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Sero-Prevalence of Brucellosis in Egypt with Emphasis on Potential Risk Factors

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Abstract: Brucellosis is still an endemic disease in human and animals of most developing countries; despite all attempts that were adopted worldwide to control the disease. This study was carried out to monitor the current prevalence of brucellosis in Egypt with emphasis on some relevant risk factors. 1935 animals raised at Lower Egypt (305 local cows, 1103 buffalo cows, 381 she camels, 36 mares, 70 ewes and 40 does) as well as 479 contact persons were subjected to serological investigations for brucellosis. Serological tests were carried out using the standard tube agglutination test (STAT) and Rose Bengal test (RBT). Results showed that the incidence of brucellosis averages 6.26% in human and 5.68% in animals. In human, the incidence was high in males (76.66%), rural inhabitants (86.66%) and occupations dealing with animals or its products (76.7%). In animals, the highest incidence of brucellosis was recorded in goat does (10.0%) and the lowest incidence in mares (2.77%) and buffalo cows (4.35 %). The rate of infection was high in all animals suffering from reduced fertility (6.32%) as compared to fertile animals (4.05 %). STAT (100%) is more sensitive than RBT (83.3%) for diagnosis of brucellosis. In conclusion, brucellosis still represents a public health hazard in Egypt, especially at rural areas. It is suggested to diagnose the infection using more than one serological test. Special care should be taken in handling of animals suffering from reduced fertility, especially goats.

Key words: Brucellosis · Human · Animals · Risk factors · Infertility · STAT · RBT

INTRODUCTION

Brucellosis is an important zoonotic disease of nearly worldwide distribution, especially in Mediterranean countries and the Middle East and it remains a significant public health concern [1].

The ultimate sources of infection with brucellosis are infected animals, mainly the major food-producing animals; bovines, ovine, caprine and porcine. Canines and equines are less important sources, but they can be very significant local sources of infection in some regions [2].

The true global incidence of human brucellosis is difficult to determine, but the incidence of the disease worldwide is estimated at more than 500,000 infections per year [3]. The reported incidence of human brucellosis in endemic areas varies widely; from <0.01 to >200 per 100,000 population [4].

Transmission of brucellosis to humans occurs through ingestion of infected animal products, direct contact with infected animals and its materials and through the inhalation of infected aerosolized particles [5].

Symptoms of brucellosis are protean in nature and non specific enough to support the diagnosis [6]. The disease is characterized by acute and chronic infections in animals leading to abortion and infertility [7]. In humans, it is a systemic, febrile illness and can be associated with chronic debilitating infection of major body organs, including bone, kidney, brain, epididymis, liver, ovary and gall bladder [8].

Due to its heterogeneous and poorly specific clinical symptomatology, the diagnosis of brucellosis always requires laboratory confirmation, either by the tedious isolation of the pathogen or by demonstration of specific antibodies. Culture provides direct evidence of the presence of the pathogen and is the gold standard [3] but blood culture sensitivity is often low, ranging from 50 to

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90 % depending on the disease stage, Brucella species, culture medium, quantity of circulating bacteria and the blood-culture technique employed [9].

The present investigation was carried out to monitor the current prevalence of brucellosis in human and contact animals, with emphasis on risk factors associated with its occurrence and to depict its public health implication in Egypt using the standard serological tests.

MATERIALS AND METHODS

Human Subjects: A total number of 479 persons was enrolled in this study; special interest was given to subjects that were suspected to suffer from brucellosis, based on history taking and clinical manifestations. Inclusion criteria included the following symptoms and signs; Fever, often rising to 40°C or more in the afternoon-a rising and falling (undulating) fever is one of the hallmarks of the disease, chills, weakness, fatigue, joint, muscle and back pain or headache, drenching sweats can occur, particularly at night, splenomegaly, hepatomegaly, coughing and pleuritic chest pain are sometimes seen. Full history was taken with special stress on age and sex. Because brucellae typically take 1-8 weeks to incubate, the history included any possible exposures in the preceding few months. Occupational history (e.g. farmer, veterinarian) that is suggestive of exposure to a source animal was considered. Other potential risk factors such as exposure to potentially contaminated foodstuffs or consumption of unpasteurized infected milk or milk products or travel to an area where the disease is endemic were also asked.

Animal Subjects: A total number of 305 local cows, 1103 buffalo cows, 381 she camel, 36 mares, 70 ewes and 40 does was clinically examined and case history was recorded. These animals were examined at the veterinary clinics and/or at small holder farms at Lower Egypt. Special interest was given to the fertility status. Animal showed normal ovarian activity (Using ultrasonography), pregnancy or normal pattern of serum progesterone level was considered fertile. Animals with case history of repeat breeding, have inactive ovaries or non detected serum progesterone level were considered to suffer from reduced fertility. She camels were examined at abattoir after slaughtering.

Blood Samples: The blood samples collected from all subjects were centrifuged at 1200 Xg for 5min. at 4°C. Serum samples were harvested and kept at-20°C until used.

Serological Tests: Serum Tube Agglutination Test (STAT): It was performed with commercial Brucella antigen (Atlas Febrile Antigens, Slide/Tube Test), according to the manufacturer's instructions. The test results were interpreted and titers equal or in excess of 1: 160 were considered significant [10].

Rose Bengal Test (RBT): RBT was performed with commercial Brucella antigen (Bio-Systems, Barcelona, Spain), according to the manufacturer's instructions. The test slide was examined macroscopically under a strong light source after 4 minutes. A positive result was indicated by the obvious agglutination pattern of the latex, in a clear solution.

Data Analysis: The collected data were statistically analyzed using SPSS program software version 17.0.

RESULTS AND DISCUSSION

In most developing countries, brucellosis is still an endemic disease in human and animals, despite of attempts that are adopted to control the disease. Although human brucellosis is a notifiable disease, it is often labeled "fever of unknown cause", the actual number of cases of brucellosis is unknown and is believed to be far more than the officially reported figures [11].

Table 1: Incidence of brucellosis among different animal species of examined farm animals with emphasis on fertility status (%)

			Positive	
Species	Fertility status	Total examined number	No.	%
Cows	F	95	6	6.32
	RF	210	18	8.57
	Total	305	24	7.86
Buffalo-cows	F	290	9	3.10
	RF	813	39	4.80
	Total	1103	48	4.35
She Camels	F	73	4	5.48
	RF	308	25	8.12
	Total	381	29	7.61
Mares	F	15	0	0.00
	RF	21	1	4.76
	Total	36	1	2.77
Ewes	F	45	2	4.44
	RF	25	2	8.00
	Total	70	4	5.71
Does	F	25	1	4.00
	RF	15	3	20.00
	Total	40	4	10.00
overall	F	543	22	4.05
	RF	1392	88	6.32
	Total	1935	110	5.68

F=Fertile. RF= Reduced fertility

In the present study, serological investigations revealed that out of 479 persons examined, 30 (6.26 %) were positive for brucellosis. Moreover, results of examination of 1935 farm animals from different species revealed that 110 (5.68%) were positive for brucellosis (Table 1).

Regarding the incidence of brucellosis in human, similar incidences (5.2-7.1 %) were previously reported [12-16]. On the other hand, a lower incidence (3.8%) was reported by Schelling *et al.* [17] and higher incidences (8.3-15%) were recorded by Zaneva *et al.* [18], Mutanda [19] and Al Sekait [20].

In animals, it was evident that the incidence was low in mares (2.77%) and buffalo cows (4.35%). On the other hand, the highest reported incidence among the examined animals was in does (10%). Moreover, it was clear that animals suffering from reduced fertility (6.32%) showed the highest incidence of brucellosis if compared with the normal fertile animals (4.05%). In this respect, Hassan *et al.* [21] reported prevalence of brucellosis in cattle (5.44%), sheep (5.41%), goats (3.55%) and buffaloes (4.11%). Ahmed *et al.* [22] reported that amongst examined livestock, 31% of goats and 42% of cattle were seropositive. Variations in the recorded results among examined farm animals could be due to the course of the disease, locality, rate of exposure to infection, reproductive status, in addition to the variety of the used diagnostic techniques.

The high incidence of brucellosis in goat does in the present study may be related to the nature of raising of this species and its rapid transfer among different localities with different hygienic measures. Also, goats were reported to have more infection with *Brucella melitensis* [11] which induced more severe pathological outcomes in human.

In the current investigation, the incidence of brucellosis was higher in animals suffering from reduced fertility if compared with fertile animals. Wither infertility predisposed to infection or vise versa it was not clear. However, it is well documented that fertility is associated with normal regular hormonal rhythm, especially of the reproductive hormones which are responsible for competent and counteract of infections [23].

Regarding the potential risk factors of the studied human cases, table 2 shows variations in the incidence due to gender, occupation and residence

It was evident from this study that there was a predominance of male (76.66%) over female distribution (23.33%). This finding is in accordance with data from surveillance system in Egypt (64.0%; ESCD [24] and 65%; Afifi *et al.* [25]) as well as in Kuwait

Table 2: Sero-positivity of human brucellosis cases on the basis of different risk factors

Variable		Positive (30)
Sex	Male	23 (76.66%)
	Female	7 (23.33%)
Occupation	House wife	7 (23.33%)
	AAPH*	23 (76.66%)
Residence	Rural	26 (86.66 %)
	Urban	4 (13.33 %)

* AAPH= Animal and animal products handlers

Table 3: Sensitivity of the used brucella tests

Variables		N0	%
STAT			
•	1/160	8	26.7
•	1/320	9	30.0
•	1/640	4	13.3
•	1/1280	9	30.0
•	total	30	100.0
RBT			
•	Positive	25	83.3
•	Negative	5	16.7

(90.5%; Shehata *et al.* [26]). Also, Jennings *et al.* [27] found that males formed 70% of cases of brucellosis. This sex distribution in the incidence of brucellosis may be due to males have more possibilities for contact with animals than females [28]. Regarding to the residence in the present study, most of cases live in rural area (86.66%). This finding is in agreement with previous studies carried out by Kozukeev *et al.* [29] and Sofian *et al.* [30] who revealed that 86 and 85.3% of cases were inhabitants of rural areas, respectively. Also, in rural areas, the disease is associated with animal husbandry and consumption of local dairy products [31]. However, Afifi *et al.* [25] showed similar distribution in both localities in all parts of Egypt whereas, animal exposure can occur in all regions and unpasteurized dairy products are widely available throughout the country.

In the current work, occupations dealing with animals or its products formed 76.7% of the studied cases. This is comparable with Bikas *et al.* [32] who found that 85.7% of cases were animal breeders. Much more, in a study conducted in Central Greece, Minas *et al.* [33] detected that people in occupations dealing with animals constituted the majority (91.5%) of patients. These occupations included farmers, slaughterhouse workers, butchers, veterinarians, cheese factory workers, cookers as well as household members as they often help their family in flock management, acquiring the infectious agent by direct contact with animals.

Table 4: Distribution of RBT results among different titers of positive STAT in the studied cases:

	RBT		
STAT	Positive (No=25)	Negative (No=5)	p
≥ 160	25 (100%)	5 (100%)	1.000
≥ 320	19 (76.0%)	3 (60.0%)	0.508
≥ 640	11 (44.0%)	2 (40.0%)	0.004*
≥ 1280	8 (32.0%)	1 (20.0%)	<0.001*

#McNemat test *Significant

Comparing results of STAT and RBT among positive human cases (30) shown in table (3) revealed that STAT (100%) is more sensitive than RBT (83.3%). This result coincides with those reported by Yildiz *et al.* [34] and Sirmatel *et al.* [35].

Mantur *et al.* [9] 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, STAT titers above 1:160 are considered diagnostic in conjunction with a compatible clinical presentation. However, in areas of endemic disease, using a titer of 1:320 as cutoff may make the test more specific.

Table (4) shows no significant difference between diagnosis of brucella by RBT and both STAT = 160 and STAT = 320, but there is a significant difference between diagnosis of brucella by RBT and both STAT = 640 and STAT = 1280.

These findings are in agreement with Franco *et al.* [36] who used the RBT as a screening test and positive results were confirmed by STAT. However, Mesa *et al.* [37] analyzed the diagnostic gain after the performance of the RBT and revealed that it was 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 have a history of infection. So the use of the RBT as the sole technique for the diagnosis of brucellosis in endemic areas should be considered very carefully in the context of patients who are exposed repeatedly to Brucella or have a history of brucellosis. The high sensitivity, together with the fact that the technique is simple and rapid (4 min), makes the RBT is ideal for screening patients for human brucellosis. Also, Junaidu and Garba [38] applied RBT, STAT and competitive ELISA for the detection of Brucella antibodies in slaughtered cattle. It was observed that of the 1711 screened serum samples 383 (22.38%), 376(21.97%) and 395(23.08%) were positive for Brucella using RBT, SAT and competitive ELISA respectively. Based on these results, it was observed that application of more than one test method might ensure more sensitivity and specificity of the diagnosis of brucellosis. Mantur *et al.* [9] detected that The RBT is often used as a rapid screening test, the sensitivity is very high (>99%), but the specificity is disappointingly low. Whenever possible, a serum that gives a positive result should be confirmed by a more specific test. The RBT is also of value in the rapid confirmation of neurobrucellosis, arthritis, epididymoorchitis and hydrocele due to Brucella if the neat is positive in CSF, synovial fluid, testicular fluid /semen and hydrocele fluid respectively

In conclusion, brucellosis still represents a significant public health problem in Egypt. Human brucellosis is related to keeping animals in house, animal husbandry and consumption of local dairy products. The data presented in this study suggested that the STAT ≥ 320 has greater diagnostic accuracy than the RBT and the combination of the 2 tests together has no statistically significant value. Great care should be taken in handling of animals suffering from reduced fertility, especially goats.

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