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Seroprevalence and Associated Risk Factors of Brucellosis among Sheep, Goats and Camels in North Western Coastal Area of Egypt

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ABSTRACT

This study was conducted to find out the seroprevalence and associated risk factors of brucellosis in northwestern coast of Egypt such important border area. In this study, 630 serum samples (272 camel, 237 sheep and 121 goat) were collected from different localities of the northwestern coast, Egypt. Serum samples were tested serologically by Rose Bengal plate test (RBPT) and competitive ELISA (cELISA). The result revealed an overall brucellosis seroprevalence of (4.04% and 3.68%) for camels, (13.50% and 23.21%) for sheep and (25.62% and 29.75%) for goats by RBPT and cELISA respectively in the northwestern coast of Egypt. Regarding to the sex of the animals nearly similar prevalence was observed (16.2%, 16%) in males and females respectively. While young animals were more likely to test positive than adult animals where prevalence rate in young animals was 18.5% and 15.9% in adult animals. The brucellosis seroprevalences of aborted and non-aborted animals were (18%, 15.5%). While our data revealed that 33% of animals with fertility problems (repeat breeders) were seropositive, prevalence of brucellosis in animals with no fertility problems was 15.5%. Finally, brucellosis is found in the northwestern coast of Egypt with relatively high percentage. More researches are needed to study the true epidemiological aspect in this important area. An effective eradication program is also needed in this area.

Keywords: *Brucellosis, sheep, goats, camels, Serological tests, North Western Coastal Area, Egypt*

INTRODUCTION

Brucellosis is a worldwide zoonotic disease that is recognized as a major cause of abortion, heavy economic losses to the livestock industry and poses serious human health hazard, it is still an uncontrolled serious health problem in many developing countries including Egypt (Mantur & Amarnath, 2008; Ocholi *et al.*, 2005; Samaha *et al.*, 2009).

Brucellosis is caused by bacteria of the genus *Brucella* which is a small Gram-negative, nonmotile, non-spore forming, aerobic, facultative intracellular coccobacilli capable of invading epithelial cells, placental trophoblasts, dendritic cells, and macrophages causing appreciable economic losses in livestock industry in the form of abortions, retained placenta, decreased milk production, orchitis in

males (Adams, 2002; Corbel, 1997; Gorvel, 2008).

Brucella infection is responsible for up to 20–25% decrease in milk production, 10 to 15% in meat production, 15% loss of calves due to abortions, 30% increase in the rate of animal replacement, and increased calving interval of to 11.5 to 20 months in domestic animals. In addition, every five infected animals abort once or become permanently infertile. Besides the loss of animal productivity, brucellosis is a zoonosis of major health public importance (Kardjadj, 2018a).

Control of brucellosis in Egypt is based on identification of infected animals, application of testing and slaughter policies, adoption of vaccination and strict quarantine measures (Refai, 2002).

Isolation of *Brucella* is the only method that allows certainty of diagnosis, but it is time consuming, laborious, in addition to the high risk of infection. Therefore, serological tests such as Rose Bengal Test (RBT), Complement Fixation Test (CFT), Enzyme Linked Immunosorbent Assay (ELISA), and Indirect Enzyme Linked Immunosorbent Assay (iELISA) are commonly used for diagnosis of *Brucella* (Glynn & Lynn, 2008; Munoz *et al.*, 2005; Refai, 2002).

Because no serological test is 100% accurate, generally, diagnosis is based on the results of two or more tests. Thus, initial testing is commonly done using a screening test, a test with high sensitivity and perhaps of less specificity. The screening tests are usually relatively inexpensive, fast and simple to perform. If a positive reaction occurs in a screening test, a confirmatory test is performed. The confirmatory test is a test, which provides good sensitivity but higher test specificity, thereby eliminating some false positive reactions. Most confirmatory tests are more complicated and more expensive to perform (Poester *et al.*, 2010).

In this study the Rose Bengal plate test (RBPT) is used as a screening test, RBPT is a prescribed test for international trade recommended by the World Organization for Animal Health. The Enzyme Linked Immunosorbent Assay (ELISA) is applied as, a confirmatory test. ELISA are also prescribed tests for trade by the OIE. ELISA tests are divided into two categories, the indirect ELISA (iELISA) and the competitive ELISA (cELISA). This study used cELISAs, which decrease non-specific reactions, by using monoclonal antibodies directed against specific epitopes of the Brucella LPS that are not shared with the LPS of other cross-reacting Gram-negative organisms (Uzal *et al.*, 1995).

All the previous studies conducted in the northwestern costal area of Egypt were in either a limited area and/or period and did not reflect the exact impact of the prevalence of brucellosis in this area. Moreover, up to date, no systemic research on the epidemiology of brucellosis that cover all the regions of the northwestern costal area of Egypt was carried out to best of our knowledge. Therefore, the objective of this study was to investigate the seroprevalence of the disease in camel, sheep and goats distributed randomly in the region and to elucidate the role of risk factors in the spread of the disease among livestock. This in turn will provide a base for the designation of the suitable control program of the disease.

MATERIAL AND METHODS

Area of study

The northwestern coast of Egypt important cities (Marsa Matrouh, El Salloum, El-Negela, Sidi Barrany, El-Hamam and Raas El-Hekma) were included in this study.

Ethic statement

Samples collection were performed under owner's consent. The Internal Ethics Review Committee of Faculty of Veterinary Medicine, University of Sadat City approved this study.

Animals

In this study, 630 animals (272 camel, 237 sheep and 121 goat) from different localities of the northwestern coast of Egypt were examined clinically according to (Jackson & Cockcroft, 2008), history focused on nature of food, mixed grazing, abortion and fertility problems.

Serum samples

A total of 630 serum samples were collected from camel (272), sheep (237) and goats (121) from different localities of the northwestem coast of Egypt as shown in table 1.

Serological Test:

A-Rose Bengal Plate test (RBPT)

Rose Bengal Plate test was carried out according to (Morgan *et al.*, 1969)**.** Sera and

antigen were left at room temperature before testing for about 15 minutes. 0.03 ml of serum (about one drop) to be tested placed on enamel plate, and then 0.03 ml of antigen was added on the serum drop. Mixing the serum and antigen well by sterile spreader. The reading was done within 4 minutes. Known positive and negative sera were included as control.

B- A competitive ELISA (COMPELISA 400®, APHA, New Haw, Addlestone, U.k.).

This test had been performed according to manufacture instructions.

Statistical analysis

Fisher's exact test was carried out to determine the prevalence of brucellosis based on the proportions of the studied population. GraphPad Prism 8 was used to determine the significance at $P<0.05$.

RESULTS

The overall Seroprevalence of Brucella antibodies in northwestern coast of Egypt

The overall seroprevalence of brucellosis was 4.04% and 3.68% in camels, 13.50% and 23.21% in sheep and 25.62% and 29.75% in goats by RBPT and cELISA respectively.

Higher seroprevalence was found in goats (25.62% and 29.75%) than in sheep (13.50% and 23.21%) and camels (4.04% and 3.68%) by RBPT and cELISA respectively.

No statistically significant differences ($p<0.05$) were recorded between the serological tests in different species. cELISA was significantly higher than RBPT in examining sheep sera (Table 2).

Seroprevalence of Brucella antibodies in camels in northwestern coast of Egypt

Among the 272 serum samples of camels screened for *Brucella* antibodies, 11 (4.04%) were positive for Rose Bengal Plate Test (RBPT) and 10 (3.68%) were positive for cELISA.

A location specific seroprevalences of 3 (6.12%), 5 (3.65%), 1 (2.04%), 2 (7.69%), and 0 (0%), based on RBPT and 4 (8.16%), 3 (2.19%), 1 (2.04%), 2 (7.69%), and 0 (0%), based on cELISA were recorded in El-Negela, Matrouh, Sidi Barrany, El-Saloum, and El-Hamam respectively (Table 3).

Seroprevalence of Brucella antibodies in sheep in northwestern coast of Egypt

Among the 237 serum samples of sheep screened for *Brucella* antibodies, 32 (13.50%) were positive for Rose Bengal Plate Test (RBPT) and 55 (23.21%) were positive for cELISA.

A location specific seroprevalences of 8 (15.09%), 5 (29.41%), 15 (13.04%), 2 (5.41%), and 2 (13.33%), based on RBPT and 9 (16.98%), 8 (47.06%), 34 (47.06%), 2 (5.41%), and 2 (13.33%) based on cELISA were recorded in El-Negela, Matrouh, Sidi Barrany, El-Hamam and Raas El-Hekma respectively (Table 4).

Seroprevalence of Brucella antibodies in goats in northwestern coast of Egypt

Among the 121 serum samples of goats screened for *Brucella* antibodies, 31 (25.62%) were positive for Rose Bengal Plate Test (RBPT) and 36 (29.75%) were positive for cELISA.

A location specific seroprevalences of 13 (31.71%), 3 (75%), 9 (20.45%), 0 (0%), and 6 (31.59%), based on RBPT and 14 (34.15%), 2 (50%), 13 (29.55%), 0 (0%), and 7 (36.84%) were recorded in El-Negela, Matrouh, Sidi Barrany, El-Hamam and Raas El-Hekma respectively (Table 5).

The influence of individual-level variables (sex and age) and previous reproductive problems (abortion and infertility) on the seroprevalence of brucellosis based on cELISA

A total of 27 (4.29%), and 20 (95.71%) animals were sampled for the age categories, which yielded 5 (18.5%), and 96 (15.9%) positives for the ages of young animals (sheep and goats from 5 months to one year, young camels from 2-5 years) and adult animals (sheep and goats above one year, adult camels above 5 years), respectively. There was no statistically significant association between age and the seroprevalence of brucellosis in animals in the northwestern coast of Egypt.

The sex specific seroprevalence revealed 16.2% and 16% for male (43) and female (587) animals, respectively. There was no statistically significant association between sex of animals

and the presence of brucellosis in the northwestern coast of Egypt.

Reproductive problems (abortion and infertility) specific seroprevalence yielded 18%, and 15.5% for animals with history of abortion and animals without history of abortion respectively. Infertile animals recorded 33% seropositivity, while fertile animals recorded 15.7%. The presence of brucellosis was associated significantly with infertility in animals OR= 3.26; 955 CI= 1.09-8.25; P<0.05) in the northwestern coast of Egypt (Table 6).

Table 1. Numbers of collected serum samples from different Species from different localities.

Locality	Species			Total
	Camel	Sheep	Goat	
	No. of samples	No. of samples	No. of samples	
El-Negela	49	53	41	143
Matrouh	137	17	4	158
Sidi Barrany	49	115	44	208
El-Saloum	26	-	-	26
El-Hamam	11	37	13	61
Raas El-Hekma	-	15	19	34
Total	272	237	121	630

Table 2. The overall Seroprevalence of Brucella antibodies

Species	Number of sera sample tested	RBPT positive (%)	cELISA positive (%)
Camels	272	11 (4.04%)	10 (3.68%)
Sheep	237	32 (13.50%)	55 (23.21%)*
Goats	121	31 (25.62%)	36 (29.75%)
Total	630	74 (11.75)	101 (16.03)

*Significant at 5% Level (p<0.05), RBPT=Rose Bengal plate test, cELISA=competitive enzyme-linked immunosorbent assay.

Table 3. Numbers and percentage of positive samples of Camel from different localities

Locality	Total No.	RBPT		cELISA	
		+ve No.	+ve %	+ve No.	+ve %
El-Negela	49	3	6.12%	4	8.16%
Matrouh	137	5	3.65%	3	2.19%
Sidi Barrany	49	1	2.04%	1	2.04%
El-Saloum	26	2	7.69%	2	7.69%
El-Hamam	11	0	0%	0	0%
Raas El-Hekma	-	-	-	-	-
Total	272	11	4.04%	10	3.68%

No. = No. of samples +ve No. = No. of +ve samples +ve % = % of +ve samples

Table 4. Numbers and percentage of positive samples of sheep from different localities

Locality	Total No.	RBPT		cELISA	
		+ve No.	+ve %	+ve No.	+ve %
El-Negela	53	8	15.09%	9	16.98%
Matrouh	17	5	29.41%	8	47.51%
Sidi Barrany	115	15	13.04%	34	29.57%
El-Saloum	-	-	-	-	-
El-Hamam	37	2	5.41%	2	5.41%
Raas El-Hekma	15	2	13.33%	2	13.33%
Total	237	32	13.50%	55	23.21%

No. = No. of samples +ve No. = No. of +ve samples +ve % = % of +ve samples

Table 5. Numbers and percentage of positive samples of goats from different localities

Locality	Total No.	RBPT		cELISA	
		+ve No.	+ve %	+ve No.	+ve %
El-Negela	41	13	31.71%	14	34.15%
Matrouh	4	3	75%	2	50%
Sidi Barrany	44	9	20.45%	13	29.55%
El-Saloum	-	-	-	-	-
El-Hamam	13	0	0%	0	0%
Raas El-Hekma	19	6	31.85%	7	36.84%
Total	121	31	25.62%	36	29.75%

No. = No. of samples +ve No. = No. of +ve samples +ve % = % of +ve samples

Table 6. Seroprevalence of Brucellosis in the northwestern coast of Egypt based on age, sex, and reproductive status.

Variables	Number of sera tested	Number of positive (%)	Odds ratio (OR)	95% CI On OR	P-value
Age					
Young animals	27	5 (18.5)			
Ault animals	603	96 (15.9)	1.26	(0.8-3.54)	0.4
Sex					
Male	43	7 (16.2)			
Female	587	94 (16)	0.82	0.5-1.6	0.6
Abortion					
Abortion history	115	21(18)			
No abortion history	515	80 (15.5)	1.8	0.75-2.22	0.2
Fertility					
Infertile animals	9	3 (33)			
fertile animals	621	98 (15.7)	3.26*	1.09-8.25	0.04
Total	630	101 (16)			

*Significant at 5% Level ($p<0.05$)

DISCUSSION

Brucellosis is considered by the Food and Agriculture Organization of the United Nations (FAO) as one of the transboundary animal diseases (TADs), FAO defined (TADs) as those that are of significant economic, trade, and food security importance for a considerable number of countries. TADs can easily spread to other countries, reach epidemic proportions, and where control, management, or exclusion is required cooperation between several countries. The North African countries are vulnerable to several TADs by virtue of its geographical location, its borders with the Sahel region, and peculiar control constraints on the budgets of the national veterinary services of each country and on the livelihoods of livestock owners across the region (Kardjadj, 2018b). Therefore, the epidemiology of such diseases, eradication constraints and control measures have to be intensively studied. The aim of our study is to give an epidemiological approach of such important disease in such important animal species in such important border area of Egypt.

As the area of the study has geographic importance to be located on the Libyan border and there is uncontrolled movement between the two countries. Furthermore, political unrest in Libya increased the potential risk of transboundary diseases spreading into neighboring border countries especially Egypt, Tunisia, and Algeria; this is mainly driven by the disruption of public health services, insecurity, and massive displacement of refugees across the borders (Oueslati, 2012).

High percentage from camel population in Egypt is in the northwestern coast. Camels raising is nearly always coupled with keeping of sheep and goats and it was found that small ruminants was incriminated in the transmission of brucellosis to camels (Radwan *et al.,* 1995). Therefore, it is important to study the prevalence of the disease in camels coupled

with sheep and goats to get an integrated picture about the epidemiology of brucellosis in the area of the study. In addition and up to our knowledge, there is lack in researches on animal brucellosis in this area.

Several national and international publications on serological investigations and on typing studies of brucellosis from 1986 to 2013 to provide insight regarding brucellosis in Egypt over the last 27 years was studied by (Wareth *et al.,* 2014). They found that the serological investigations within the national surveillance program give indirect proof for the presence of brucellosis in cattle, buffaloes, sheep, and goats in 22 of 27governorates. Ismailia, Red Sea, North Sinai, South Sinai, and Matrouh did not report seropositive animals. The number of animals tested was always very low when compared to the total number of animal stocks in Egypt according to the Food and Agriculture Organization (FAO) so it cannot be excluded that sampling is biased. Therefore, they concluded that brucellosis is present in all governorates in cattle, buffaloes, goats, and sheep .but a comprehensive, evidence-based assessment of officially available data on animal brucellosis is missing for the northwestern coast of Egypt.

In this study, the overall seroprevalence of camel brucellosis in the northwestern coast of Egypt was (4.04%, 3.68 %) by RBPT and cELISA respectively. The highest prevalence was recorded by cELISA in El-Negela (8.16%) and the lowest prevalence was recorded in El-Hamam (0%).

These results are inagreement with that recorded by (Hosein *et al.,* 2016) who recorded an 4.17% and 3.73% overall seroprevalence of *Brucella* antibodies in camel sera from different localities in Egypt during 2014-2015, as detected by the RBPT and c-ELISA respectively.

The overall seroprevalence of sheep brucellosis recorded in this study was (13.50%, 23.21%) by RBPT and cELISA respectively, the highest prevalence was recorded by cELISA in Matrouh (47.51%) while, the lowest prevalence was recorded in El-Hammam (5.41%). A lower seroprevalence of sheep brucellosis was recorded in Matrouh (11%) by Diab *et al.*, 2018, Kafr El Sheikh (12.2%) by (Hegazy *et al.,* 2011) and Alexandria (6%) (Hosein *et al.,* 2016). Lower seroprevalence of sheep brucellosis in countries other than Egypt were also recorded by (Ferede *et al.,* 2011; Horton *et al.,* 2014; Patel *et al.,* 2017; Rahman *et al.,* 2011; Tsehay *et al.,* 2014) by rates of 0.74%, 3.08%, 4% , 7% and 8.70 respectively. The differences in prevalence of brucellosis may be attributed to time and place of sampling in addition to people habits in reporting cases.

While our results were nearly similar to that found by (Mahboub *et al.,* 2013) and (Nagati & Hassan, 2016) by rates of, 18.09% and 16.4% respectively. On the contrary, higher prevalence of sheep brucellosis were recorded by (Al-Majali *et al.,* 2007) 33.1%; (Ahmed *et al.,* 2010) 24% ; (Kaoud *et al.,* 2010) 26.6%.

Our data revealed an overall seroprevalence of goat brucellosis in the northwestern coast of Egypt was (25.62%, 29.75%) by RBPT and cELISA respectively, the highest prevalence recorded by cELISA was in Matrouh (50%), while, the lowest prevalence was recorded in El-Hammam (0%). A lower seroprevalence of *Brucella* antibodies in goats (7 %) was reported in Alexandria Province in 2016 by (Haggag *et al.,* 2016).

Rates of seropositivity were higher in goats than in sheep , that agree with previous studies (Ahmed *et al.,* 2010; Arashdeep *et al.,* 2010) who reported seroprevalence rates in sheep and goats of 3.3 and 5.8%, respectively .That may be due to that goats are the classic and natural host of *B. melitensis* (Aparicio, 2013).

As mentioned above the area of the study located on the Libyan border. The uncontrolled movement between the two countries and political unrest in Libya increased the transboundary diseases spreading into Egypt, adding to this by taking animals history during sampling in our study, there were some animals brought from Libya, so brucellosis seroprevalence in Libya must be kept in mind when studding animals brucellosis in the northwestern coast of Egypt. Brucellosis seroprevalence in Libya was 24% in sheep, 31% in goats and 14% in camels (Ahmed *et al.,* 2010).

Relatively high prevalence in the area of study (3.68% in camels, 23.21% in sheep and 29.75% in goats by cELISA) may be due to husbandry methods, faulty management practices adopted

by farmers in this region as large-scale animal grazing, sharing the same pasture with different species, which contributes to easy spread of infection and a higher population density of livestock. Also, lack of researches and illegal animal movement, communal clashes, unreported outbreaks, poor vaccination coverage, absence of brucellosis control programme in this region all of this may have contributed to the establishment and maintenance of brucellosis and increasing the prevalence.

Regarding to the sex of the animals examined in this study nearly similar prevalence was observed in males and female, while, males had slightly higher prevalence rate than females, as 16.2% of the males examined were positive using cELISA, but 16% of the females examined were positive using cELISA. These results agreed with that of (Okoh, 1979) who found that 3 out of 232 camels were positive reactors to *brucella*, all of these 3 camels were males with an incidence of 1.5%, these results were also in agreement with (Damir *et al.*, 1984) who reported an incidence of 5.6% in males and 4.5% in females. This study results also agreed with previous study by (Radwan *et al.*, 1992) who suggest a similar susceptibility to brucellosis among male and female camels of different age groups

On the other hand, the obtained results were in contrast with that reported in Egypt by El-Nahas (1964) (2% in males and 4% in females), Ayoub et al. (1978) (14% in males and 25% in females), Ahmed et al. (1999) (9.2% in males and 13.7% in females). The higher prevalence in females than in male were also observed in numerous previous studies (Blasco *et al.*, 1994; Haggag *et al.*, 2016; Hosein *et al.*, 2016; Musa & Shigidi, 2001; Omer *et al.*, 2010).

The nearly similar prevalence rate in males and females in the current study may be attributed to the similar management between males and females as reported by (Al-Rawahi, 2015) who also concluded that the seroprevalence of brucellosis was not affected by the gender of the sampled animals. Also may be due to the continuous movement of males either during grazing or during the trading activities or during mating time, which make males more susceptible to the infection.

In this study, young animals (sheep and goats from 5 months to one year, young camels from 2-5 years) were more likely to test positive than adult animals where prevalence rate in young animals was 18.5% and 15.9% in adult animals, which disagreed with previous studies (Ahmed & Munir, 1995; Alrawahi *et al.*, 2019; Amin *et al.*, 2005). Also, disagreed with (Arashdeep *et al.*, 2010) who mentioned that the seroprevalence was highest in goats aged 1-2 years, followed by those aged more than 2 years and those less than one year (8.9, 4.8 and 2.4%, respectively). While Radwan et al. (1992) suggest a similar susceptibility to brucellosis among male and female camels of different age groups due to similar susceptibility between young and adult to brucellosis. The higher prevalence rate recorded in this study in young animals than adults may be attributed to smaller sample size of young animals in comparison with adults.

Regarding to the relation between brucellosis and the previous reproductive problems (abortion and fertility status) prevalence of brucellosis in aborted animals was 18%. While prevalence of brucellosis in non-aborted animals was 15.5% by cELISA. A lower prevalence of *Brucella* antibodies in aborted animals was detected by (Rahman *et al.*, 2006) who detected 15% by RBT and 10% by tube agglutination test (TAT) and (Ibrahim & Habiballa, 1975) who reported a 14.2 % prevalence of brucellosis in cows that had previously aborted. On the other hand a higher seroprevalence was founded by (Kumar *et al.*, 2005) who detected prevalence of 33.87% in animals with a history of abortion,11.63% in those without such a history. The relation between brucellosis and abortion was also previously described by (Muma *et al.*, 2007; Schelling *et al.*, 2003) who determined that cows infected with *Brucella* are three to four times more likely to abort than unexposed cows.

While our data revealed that 33% of animals with fertility problems (repeat breeders) having brucellosis, prevalence of brucellosis in animals with no fertility problems was 15.5% by cELISA. The prevalence was duplicated in animals with fertility problems than fertile animals. A lower prevalence of brucellosis in repeat breeding cases 1.45 % was reported by (Rahman *et al.*, 2006).

The high prevalence of *Brucella* seropositivity recorded in the northwestern coast of Egypt in the current study might suggests the need for

more researches, further examination, studying risk factor associated with infection, isolation of *Brucella* in this area and actions needed to be taken to control brucellosis in this region.

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