



# Seroprevalence and Associated Risk Factors of Brucellosis in Sheep and Human in Four Regions in Matrouh Governorate, Egypt

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## ABSTRACT

Brucellosis is a worldwide zoonosis that has major public health concern in Egypt. The present work was conducted to investigate the seroprevalence of brucellosis in sheep and human in four localities in North Western region of Egypt, on basis of the Rose Bengal plate test (RBPT) and further confirmation by complement fixation test (CFT). A total of 2471 sheep serum samples and 371 human samples were collected. The prevalence of brucellosis in sheep and human by using RBPT were 11% (272/2471) and 24.3% (90/371), respectively while by CFT were 10.56% (261/2471) and 22.91% (85/371). There was significant relationship between age and in infection rate in sheep ( $P < 0.01$ ), with higher percentage of infection was indicated in age group over than 24 months by 14.19% (264/1860) followed by age group less than 24 month and over 12 months by 2.39% (8/335). On studying the relation between locality and infection rate there was no significance in human samples while in sheep it was significant ( $P < 0.01$ ) with higher percentage of infection found in Siwa region by 20.30% (94/463) in sheep and in human by 27.6% (27/98). Concerning season there is highly significant relationship between season and percent of infection with *Brucella*, the high percent of infection found in human and sheep by 43.1% (62/144) and 16.51% (123/745) respectively and lower percent found in spring months by 8% in sheep. From our result, it is concluded that RBPT and CFT used as screening tests for detection the prevalence of species in serum samples, *Brucella* infection is found with high percent in north, west region of Egypt, which need further examination and studying another risk factor associated with infection and isolation of *Brucella* in this area.

**Key words:** Brucellosis, Complement fixation test, Human brucellosis, Rose Bengal plate test, Sheep

## INTRODUCTION

Brucellosis is a highly contagious zoonosis caused by genus *Brucella* affecting both humans and animals (Schelling et al., 2003). Sheep brucellosis divided into typical zoonotic brucellosis that caused by *Brucella melitensis* and non-zoonotic ram epididymitis that caused by agent *B. ovis* (Acha and Szyfres 2003). Sheep and goats are primary hosts for *Brucella melitensis* (*B. melitensis*) which is common *Brucella* species in humans (Godfroid et al., 2011). Direct contact with fluids from infected animals in birthing products and other bodily fluids such as urine is the major rout of infections between animals and humans. Symptoms of *B. melitensis* in animals include abortions, stillbirths, infertility and decreased production (Corbel, 2006). While Major symptoms of human brucellosis are undulant fever, headache, muscle pain, lumbar pain and arthritis (Acha and Szyfres 2003; Pal et al., 2017).

Despite of continuous effort for zoonotic brucellosis control, that represent a major public health threats, it remains endemic in the vast majority of middle eastern countries, accused of tens of thousands of new cases yearly (Pappas and Memish 2007; Patel et al., 2017). There are about half a million new human cases of brucellosis occur every year worldwide making it the most common zoonosis (Seleem et al., 2010). Transmission of brucellosis to human occurs through ingestion of the infected product, direct contact with infected animals and its materials and through inhalation of the infected particles (Dieckhaus and Kyebambe 2017). The causative agent has a very low infectious dose, only 10 organisms of *Brucella melitensis* initiate infection (Lopes et al., 2010). Rose Bengal plate test (RBPT) is simple, good, rapid and easy to implement and can be used as herd screening test at remote places (Gul and Khan 2007; Teng et al., 2017). Moreover, Complement Fixation Test (CFT) used as a confirmatory test for diagnosis of brucellosis (Ashraf et al., 2014).

Therefore, this study was intended to study the seroprevalence of brucellosis in sheep and humans in four localities in Matrouh Governorate, Egypt by using Rose Bengal Plate Test and confirmed by complement fixation test.



## Statistical analysis

Chi square test was used in statistical studies. The P value is the probability of the event occurring by chance if the null hypothesis is true, P-values 0.0001 (<0.01)

## Ethical approval

All procedures performed in this study including collection of human serum samples and animals were in accordance with the Egyptian ethical standards of the national research committee. All human subjects gave their consent for the collection of the serum samples, with the agreement that any identifying details of the individuals should not be published.

## RESULTS

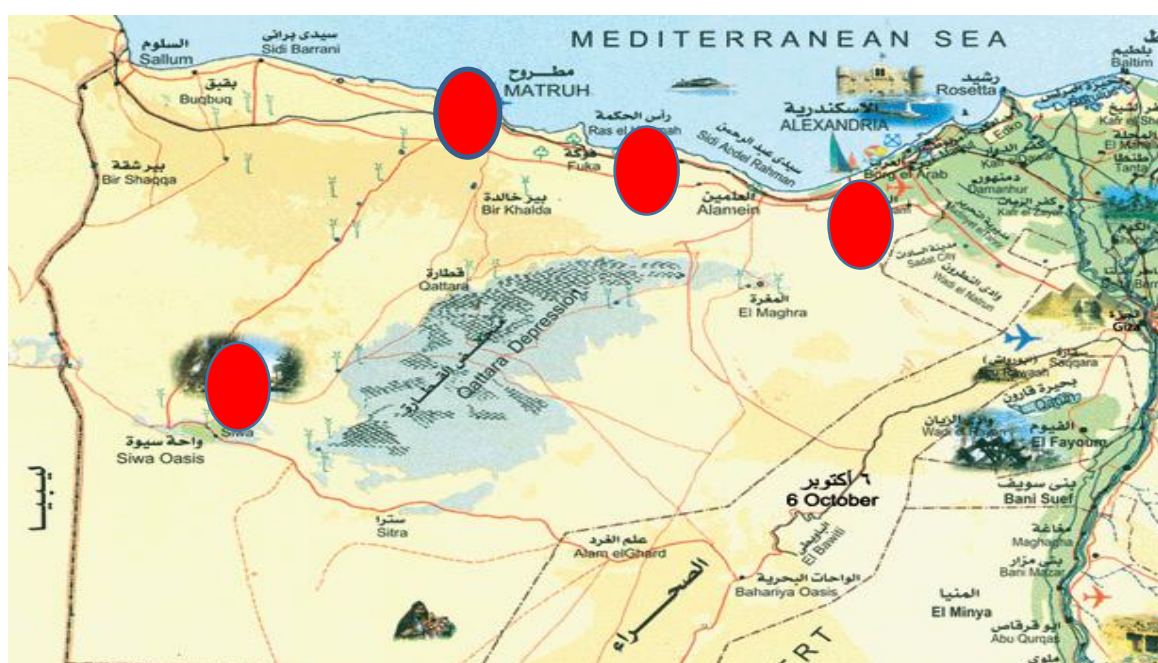
In table 2 and figure 1, the overall seroprevalence of brucellosis were 11% (272/2471) and 10.56% (261/2471) in sheep serum samples using RBPT and CFT respectively while in human samples were 24.3% (90/371) and 22.91% (85/371). In table 3 and graph 1, studying age as risk factors in infection with brucellosis theirs is highly significant between age of sheep and infection with *Brucella*  $P < 0.01$ , the high percent of infection found in age group over than 24 months by 14.19% (264/1860) followed by age group less than 24 months and over 12 months by 2.39% (8/335). In table 4 and graph 2, studying locality as other risk factors, theirs is highly significance between locality and percent of infection with brucellosis in sheep  $P < 0.01$  while in human samples there is no significance. The high percent of infection found in Siwa region by 20.30% (94/463) in sheep and in human by 27.6% (27/98).

In table 5 obtained the high percent of infection found in human females by 13.6% (21/154) and in sheep by 11.35% (259/2282). In table 6 in studying human the high percent of infection found in contact animals with sheep by 31.5% 68 (216) compared with non-contact human in which the percent of infection were 14.2% (22/155) while table 7, graph 3, studying a season as a risk factors explain that theirs highly significance between season and percent of infection with *Brucella*, the highest percent of infection found in human and sheep in winter by 43.1% (62/144) and 16.51% (123/745) respectively and lower percent found in spring months by 8% in sheep and no examination to human in spring months.

**Table 2.** Overall prevalence of *Brucella melitensis* by rose Bengal plate test and complement fixation test in Barki and Rahmani sheep at four areas of Matrouh governorate between April 2016 to February 2017

Test	Total No	RBPT		CFT	
		Positive No	%	Positive No	%
Sheep	2471	272	11	261	10.56
Human	371	90	24.3	85	22.91

RBPT; Rose Bengal plate test, CFT; Complement fixation test

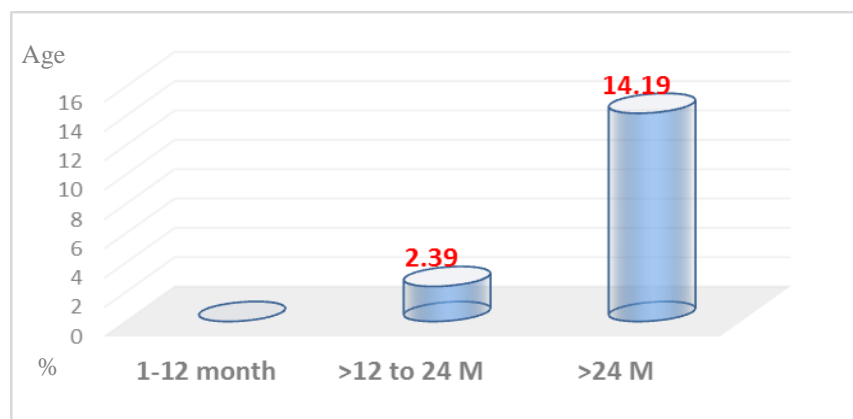


**Figure 1.** Map for Matrouh governorate, Egypt showing four areas of study between April 2016 to February 2017

**Table 3.** Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in Barki and Rahmani sheep at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Age (months)	Total	Positive	%
1-12	276	0	0
>12 to 24	335	8	2.39
>24	1860	264	14.19
Total	2471	272	11
Chi- square		78.82**	
P value		0.0001 (<0.01)	

\*\* Highly significant

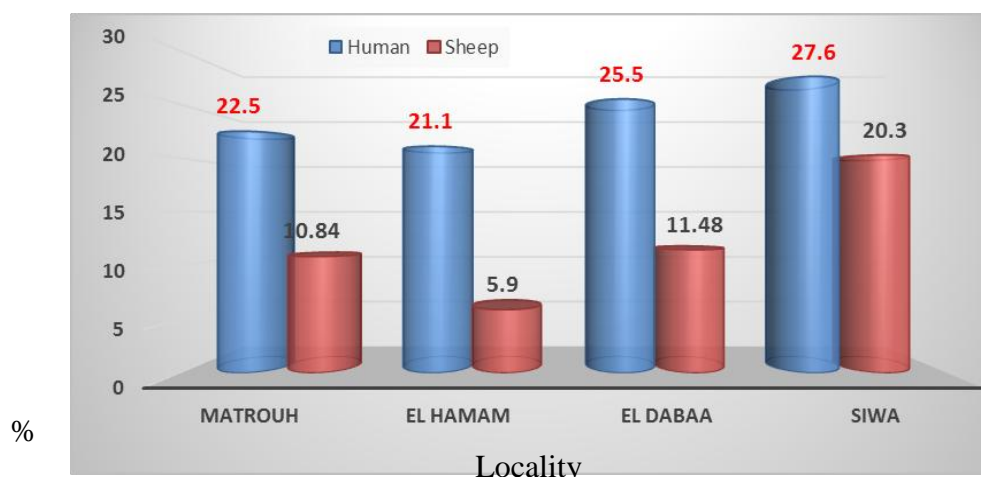


**Graph 1.** Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in Barki and Rahmani sheep at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

**Table 4.** Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Locality	Human serum samples			Sheep serum samples		
	Total	Positive	%	Total	Positive	%
Matrouh	89	20	22.5	526	57	10.84
El Hamam	90	19	21.1	881	52	5.90
El dabaa	94	24	25.5	601	69	11.48
Siwa	98	27	27.6	463	94	20.30
Total	371	90	24.3	2471	272	11
Chi- square		1.3 <sup>NS</sup>			64.43**	
P-value		0.73			0.0001 (<0.01)	

NS: Non-Significant; \*\*: Highly significant



**Graph 2.** Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

**Table 5.** Prevalence of *Brucella melitensis* in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017 by rose Bengal plate test in relation to sex

Sex	Samples	Human serum samples			Sheep serum samples		
		Total	Positive	%	Total	Positive	%
Male		217	69	31.8	189	13	6.88
Female		154	21	13.6	2282	259	11.35
Total		371	90	24.3	2471	272	11
<i>Chi- square</i>		16.17**			3.56 <sup>NS</sup>		
<i>P-value</i>		0.0001 (<0.01)			0.06		

NS: Non-Significant; \*\*: Highly significant

**Table 6.** Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to history of human contact with sheep in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

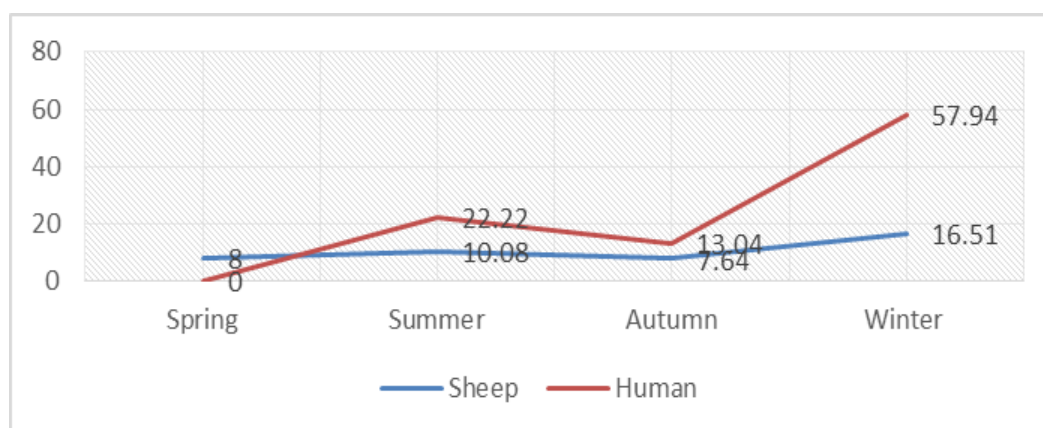
Item	Total	Positive <i>Brucella melitensis</i>	%
Contact	216	68	31.5
Non -contact	155	22	14.2
<i>Chi- square</i>		14.68**	
<i>P value</i>		0.0001 (<0.01)	

\*\* Highly significant

**Table 7.** Prevalence of *Brucella melitensis* by rose Bengal plate test according to season in serum samples of Barki and Rahmani sheep and human from four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Season	Species	Sheep			Human		
		Total	Positive	%	Total	Positive	%
Spring		400	32	8	-	-	-
Summer		645	65	10.08	107	16	15
Autumn		681	52	7.64	120	12	10
Winter		745	123	16.51	144	62	43.1
Total		2471	272	11	371	90	24.3
<i>Chi- square</i>		35.19**			46.01**		
<i>P-value</i>		0.0001 (<0.01)			0.0001 (<0.01)		

\*\* Highly significant



**Graph 3.** Prevalence of *Brucella melitensis* by rose Bengal plate test in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

## DISCUSSION

Brucellosis is a zoonosis of veterinary, major public health and economic importance in most unindustrialized countries including Egypt (Afifi et al., 2005; Asiimwe et al., 2015). The diagnosis is based mainly on the serologic testing, as it is fast and simple in addition to the fact that culture techniques are not available in laboratories in endemic countries (Young, 1995). RBPT and CFT have been used for many decades, confirming to be successful for eradicating brucellosis in some countries (Garin-Bastuji et al., 1998). Therefore, in the present work RBPT were used for determination of seroprevalence of brucellosis in both sheep and human in North West region of Egypt. Results in table 2 indicated that the overall prevalence of brucellosis by RBPT in sheep were 11% (272/2471). Our results were nearly similar to that found by Hegazy et al. (2011) who confirmed that seroprevalence among sheep were 12.2%, Hussain et al. (2014) and who concluded that the overall seroprevalence of ovine brucellosis was 10.0%. The differences in prevalence of brucellosis may be attributed to time and place of sampling in addition to people habits in reporting cases.

On the contrary, higher prevalence were recorded by Al-Majali et al. (2007) 33.1% Ahmed et al. (2010), Kaoud et al. (2010), Mahboub et al. (2013) and Nagati and Hassan (2016) by rates of 24%, 26.6%, 18.09% and 16.4% respectively. On the other hand, lower results were documented by Ferede et al. (2011), Rahman et al. (2011), Horton et al. (2014), Tsehay et al. (2014) and Patel et al. (2017) by rates of 0.74%, 3.08%, 4% , 7% and 8.70 respectively.

RBPT is the ideal screening test for human brucellosis. This is for the reason that it is highly sensitive, simple and rapid technique (Smits and Kadri 2005; Teng et al., 2017). In our study, the overall prevalence of human brucellosis by RBPT as illustrated in table 2 was 24.3% (90/ 371). Nearly similar results were obtained by Fouad et al. (1996) (26%), Kumar et al. (1997) (28.57%) and De Massis et al. (2005) who concluded that Human brucellosis is more widespread in areas where *Brucella* is prevalent in sheep. Hussain et al. (2014) confirmed that nomadic life is characterized by some factors that favor brucellosis infection such as regular migration, inadequate health services, close animal contact, poor hygienic procedures and ingestion of raw animal products.

Lower results were obtained by Afifi et al. (2007) (11%), Hassanain and Ahmed (2012) (6.26%), Hussain et al. (2014) (6%), Ali et al. (2015) (8.6%) , Nagati and Hassan (2016) who declared that the seroprevalence of brucellosis among human was 15.2% and Tsegay et al. (2017) (4.7%). However, higher results were recorded by Ahmed et al. (2010) who founded that the overall seroprevalence of human brucellosis in Libya were 40%.

Data presented in table 3 showed that there is a highly significant difference between age groups, with higher percent of infection in age group more than 24 Month (14.19%) than other groups (2.39%) the obtained data were agreed with Abdallah et al. (2015) and Alhamada et al. (2017) who confirmed that seropositivity were significantly higher older animal ages. On the other hand, the infection rate in human were highly significant in male than female. Our results were agreed with results obtained by Afifi et al. (2005), Khan et al. (2009), Shahid et al. (2014).

A total of 2471 and 371 sheep and human samples were collected from four different localities. Data presented in table 4 showed that however, there a difference in the infection rates in four localities, statistical analysis showed that significant differences found only in sheep samples. Ecological dissimilarity has been reported to influence the seroprevalence of brucellosis (Rahman et al., 2011). Prevalence of brucellosis in relation to sex were illustrated in table 5 and showed that the infection rate in sheep were higher in female (11.35%) than male (6.88%). However statistical analysis showed no significant difference. These results were agreed with that obtained by Ferede et al. (2011), Hussain et al. (2014), Tsehay et al. (2014) and Ali et al. (2015). Rams could be lower than ewes as they may be culled or sold faster. In addition to erythritol and sex hormone in ewes (Rahman et al., 2011).

On the other hand, the infection rate in human were highly significant in male than female. Our results were agreed with results obtained by Afifi et al. (2005), Khan et al. (2009), Shahid et al. (2014). Analysis of the seasonal variation of brucellosis illustrated in table 7 and showed a highly significant differences with the highest infection rate during winter season in both human and sheep. Our results disagreed with Al-Ballaa et al. (1994) who founded that the predominance of cases occurring during spring, summer and early fall and De Massis et al. (2005) who confirmed that the seasonal occurrence of human cases of brucellosis showed a peak in summer.

## CONCLUSION

High prevalence of brucellosis in sheep and human in North West region of Egypt, representing major public health threats. We need further investigation including isolation and molecular identification and further analysis of other possible risk factors associated with *Brucella* infection.

## DECLARATIONS

### Competing interests

All authors have no conflict of interest.

## Author's contributions

Mohamed S. Diab, Yasser Elnaker, Nermin Awade, Eman Khalifa and Sherif Zidan conceived and designed the experiments. Yasser Elnaker, Nermin Awade, Eman Khalifa performed the experiments. Sherif Zidan and Yasser Elnaker analyzed the data. Mohamed S. Diab, Sherif Zidan, Nermin Awade and Eman Khalifa contributed reagents/materials/ analysis tools. Yasser Elnaker, Mohamed S. Diab and Nermin Awade wrote the paper.

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