

Seroepidemiological Study on Human Brucellosis in Assiut Governorate

¹Asmaa A.A. Hussein; ¹Amal S.M. Sayed and ²Mohamed A. El Feki

¹Animal Hygiene & Zoonoses Department, Faculty of Veterinary Medicine, ²Microbiology & Immunology Department, Faculty of Medicine, Assiut University, Assiut, Egypt.

Brucellosis is the most important zoonotic disease constituting a public health problem in Assiut Governorate, hence this study was carried out to determine the prevalence of brucellosis among humans in Assiut Governorate. A total of 7154 peripheral blood samples were collected from patients with fever at Assiut Fever Hospital during the period from 2002-2003. A full detailed anamnestic and clinical assessment in the form of questionnaire was designed for each individual to determine the risk factors with specific emphasis to age, sex, residence and occupation. All serum samples were screened for *Brucella* antibodies by slide agglutination test. Positive sera were further analyzed by standared tube agglutination test. Enzyme linked immunosorbent assay (ELISA) was carried out to detect IgM and IgG *Brucella* antibodies. Statistical analysis was performed and correlation coefficient was done between all risk factors. Results declared that the prevalence of brucellosis was $(1.29 \pm 0.004 \%)$ and $(1.22 \pm 0.002 \%)$ as detected by agglutination and ELISA, respectively. IgM antibodies were estimated in 9.8 % of the examined patients, while IgG antibodies were found in 30.4 % of the examined patients, moreover both IgM and IgG antibodies were detected in 54.3 % of the examined patients. The prevalence of brucellosis was significantly ($P < 0.05$) affected by sex, where the rate of detection was higher among females ($1.76 \pm 0.009 \%$) than males ($1.05 \pm 0.004 \%$) as detected by agglutination test .On the other hand, the prevalence rate based on ELISA was $(1.64 \pm 0.39 \%$ and $1.01 \pm 0.89 \%$) for females and males, respectively. Prevalence of brucellosis was higher in rural areas ($1.3 \pm 0.005 \%$ & $1.25 \pm 0.009 \%$) than in urban areas ($1.23 \pm 0.001 \%$ & $1.12 \pm 0.01 \%$) as detected by agglutination test and ELISA, respectively. The prevalence of brucellosis varied significantly between different occupational and age groups. Public health impact of brucellosis is discussed and suggestive measures for control are explained.

Brucellosis is the most important zoonosis in terms of social and economic impacts, despite the control measures undertaken by national authorities in many developing countries (Acha and Szyfres, 2001). Half a million of new human cases are reported annually worldwide, however the World Health Organization announced that these numbers are greatly underestimate the true incidence of human disease as the actual number of cases is estimated to be at least 10 times the figures officially announced (WHO, 1997 and Seimenis, 2002).

Brucellosis has been recognized as a global problem of wild and domestic animals, especially cattle, sheep and goats causing a decrease in reproductive efficiency and abortion (Rijpens et al., 1996).The incidence

of human disease is closely tied to the prevalence of infection in animals, where transmission occurs to human by exposure to infected animals and their infectious secreta and excreta during septic abortion, at the time of slaughter and the more frequently through consumption of raw animal products especially milk and dairy products (Wallach, 1994 ; Matar et al., 1996 and Araj , 2000).

In Egypt, brucellosis is still an endemic serious disease among domestic animals and human; inspite of attempts that were implemented in the country to control the disease either through bilateral projects with some agencies or international organizations. Many authors reported the incidence of brucellosis among animals (Mahmoud, 1990; Refai, 1994; Abdel Hafeez et al., 2001 & Ibrahim et al., 2002) and humans (Soliman,

1998 & Abdel Hafeez et al., 2001). The aim of this work was to investigate the prevalence of brucellosis among patients with fever of unknown origin, admitted to Assiut Fever Hospital and determine the possible risk factors in acquiring the infection.

Materials and Methods

Samples collection

A total of 7154 peripheral blood samples from patients with fever of unknown origin were screened at Fever Hospital in Assiut Governorate in the period from 2002-2003. A full detailed anamnestic and clinical assessment in the form of questionnaire was designed for each individual to determine the risk factors with specific emphasis to age, sex, residence and occupation.

Serological examination

All serum samples were first screened for brucella antibodies by slide agglutination test (SASTM, SA Scientific Inc.) according to manufacturer's procedure. Positive reaction sera were further analyzed by standered tube agglutination test, according to manufacturer's procedure (SASTM, Scientific Inc.). A titer of 1/80 or above indicates positive reaction.

Enzyme linked immunosorbent assay (ELISA) was carried out to detect IgM and IgG brucella antibodies by using ELISA IgG/IgM kit (Genzyme Virotech. GmbH) according to Alton et al., (1988).

Statistical analysis

The data were expressed as the means \pm SE for the examined factors, then analyzed by using analysis of variance (ANOVA) and means \pm SE were tested at least significant difference (LSD). Correlation coefficient was done between all risk factors. All tests were done by using PC-Stat computer program.

Results

Table 1 shows the prevalence of brucellosis among patients with fever of unknown origin. The percent detection by agglutination and ELISA tests was 1.29 ± 0.004 and 1.22 ± 0.002 , respectively. All sera showed positive agglutination by SAT and positive ELISA except for five cases (5.4 %) with false negative results. There was no significant difference between results obtained by SAT and ELISA.

Table 1. Seroprevalence of brucellosis among patients with fever of unknown origin

No. of examined samples	Agglutination test		ELISA	
	No.	%	No.	%
7154	92	1.29 \pm 0.004	87	1.22 \pm 0.002 ^{ns}

ns: non significant

IgM antibodies were detected in 9.8 % of the examined patients, while IgG antibodies were

found in 30.4 %. Both of IgM and IgG antibodies were detected in 54.3 %, (Table 2).

Table 2. Prevalence of *Brucella* IgM and IgG antibodies among patients with fever of unknown origin.

No. of examined samples	Types of antibodies					
	IgM		IgG		*IgM & IgG	
	No	%	No	%	%	No
92	9	9.8	28	30.4	50	54.3

*Both IgM & IgG antibodies detected in the same patient.

The prevalence of brucellosis was significantly ($P < 0.05$) affected by sex (Table 3), where the rate of detection was higher among females (1.76 ± 0.009 %) than males (1.05 ± 0.004 %) as detected by agglutination

test, while the prevalence by ELISA was (1.64 ± 0.39 %) and (1.01 ± 0.89 %) for females and males, respectively with no significance difference.

Table 3. Occurrence of brucellosis in patients with fever of unknown origin according to sex.

Sex	No. of samples	Agglutination		ELISA	
		No.	%	No.	%
Male	4769	50	1.05±0.004 ¹	48	1.01±0.89 ¹
Female	2385	42	1.76±0.009 ²	39	1.64±0.39 ¹
Total	7154	92	1.29	87	1.22

-Means in the same column with the same superscript letters are not significantly different.

^{1,2}P < 0.005

Table 4 shows that the prevalence of brucellosis is higher in rural areas (1.3 ± 0.005 % & 1.25 ± 0.009 %) than in urban areas (1.23 ± 0.001 % & 1.12 ± 0.01 %) as detected by agglutination test and ELISA respectively, and it was not statistically different.

Table 4. Ecological distribution of brucellosis among patients in rural and urban areas

Residence	No. of samples	Agglutination		ELISA	
		No.	%	No.	%
Rural	5365	70	1.3±0.005 ¹	67	1.25±0.009 ¹
Urban	1789	22	1.23±0.001 ¹	20	1.12±0.01 ¹
Total	7154	92	1.29	87	1.22

-Means in the same column with the same superscript letters are not significantly different.

Among occupational groups, the prevalence of brucellosis varied in significance (Table 5), where *Brucella* infection increased among students (1.67±0.06 % & 1.33 ± 0.001 %), followed by children (1.51 ± 0.009 % & 1.26 ± 0.004 %) then workers (1.27 ± 0.006 % & 1.14 ± 0.002 %) and farmers and their families (1.21 ± 0.002 %) as detected by both agglutination test and ELISA, respectively.

Table 5. Sero-prevalence of brucellosis among occupational groups of patients with fever of unknown origin.

Occupational group	No. of samples	Agglutination		ELISA	
		No.	%	No.	%
Children	795	12	1.51±0.009 ¹	10	1.26±0.004 ¹
Students	600	10	1.67±0.06 ²	8	1.33±0.001 ²
Farmers & their families	4969	60	1.21±0.002 ³	60	1.21±0.002 ¹
Workers	790	10	1.27±0.006 ³	9	1.14±0.002 ³
Total	7154	92	1.29	87	1.22

-LSD is 0.1248 at P<0.05 and 0.1817 at p<0.01 in agglutination test while, it is 0.005 and 0.008 at P<0.05 and P< 0.01 in ELISA, respectively.

-Means in the same column with the same superscript letters are not significantly different.

The prevalence of brucellosis among different age groups, showed a significant varied values as tabulated in Table 6, where *Brucella* infection increased among patients of age groups, (51-60 yrs) with a rate of (1.85 ± 0.009 %) followed by age group (14 - 20 yrs) with a rate of (1.72 ± 0.009 %), then age group (7 m - 13 yrs) with a rate of (1.51 ±

0.009 %) and (1.26 ± 0.004 %) as detected by agglutination test and ELISA, respectively. However, both of the age groups (21-30) and (31-40) revealed nearly the same prevalence

with both tests, while the lowest prevalence rate was concerned with age group (61-70 yrs) with a rate of (0.73 ± 0.009 %) as estimated by both tests.

Table 6. Age-wise prevalence of brucellosis among patients with fever of unknown origin.

Age group	No. of samples	Agglutination		ELISA	
		No.	%	No.	%
7months-13 yrs	795	12	1.51 ± 0.009^1	10	1.26 ± 0.004^1
14-20 yrs	757	13	1.72 ± 0.009^2	13	1.72 ± 0.009^2
21-30 yrs	1395	18	1.3 ± 0.009^3	17	1.22 ± 0.1^1
31-40 yrs	1007	13	1.3 ± 0.009^3	13	1.3 ± 0.009^1
41-50 yrs	2000	20	1 ± 0.009^4	18	0.9 ± 0.1^3
51-60 yrs	650	12	1.85 ± 0.009^2	12	1.85 ± 0.009^2
61-70 yrs	550	4	0.73 ± 0.009^5	4	0.73 ± 0.009^4
Total	7154	92	1.29	87	1.22

-LSD is 0.1727 at $P < 0.05$ and 0.2397 at $p < 0.01$ in agglutination test while, it is 0.1617 and 0.2245 at $P < 0.05$ and $P < 0.01$ in ELISA, respectively.

-Means in the same column with the same superscript letters are not significantly different.

Discussion

Worldwide, millions of individuals are at risk of acquiring brucellosis especially in developing countries, where the infection in animals has not been brought under control, which may be due to mismanagement on animal quarantine, eradication of infected animals or vaccination in the poor areas (Wang et al., 1998). It has been estimated that the incidence in humans ranges widely between different regions, with values of up to 200 cases per 100,000 populations (Orduna et al., 2000).

Clinical picture of brucellosis in man is very heterogenous and nonspecific which may be represented either by subclinical or atypical infection in both the acute and chronic stages. This makes the diagnosis of brucellosis always requires laboratory confirmation, either by isolation of the pathogen or by demonstration of specific antibodies (Colmenero et al., 1996; Morata et al., 2003). Serological diagnosis however is recommended as the best mean of diagnosis

since positive results of blood cultures are obtained only during the acute stage.

Furthermore, handling of these microorganisms represent a high risk for laboratory personnel (Young et al., 1995; Baldi et al., 1999; Cassataro et al., 2002). The most widely used serological tests for diagnosis of brucellosis are agglutination tests, however indirect enzyme linked immunosorbent assay (IELISA) was documented as the most sensitive test (WHO, 1986; Corbel and MacMillan, 1998).

The low incidence of brucellosis reported in this study may be attributed to the low incidence of brucellosis reported among animals in Assiut Governorate due to vaccination programs of farm animals. In addition, the dry hot weather of Assiut Governorate is considered improper environment for *Brucella* microorganisms to survive for long periods and consequently limit the spread of infection (Seddek, 1999; Abdel Hafeez et al., 2001).

Higher prevalence rates were reported by Amer, 1989 (18.75 %); Mahgoub, 1995 (17.5

); Soliman, 1998 (10.9 %) and Saleh et al., 2003 (15.4 %). However, lower rates were detected by Mousa et al., 1987 (0.08 %); Dajani et al., 1989 (0.04 %); Masoumi et al., 1992 (0.95 %); Awad, 1998 (0.008 %) and Abou Eisha, 2000 (0.9 %).

The variation in prevalence rates of brucellosis among population in different geographical locations and countries may be due to variation in existence of disease among animals, occupational contact and social habits of different population and this explanation agrees with that reported by Alton, (1990) and Abou-Shehadai et al., (1991).

Most patients with acute brucellosis produce antibodies of IgM within a few days of onset of the disease, followed and superseded rapidly by IgG and to a lesser extent by IgA antibodies, therefore, the detection of *Brucella* - specific immunoglobulin M (IgM) antibodies allows early diagnosis of patients with brucellosis and also may help to discriminate between patients with acute and chronic brucellosis. Moreover, in countries where the disease is highly endemic, a large proportion of the population may have persistent brucella-specific IgG antibodies, hence under such conditions, the detection of specific IgM antibodies is important to diagnose brucellosis in early phase (Smits et al., 1999).

IgM antibodies were estimated in 9.8 % of the examined patients, while IgG antibodies were found in 30.4 % of the examined patients, moreover both of IgM and IgG antibodies were detected in 54.3 %. These findings are in agreement with that reported by Diaz et al., (1991) and Ariza et al., (1992).

Epidemiology of human brucellosis differs between endemic and non-endemic areas in terms of age, sex, season and other risk factors (Araj, 2000). In our study, the prevalence of brucellosis was significantly ($P < 0.05$) affected by sex, when the detection was made by agglutination test, while the prevalence

detected by ELISA for females and males, was non-significance. The relatively higher incidence reported among females than males in this study may indicate that females may be highly exposed to the risk of infection through direct contact with animals, consumption of raw milk and milk products as well as some risky habits that occur in rural areas such as skinning of stillborn lambs and kids as well as crushing the umbilical cord of newborn lambs and kids with their teeth (Kolar, 1987). However, Abou Eisha, (2001) reported higher incidence in males (3.5 %) than females (2.6 %) with no significance difference statistically. In addition, Saleh et al., (2003) detected similar results with prevalence rate 12.5 % among females and 16.1 % among males. Furthermore, it has been reported that, the ratio of infection in males and females differs in relation to regions being 2:1 in endemic areas and 10:1 in non-endemic areas (Araj, 2000).

Our results revealed that the prevalence of brucellosis was non significantly higher in rural areas than in urban areas. These findings are in agreement with that reported by Smits et al., (1999) who concluded that the higher prevalence in rural areas may be due to close contact of individuals with livestock. However the occurrence of brucellosis in urban settings may be explained by the role of consumption of raw infected milk and milk products, in addition to eating inadequately cooked meat, bone marrow, liver and spleen harboring microorganisms (Young, 1995). Concerning occupational groups, it has been estimated by both agglutination test and ELISA that the prevalence of brucellosis varied significantly, where *Brucella* infection increased among students, followed by children then workers and farmers and their families. The low incidence reported among farmers and their families does not reflect the true incidence of brucellosis among this group because not all of the farmers and their families attend fever hospital when they are

sick and many of them may have symptomatic treatment and consequently the infection changed from acute to chronic form.

Regarding the prevalence of brucellosis among different age groups, it has been reported a significant varied value by agglutination test and ELISA, where *Brucella* infection increased among patients of age groups, (51-60 yrs) followed by age group (14-20 yrs), then age group (7 m-13 yrs). However, both of the age groups (21-30) and (31-40) revealed nearly the same prevalence with both tests while the lowest prevalence rate were concerned with age group (61-70) estimated by both tests. It has been reported that brucellosis occurs predominantly in adults in non-endemic areas (mostly as workrelated) compared to a ratio of 2:1 in adults and children in endemic areas which is mostly related to ingestion of unpasteurized milk or its dairy products (Araj, 2000).

Prevention of human brucellosis focuses mainly on elimination of infection among farm animals. Cooperation is recommended between public health and veterinary officials to overcome the failure of controlling disease among both animals and human. Awareness of public as well as high risk group about danger of acquiring the infection and early diagnosis is the best way for management of the disease. Better training for medical staff is recommended for proper judging on the results of agglutination test and how to deal with doubtful cases to overcome the chronicity of the disease.

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