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THE USE OF ELISA FOR DIAGNOSIS AND EPIDEMIOLOGY OF BRUCELLA INFECTION IN HUMANS IN ASSIUT GOVERNORATE

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SUMMARY

The prevalence of brucella in fection among humans in Assiut Governorate was stimated b using Rose Bengal plate test (TAT), and Enzyme linked immunosobent assay (ELISA). The seroprevalence of brucellosis among humans in Assiut Governorate was 32.3 %. Subjects professionally exposed to livestock including farmers (40.3 %) and veterinarians (18.2 %) represent a high risk group for brucella infection. The positive butcher case recorded in our study reflects the possibility of abattoir workers to attain the infection. Infections of the occupational groups including students (44.4 %), children (40 %), manual workers (14.3 %) and others (66.7%) explain the role of consumption of raw milk and milk products as well as inadequately cooked meat, liver and spleen in spreading of that zoonosis among humans. A higher brucella prevalence was recorded in men (36.6 %) than in women (26. 8 %). Also, the rural population shows a higher infection rate (38.7 %) than the urban one (14.7 %) and this may be attributed to the local dietary customs and habits. Intensification of the infection (40 %) among the age group less than 16 years gives a spot light on the serious role of raw milk and milk products in the epidemiology of brucellosis among humans in Assiut Governorate.

INTRODUCTION

Brucellosis is an animal disease in origin, most of human cases, worldwide, are ascribed to animal exposure either directly or indirectly. Therefore, the incidence of human brucellosis is a good index of the presence of infection in animal population (Young, 1983). WHO (1992) stated that the human prevalence of brucellosis per 10^5 is higher

than 10 in Tunisia, Jordon, Kuwait, Oman and Iran; from 5 to 10 in Djibouti, Syria, Spain, and Turkey; and between 1 to 5 in Algeria, Israel Iraq, Mexico, Italy, Greece, and Yugoslavia. OIE (2000) reported that the annual incidence of brucellosis in people in the Mediterranean and Middle East Countries varies from 1 to 78 cases per 100,000. In the United States, the Centers for Disease Control and Prevention (CDC, 2001) recorded approximately 100 cases each year during the past 10 years, with most cases in the Southwest region. In Egypt, there are only few reports concerning the epidemiology of the disease. Kamel and Zaghloul (1985) detected brucella antibodies in 8 (5.06 %) by using tube agglutination test out of 158 human sera with titer varying from 1 / 80 to 1 / 2560 and 3 (1.9 %) were suspicious. Furthermore, El-Gohary and Hatıab (1992) in El-Behera Governorate estimated an incidence of 11.3 % and 8.1 % brucella positive reactors among 62 humans by using RBPT and TAT respectively. Moreover, El-Gibaly (1993) revealed the detection of brucella antibodies in 20.9 % of 1133 human blood sera from different groups [out patients (22.3 %), in patients (20.5 %) and animal attendants (22.2 %)]. Two years later, Mahgoup (1995) detected an incidence of 32.4 %, 23.5 %, 11.5 %, 10.0 %, and 8.9 % brucella infection among animal husbandry workers, veterinarians, butchers, veterinary assistants, and milkers respectively. Abou-Eisha (2000) estimated a percentage of 0.9 % brucella positive reaction among human patients admitted in El-Arish Fever hospital and others were involved in caring for camels, sheep and goats. In another study, Abou-Eisha (2001) used Rose-Bengal and serum agglutination tests to record a prevalence of 5.1 % brucella infection of 1316 human sera from Suez Canal area. Meanwhile, Abdel-Hafeez et al. (2001) estimated a brucella seroprevalence in 12.23 % of human sera collected from three different categories (veterinarians, officials, and workers) in Assiut Governorate. In a recent study, Saleh et al. (2003) recorded an incidence of 40 (11 %), 37 (10.2 %), and 41 (11.3 %) among 363 workers in different dairy farms by using Rose-Bengal plate test, Serum agglutination test and indirect ELISA (Enzyme-linked immunosorbent assay) respectively. Finally, Samaha et al. (2004) detected brucella antibodies in 8 %, 8 %, 5 % and 5 % of 100 examined human sera by using BA-PAT, RBPT, Riv.T., and TAT respectively. This study attempts to investigate the seroprevalence of brucellosis among humans in Assiut Governorate as well as the evaluation of the used serological tests in comparison with ELISA.

MATERIALS and METHODS

The present study was carried out during the period between May, 2003 to June, 2004 in the department of Animal Hygiene and Zoonoses, faculty of Vet. Med., Assiut University and the department of Microbiology and Immunology, faculty of Med., Assiut University.

Source of specimens

A sum of 127 subjects, including (10^4) patients with a history of pyrexia attending the fever hospital in Assiut Governorate as well as (23) of apparently healthy individuals. A questionnaire was designed for each individual to determine the risk factors assessments regarding age, sex, residence, drinking raw milk, consumption of raw milk products, contact with livestock, caring for breeding livestock, handling parturient animals and approach to an aborted case.

Collection of samples

5 ml blood were collected from each human subject by using a vacutainer tube. Samples were kept overnight at 4°C to allow the separation of serum then centrifuged at 3000 r.p.m for 10 minutes. The collected sera were coded and kept at -20°C up to the time of the test.

Serological examination of samples:

All sera were subjected to Rose-Bengal plate test (RBPT) according to Morgan et al. (1969), tube agglutination test (TAT) Alton et al. (1975), and Enzyme linked immunosorbent assay (ELISA) according to Ariza et al. (1992) and Baldi et al. (1996). The used antigens were obtained from the veterinary sera and vaccine research institute (VSVRI), Abassia, Cairo, 11517, Egypt. The ELISA kits [Brucella ELISA IgG (Vircell No. G1003) and Brucella ELISA IgM (Vircell No. M1006)] were obtained from Vircell Company, Web site: HYPERLINK "http://www.vircell.com" http://www.vircell.com .

RESULTS

The obtained results were illustrated in Table (1) to Table (6).

Table (1): Seroprevalence of Brucellosis among humans in Assiut Governorate.

Number of tested blood samples	RBPT				TAT						ELISA			
	+ve cases		-ve cases		+ve cases		±ve cases		-ve cases		+ve cases		-ve cases	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
127	44	34.6	83	65.4	38	29.9	6	4.7	83	65.4	41	32.3	86	67.7

Table (2): Estimation of Brucellosis among humans by using the enzyme-linked immunosorbent assay (ELISA).

Number of tested blood samples	IgM				IgG				IgM &IgG				Total number			
	+ve cases		-ve cases		+ve cases		-ve cases		+ve cases		-ve cases		+ve cases		-ve cases	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
127	14	11	113	89	16	12.6	111	87.4	11	8.7	116	91.3	41	32.3	86	67.7

Table (3): Prevalence of brucella positive reactors among professional groups.

Occupation	Total	Positive cases											
		RBPT		TAT		ELISA							
		No.	%	No.	%	IgM		IgG		IgM&IgG		Total	
						No.	%	No.	%	No.	%	No.	%
Farmers	62	24	38.7	22	35.5	5	8	13	21	7	11.3	25	40.3
Veterinarians	11	3	27.3	1	9	0	0	1	9	1	9	2	18.2
Veterinary attendants	6	0	0	0	0	0	0	0	0	0	0	0	0
Technician & medical services employees	9	2	22.2	1	11.1	0	0	0	0	0	0	0	0
Housewives	4	0	0	0	0	0	0	0	0	0	0	0	0
Students	9	5	55.6	5	55.6	3	33.3	0	0	1	11.1	4	44.4
Children	15	7	46.7	6	40	3	20	1	6.7	2	13.3	6	40
Manual workers	7	1	14.3	1	14.3	0	0	1	14.3	0	0	1	14.3
Others	3	1	33.3	2	66.7	2	66.7	0	0	0	0	2	66.7

N.B: One butcher case was obtained from fever hospital and gave positive reaction with RBPT & ELISA IgM.

Table (4): Seroprevalence of Brucellosis among humans according to sex.

Sex	Total	Positive cases					
		RBPT		TAT		ELISA	
		No.	%	No.	%	No.	%
Male	71	27	38	24	33.8	26	36.6
Female	56	17	30.4	14	25	15	26.8
Total	127	44	34.6	38	29.9	41	32.3

Table (5): Ecological distribution of brucella seropositive human cases according to residence.

Residence	Total	Positive cases					
		RBPT		TAT		ELISA	
		No.	%	No.	%	No.	%
Rural	93	39	41.9	33	35.5	36	38.7
Urban	34	5	14.7	5	14.7	5	14.7
Total	127	44	34.6	38	29.9	41	32.3

Table (6): Age distribution of the brucella seropositive human cases.

Age group	Total	Positive cases					
		RBPT		TAT		ELISA	
		No.	%	No.	%	No.	%
Less than 16 years	20	8	40	7	35	8	40
From 16 to 30 years	34	13	38.2	12	35.3	12	35.3
From 31 to 50 years	49	16	32.7	12	24.5	15	30.6
Above 50 years	24	7	29.2	7	29.2	6	25
Total	127	44	34.6	38	29.9	41	32.3

DISSCUSSION

Brucellosis is a widely prevalent zoonotic disease, caused by different species of the Genus Brucella. The persistence of animal reservoir of infection , low physician awareness , poor availability of diagnostic facilities as well as absence of regional databases, contribute toward the perpetuation of this zoonosis. (Handa et al., 1988).

The seroprevalence of brucellosis among humans was 34.6 %, 29.9 % and 32.2 % by using RBPT, TAT, and ELISA respectively (Table 1). A variable incidence of brucellosis among humans was recorded by many workers as Kamel and Zaghloul, 1985 (5.06 %); El-Gohary and Hattab, 1992 (11.3 %); El-Gibaly, 1993 (20.9 %); Abou-Eisha, 2000 (0.9 %); Abou-Eisha, 2001 (5.1 %); Abdel-Hafeez et al., 2001 (12.23 %) and Saleh et al., 2003 (11.3 %).

A total number of positive reactors by using RBPT and TAT was 44 (34.6 %) and 38 (29.9 %) respectively (Table 1). This discrepancy between the results recorded by both tests may be accredited to the prozone phenomenon which occurs sometimes in agglutination tests as a result of antibody excess or blocking by non agglutination IgG and IgA isotypes (Saleh et al., 2003). On the other hand, the presence of 6 patients (4.7 %) showed positive reaction with RBPT and inconclusive reaction with TAT (titer 1/20) may be ascribed to the presence of latent infection (Saleh et al., 2003).

The present work revealed that 41 (32.3 %) of 127 subjects under investigation proved to be ELISA positive reactors. 11% of these cases were positive for IgM, 12.6 % for IgG and 8.7% for both IgM &IgG (Table, 2). ELISA test provides a profile of immunoglobulin classes for the diagnosis of acute and chronic brucellosis and it is considered by many researchers as the test of choice especially in chronic and complicated cases (Araj et al., 1986 and Araj et al., 1988). The value of brucella specific immunoglobulins, as determined by ELISA, were assessed in the search of diagnostic and prognostic markers. In relation to diagnosis, patients with acute brucellosis showed elevation in IgM (Magee, 1980). The elevation of both IgM and IgG reflects to a great extent the subacute infection. This view is supported by the finding of Araj et al., (1986) who recorded the elevation of IgG (100 %) and IgM (86 %) in sera of subacute patients. While, those of chronic brucellosis showed an elevation in IgG2, IgA, IgE, IgG1 and IgG4 (Araj, 2000).

The occurrence of brucella infection among the individuals whose duties bring them in close contact with infected animals (Table 3) as farmers (40.3 %) and veterinarians (18.2 %) gives a good support that the disease is primarily an occupational hazard (Henderson and Hill, 1972). Cases

of abortion and placental retention as well as aerosols liberated from animal excreta and aborted materials represent an actual hazard to veterinarians (Kamel and Zaghloul, 1985 and Saleh et al., 2003). Moreover, the accidental splashing of attenuated live brucella vaccine into the eyes has been reported as a cause of brucellosis in veterinarians (Williams, 1982). While, farmers contract infection mainly through direct or indirect contact with infected animals and their discharges (uterine discharge, placenta, urine and manure) (Saleh et al., 2003).

The positive butcher case (Table, 3) recorded in our study reflects the possibility of abattoir workers infection from the cutting of infected carcasses and contact with animal and fresh meat. Splashing of animal's blood, lymph node or other body fluids into worker conjunctiva can be considered as a method of transmission of the disease (Saleh et al., 2003). Also, they may contract the disease by inhalation route (Kufman et al., 1980 and Manson-Baher and Bell, 1987).

The existence of brucella infection among students (44.4 %), children (40 %), manual workers (14.3 %) and others (66.7 %) explains the role of consumption of raw infected milk and milk products as well as inadequately cooked meat, liver and spleen in the stage of bacteraemia resulting in spreading of that zoonosis among humans.

The illustrated results in Table (4) explain that a higher brucella seroreactors were recorded in men (36.6 %) than in women (26.8 %). These findings reflect the relationship between brucella infection and occupational exposure. Men had a greater chance of coming in contact with animals than women through their work in abattoirs, veterinary field or as livestock handlers (Kulshershtha et al., 1978; Rahman et al., 1983 and Rana et al., 1985). Concerning the ecological distribution of brucellosis according to residence (Table 5), it was clear that rural group (38.7 %) showed a higher infection rate than the urban one (14.7 %). The relative high attack rate among rural population may be related to their involvement in caring for domestic animals as well as consumption of raw milk and milk product, eating of home-made cheese (Karish) and uncooked vegetables which may be contaminated with excreta of infected animals. Some risky habits that occur in the rural area such as skinning of stillborn lambs and kids and aborted fetus as well as the crushing the umbilical cord of newborn lambs and kids with the teeth may be responsible for the persistence of infection among rural population (Kolar, 1987).

Intensification of the infection (40 %) among the age group less than 16 years (Table 6), gives a spot light on the serious role of raw milk and milk products in the epidemiology of brucellosis among humans in Assiut Governorate. The relative high infection rate (35.3%) among the age group 16-30 years may be attributed to the fact that the majority of workers engaged in abattoirs,

veterinary care rearing, milking and attending animals aged 20 to less than 40 year (Saleh et al., 2003).

Brucellosis is one of the most important zoonotic diseases which affect animals and humans. Consequently, the control of human brucellosis depends on minimizing the infection rate among animals. Prevention and control of brucellosis among livestock must be principally based on preventive and hygienic measure to protect farm animals from infection access, strict sanitary measure and elimination of diseased animals from infected herds. On the other hand, the role of milk and dairy products as a source of brucella infection among our population cannot be ignored. It is necessary to educate the public on the danger of contracting the infection through milk and milk products and encourage the consumption of pasteurized, sterilized or boiled milk only.

Finally, there is an urgent need to encourage physicians to work in an effort to detect early the disease and control the spreading of infection. The early detection of infection is possible through the detection of specific IgM. ELISA test has also several advantages over the other serological tests as It shows a higher sensitivity and specificity which leads to better serological follow-up of patients under treatments and gives an objective reading with a very high reproducibility of the antibody titer determination.

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