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**BRUCELLOSIS AMONG PERSONS AT HIGH-RISK
OCCUPATION IN SUEZ CANAL AREA, EGYPT**
(With 4 Tables)

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مرض البروسييلوسيس بين الأشخاص أصحاب المهن الأكثر خطرا للتعرض
للإصابة في منطقة قناة السويس، مصر

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لتحديد انتشار الإصابة بالبروسيلا بين الأشخاص أصحاب المهن الأكثر خطرا للتعرض للإصابة تم فحص ١٣١٦ شخص (٨٨٦ أكثر خطرا للتعرض و ٤٣٠ من المقيمين في المدن) من محافظة الإسماعيلية وبور سعيد باستخدام اختبار الـ روز بنجال (اختبار التليد السريع) واختبار التليد البطيء الأنبوبي ووجد أن النسبة الكلية لانتشار الإصابة هي ٥٠.١%، وكانت أعلى معدلات للإصابة بين المساعدين البيطريين (٤٤.٤%) والأطباء البيطريين (٢٣.٥%) ثم العاملين في المجازر (١١.٦%) والمزارعين وأسرهم (٣.١%). وكان أقل معدل للإصابة بين المقيمين في المدن والغجر ملامسين لحيوانات المزرعة (١.٦%). ولا يوجد اختلاف في نسبة الإصابة بين الذكور والإناث بالنسبة للمزارعين والمقيمين بالمدن. بينما المهنيين الآخرين الذين تم فحصهم كانوا كلهم ذكور وأعمارهم تتراوح بين ٢٨ إلى ٥٣ سنة ونسبة الإصابة بينهم كانت تزداد بزيادة أعمارهم. وبالنسبة لعزل البروسيلا تم عزل عترة البروسيلا ميلينتس النوع الثالث من دم عدد ٤ أشخاص إيجابيين للاختبارات السيرولوجية السابقة. وأتضح من هذه الدراسة إن الأكثر خطرا للتعرض للإصابة بالبروسيلا هم الأشخاص الذين يساعدوا في ولادة الحيوانات، وأن عترة البروسيلا ميلينتس هي المسبب الأكثر لمعظم حالات الإصابة بهذا المرض بين الأميين، وهذا بدوره يبين مدى أهمية الأغنام والماعز في نشر الإصابة بين الحيوانات والإنسان والإجراءات التي يجب أن تتخذ للتحكم ومنع الإصابة بهذا المرض خاصة في الأغنام والماعز.

SUMMARY

Brucellosis is considered one of the most important zoonotic diseases constituting a public health problem throughout the world, particularly in

the developed countries. This study was to determine the prevalence of brucella infections among persons at high-risk occupation. A total of 1316 persons (886 at high-risk and 430 of city dwellers), from Ismailia and Port Said provinces, were first screened for brucella antibodies by Rose Bengal test (RBT) to measure the exposed rate. Reactive sera were further analyzed by the standard tube agglutination test (STAT). The prevalence of brucella infections among the examined persons was 5.1% (67 out of 1316 human sera). The highest infection rates were recorded among veterinary assistants (44.4%) followed by veterinarians (23.5%) then abattoir workers (11.6%), farmers and their families (3.1%), and lastly the city dwellers (1.6%) that were not in contact with the farm animals. The seroprevalence among farmers and city dwellers was not influenced by sex. All isolates were identified and biotyped as *Brucella melitensis* biovar3 (4 isolates) that isolated from human blood of four seropositive persons at high-risk occupation which had STA-antibody titers of 1:1280. The current study found a very high risk associated with assisting in animal parturition but no significant risk associated with other direct animal contact. Such findings could be used as means to locate cases of human brucellosis and design measures to control brucellosis in man and animals.

Key word: *Brucellosis, high-risk occupation, Suez Canal area.*

INTRODUCTION

Despite long standing efforts for control and eradication of brucellosis, this disease remains a zoonotic disease in the Middle East countries. Human infection by brucella organisms has been caused by four species: *Brucella melitensis*, *B. abortus*, *B. suis* and *B. canis* (FAO/WHO, 1986 and Young, 1991). In the Middle East, *B. melitensis* accounts for most human cases (Dajani *et al.*, 1989 and Al-Balla *et al.*, 1994). The major route of human infection in endemic areas is ingestion of unpasteurized milk or its products. In non endemic areas, occupational exposure through direct contact with infected livestock, or brucella culture represents the major route of transmission, via the respiratory tract, conjunctiva and skin abrasion (Madkour and Gargani, 1989 and Young, 1991). Seroprevalence of brucellosis is higher in individuals working in livestock and meat processing industries, veterinary services and laboratory workers handling brucella culture (FAO/WHO, 1986). In

Egypt, earlier studies on animals have shown high prevalence of positive reactors ranging from 7.2 to 38.4% in cattle; 1.8 to 15.3% in buffaloes; 3.1 to 8.6% in sheep; 3.7 to 11.3% in goats and 1.7 to 12.3% in camels (Abou-Eisha, 1986; Mahmoud, 1991; El-Bauoumy, 1993; El-Sheery, 1993; Soliman, 1993; Youssif, 1994; El-Gawad, 1996; Atwa, 1997; Zayed, 1997; Soliman, 1998 and Abou-Eisha, 2000). Moreover, the increasing importation of animals and establishment of big farms are accompanied with increased rate of brucella reactors (Selim, 1987; Hamdy, 1989 and Refai, 1994). These findings indicate that the prevalence of brucella infections in animal reservoirs provides a key to its occurrence in humans (Seroka and Seroka, 1993).

In view of the little information on the prevalence of brucellosis among persons who are in close contact with animals in Suez Canal area, this study was undertaken to determine brucella infections among persons at high-risk occupation.

MATERIAL and METHODS

Study population:

This study was carried out in Ismailia and Port Said Provinces in the period from February 2000 to April 2001. The study population consisted of farmers and their families, abattoir workers, veterinarians, veterinary assistants and others were not in contact with animals (city dwellers).

Sampling:

A total of 1316 blood samples were collected from persons of veterinary health units and slaughter houses; persons submitted to the transported health units in the different villages of Ismailia province and patients of Ismailia and Port Said fever hospitals. These individuals were requested to answer a series of questions in order to obtain more information such as name, address, age, sex, occupation and their feeding habits. Each sample was marked and identified, then transferred to the laboratory of the Faculty of Veterinary Medicine, Suez Canal University, for preparation and maintenance of the serum.

Serologic examination:

All serum samples were first screened for brucella antibodies by Rose Bengal test using *Brucella abortus* Rose Bengal stained that commercially supplied by BioSystem, S.A. Reactive sera were further analyzed by Standard tube agglutination test (STAT), using standard

Brucella abortus agglutination concentrate (Serum and Vaccine Res. Inst. Abbasia, Cairo, Egypt), in which a titer of 1/40, i.e. 50% agglutination (80 I.U./ml) or above indicates positive reaction (Alton et al., 1975).

Bacteriological examination:

Human blood samples were collected aseptically from the seropositive cases for brucella. These blood samples were bacteriological examined for brucella organisms using direct culture and subculturing from broth. *Brucella* agar medium containing antibiotics was used, incubated at 37 C in an atmosphere of 10% CO₂ for 6 days then examined for brucella Colonies. Isolates were identified as *Brucella* species by their colony morphology, Gram stain, urease and oxidase reactions, H₂S production, growth on dyes: thionin&basic fuchsin and agglutination with brucella specific antisera (Central Vet. Lab. Weybridge, England) (Alton et al., 1988).

RESULTS

Table 1: Prevalence of brucella antibodies among persons according to their occupations.

Occupations	Total No. examined	Seropositive cases (%)	
		RBT +ve No (%)	STAT +ve No (%)
-Farmers and their families	755	23(3.1)	16(2.1)
-Abattoir workers	43	5(11.6)	4(9.3)
-Veterinarians	34	8(23.5)	8(23.5)
-Vet. Assistants	54	24(44.4)	22(40.7)
-City dwellers (not in contact with animals)	430	7(1.6)	5(1.2)
Total	1316	67(5.1)	55(4.2)

Difference in brucella positive reactors between personnel groups who were in close contact with animals (6.8%) and city dwellers (1.6%), was statistically significant ($\chi^2=15.8$, $P<0.01$). However, there was no significant difference in positive reactors between farmers and their families, and city dwellers ($\chi^2 =2.2$, $P > 0.05$).

Table 2: Occurrence of brucella antibodies among farmers and their families according to their age and sex.

Age/years	Total No. exam.	Sex				total +ve(%)
		Males		Females		
		No.	+ve(%)	No.	+ve(%)	
<10	49	21	0(0.0)	28	1(3.6)	1(2.04)
10-19	178	75	2(2.7)	103	3(2.9)	5(2.8)
20-29	181	96	4(4.2)	85	2(2.4)	6(3.3)
30-39	176	81	5(6.2)	95	3(3.2)	8(4.6)
40-49	93	50	1(2)	43	1(2.3)	2(2.2)
> 50	78	49	1(2.04)	29	0(0.0)	1(1.3)
Total	755	372	13(3.5)	383	10(2.6)	23(3.1)

$\chi^2 = 0.5$ (P > 0.05)

Table 3: Occurrence of brucella antibodies among city dwellers according to their age and sex.

Age/years	Total No. examined	Sex				total +ve(%)
		Males		Females		
		No.	+ve(%)	No.	+ve(%)	
<10	18	11	0(0.0)	7	0(0.0)	0(0.0)
10-19	58	24	1(4.2)	34	1(2.9)	2(3.5)
20-29	96	51	0(0.0)	45	2(4.4)	2(2.1)
30-39	115	65	1(1.5)	50	0(0.0)	1(0.9)
> 40	143	81	1(1.2)	62	1(1.6)	2(1.4)
Total	430	232	3(1.3)	198	4(2.02)	7(1.6)

$\chi^2 = 0.4$ (P > 0.05)

Table 4: Distribution of the seropositive persons for brucellosis according to STA- antibody titers.

Occupation	Total No. exam.	STA-antibody titers							
		40 No(%)	80 No(%)	160 No(%)	320 No(%)	640 No(%)	1280 No(%)	2560 No(%)	
-Farmers and their families	755	2(12.5)	3(18.8)	5(31.3)	3(18.8)	1(6.3)	2(12.5)	0(0.0)	
-Abattoir workers	43	0(0.0)	0(0.0)	2(50)	2(50)	0(0.0)	0(0.0)	0(0.0)	
-Veterinarians	34	0(0.0)	0(0.0)	1(12.5)	3(37.5)	2(25)	1(12.5)	1(12.5)	
- Vet. assistants	54	0(0.0)	2(9.1)	6(27.3)	3(13.6)	7(31.8)	3(13.6)	1(4.6)	
-City dwellers (not in contact with animals)	430	0(0.0)	2(40)	2(40)	1(20)	0(0.0)	0(0.0)	0(0.0)	

The prevalence of brucella infections among the examined persons according to their occupation is summarized in Table (1). Of 1316 human sera tested, 67 (5.1%) and 55 (4.2%) samples were found positive for brucellosis with RBT and STAT respectively. The highest rates of brucella infections were recorded among veterinary assistants (44.4%) followed by veterinarians (23.5%) then abattoir workers (11.6%), farmers and their families (3.1%), and lastly the city dwellers (1.6%). Generally, there was significant difference between personnel who were in close contact with animals (farmers, veterinarians, veterinary assistants and abattoir workers) and the others were not in contact with animals (city dwellers) ($P < 0.01$). On the other hand, although brucella infection rate among farmers and their families (3.1%) was higher than that recorded among city dwellers (1.6%). Statistically there was no significant difference ($P > 0.05$).

The prevalence of brucella infections among the farmers and their families according to their age and sex is summarized in Table (2). The highest infection was recorded among age group 30-39 years (4.6%), while it was ranged from 1.3 to 3.3% among the other age groups. Although the prevalence of brucella infections among males (3.5%) was higher than that recorded in females (2.6%), there was no significant difference ($P > 0.05$).

Regarding the age of city dwellers (Table, 3), the infection rate ranged from 0.9% among the age group 30-39 years to 3.5% among the age group 10-19 years. There was no infection determined among the age group <10 years. There was no significant difference in infection rate between males and females ($P > 0.05$).

In Table (4): The high STA-antibody titers (1:1280), of brucella seropositive cases were recorded among veterinarians (25%) followed by veterinary assistants (18.2%) then farmers and their families (12.5%). These STA-antibody titers were not recorded among abattoir workers and city dwellers. Mild STA-brucella titers (1:320 - 1:640) of seropositive cases were recorded in 62.5% of veterinarians, 50% of abattoir workers, 45.5% of Veterinary assistants, 25% of farmers and their families and 20% of city dwellers. Low STA-antibody titers (1:40 - 1:160) of reactors were recorded among 80% of city dwellers, 62.5% of farmers and their families, 50% of abattoir workers, 36.4% of veterinary assistants and 12.5% of veterinarians.

Bacteriologically, all isolates were identified and biotyped as *Brucella melitensis* biovar 3. Only four isolates of this strain isolated

from human blood of seropositive persons (2 veterinary assistants, one veterinarian and one farmer), had STA-antibody titers of 1:1280.

DISCUSSION

The human infection with brucella is generally a reflection of the level of activity of the infective agent in the animal reservoir. In this study, it was found that 67 (5.1%) out of 1316 persons (886 at high-risk and 430 of city dwellers) were positive for brucellosis. These results were lower than those previously reported in Egypt by Amer, 1989 (18.75%) and Soliman, 1998 (10.9%). However, Abou-Eisha (2000) in North Sinai, found that the percentage of human reactors for brucellosis was 0.9. A significantly higher seroprevalence of brucellosis among high-risk persons compared to city dwellers was found in the present study ($P < 0.01$). The highest prevalence of brucella infections was recorded among veterinary assistants followed by veterinarians and abattoir workers. These results were in agreement with those previously reported (Cooper, 1992, Al-Sekait, 1993; Abo-Shehada *et al.*, 1996; Araj and Azzam, 1996; Kumar *et al.*, 1997 and Seroka, 1999). It was suggested that the infected tended to participate more in animal parturition and had contact with blood of infected animals (Mishal *et al.*, 1999).

In this study, the total seroprevalence of brucellosis among personnel at high-risk occupation (Veterinarians, Veterinary assistants and abattoir workers) was lower than those reported in Egypt by Amer, 1989 (92.4%) and Hamdy, 1989 (61.6%). In Jordan, Abo-Shehada *et al.* (1991) found that 54.5% of practicing veterinarians were seropositive for brucella.

From the aforementioned results, the prevalence of brucellosis can vary among populations from different geographic locations and countries, mostly due to variation in occupational contact and the type of test use (Alton, 1990 and Abo-Shehada *et al.*, 1991).

In the present study, there was no significant difference in brucella infection rate between farmers and their families, and city dwellers. The ingestion of raw milk and soft cheese (Karish) that made from unboiled or unpasteurized milk are the main source of brucella infection for both farmers and city dwellers (Al-Balla, 1995 and Makhseed *et al.*, 1998).

Regarding the age of the examined persons, the prevalence of brucella infections increased among farmers of age group 30-39 years

and city dwellers of age group 10-19 years. There was no significant difference in the prevalence of brucella infections between males and females in both farmers and city dwellers. These results were in agreement with those previously reported by Abo-Shehadeh *et al.*, 1996, while all of the other examined personnel were males aged 28-53 years. The brucella infections among them increased with increasing their ages.

In this study, the highest STA-brucella titers were recorded among the seropositive veterinary assistants and veterinarians. These results indicated that, when the direct contact with animals was considered, a very high risk associated with assisting in animal parturition but no significant risk associated with other direct animal contact (Cooper, 1992; Kumar *et al.*, 1997 and Seroka, 2000).

Although the present serosurvey suggests the involvement of *Brucella* spp. as an aetiological agent, it should be noticed that antigen preparations of brucella may cross-react serologically with other organisms in similar titers including *Yersinia enterocolitica* serotype 0:9, *Vibrio cholerae*, *Francisella tularensis*, *E. coli* serotype 0157:h7 and *Salmonella* serotype 0:30 (Heizmann *et al.*, 1985 and Moyer *et al.*, 1991).

The isolation of brucella organism is the only definitive means of diagnosis. In this study, only four isolates of *Brucella melitensis* biovar3 were isolated from human blood of four seropositive cases (two veterinary assistants, one veterinarian and one farmer), had STA-antibody titers of 1:1280. On the other hand it was failed to isolate brucella organisms from human blood of the other seropositive cases. This may be attributed to the wide spread use of antibiotics and the low STA- antibody titers among most of the other seropositive cases. It is suggested that the best chance for brucella isolation could be obtained from man with high blood titers (Mikolich and Boyce, 1990 and Shehaby *et al.*, 1990).

Brucella melitensis is considered the indigenous strain prevalent in sheep and goats in Egypt (El-Gibaly *et al.*, 1993 and El-Sheery, 1993). The extent of sheep and goat flocks movement between seasonal pastures is considered as the main factor that increases the exposure potential of individual animal or herds to brucellosis and may be the main obstacle in the control and prevention of this disease in Egypt. The obtained results, in this study, should draw an attention to the serious role played by sheep and goats in transmission of the disease to man and other animals which may act as disseminator of *Brucella melitensis*. This constitutes a serious public health problems (Mishal *et al.*, 1999; Abu-Shaqra, 2000 and Reviriego *et al.*, 2000).

In conclusion, this study revealed a high prevalence of brucella infections among persons at high-risk occupation especially those associated with animal parturition and *Brucella melitensis* was accounted for most human cases. Such findings could be used as means to locate cases of human brucellosis, initiate public education and design measures to control brucellosis with giving more attention to sheep and goats. The interchange of information and surveillance data between veterinary and health service is mostly essential for prevention of the disease in man and animals.

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