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Seroprevalence of Ovine and Caprine brucellosis and associated risk factors in Dakahlia and Damietta Governorates

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ABSTRACT

The study was carried out to get clear image about the seroprevalence and risk factors associated with occurrence of brucellosis in sheep and goats. Total 2325 serum samples were collected from sheep and goats having above 6 months of age during April, 2021 to April, 2022 and screened by Rose Bengal Plate Test (RBPT) and Buffered Acidified Plate Antigen Test (BAPAT) and were furtherly confirmed with CFT. Out of total 2325 sera samples, 278 (11.95%) were positive. The seroprevalence was found higher in Rahmani sheep followed by Assaf, Barki, Romaov and Texel breeds. Also, the seroprevalence was found higher in Boer goats followed by Shami goats. Moreover, the seroprevalence was higher in female than male by the two tests. In addition, the seroprevalence was higher found in adult age group than young age group; mixed flock than single flock; unorganized rearing practice (mobile flocks) than organized and with abortion history than without abortion history. So control programs should be applied.

INTRODUCTION

Brucellosis is a highly contagious zoonotic disease that causes significant financial harm

in sheep and goat herds due to abortion and fertility problems. In addition, it causes Malta fever in humans. Brucellosis is endemic in

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Egypt and continues to be an uncontrollable problem throughout the country (Hegazy et al. 2011).

The bacterium is transmitted by sexually adult animals with predilection of placenta and fetal fluid. Brucellosis is characterized by abortion, with excretion of the organisms in uterine discharge and in milk. In female animals, the bacteria are localized in the udder followed by excretion via milk and in male animals orchitis andepididymitis can lead to infertility (Seyyed-Gholizadeh, 2013). Various serological surveys of sheep and goat brucellosis revealed varying levels of infection in various governorates in Egypt (Abdel-Hamid et al. 2017; Eltholth et al., 2017; Wareth et al. 2014). Besides that, B. melitensis biovar 3 is the most prevalent cause of brucellosis in sheep and goats in Egypt (Abdel-Tawab et al. 2018; El-Diasty et al. 2021; El-Diasty et al. 2022; Hegazy et al. 2022; Hosein et al. **2021)**. The serological tests are the most useful epidemiological tool for diagnosis of brucellosis in animals and humans (Chothe and Sakena, 2011). Currently, rapid, simple and low-cost assays as RBPT and BAPAT were very useful as a screening assays for detection of Brucellosis (El-Kholy 2007, Sareyyupoglu et al. 2010). Furthermore, Complement Fixation Test (CFT) can be used as a confirmatory test for diagnosis of brucellosis (Ashraf et al. 2014).

Sheep holder increased in Egypt day by day preferring the rearing of sheep all over the country due to their ability to graze on a large scale and their need for little care (FAOSTAT, 2018). In the same line sheep is considered a great threat to the other animal population and human as a source of brucellosis due to the sheep holder and the rearing system to rear sheep in close contact with other animal species, (El-Diasty et al. 2021).

Hence, the goal of the current work was to determine seroprevalence of brucellosis in sheep and goats by using serological tests as RBPT, BABAT and confirmed with CFT. In addition to assess the risk factors contributed to brucellosis.

MATERIALS and METHODS

Study area:

The assessment of seroprevalence and risk factors associated with ovine and caprine brucellosis was conducted in different localities in Dakahlia (Belqas, Shirbin, and Mansoura) and Damietta (Damietta, Faraskour, and Kafr Saad) governorates. These governorates are characterized by agricultural activities and a high density of animal population and about 60% of the inhabitants who live in rural communities are in close contact with their livestock.

Study periods:

The study was conducted during April 2021 to April 2022.

Animals used for the epidemiological study:

A total of 2325 animals (1670 sheep and 655 goats) of both sexes with different breeds (Rahmani, Assaf, Barki, Romanov, Texel, Boer, and Shami), different ages, and different rearing systems (organized and mobile flock) were used in this study. They were collected from different localities in Dakahlia and Damietta Governorates. These animals haven't previously been vaccinated against brucellosis.

Serum sample collection:

Total 2325 serum samples of sheep and goats were collected from different localities in Dakahlia and Damietta Governorates. The information regarding risk factors of brucellosis was noted in previously decided questionnaire. The risk factors like breed, sex, age, flock type, animal rearing practice and abortion history were recorded. Age of animals was categorized into < 1, 1, -2, 2, -4, and >4 years; flock type into single or mixed rearing (more than one species kept together) and animal rearing practice into organized and unorganized farms (mobile farms). Whole blood was collected from the jugular vein of each animal and then the blood was stored at room temperature over night until the serum was separated. The sera were stored at -20°C in the laboratory until tested by RBPT and BABAT as a screening tests for the presence of Brucella antibodies and the positive samples confirmed with CFT.

Serum examination:

The RBPT and BAPAT both of them were done according to the protocol described by **Alton et al. 1988.** The RBPT and BAPAT antigen purchased from the Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo 11517, Egypt.

The positive serum samples by RBPT and BAPAT were retested using CFT. The test antigen obtained from veterinary serum and vaccine research institute Abbasia, Egypt, and the CFT was done according to **Alton et al.,(1988)**.

RESULTS

1. Seroprevalence of brucellosis in sheep and goats at Dakahlia and Damietta Governorates:

As shown in **Table (1)**, the results cleared that the overall seroprevalence of brucellosis among the examined sheep and goat flocks was (11.95%) using RBPT and BAPAT and further confirmed with CFT. Briefly, the total seroprevalence of brucellosis among the examined sheep was (12.75%). On the other hand, the total seroprevalence of brucellosis among the examined goats was slightly lower (9.9%).

Table 1. Seroprevalence of brucellosis in the examined sheep and goats at Dakahlia and Damietta Governorates

Animal species	Total No. of examined animals	No. of positive samples	Prevalence (%)
Sheep	1670	213	12.75%
Goats	655	65	9.9%
Total	2325	278	11.95%

2. Risk factors associated with brucellosis: The effect of breed on the prevalence of

brucellosis in sheep and goats:

On studying the seroprevalence of brucellosis at the breed level in sheep (Table 2, Plate 1 and 2), the results showed that the prevalence of brucellosis was higher in Rahmani sheep (16.15%), followed by Assaf at (12.07%), Barki at (10.77%), Romanov at (7.65%), and Texel (0%). On the other side in goats, the prevalence of brucellosis was higher in Boer (13.5%) followed by Shami (4.31%).

Table 2. Seroprevalence of brucellosis at the breed level in the examined sheep and goats.

Species	Animal breed	Total no. of serum samples	No. of positive samples	Prevalence (%)
Sheep	Rahmani	780	126	16.15%
-	Assaf	265	32	12.07%
	Barki	390	42	10.77%
	Romanov	170	13	7.65%
	Texel	65	-	0%
Goats	Boer	400	54	13.5%
	Shami	255	11	4.31%





Assaf Sheep

Barki Sheep



Romanov Sheep

Plate (1): Showing different breeds of sheep (Rahmani, Assaf, Barki, and Romanov).



Texel sheep







Shami goats

Plate (2): Showing different breeds of sheep and goats (Texel sheep, Boer goats, and Shami goats).

The effect of locality on the prevalence of brucellosis in sheep and goats:

The seroprevalence of brucellosis in sheep and goats varies between different localities, **Table** (3). At the Dakahlia Government, the results showed that the prevalence of brucellosis in sheep was higher in Shirbin city at (14.63%), followed by Belqas at (13.55%), and Mansoura at (12.38%). On the other side, the seroprevalence of brucellosis in goats was higher in Mansoura at (28%), while in Shirbin and Belqas the prevalence was at (8.28% and 6.88%). At Damietta government, the results showed that the prevalence of brucellosis in

sheep was higher in Damietta city (13.39%), followed by Faraskour (8.57%), and Kafr Saad (3.40%). On the other side, the seroprevalence of brucellosis in goats was higher in Damietta city 18.88%, followed by Faraskour (15.55%), and in Kafr Saad, there were no positive reactors detected in goats.

Governorate	Species	Locality	Total no. of serum sam- ples	No. of posi- tive samples	Prevalence (%)
Dakahlia	Sheep	Belqas	1055	143	13.55%
Governorate		Shirbin	205	30	14.63%
		Mansoura	105	13	12.38%
		Total	1365	186	13.63%
	Goats	Belqas	305	21	6.88%
		Shirbin	157	13	8.28%
		Mansoura	25	7	28%
		Total	487	41	8.42%
Damietta	Sheep	Damietta	112	15	13.39%
Governorate		Faraskour	105	9	8.57%
		Kafr Saad	88	3	3.40%
		Total	305	27	8.85%
	Goats	Damietta	90	17	18.88%
		Faraskour	45	7	15.55%
		Kafr Saad	33	-	0%
		Total	655	24	3.66%

Table 3. Seroprevalence of brucellosis at the locality level in the examined sheep and goats.

The effect of age on the prevalence of brucellosis in sheep and goats:

As shown in **Table (4)**, in studying the relationship between age and brucellosis prevalence in sheep, the seroprevalence was higher in the 2- 4 year age group at (17.86%) followed by more than in the 4-year age group at (13.68%), also in 1 to 2 year age group the prevalence was (4.51%), and in below 1 year of age group was (3.75%). While seroprevalence of brucellosis in goats to be associated with age was low prevalence in the below 1 year of age group (1.85%), followed by the 1-2 year age group (6.92%), and in the 2-4 year age group, the prevalence was (12.8%). The

seroprevalence of brucellosis was noticed to be associated with age a low prevalence in young ages (less than 1 year) than the adults (more than 4 years of age). Our results are following **Sadhu** *et al.*, (2016) who found that seroprevalence of brucellosis in goats was higher in more than 4-year age group (13.71%) followed by in the 3 to 4-year age group (11.02%), 2 to 3 year age group (6.87%). This might be because sexually matured animals are susceptible to infection with brucellosis which increases with sexual maturity due to the effect of erythritol and sex hormones on the occurrence of the disease (Constable *et al.*, 2017).

Species	Age (year)	Total no. of serum sam- ples	No. of positive samples	Prevalence (%)
Sheep	<1	213	8	3.75%
1	1-2	332	15	4.51%
	2-4	862	154	17.86%
	>4	263	36	13.68%
	Total	1670	213	12.75%
Goats	<1	54	1	1.85%
	1-2	130	9	6.92%
	2-4	292	32	10.9%
	>4	179	23	12.8%
	Total	655	65	9.9%

Table 4. Seroprevalence of brucellosis at the age level in the examined sheep and goats.

The effect of flock type on the prevalence of

brucellosis in sheep and goats:

Diseased animals in the mixed-type herd would frequently increase the rate of contact with the rest of the healthy animals in flocks. This was clear in our study in **Table (5)**, as in sheep flocks seroprevalence of brucellosis in mixed type was higher (16.05%) than in single type (11.61%). Also in goat flocks, the seroprevalence of brucellosis in mixed type was higher (19.02%) than in single type (6.91%). The overall prevalence of brucellosis in mixed rearing was higher (16.86%) than in single type (10.27%).

Table 5. Flock type and their association with the occurrence of brucellosis in the examined sheep and goats

Species	Type Of flock	Total no. of serum samples	No. of positive samples	Prevalence (%)
Sheep	Mixed rearing	430	69	16.05%
	Single rearing	1240	144	11.61%
Goats	Mixed rearing	163	31	19.02%
	Single rearing	492	34	6.91%
Total	Mixed rearing	593	100	16.86%
	Single rearing	1732	178	10.27%

The effect of animal rearing practice on the prevalence of brucellosis in sheep and goats:

In the unorganized sheep and goat flocks (mobile flocks), the seroprevalence of brucellosis was high at (19.02%) and (14.16%) in sheep and goat flocks than in organized farm flocks at (0%). The overall prevalence of brucellosis in mobile farms was greater (17.60%) than in organized farms (0%), **Table (6)**.

, proper removal of aborted matters, and disposal of foci of infection could increase the spread of infection in unorganized mobile herds compared to organized ones.

Type of Farming	Total no. of serum samples	No. of positive samples	Prevalence (%)
Organized farm	550	-	0%
Mobile farming	1120	213	19.02%
Organized farm	196	-	0%
Mobile farming	459	65	14.16%
Organized farm	746	-	0%
Mobile farming	1579	278	17.60%

Table 6. Animal rearing practice and their association with the occurrence of brucellosis in the examined sheep and goats.

The effect of abortion history on the prevalence of brucellosis in sheep and goats:

The occurrence of brucellosis was relatively associated with abortion (**Table 7, Figure 1**), in sheep, the seroprevalence of brucellosis was higher with an abortion history at (80.61%) than without an abortion history at (8.87%). While in goats the seroprevalence was higher in abortion history at (72.41%) than without abortion history at (5.41%).



Figure 1 showing aborted lamb.

Table 7. Abortion history and their association with the occurrence of brucellosis in the examined sheep and goats.

Species	Abortion history	No. of serum samples	No. of positive samples	Prevalence (%)
Sheep	Yes	98	79	80.61%
	No	1296	115	8.87%
Goats	Yes	29	21	72.41%
	No	536	29	5.41%
Total	Yes	127	100	78.74%
	No	1832	144	7.86%

The effect of sex on the prevalence of brucellosis in sheep and goats:

In studying the occurrence of brucellosis and its association with the sex of animals (**Table 8**), in sheep flocks, the seroprevalence was higher in female sheep (13.91%) than in males (6.88%). While in goats the seroprevalence was higher in male goats (16.67%) than in females (8.85%).

Table 8.	Sex and	their ass	sociation	with the	occurrence	of bruce	ellosis	in th	ne examined	sheep	and g	goats.
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Species	Sex	Total no. of serum samples	No. of positive samples	Prevalence (%)
Sheep	Male	276	19	6.88%
	Female	1394	194	13.91%
	Total	1670	213	12.75%
Goats	Male	90	15	16.67%
	Female	565	50	8.85%
	Total	655	65	9.9%
Total	Male	366	34	9.28%
	Female	1959	244	12.6%
	Total	2325	278	11.96%

DISCUSSION:

The present study aimed mainly to examine the serological prevalence of brucellosis in sheep and goat flocks in different localities in Dakahlia and Damietta governorates using RBPT and BAPAT and further confirmed with CFT. In sheep and goats, the difference in the prevalence of brucellosis may be attributed to the geographic distribution, farming practices, and the susceptible age of sheep and goats in the region. Environmental conditions and management practices also suggestively affect the incidence of infection (El-Diasty et al., 2021). The total seroprevalence of brucellosis among the examined sheep and goats was (12.75 and (9.9%), respectively. The current findings are in agreement with (Adam et al., 2018) who tested 426 goat serum in El-Gedarif state, Eastern Sudan, and noticed that the overall seroprevalence of brucellosis was 10.8%. Also, Kosgei et al., (2014) found that 8.23% (6/73) of the sheep and goats serum samples, in the Baringo county government in Keyna, were positive for Brucella antigens. In contrast, Diab et al., (2020) examined serum samples collected from 20 sheep and 18 goats at different localities in Egypt, the prevalence was 5/20 in sheep (25%), and 9/18 in goats (50%). In Qalyobia Governorate, serological examination of 610 sheep and 460 goats for brucellosis showed that the seroprevalence of brucellosis was (9.5% and 7.8%) in sheep and goats, respectively (Lobna, 2006). In Sharkia Governorate, the incidence of Brucellosis in sheep was (11.54%) and in goats was (4.08%). B. melitensis biovar 3 was isolated from milk samples and tissue specimens of 17 ewes seropositive to brucellosis (Saleh et al., 2006). In Dakahlia and Damietta Governorates, the total incidence of Brucella infections among ewes was (6.98%) (Hashem et al., 2020). Also, the occurrence of other sources of infection on the farm, such as carriers such as dogs and cats (Wareth et al., 2017), the occurrence of latent infections (El-Diasty et al., 2018), and the survival of the organisms in the pasture for long periods affect the prevalence of brucellosis.

Brucellosis is not breed-specific (Ajogi *et al.*, 2002). The seroprevalence of brucellosis at the breed level in sheep was higher in Rahmani sheep (16.15%), followed by Assaf at (12.07%), Barki at (10.77%), Romanov at (7.65%), and Texel (0%). On the other side in goats, the prevalence of brucellosis was higher in Boer (13.5%) followed by Shami (4.31%). Thus, these differences may be due to the sample size differences among different breeds.

The seroprevalence of brucellosis in sheep and goats varies between different localities. This discrepancy in the prevalence in different regions is mainly due to environmental causes, management systems, the entrance of new animals without routine serological testing, and the absence of proper detection and removal of animals with high abortion rates, stress which may increase susceptibility to infection, time of periodical investigation and lack of knowledge of the owner (Hamidullah *et al.*, 2009).

On studying the relationship between age and brucellosis prevalence in sheep, the seroprevalence was higher in the 2-4 year age group at (17.86%) followed by more than in the 4-year age group at (13.68%), also in 1 to 2 year age group the prevalence was (4.51%), and in below 1 year of age group was (3.75%). While seroprevalence of brucellosis in goats to be associated with age was low prevalence in the below 1 year of age group (1.85%), followed by the 1- 2 year age group (6.92%), and in the 2-4 year age group (10.9%), also in more than 4 year age group, the prevalence was (12.8%). The seroprevalence of brucellosis was noticed to be associated with age a low prevalence in young ages (less than 1 year) than the adults (more than 4 years of age). Our results are following Sadhu et al., (2016) who found that seroprevalence of brucellosis in goats was higher in more than 4-year age group (13.71%) followed by in the 3 to 4-year age group (11.02%), 2 to 3 year age group (6.87%). This might be because sexually matured animals are susceptible to infection with brucellosis which increases with sexual maturity due to the effect of erythritol and sex hormones on the occurrence of the disease (Constable et al., 2017).

Diseased animals in the mixed-type herd would frequently increase the rate of contact with the rest of the healthy animals in flocks. So in sheep flocks, the seroprevalence of brucellosis in mixed type (**Table 5**) was higher than in single type. Also in goat flocks, the seroprevalence of brucellosis in mixed type was higher than in single type. The overall prevalence of brucellosis in mixed rearing was higher than in single type. The findings of the present study were following the results of Asmare *et al.*, (2013) who recorded a greater prevalence of brucellosis in cattle, sheep, and camels that were kept together with goats. Moreover, Megersa *et al.*, (2011) found that the prevalence of seropositive reactors was higher in large and mixed herds. This could be due to the raising of sheep and goats together in a mixed-type flock.

In this study, unorganized mobile flocks had higher seroprevalence compared to organized flocks (Table 6). That was following Sadhu *et al.*, (2016) who reported that seroprevalence was high in the unorganized flocks (mobile flock) at 8.03% than in organized farm flock at 0%. These variations might be due to differences in management and production protocols. Also, repeated mixing of herds, imperfect separation of diseased herds, and lack of hygiene measures including the use of isolated lambing areas, proper removal of aborted matters, and disposal of foci of infection could increase the spread of infection in unorganized mobile herds compared to organized ones.

The occurrence of brucellosis was relatively associated with abortion (**Table 7**). in sheep, the seroprevalence of brucellosis was higher with an abortion history than without an abortion history. While in goats the seroprevalence was higher in abortion history than without abortion history. This result was in agreement with the findings of **Rahman** *et al.*, (2011), and **Tesfaye** *et al.*, (2012) who recorded a substantial correlation between the abortion rate and the incidence of brucellosis in goats and sheep.

On studying the occurrence of brucellosis and its association with the sex of animals (**Table 8**). In sheep flocks, the seroprevalence was higher in female than in males. While in goats the seroprevalence was higher in male than in females. Brucellosis is not known as sex-specific (Ajogi *et al.*, 2002), but the detection of slightly high seroprevalence in females than in males may be due to the presence of suitable factors such as sex hormones and erythritol which aids in the growth of *Brucella* organisms. Erythritol is a sugar manufactured and localized in the placenta and indorses the growth of virulent strains of *Brucella* organisms (Smith et al., 1962).

CONCLUSION

This study was carried out in Dakahlia and Damietta govrnorats, Egypt. This study showed that brucellosis exists in sheep and goats of this area in an endemic mannar and may serve as source of brucella infection to human being either by consumption of raw milk and meat or by improper handling of placenta and aborted fetus. Brucellosis causes the high economic losses to livestock sector due to loss of animal protein and also serves as hazard to human health. So that, timely awareness programme should be facilitated. Adaptation of proper prevention and control strategies are also needed. The use of Rev-1 vaccine to protect small ruminants against brucellosis and to protect females from abortion would decrease the prevalence in regions where the disease occurs. There are several risk factors like age, flock type, animal rearing practice, abortion history are related with occurrence of brucellosis.

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Conflict of Interest:

The authors have no conflict of interest to declare.

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