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Brucellosis at the Human-Animal Interface in Kafrelsheikh Governorate, Egypt

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ABSTRACT

Key words: Brucellosis, Human-Animal interface, Control, Egypt

Brucellosis is an endemic disease in Egypt among humans and animals. Accurate estimates of disease prevalence are the cornerstone to apply and study the efficiency of any control program. In this study, a cross sectional study was carried out at one area of the Nile Delta with high livestock density; Kafrelsheikh district to study the disease prevalence in cattle and humans. A total of 149 milk samples randomly collected from cattle in this district were tested using ELISA against *Brucella* spp. antibodies. The prevalence of brucellosis was estimated at 7% (95% CI: 2.9 – 11.1%). The within village seroprevalence was ranged from 0% to 46.5%. About half of the visited villages had at least one infected cattle; 44.8% (95% CI: 13.0 % – 75.9%). There is a positive strong correlation (> 0.7) between the prevalence of brucellosis among sheep and cattle on village flock level ($P < 0.02$). In humans, the prevalence of *Brucella* was estimated at 1.25% (95%CI: 0% – 3.45%) in Kafrelsheikh district. Hospital-based incidence rate of human brucellosis at the governorate level was 0.54/100000 population; 0.75/100000 population for male and 0.38/100000 population for females. Half of the reported human cases with brucellosis in the governorate have no contact with animals, while 35.7% of them owned a seropositive animal in their households. Continuous integrated surveillance at human-animal interface, revising the current national control program in animals with suggestion of vaccination to be in place, restriction of raw milk marketing and enhancement of public health education regarding brucellosis risks are recommended measures to reduce brucellosis prevalence among human and animal population of the study region.

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1. INTRODUCTION

Brucellosis is one of the most common zoonotic diseases worldwide. The common routes of transmission to humans are consumption of unpasteurized dairy products or through direct contact with infected animals, placentas or aborted fetuses (Díaz-Aparicio, 2013). 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 (Corbel, 2006). Brucellosis has major economic ramifications due to time lost by patients from normal daily activities and losses in animal production (Corbel, 2006; Roth et al., 2003).

In Egypt, accurate estimates of the prevalence and/or incidence of human brucellosis are sparse. In a hospital based survey carried out over a 2 year period, of 270 patients with Acute Febrile Illness (AFI) admitted to two hospitals in Cairo 5% were positive for *Brucella* spp. (Montasser et al., 1991). Between March 1999 and October 2003, blood samples were collected from 10,130 AFI patients from 13 out of 109 infectious disease hospitals all over Egypt, 3% and 11% were positive for *Brucella* spp. by culture and serology, respectively (Afifi et al., 2005). These hospital-based surveys are limited because not all patients seek medical care from the public hospitals, consequently estimates from such studies do not reflect the actual incidence and/or prevalence of the disease in the population. In 2007, blood samples were collected from 100 human cases with a history of contact with animals at dairy farms or abattoirs and 5% to 8% were seropositive for *Brucella* spp. (Samaha et al., 2009). High numbers of seropositive results from the last study reflect the fact that samples were collected from groups with a high risk of occupational exposure to *Brucella* spp. via direct and/or indirect contact with infected animals. The annual incidence of human infection in Egypt is thought to be increasing from 0.5/100,000 in 1994 to 1.9/100,000 population in 1998 (Refai, 2002). In 2000, it was estimated to be 18/100,000 in Bilbeis district, Sharkia Governorate (Crump et al., 2003) and recently it has been estimated to be 64/100,000 in 2002 and 70/100,000 population in 2003 in Fayoum Governorate and 70% were males (Jennings et al., 2007). Accurate estimates for the prevalence of brucellosis in ruminants are scarce in Egypt, however recent studies indicated that brucellosis is endemic with high prevalence in spite of the current brucellosis control programme in place since 1985 (Samaha et al., 2008; Samaha et al., 2009; Hegazy et al., 2011; Wareth et al., 2014; Hegazy et al., 2016). Other studies concluded that the national program under current regulations was not effective in control of brucellosis among animals in Egypt (Hegazy et al., 2009; Eltholth et al., 2016).

The aim of the current study was to estimate the prevalence of brucellosis in cattle and humans in Kafrelsheikh district and to investigate the epidemiological pattern of such disease among humans and animals.

2. MATERIAL AND METHODS

2.1. Study design:

A cross sectional study was conducted during the period between July and December of 2014 to estimate human brucellosis prevalence and incidence rate based on private lab samples and official record of confirmed cases reported to Kafrelsheikh directorate of the Ministry of Health, respectively. The prevalence of brucellosis among cattle with regard to individual, village and village flock levels was conducted. Spatial association between sheep and cattle brucellosis at village flock level was highlighted.

2.2. Sample and data collection:

2.2.1. Humans:

Three private laboratories in Kafrelsheikh district were visited during the period of study. All human serum samples at the date of lab visit were collected to be examined for seropositivity against brucellosis. A total of 95 (49 males and 46 females) serum samples were collected and stored at – 20°C until being examined serologically.

The records of all confirmed cases reported to Kafrelsheikh directorate of the Ministry of Health from different fever hospitals in the governorate during the year of 2014 were collected. The total number of reported human cases was 18 cases. Data for confirmed human cases of brucellosis in the study area was obtained including the sex of the case and location (village/city). Data of Brucella seropositivity in cases owned livestock was obtained from Kafrelsheikh veterinary directorate of the Ministry of Agriculture. As a part of the national control programme of brucellosis, all confirmed human cases in the governorate's fever hospitals were reported to Kafrelsheikh veterinary directorate. In response to this reporting, a team of assigned veterinarian visited the cases' home for examination and slaughtering of seropositive livestock if existed.

2.2.2. Cattle:

This study is targeting cattle that are kept at households. A simple random study design was used, with individual cattle as primary sampling units. The total number of cattle was obtained from the census of animal population at Kafrelsheikh governorate 2010 (Statistics of livestock, 2011). The sample size was estimated using Win episcope 2. program as 150 animals. This number was divided equally on the major 10 villages of Kafrelsheikh governorate. All of these villages, with exception of one due difficulty in milk sampling in this village, were selected before by the authors in another study for estimation of seroprevalence of brucellosis among sheep in Kafrelsheikh district (Hegazy et al., 2016). Within each village; 15 milk samples were collected from individual household cattle by simple random sampling. A total of 149 milk samples were collected from the 10 villages in Kafrelsheikh district and preserved at -20°C till being examined serologically.

2.3. Serological examination:

Frozen serum and milk samples were transferred under refrigeration to Brucellosis Research lab, Animal Health Research Institute, Egypt for serological examination as follows:

2.3.1. Humans:

Collected serum samples were examined by indirect ELISA for detection of *Brucella* IgG antibodies using a commercial kit; SERION ELISA classic *Brucella* IgG (Serion/Virion, Germany) according to the manufacturer's instructions.

2.3.2. Cattle:

Milk samples were examined by indirect ELISA using commercial kits; BRUCELISA–160M (APHA Scientific, UK) according to the manufacturer's instructions. For optimum sensitivity and specificity, defatted samples was used by centrifugation of the milk samples for 15 minutes at 3000 rpm and the sample was taken from below the fatty layer.

2.4. Epidemiological investigation:

2.4.1. Prevalence of brucellosis among humans:

The seroprevalence of brucellosis among the 95 collected human serum samples was obtained by dividing the number of seropositive samples on the total number of examined samples then multiply the result by 100. The true prevalence of brucellosis among humans was calculated as shown below in true prevalence estimation of cattle.

The apparent seroprevalence (AP), true prevalence (TP) and confidence interval (CI) for TP of human brucellosis among the 95 collected human serum samples were estimated as follows (Thrusfield, 2007):

AP = (Number of humans seropositive to ELISA / Number of examined humans) x 100.

TP = AP+Se-1 / Se+Sp-1. Where Se and Sp are the sensitivity and specificity of ELISA; 84% and 100 %, respectively (Gomez et al., 2008).

$CI = p \pm Z * \sqrt{\frac{p*(1-p)}{n}}$. Where p is the prevalence, n is the number of samples and Z = 1.95.

The yearly cumulative incidence rate of brucellosis among human population on the governorate level was calculated according to the reported official cases to Kafrelsheikh directorate of the Ministry of Health from fever hospitals in the governorate as follows (Thrusfield, 2007):

Yearly cumulative incidence per 100000 population = (number of disease onsets /number of population at risk)*100000. Where the total number of human population of Kafrelsheikh governorate was 3172753; 1567945 female and 1604808 male (CAPMAS, 2015).

2.4.2. Prevalence of brucellosis among cattle:

The AP, TP and CI of brucellosis among cattle for individual were calculated as shown in prevalence estimation of humans. The sensitivity and specificity of ELISA testing of milk samples were 98% and 98%, respectively (Hegazy et al., 2011). The village flock true seroprevalence (VFTP) for each of the 10 studied villages was calculated as VFTP = (Village flock AP + Sp - 1) / (Se + Sp – 1). The proportions of seropositive villages, which have at least one seropositive milk sample, were calculated accounting for the sensitivity and specificity of the serological tests at the village level for milk samples ($VTSe_{ELISA}$ and $VTSp_{ELISA}$); 98% and 88%, respectively (Hegazy et al., 2011).

2.4.3. Spatial correlation of brucellosis prevalence in sheep and cattle at village flock level in Kafrelsheikh district:

The correlation between the seroprevalence of brucellosis among sheep and cattle on the same villages (9 out of the 10 investigated villages for cattle brucellosis) was carried out on SPSS 21.0 software (IBM, USA). The correlation coefficient (r^2) was calculated and interpreted as follows; $r^2 >$ 0.8 were considered as very strong, 0.6–0.79 as strong, 0.4–0.59 as moderate, 0.2–0.39 as weak, and < 0.2 as very weak (Swinscow, 1997). The significance level of correlation was also studied.

3. RESULTS

The TP of human brucellosis was estimated at 1.25% and the 95% CI: 0% – 3.45%. Data from Ministry of Health records indicated that 18 cases were confirmed with brucellosis in Kafrelsheikh governorate in 2014 (Table 1). Out of the 18 cases, 14 cases were visited by veterinary authorities, while the rest were not reached due to mistakes in either names or addresses of the cases reported by the Kafrelsheikh directorate of the Ministry of Health. Five of the 14 cases (35.7%) have *Brucella* seropositive animals in their households, while 7 cases (50%) have no history of animal contact. The yearly cumulative incidence rate at the governorate level was estimated at 0.54/100000 population. In addition, the incidence rate was estimated at 0.38/100000 population and 0.75/100000 population among females and males at the governorate, respectively. The TP of *Brucella* in milk samples was estimated at 7% (95%CI: 2.9–11.1%). The village flock TP ranged from 0% to 46.5% as shown in Table 2. The TP of villages with at least one seropositive cattle for brucellosis was estimated at 44.8% (95%CI: 13.0%–75.9%) with 5 out of 10 villages having at least one cattle that tested positive. There was a significant strong spatial correlation (> 0.7) between the seroprevalence of brucellosis among sheep and cattle ($P < 0.02$) at the village flock level (Figure 1).

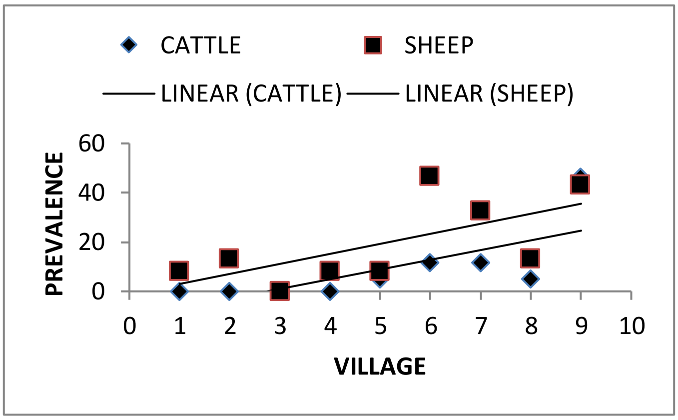
Table 1. Description of Human cases with brucellosis in Kafrelsheikh governorate with regard to sex, location and *Brucella* seropositivity in owned livestock

Case No.	Sex	Location		Visits	Owned Animals		
		District	V. / C.		Species	No.	+ve Sera
1	M.	Kafrelsheikh	V.	+			
2	M.	Kafrelsheikh	V.	+	Sheep	1	Positive
					Goat	1	Positive
3	M.	Kafrelsheikh	C.	+			
4	M.	Kafrelsheikh	C.	+			
5	F.	Kafrelsheikh	V.		Not reached		
6	M.	Qillin	V.	+	Cow	1	
7	M.	Qillin	C.	+			
8	M.	Billa	V.	+	Sheep	12	Positive
					Goat	2	Positive
9	F.	Billa	C.	+			
10	F.	Sidi-Salim	V.	+			
11	M.	Sidi-Salim	V.	+	Cow	1	Positive
					Buffalo	1	
12	F.	Sidi-Salim	V.	+	Cow	1	
13	M.	Sidi-Salim	V.	+			
14	M.	Sidi-Salim	V.	+	Cow	2	Positive
15	M.	Sidi-Salim	V.	+	Cow	1	Positive
16	F.	Sidi-Salim	V.		Not reached		
17	M.	El-Hamoul	V.		Not reached		
18	F.	Baltium	V.		Not reached		

Table 2. The village flock prevalence among cattle of different villages of Kafrelsheikh District

No.	Village Name	Seropositive Cattle	
		No.	TP
1	Elhamra	0	0%
2	Elshamarka	0	0%
3	Rowina	0	0%
4	Meseer	0	0%
5	Mahlet Mousa	1	4.9%
6	Elnataf	2	11.8%
7	Sandala	2	11.8%
8	Elkafr Elgedid	1	4.9%
9	Defria	7	46.5%
10	Amia	0	0%
	Total	**13**	**7%**

Figure 1. Spatial correlation between the village flock brucellosis prevalence among sheep and cattle in Kafrelsheikh district.



*Correlation coefficient (r^2)= 0.744 indicating a significant strong positive correlation (P < 0.02).

4. DISCUSSION

Brucellosis is one of the most important neglected tropical diseases (WHO, 2006). There are some countries, which were able to get rid of the disease, but on the other hand most of developing countries are still endemic with brucellosis among humans and animals. In Egypt, the control measures were unsuccessful and one of the reasons of this failure is the incomplete adherence to the planned measures mainly due to the economic implications (Hegazy et al., 2009; Hegazy et al., 2011). This results in the endemicity of such disease among humans and animals in the region. The aim of this study was to fill some data gaps in the epidemiology of brucellosis at humans-animals interface in Kafrelsheikh governorate of Egypt.

The true prevalence of brucellosis in this study among cattle was estimated at 7%, which was comparable was a report in Kenya (8%) by Osoro et al. (2015). Lower rates were recorded in Gharbia Governorate of Egypt (5.2%) by El-Sherbini et al. (2007) and in Ethiopia (3.5%) by Megersa et al. (2011), however higher rates (16%) were reported in Delta region of Egypt (Wareth et al. 2014). The obtained results showed that at least 7% of the milk distributed in the governorate could be a risk for human. It is expected that the percentage of market milk with brucellosis may exceed this percentage as most of farmers and retail sellers mix the milk of different animals species before selling it. The findings of Hegazy et al. (2011) supported this theory as higher prevalences (12.2%) were reported in milk samples from same region when samples were taken from milk tanks. Clustering of milk samples from different animals in same milk tank may be resulted in increased prevalence rate in milk samples.

The village flock seroprevalence of cattle brucellosis ranged from 0 to 46.5 %. Same wide-range of intra-village prevalences was reported in another study in Egypt (0-16%) by El-Sherbini et al. (2007) and in Ethiopia (0-100%) by Megersa et al. (2011). Data of village flock prevalences could help the policy makers in choosing the appropriate control measures based on village prevalence. Villages with high prevalences could use the test and slaughter or vaccination strategies, however these strategies should be accompanied by educational campaigns to enhance their effectiveness. On the other hands, villages with no *Brucella* infection could apply a preventive measure called “zoning” as previously recommended by FAO/WHO report for Middle East (Hegazy et al., 2011). In this system, quarantine should be applied on these villages to prevent entrance of infection and also the animals’ offspring in these villages could be used for building farms in new areas. Yet, regulation of animal movement is necessary for this system to be effective.

This study showed that 44.8% of the villages have at least one seropositive cattle to brucellosis. This result was higher than that reported in Ethiopia (31.3%) by Megersa et al. (2011). These high rates of villages with *Brucella* infection indicated that the current test and slaughter policy of the national control program might not be the most suitable choice for brucellosis control in Egypt. Several studies in Egypt also highlighted the failure of the current national programme in controlling *Brucella* infection among animals (Hegazy et al., 2009; Eltholth et al., 2016; Hegazy et al.. 2016). The high rate of infected villages (around 50%) requires more animals to be tested and slaughtered every year, which could be an economical obstacle especially in developing country as Egypt. Hence, mass vaccination could provide a more reliable choice in Egypt. Our previous study (Hegazy et al., 2016) on sheep brucellosis in same district showed very high village prevalence (95.5%). Spatial correlation was tested between the two species at the village flock level and the results showed a significant strong correlation (> 0.7). This correlation was statistically significant (p < 0.02), which means that the increase in the prevalence of sheep brucellosis in any village in the district will result in higher prevalences among cattle of the same village. Sheep are raised mainly in large movable flocks, which may come in contact with household animals rested at fields’ shelters or drinking from water canals, hence infection in sheep flock may be considered a major source for *Brucella* infection in cattle in close proximity with these flocks. Unfortunately, the last study also showed complete lack for control measures regarding sheep brucellosis, which means a continuous prevalence will be expected in the district (Hegazy et al., 2016). This study could direct policy maker for controlling brucellosis in sheep in addition to the current efforts that are all directed to cattle.

The human brucellosis prevalence was estimated at 1.25% in Kafrelsheikh district. This result was comparable with findings of El-Sherbini et al. (2007) who reported a prevalence of 1.7% among humans in Gharbia Governorate, Egypt. Higher rates (5-8%) were reported in dairy farms and abattoirs workers by Samaha et al. (2009), which may be attributed to the high risk of occupational infection among these individuals due to prolonged contact with animals or its products.

Hospital based annual incidence rate of human brucellosis per 100000 population was 0.54. This result agreed with findings of Refai (2002) who reported that incidence rate of human brucellosis in Egypt ranged from 0.5 – 1.9 /100,000 population. The human incidence rate reported in this study is one of the highest in the world (Pappas et al., 2006), however it is highly underestimated, as other healthcare providers were not included in the investigation. High incidence rates of 18 and 70 /100000 population were recorded by Crump et al. (2003) and Jennings et al. (2007), respectively. Both studies included records of community healthcare providers as private hospitals, physicians' clinics and rural health units. In addition, Jennings et al. (2007) reported that reliance on hospital-based incidence rate alone would underestimate the actual figure by 12–18 times. Brucellosis incidence rate in male (0.75 / 100000) was almost twice the incidence rate in female (0.38 / 100000) of Kafrelsheikh governorate, which agreed with findings of Jennings et al. (2007). This is usually attributed to the possible higher rates of infection exposure in males during work or social activities. Several studies reported that close contact with animals at households and consumption of raw milk were associated with human brucellosis (El-Sherbini et al., 2007; Jennings et al., 2007; Megersa et al., 2011; Osoro et al., 2015). In agreement with these studies, 35.7% of human cases in Kafrelsheikh governorate owned a seropositive animal for brucellosis in their households. Moreover, 50% of the cases did not own animals therefore they may be contracted infection by other routes especially the consumption of raw milk or dairy products as reported in these studies. The relatively high rate of *Brucella* prevalence in milk reported in this study (7%), mixing of infected milk with clean milk in large tanks before marketing and uncontrolled marketing of raw milk and dairy products in local informal markets in Kafrelsheikh governorate may result in increasing the risk of brucellosis to those cases with no history of animal contact. It is, therefore, strongly recommended to advice the policy makers for panning the marketing of non-heat treated milk and dairy products in Egypt.

In conclusion, this study provides fundamental information regarding the prevalence of brucellosis in humans and cattle of Kafrelsheikh governorate. A continuous integrated disease surveillance that involves both humans and animals is recommended for better understanding of brucellosis epidemiology and designing appropriate preventive measures. Policy makers have to consider vaccination as an alternative to the current test and slaughter control program and to strengthen the measures for surveillance and control of sheep brucellosis as a major source of *Brucella* infection in the country. Restriction of raw milk marketing and continuous health education to increase the awareness of brucellosis may help in decrease the risk of *Brucella* infection among residents of the study region.

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