella* infection. The sera of these blood specimens were first screened by rose bengal plate test (RBPT) and those giving positive reaction were retested by the standard tube agglutination test (SAT), EDTA modified SAT and rivanol test to determine their titers. The results for clinical specimens showed that 89.70%, 82.35%, 66.18% and 58.82% were positive using RBPT, SAT, EDTA modified SAT and rivanol test, respectively. The respective percentages of brucellosis in buffalo were 44.70%, 43.42%, 43.42% and 43.24%; while the respective percentages of brucellosis in cattle were 46.90%, 43.45%, 39.31% and 37.93%. In addition, serological examination of 191 sheep revealed that 60.20%, 56.54%, 53.40% and 51.83% were positive using RBPT, SAT, EDTA modified SAT and rivanol test, respectively

Conclusion: The results give clear evidence for: (i) the real picture of brucellosis surveillance among human cannot be reflected using single serodiagnostic test, (ii) In comparison to human, serodiagnosis of *Brucella* among animals is less dependent on test type and such dependency took the order sheep > cattle > buffalo, (iii) serodiagnosis of *Brucella* among buffalo had nearly no dependency on test type.

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Introduction

The genus *Brucella* is aerobic, facultative intracellular, Gram negative coccobacilli [1]. The main pathogenic species worldwide are *B.abortus*, *B.melitensis* and *B.suis* which cause abortion in their natural hosts resulting in huge economic losses. They also account for most cases of human brucellosis [2]. Brucellosis is a zoonotic disease that is widely distributed throughout the developing world and has been recognized as a global problem of wild and domestic animals causing a decrease in reproductive efficiency and abortion [3]. The incidence of human disease is closely tied to the prevalence of infection in animals with half a million of new human cases reported annually worldwide [4] and considered as an important health problem in Egypt and an important cause of acute febrile illness (AFI) [5-7]. Diseased animals excrete

Brucella through the urine, milk, placenta and the products of miscarriages. In this way, the bacteria disseminated and infect other animals and humans [8]. Transmission of the infection to humans occurs following direct contact with infected animals and their secretions during septic abortion or at the time of slaughter. Infection can occur via injured skin, inhalation or inoculation into the conjunctival sac of the eyes. Foodborne infection is more frequently via the ingestion of unpasteurized dairy products [9-11]. Acute signs and symptoms of human brucellosis mainly include undulating fever, sweats, headache, myalgia, anorexia, back pain, fatigue and other clinical manifestations such as splenomegaly, hepatomegaly and spondylitis [12]. Complications of human brucellosis may include infective endocarditis [13], splenic, liver and pulmonary abscesses [14] with splenomegaly or hepatomegaly [15], osteoarticular manifestations, genitourinary complications, neurological findings, mucocutaneous manifestations [16], deep vein thrombosis [17], meningitis [18], nephritis [19] and ocular manifestations [20]. *Brucella* bacteremia can result in abortion in pregnant women, especially during the early trimesters. Abortion is a frequent complication of brucellosis in animals, where placental localization is believed to be associated with erythritol, a growth stimulant for *Brucella* [21]. Although not routinely diagnosed, brucellosis is reported in all domestic animals in the Near East region, including Egypt. The highest incidence of human brucellosis is reported in Saudi Arabia, Iran, the Palestinian Authority, Syria, Jordan, and Oman. The most common *Brucella* species reported in Egypt is *B.melitensis* [22]. In this article, a number of serodianostic techniques were used for detection of *Brucella* infection among humans and different animal species. The percentages of disease detection of the applied tests among investigated cases were calculated and provided.

Materials and Methods

Specimen collection

Blood specimens were obtained from 68 patients from Abbasia Fever Hospital (25 patients) and different private laboratories (43 patients) who showed suspected brucellosis, depending upon history and/or clinical signs as well as from different investigated animals, both apparently healthy animals and suggestive infected cases (suffering from abortion), from different farms. These investigated animals comprised 76 buffalo (1 herd), 145 cattle (3 different herds) and 191 sheep (2 different herds). For clinical specimens, the treatment/therapy history was not considered during specimens collection since the study focused primarily on serodiagnosis of suspected cases and not concerned with the impact of therapy on the incidence/prevalence of brucellosis. While in case of animals, the infected ones after establishment of diagnosis are killed and no treatment/therapy is recommended.

Chemicals

Rose bengal plate test antigen, standard *Brucella* concentrated antigen for standard tube agglutination test, rivanol test antigen and rivanol reagent were provided by Central Veterinary Lab., Newhow, Weybridge, Surrey KT 15, England. Ethylene diamine tetra acetic acid (EDTA) was purchased from El-Nasr pharmaceutical chemicals Co. (ADWIC), Abuzaabal, Qalyubiyah, Egypt. 0.5% phenol saline solution was used for SAT and EDTA modified SAT.

Preparation of sera

Blood specimens were transferred to sterile dry vacutainer tubes which were left at room temperature for about one hour to facilitate blood clotting before they were transferred to the laboratory. In the laboratory, vacutainer tubes were kept in refrigerator (4°C) overnight to help serum separation and the clear sera that oozed from the clotted blood specimens were aspirated by sterile Pasteur pipettes and put in sterile screw capped tubes to be stored in the deep freezer (-20°C) until being tested. For some blood specimens, centrifugation at 3000 rpm for 10 minutes was applied to obtain clear sera.

Serological tests

The sera of blood specimens were first screened by RBPT and those giving negative results were discarded, whereas sera giving positive reaction were retested by the SAT, EDTA modified SAT and rivanol test to determine their titers. RBPT, SAT and rivanol test were carried out as described by Alton *et al*. [23] and EDTA modified SAT was done as described by MacMillan and Cockrem [24].

Results

The results for clinical specimens showed that 61 (89.70%), 56 (82.35%), 45 (66.18%) and 40 (58.82%) were positive using RBPT, SAT, EDTA modified SAT and rivanol test, respectively (**Table 1**). Serum specimens that showed a titer of 1/80 for SAT and EDTA modified SAT were considered as suspicious cases for human brucellosis. The respective percentages of brucellosis in buffalo were 34 (44.70%), 33 (43.42%), 33 (43.42%) and 32 (43.24%); while the respective percentages of brucellosis in cattle were 68 (46.90%), 63 (43.45%), 57 (39.31%) and 55 (37.93%). In addition, serological examina-

Table 1. Serological results of clinical blood specimens.

Source of specimen collection	No. of specimens	Number and percentage(a) (%) of specimens showing Brucella infected cases.(b)			
		RBPT	SAT	EDTA modified SAT	Rivanol test
Private laboratories	43	38 (88.37%)	35 (81.39%)	29 (67.44%)	27 (62.79%)
Abbasia Fever Hospital	25	23 (92%)	21 (84%)	16 (64%)	13 (52%)
Overall data	68	61 (89.7%)	56 (82.35%)	45 (66.18%)	40 (58.82%)

(a) Percentage was calculated depending upon the number of positive specimens obtained with each test relative to the total number of specimens of each source and the number of positive specimens relative to the total number of specimens of the two sources in case of overall results.
(b) As stated by Alton *et al.* [23] *Brucella* infected cases were those showed positive results with RBPT, those gave titer of ≥ 1/160 with both SAT and EDTA modified SAT as well as those gave positive results with any titer for rivanol test.

Table 2. Serological results of animal specimens.

Animal type	Locality	No. of herds	Animal No. per herd(s)	Number and percentage(a) (%) of specimens showing Brucella infected cases.(b)			
				RBPT	SAT	EDTA modified SAT	Rivanol test
Buffalo	El Menoufia	1	76	34 (44.7%)	33 (43.42%)	33 (43.42%)	32 (43.24%)
Cattle	Sharkia	1	74	29 (39.19%)	29 (39.19%)	27 (36.48%)	27 (36.48%)
	Sharkia	1	37	16 (43.24%)	13 (35.14%)	12 (35.14%)	11 (29.74%)
	Kafer El Sheikh	1	34	23 (67.65%)	21 (61.76%)	18 (52.94%)	17 (50%)
Overall data for cattle		3	145	68 (46.9%)	63 (43.45%)	57 (39.31%)	55 (37.93%)
Sheep	El Kalyobia	1	53	21 (39.62%)	20 (37.73%)	19 (35.84%)	20 (37.73%)
	El Menoufia	1	138	94 (68.11%)	88 (63.76%)	83 (60.14%)	79 (57.24%)
Overall data for sheep		2	191	115 (60.2%)	108 (56.54%)	102 (53.4%)	99 (51.83%)

(a) Percentage was calculated depending upon the number of positive specimens obtained with each test relative to the total number of specimens of each herd and the number of positive specimens relative to the total number of specimens of each animal type in case of overall results.
(b) As stated by Alton *et al.* [23] *Brucella* infected cases were those showed positive results with RBPT, those gave titer of ≥ 1/40 with both SAT and EDTA modified SAT as well as those gave positive results with any titer for rivanol test.

Table 3. Serological results in titer for SAT, EDTA modified SAT and rivanol test of clinical blood specimens.

Source of specimen collection	No. of specimens	Titer obtained with									
		SAT			EDTA modified SAT			Rivanol test			
		< 1/80	1/80	≥ 1/160*	< 1/80	1/80	≥ 1/160*	1:25*	1:50*	1:100*	1:200*
Private laboratories	38	1	2	35	2	7	29	2	7	13	9
Abbasia Fever Hospital	23	0	2	21	3	4	16	1	3	6	2
Overall data	61	1	4	56	5	11	45	3	10	19	11

* Titers represent *Brucella* infected cases according to Alton *et al.* [23].

Table 4. Serological results in titer for SAT, EDTA modified SAT and rivanol test of animal serum specimens.

Animal type	Locality	No. of herds	Animal No. per herd(s)	No. of specimens	Titer obtained with									
					SAT			EDTA modified SAT			Rivanol test			
					< 1/20	1/20	≥ 1/40*	< 1/20	1/20	≥ 1/40*	1:25*	1:50*	1:100*	1:200*
Buffalo	El Menoufia	1	76	34	0	1	33	1	0	33	2	17	4	9
Cattle	Sharkia	1	74	29	0	0	29	0	2	27	4	18	3	2
	Sharkia	1	37	16	2	1	13	3	1	12	1	5	3	2
	Kafer El Sheikh	1	34	23	0	1	22	2	3	18	2	9	5	1
Overall data for cattle		3	145	68	2	2	64	5	6	57	7	32	11	5
Sheep	El Kalyobia	1	53	21	1	0	20	1	1	19	2	9	5	4
	El Menoufia	1	138	94	2	4	88	4	7	83	5	33	22	19
Overall data for sheep		2	191	115	3	4	108	5	8	102	7	42	27	23

* Titers represent *Brucella* infected cases according to Alton *et al.*[23].

tion of 191 sheep revealed that 115 (60.20%), 108 (56.54%), 102 (53.40%) and 99 (51.83%) were positive using RBPT, SAT, EDTA modified SAT and rivanol test, respectively (**Table 2**). Serum specimens that showed a titer of 1/20 for SAT and EDTA modified SAT were considered as suspicious cases for animal brucellosis. The titer results for SAT, EDTA modified SAT and rivanol test for clinical and animal specimens are shown in **Table 3** and **Table 4**, respectively.

Discussion

The serological diagnosis showed high prevalence of human brucellosis among suspicious human patients. The high prevalence of human brucellosis in this study compared to that reported by Fouad *et al.* [25] (26%) and Refai [22] (11%) was due to that the patients involved in this study were selected based on clinical evidence and/or personal history for

brucellosis. However, 58% seropositive cases were recorded between family members of infected cases in Saudi families [26] and 84.9% prevalence was reported by Nimri [27]. In addition, Kazemi *et al.* [28] reported 80.76% seropositive individuals among suspicious human patients.

Our results also showed high prevalence of brucellosis between different investigated animals and this was because that all herds used in the study were suffered from history of brucellosis. This high prevalence was agreed with that reported by Chauhan *et al.* [29] (44%) and Nasir *et al.* [30] (35.40%) for buffalo and with that reported by Genc *et al.* [31] (55.2%); Otlu *et al.* [32] (34.64%) and Sahin *et al.* [33] (39.5%) for cattle. In addition, the high prevalence of sheep brucellosis in this study was agreed with that reported by Al-Talafhah *et al.* [34] (56%); Gupta *et al.* [35] (59%); Nashwa *et al.* [36] (31.3%); Otlu *et al.* [37] (40.1%) and Celebi and Ataby [38] (36.7%).

The results revealed that the number of positive reactions with rivanol test were found to be < EDTA modified SAT < SAT < RBPT. The high prevalence rate obtained with RBPT may be attributed to the high sensitivity and low specificity of the test. RBPT is mainly used for screening purposes which is rapid, simple and sensitive test but has low specificity and is usually followed by one of more specific confirmatory assays [30]. Several serological assays have been developed to diagnose brucellosis including SAT which stills the most reliable method [39]. Gall and Nielsen [40] reported that SAT had higher specificity (95.7%) but lower sensitivity (75.9%) than RBPT which had sensitivity and specificity of 81.2% and 86.3%, respectively. Thus, in the present study, sera that gave positive results with RBPT were retested using the more specific test (SAT). On the other hand, false-positive reactions can also be seen in the SAT and they occasionally result from cross-reactions with antibodies to *Salmonella spp., Yersinia spp., Vibrio cholera, Francisella tularensis* or *Escherichia coli* O:157 [41] resulting in doubtful reactions (with titer of 1/80 or 1/20 for clinical and animal specimens, respectively). Macmillan and Cockrem [24] stated that agglutination reaction was sufficiently affected by the action of EDTA. It was reported that non specific reactions with *Brucella* could be reduced by addition of EDTA [42, 43]. Thus, EDTA modified SAT showed less positive reactions than that obtained with SAT; however, doubtful reactions could also be observed for EDTA modified SAT. Rivanol test is useful in detection of chronic cases that mainly contain IgG. The rivanol test detects principally IgG1, and to a lesser extent IgG2, because initial treatment of sera with rivanol solution removes IgM by precipitation, reduces the reactivity of IgG2 and promotes the reactivity of IgG1. This gives the rivanol test low sensitivity but high specificity [44]. Thus, rivanol test showed the least number of positive reactions. In conclusion, the results showed that: (i) the real picture of brucellosis surveillance among human cannot be reflected using single serodiagnostic test, (ii) In comparison to human, serodiagnosis of *Brucella* among animals is less dependent on test type and such dependency took the order sheep > cattle > buffalo, (iii) serodiagnosis of *Brucella* among buffalo had nearly no dependency on test type.

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