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Using Competitive ELISA for Accurate Diagnosis of Brucellosis in Humans in Alexandria Province with Emphasis on the Disease Epidemiology

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Abstract

A total of 300 human serum samples were examined by RBPT and c. ELISA to estimate the prevalence of brucellosis in humans in Alexandria Province and to investigate the epidemiological pattern of such disease among humans. It was found that the overall seroprevalence of brucellosis in humans through RBPT and c. ELISA was 14.67, and 11.67%, respectively. Statistical analysis showed that there was a significant association at (P<0.0001) between the seroprevalence of brucellosis among human beings in relation to place of residence where the highest prevalence was noticed in El-Aameria (18.40%) followed by Gharb (17.86%), Borg El Arab (16.66%), Sharq (13.33%), El Montazah (13.24%) and Wasat (12.5%). On contrary, all the examined samples that were collected from El- Gomrok were found to be negative. In addition, the results clarified that 25% of the examined samples collected from patients complaining of fever were found to be positive for brucellosis. On the other side, only 7.78% of the examined samples of apparently healthy individuals were found to be positive for brucellosis with significant association. Sex based seroprevalence showed that males' prevalence (16.67%) was higher than that in female (12.12%) with significant association at (P < 0.05). Also, age based seroprevalence showed that the highest prevalence was observed in the age group (20 - < 40 years) (19.75%) followed by the age group (< 20 years) (13.48%) then the age group (40 - < 60 years) (12.79%) and lastly the age group (> 60 years) (11.36%) with non-significant association between different age groups and prevalence of human brucellosis with significant association at (P < 0.01). Moreover, the highest seroprevalence was observed in farmers (16.30%) followed by housewives (14.25%), animals' attendants (14.10%), veterinarians (11.11%), slaughterhouse workers (8.33%) and finally veterinary students (0.59%) with significant association at (P < 0.01) between different occupations and prevalence of human brucellosis. Under the conditions in the current study and according to the data obtained, it is concluded that brucellosis is still remaining a problem in Alexandria province and it was threatening human population through direct and indirect transmission. Also, the prevalence of the disease is significantly associated with health status, sex, age and occupation while it is non-significantly associated with manner of milk consumption, locality and the type of animal contact.

1. INTRODUCTION:

Brucellosis as a global zoonotic disease remains a significant public health problem in many regions around the world, especially those in the Asia and Middle East (Chegeni et al., 2014). Brucellosis was described in Mediterranean African countries more than 100 years ago (Rafai, 2002). It is caused by Gram negative bacteria belonging to the genus *Brucella* (Cloeckaert et al., 2003). The genus *Brucella* consists of 10 species: *B. abortus, B. canis, B. suis, B. ovis, B. neotomae, B. melitensis, B. ceti, B. pinnipedialis, B. microti,* and *B. inopinata.* Brucella species show a host preference, but some strains can be transmitted among a variety of animals, including humans (Kang

et al., 2011). Brucella can be transmitted to humans through direct contact with infected animals, indirectly by the ingestion of raw milk products, and during the handling of strains or infected material in the laboratory. Also, uterine discharge and placenta expelled from infected animals are the main sources of transmission to humans and animals (Corbel, 2006). Human brucellosis is mainly an occupational disease affecting animal caretakers, livestock farmers, artificial inseminators, abattoir workers, meat inspectors and veterinarians due to frequent exposure to infected animals. The common routes of transmission to humans are consumption of unpasteurized dairy products or through direct contact with infected animals, placentas or aborted fetuses. The most common symptoms are fever, sweating, fatigue, weight loss, headache, and joint pain. Some cases may have neurological complications, endocarditis and testicular or bone abscess formation (Refai, 2002). To enhance efficiency of brucellosis-specific prophylaxis, early detection of brucellosis by highly sensitive and specific methods is needed. Rapid detection of Brucella by recent diagnostic seroprevalence and molecular methods may lead to an earlier diagnosis and could improve disease control (Kang et al., 2011). The Rose Bengal Plate Test (RBPT) is a rapid slide-type agglutination assay performed with a stained *B. abortus* suspension at pH 3.6–3.7 and plain serum. Because of its simplicity, it is often used as a screening test in human brucellosis and would be optimal for small laboratories with limited means. However, there is confusion about the value of this test so that present WHO guidelines recommend that RBPT results be confirmed by other tests (Franco et al., 2007). Portanti et al. (2006) recommended and validated competitive Enzyme Linked Immunosorbent Assay (c-ELISA) for detection of Brucella antibodies and found out that C-ELISA didn't show cross-reaction when testing positive sera for antibodies to some Enterobacteriaceae. Also, Etman et al., (2014) recommended the use of c. ELISA as a confirmatory test. The prevalence of human brucellosis was estimated by Abd- El Ghany, (1999), Abd El-Hafeez et al., (2001), Abou Eisha (2001), Habib et al. (2003), Afifi et al., (2005), Haggag and Samaha (2007), El Mabrouk, (2013), Chegeni et al., (2014), Zolzaya et al., (2014), Assenga et al., (2015), Tumwine et al., (2015) and El-Monir et al., (2016). The objectives of this study are to use the advanced diagnostic seroprevalence; C. ELISA in parallel with RBPT to assess the prevalence of human brucellosis in Alexandria province with special emphasis on the epidemiology of brucellosis in this area and possible control measures that should be taken will be used in parallel.

2. MATERIAL AND METHODS

2.1. Collection of samples

A total of 300 serum samples were collected from patients attending El- Hadara Fever Hospital suffered from fever of unknown causes and apparently healthy individuals attending private laboratories in Alexandria seeking for medical advice. It was carried out according to Alton et al., (1988) by allowing 5 ml of blood to flow freely from radial vein of human beings by using sterile dry special double ended needle into a sterilized vacutainer tube in which blood samples were left at room temperature for 30 minutes, then centrifuged at 3000 rpm for 10 minutes and placed in the refrigerator for 24 hours and when the colt retracted, clear serum was obtained by using sterile Pasteur pipette, then kept in Ependorff tubes and labeled. They were stored at -20° C in the deep freezer till examined serologically in the laboratory of Animal Hygiene and Zoonoses Department, Faculty of Veterinary Medicine, Alexandria University. Full history from each patient was taken including age, sex, residence, type of animal contact, manner of milk production and health condition.

2.2. Antigens for serological tests:

2.2.1. Brucella abortus antigen for RBPT:

This is a Rose Bengal stained *B. abortus* strain 99 cells in lactate buffer (pH 3.65). It was obtained from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt.

2.2.2. SVANOVIR® BRUCELLA-AB C-ELISA

The SVANOVIR® competitive Enzyme Linked Immunosorbent Assay (C. ELISA) for detection of serum antibodies to *Brucella abortus* and *melitensis* is a multi-species assay allowing detection of Brucella specific antibodies in both domestic and wildlife species.

2. 3.Methods:

2.3.1. Rose Bengal plate test (RBPT):

It was performed following the procedure described by **Aldomy et al. (2009).**

2.3.2. SVANOVIR® BRUCELLA-AB C. ELISA:

Serum samples were examined according to the manufacturer's instructions.

2.4. Statistical analysis:

The statistical analysis was carried-out using Chi^2-test for study the prevalences of certain parameters among different studied tests, sex and locality according to **SAS, (2004).**

3. RESULTS

Table (1): Seroprevalence of brucellosis among examined humans through RBPT and c. ELISA

Test	No. of examined samples	Positive	%
RBPT	300	44	14.67
c-ELISA	300	35	11.67
Chi^2 value		6.55**	

**= Significant at (P<0.0001)

Table (2): Seroprevalence of brucellosis among examined humans by RBPT in relation to place of residence in Alexandria province

Place of residence	No. of the examined samples	Positive	%	Chi^2
El Montazah	68	9	13.24	
Sharq	45	6	13.33	
Wasat	24	3	12.50	
Gharb	28	5	17.86	**13.44****
El-Gomrok	20	0	0.00	
El- Aameria	67	13	18.40	
Borg El Arab	48	8	16.66	
Total	**300**	**44**	**14.67**	

** = Significant at (P < 0.01)

Table (3): Seroprevalence of brucellosis among examined humans by RBPT in relation to their health status

Health status	No	Positive	%	Chi^2
Apparently healthy	180	14	7.78	
Patients	120	30	25.00	**7.89 ****
Total	**300**	44	**14.67**	

** = Significant at (P < 0.01)

Table (4): Seroprevalence of brucellosis among examined humans by RBPT in relation to sex

Sex	No	Positive	%	Chi^2
Females	132	16	12.12	
Males	168	28	16.67	**5.56***
Total	**300**	**44**	**40.67**	

* = Significant at (P < 0.05)

Table (5): Seroprevalence of brucellosis among examined humans by RBPT in relation to age groups

Age groups (Years)	No	Positive	%	Chi^2
< 20	89	12	13.48	
20 - < 40	81	16	19.75	
40 - < 60	86	11	12.79	**12.33****
> 60	44	5	11.36	
Total	**300**	44	**14.67**	

** = Significant at (P < 0.01)

Table (6): Seroprevalence of brucellosis among examined humans by RBPT in relation to location

Sex	No	Positive	%	Chi^2
Rural	193	30	15.54	
Urban	107	14	17.08	**3.25 NS**
Total	**300**	44	**14.67**	

NS= Non-significant at (P<0.05)

Table (7): Seroprevalence of brucellosis among examined humans by RBPT in relation to occupation

Location	No	Positive	%	Chi^2
Veterinarian	18	2	11.11	**18.55****

Animal attendants	78	11	14.10
Slaughterhouse workers	24	2	8.33
Farmers	92	15	16.30
Housewives	35	5	14.25
Veterinary Students	17	1	0.59
Others	36	8	22.22
Total	**300**	**44**	**14.67**

** = Significant at (P < 0.01)

Table (8): Seroprevalence of brucellosis among examined humans by RBPT in relation to milk consumption

Sex	No	Positive	%	Chi^2
Regular	120	16	13.33	**4.55 NS**
Irregular	144	23	15.97	
Rarely	36	5	13.88	
Total	**300**	**44**	**14.67**	

NS= Non-significant at (P > 0.05)

Table (9): Seroprevalence of brucellosis among examined human beings by RBPT in relation to type of animal contact

Animal contact	No	Positive	%	Chi^2
Contact	219	31	14.15	
Non-contact	81	13	16.05	**3.55 NS**
Total	**300**	**44**	**14.67**	

NS= Non-significant at (P> 0.05)

4. DISCUSSION:

Although many countries have eradication programs for controlling brucellosis, economic losses can be heavy due to abortion and infertility and subsequent culling so herds should be monitored for the presence of infection. Despite eradication programs, including vaccination, testing and slaughter, brucellosis remains a major zoonosis worldwide. The current study was carried out to estimate the seroprevalence of brucellosis in humans in Alexandria Province and to investigate the epidemiological pattern of such disease.

The data presented in Table (1) showed the Seroprevalence of brucellosis among examined human beings through different serological tests in Alexandria province. It was found that the prevalence of brucellosis was 14.67 and 11.67%, respectively. Statistical analysis (Chi^2 value= 6.55) showed that there was a significant association at (P<0.0001) between the results of RBPT and c. ELISA. The recorded prevalence according to the results of RBPT (14.67%) was nearly similar to that obtained by Abd- El Ghany, (1999) (13.3 %), Abd El-Hafeez et al., (2001) (12.23%) and Haggag and Samaha (2007) (14 %). On the other hand, it was extremely higher than that recorded by Assenga et al., (2015) (0.6 %) and El-Monir et al., (2016) (1.25%) and it was higher than that recorded by Abou Eisha (2001) (5.1%), Afifi et al., (2005) (11%) and El Mabrouk, (2013) (4 %); on contrary, it was lower than that recorded by Chegeni et al., (2014) (29.5%), Zolzaya et al., (2014) (27.3%) and Tumwine et al., (2015) (17.0 %). Nevertheless, it was very low when compared with the result recorded by Habib et al. (2003) (37.6%). This variation in prevalence of brucellosis in humans in the current work and others may be attributed to different geographic locations, variation in occupational contact and the type of used tests. The obtained result in the current study was supported by that of Meky et al., (2007) enrolled 72 confirmed cases of brucellosis from Alexandria province in the study and interviewed them using a structural questionnaire. They proved that working with animals, breeding goats and eating ice-cream bought from street vendors were significantly associated with the affections and they concluded that contact with infected animals and their products was the most important method of transmission and Tumwine et al. (2015) who found that prevalence of human brucellosis was parallel with animal prevalence.

Seroprevalence of brucellosis among examined human beings depending on the results of RBPT in relation to place of residence in Alexandria province was illustrated in Table (2) was showed that the highest prevalence was noticed in El- Aameria (18.40%) followed by Gharb (17.86%), Borg El

Arab (16.66%), Sharq (13.33%), El Montazah (13.24%) and Wasat (12.5%). On contrary, all the examined samples that were collected from El-Gomrok were found to be negative. Statistical analysis (Chi^2 value= 13.44) showed that there was a significant association at (P<0.0001) between the seroprevalence of brucellosis among examined human beings in relation to place of residence in Alexandria province. It was clear that there was a relationship between the recorded higher comparable prevalence of brucellosis in livestock and humans in El- Aameria and Borg El Arab highlighting the zoonotic potential of brucellosis in this geographic area and clarifying the need for more attention in planning a control program in order to reduce that prevalence. This finding disagreed with Zolzaya et al. (2014) who found that human seroprevalence was not associated with small ruminant and cattle seroprevalence at the nomadic camp level.

Seroprevalence of brucellosis among examined human beings by RBPT in relation to their health status was presented in Table (3). It was clarified that 25% of the examined samples collected from patients complaining of fever were found to be positive for brucellosis. On the other side, only 7.78% of the examined samples of apparently healthy individuals were found to be positive for brucellosis. Statistical analysis showed significant association at (P < 0.01) explaining the effect of health status on the prevalence of brucellosis. This result was supported by that of Chegeni et al. (2014) who examined 312 patients with clinical feature of brucellosis and found that 92 patients had titers of 1:80 or higher in STAT, Sannikova et al., (2014) who analyzed the epidemiological situation of brucellosis and found that 78.5% of the patients seeking medical advice and as high as 79.9% of those being covered by serological examination of groups at risk for brucellosis were detected and El-Monir et al., (2016) who estimated that hospital - based incidence rate of human brucellosis at the governorate level was 0.54/100000 population.

Seroprevalence of brucellosis among examined humans depending on the results of RBPT in relation to sex was recorded in Table (4) and showed that males' prevalence (16.67%) was higher than that in females (12.12%) and statistical analysis showed significant association at (P < 0.05) between sex and the prevalence of brucellosis. This result disagreed with El Mabrouk, (2013) who found non-significant association between prevalence of brucellosis in males and females. The higher prevalence in males agreed with Hassanain and Ahmed (2012) (males, 76.66%), Chegeni et al. (2014) (males, 54.3% and females, 45.7%), Ghoneim et al., (2014) (males, 23.3% and females 17.5%) and Tumwine et al., (2015) who recorded that the prevalence was highest among males (20.5 %). Also, El-Monir et al., (2016) estimated that the hospital - based incidence rate of human brucellosis at the governorate level was 0.75/100000 population for males and 0.38/100000 population for females. On contrasts, it disagreed with those recorded by Habib et al., (2003), Nossair, (2005), Troy et al., (2005) and Zolzaya et al. (2014) who found that more women than men were seropositive.

The effect of age on the prevalence of human brucellosis was illustrated in Table (5) and pointed out that Chi square analysis of the obtained result of the age wise positivity of the detection of Brucella antibodies in human serum samples showed significant relationship at (P < 0.01) between the different age groups. The highest prevalence was observed in the age group (20 - < 40 years) (19.75%) followed by the age group (< 20 years) (13.48%) then the age group (40 - < 60 years) (12.79%) and lastly the age group (> 60 years) (11.36%). The increased prevalence in the age group (20 - < 40 years) may be attributed to this age group represents the most active age of work. It was in agreement with Abou Eisha, (2001) who reported that the prevalence of Brucella infection increased among the age group 30-39 years, El Mabrouk, (2013) who found that the highest prevalence was found in the age group (30 - <45 years) (7.5 %) followed by the age group (45 - 65 years) (4.5 %) and lastly the age group (15- <30 years) (0.0 %) with non-significant association and Nasinyama et al. (2014) who found that there was no association between sero-positivity with age. On contrast, it disagreed with the results obtained by Tumwine et al., (2015) who noticed that the elderly - above 60 years (22.2 %) was the highest age group.

Seroprevalence of brucellosis among examined human beings depending on the results of RBPT in relation to location was illustrated in Table (6). It was noticeable that the seroprevalence of brucellosis was 15.54% (30 out of 193) in those inhabiting rural areas and 17.08% (14 out of 107) in those inhabiting urban areas. There was non-significant association at (P<0.05) between prevalence in urban and rural areas. Ahmetagić et al. (2015) noticed that in the majority of cases, the children were from rural parts of the country. Also, Tumwine et al., (2015) found that higher prevalence was recorded in those inhibited rural areas.

Seroprevalence of brucellosis among examined human beings depending on the results of RBPT in relation to occupation was presented in Table (7). It was shown that the highest seroprevalence was

observed in farmers (16.30%) followed by housewives (14.25%), animals' attendants (14.10%), veterinarians (11.11%), slaughterhouse workers (8.33%) and finally veterinary students (0.59%) with significant association at ($P < 0.01$) between different occupations and prevalence of human brucellosis. The recorded prevalence in farmers, animals' attendants and veterinarians highlighted the role of direct animal contact as risk factors affecting the transmission of brucellosis to human while the higher recorded prevalence in housewives may be attributed to the direct animal contact if they inhibited rural areas and reared livestock or may be attributed to indirect transmission through ingestion of non-pasteurized milk or milk products.

Seroprevalence of brucellosis among examined human beings depending on the results of RBPT in relation to milk consumption was presented in Table (8). It was found that the highest seroprevalence was found in those who irregularly drinking milk (15.97%), followed by those who rarely drinking milk (13.88%) and lastly those who drink milk regularly (13.33%). Statistical analysis showed non-significant association at ($P > 0.05$) between the manner of milk consumption and the prevalence of brucellosis in human.

The recorded prevalence in those who drank mill even in irregular may confirmed the role that might be played by inefficient heat treated milk or milk products in transmission of brucellosis to man. This was supported by the results of Nasinyama et al., (2014) who found that the sero-prevalence of brucellosis among consumers of unpasteurized milk in Kampala Districts was 9%, Ahmetagić et al., (2015) who decided that consumption of unpasteurized dairy products from farms where brucellosis had been already established was an significant risk factor of children brucellosis in Bosnia and Herzegovina, Tumwine et al., (2015) who recorded higher prevalence in those consuming locally processed milk products and Ikeda and Nagamine, (2016) who reported a case study of a 62-year-old man who presented with symptoms of intermittent fever that persisted after returning from a trip to France and he had eaten natural cheese.

Data presented in Table (9) showed that the seroprevalence of human brucellosis was higher in those who were not in direct contact with farm animals (16.05%) than those who were in direct contact with farm animals (14.15%). Statistical analysis showed non-significant association at ($P > 0.05$) between the type of animal contact and the prevalence of brucellosis in human. This result was in harmony with El-Monir et al., (2016) who noticed that about the half of the reported human cases with brucellosis in Kafrelsheikh governorate had no contact with animals. On contrary, it disagreed with the results of Nasinyama et al., (2014) who found that the sero-prevalence of brucellosis among exposed cattle keepers in Mbarara in Kampala Districts was 5.8% and Tumwine et al. (2015) who found that prevalence of human brucellosis was parallel with animal prevalence.

Based on the obtained results, it is concluded that brucellosis is still remaining a problem in Alexandria province and it was threatening human population through direct and indirect transmission. In addition, it was found that the prevalence of the disease is significantly associated with health status, sex, age and occupation while it is non-significantly associated with manner of milk consumption, locality and the type of animal contact. Moreover, the current status of brucellosis in the current study condition was revealed and the results may help in prevention and control strategies against brucellosis.

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