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# Serological and Molecular Epidemiological Study on Brucellosis in Camels and Human in Matrouh Province

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## ABSTRACT

A cross-sectional study was carried out in Matrouh Province. A total of 100 camels and 100 human serum samples were examined serologically for brucellosis using RBPT, BAPAT, and CFT. In addition, multiplex PCR was carried out as further confirmation. The overall prevalence of brucellosis in camels by RBPT, BAPAT, CFT, and PCR were 10, 10, 9 and 9%, respectively while they were 17, 15, 14 and 13% for human samples with non-significant statistical association between them. Molecular characterization of seropositive samples of camels as tested by multiplex PCR clarified that B. abortus was the highest detected Brucella spp. while B. melitensis was the highest detected in humans. Females and older camels were more affected and the highest prevalence rate was observed during winter season. Concerning humans, the results of testing were 17, 15, 14, and 13%, respectively. It was noticed that males were more seropositive than females and age group 20 - 40 years appeared to be the most group at the risk than younger or/and older ones. On studying the effect of locality, it was clear that the highest seroprevalence was recorded in Sallum (26.67%). Finally, winter season showed the highest seasonal prevalence of human brucellosis. Conclusively, brucellosis is alarming in Matrouh Province so there was an urgent need for implementing a proper control program for brucellosis in camels and more attention should be paid towards improving the animal health delivery system in those Provinces that are large in size and share borders with other countries. Keywords: Brucellosis, camels, human, serology, PCR

## 1. Introduction

Old world camels are even-toed ungulates belonging to the genus camelus which distinguishes two species: the two-humped Bactrian camel (Camelus bactrianus) and the one-humped Arabian camel (Camelus dromedarius), also known as dromedary (henceforth referred to as 'camel') (Schwartz and Dioli, 1992). In many developing countries of Asia and Africa, camels are the most important source of income for the nomadic population. Both species are not only kept as working animals, but also as providers of milk, meat, wool, leather and fuel. With increasing urbanization, camel milk, a major component of the diet in many pastoralist societies, has gained a wider market and commercialization and consumption of camel milk is on the rise (Farah et al., 2007).

Brucellosis was reported in camels as early as 1931 (Solonitsuin, 1949). Camels are not known to be primary hosts of Brucella, but they are susceptible to both B. abortus and B. melitensis. In addition, brucellosis in camels is an insidious disease, since it hardly provokes any clinical signs, and may furthermore cause problems in the laboratory due to the lack of sufficiently validated tests (Cooper, 1991).

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Brucella spp. can enter the body through the lungs, the digestive tract, mucous membranes, and intact skin. Once in the blood stream, the organism disseminates to multiple organs, thereby displaying an affinity for reticuloendothelial tissues, such as liver, spleen, the skeletal and hematopoietic system (Greenfield et al., 2002).

The clinical picture of brucellosis in camels can vary from asymptomatic to abortion. Dams can develop ovario-bursal adhesions, hydrobursitis, and granulomatous endometritis. Placental retention, infertility, and delayed sexual maturity have also been reported. Males may suffer from orchitis and arthritis accompanied by acute lameness (Musa et al., 2008).

Brucellosis is most likely one of the oldest recognized diseases of mankind and under control in most developed countries, the containment of this zoonosis has been ignored elsewhere as it mostly affects the poor (Musa, and Shigidi, 2001).

Although asymptomatic infections regularly occur in humans, this multisystemic disease frequently presents with a wide range of symptoms. It usually begins as an acute febrile disease with nonspecific flu-like symptoms, such as fever, headache, malaise, back pain, myalgia, drenching night sweats, and generalized ache. Undulant fever may develop which can last up to 12 months. Neurological signs can occur in up to 5% of cases. Fatalities can be observed due to the development of endocarditis (Sprague et al., 2012). So the aims of this study were detection of Brucella spp. by multiplex PCR technique and serological tests (RBPT, BAPAT, and CFT) of Brucella spp. from camels as well as humans in Matrouh Province.

## 2. Material and methods

2.1. Study area and period:

The study was carried out in Matrouh Province for a period of 9 months from July 2019 to March 2020. The study population consisted of camels and humans. All samples were tested in the laboratory of Microbiology Department, Faculty of Veterinary Medicine, Matrouh University. 2.2. Samples:

A total of 100 camels and 100 human blood samples were randomly collected from different localities in Matrouh Province. About 5 ml of blood samples were collected aseptically in a sterile tube (2 ml in a plain tube for serological tests and 3 ml in a tube containing EDTA anticoagulant for molecular diagnosis) and the full history of each animal was recorded including sex, age, season and locality.

To obtain serum, samples were left for 30 minutes at room temperature for clotting then centrifuged at 3000 rpm /15 minutes then the clear serum was obtained by using sterile Pasteur pipettes then kept in Eppendorf tubes and labeled. All the serum samples were stored at -20°C until tested. 2.3. Serological testing:

1.Rose Bengal Plate Test (RBPT) was carried out according to Aldomy et al., (2009).

2.Buffered Acidified Plate Antigen Test (BAPAT) was carried out according to Farahat et al., (2019).

3.Complement Fixation Test (CFT) was carried out according to Wanger et al., (2017).

2.4. Multiplex PCR:

Positive RBPT samples were tested for further confirmation using a PCR assay that targeting the bcsp31 gene specific for genus Brucella, IS711 element downstream of the alkB gene specific for B. abortus, and the IS711 element downstream of BMEI1162 specific for B. melitensis (Probert et al., 2004).

2.4.1. DNA extraction:

Extraction of DNA from blood was carried out according to the technique recommended by O'Leary et al. (2006).

2.4.2. Oligonucleotide primers used for detection of Brucella:

The target gene sequences of the used primers and band sizes were tabulated in the following table;

Target gene	Oligonucleotide sequence $(5' \rightarrow 3')$	Band size (bp)
bcsp31, Brucella spp. (F) bcsp31, Brucella spp. (R)	5' GCTCGGTTGCCAATATCAATGC '3 5' GGGTAAAGCGTCGCCAGAAG '3	223 (Zerva et al., 2001)
BMEI1162 gene, B. melitensis (F) BMEI1162 gene, B. melitensis (R)	5' AACAAGCGGCACCCCTAAAA '3 5' CATGCGCTATGATCTGGTTACG '3	279 (Mutnal et al., 2007)
alkB gene, <i>B.abortus</i> (F) alkB gene, <i>B.abortus</i> (R)	5' GCGGCTTTTCTATCACGGTATTC '3 5' CATGCGCTATGATCTGGTTACG '3	495 (Song et al., 2019)

2.4.3. Cycling condition of PCR:

Description of cycling conditions was presented in the following table;

Steps	Temperature	Duration	No. of cycles
Initial PCR	95°C	3 min.	1 hold
activation step			
Denaturation	95°C	90 sec.	35 cycles
Primer annealing	65°C	1 min.	
Extension	72°C	2 min.	
Final extension	72°C	5 min.	1 hold
Cooling	Hold at 4°C till		
	further processing		

#### 2.5. Statistical analysis:

The statistical analysis was carried-out using the Chi2 test to study the significant differences in the detection rate of antibodies among different groups studied according to SPSS 16.0 according to Norusis (2008). A probability (p) value (P < 0.05) was considered statistically significant.

## 3. Results

Table (1): Seroprevalence of brucellosis in camels and human in Matrouh Province as examined by different serological tests and PCR

	RBP	Т	BAP	AT	CF	Г	PCI	R	Chi <sup>2</sup>
	Positi	%	Positi	%	Posit	%	Posit	%	value
	ve		ve		ive		ive		
Cam	10	10.	10	10.	9	9.0	9	9.0	X <sup>2</sup> =5.5
els		0		0					21
(n=1									p=0.13
00)									7NS
Hum	17	17.	15	15.	14	14.	13	13.	$X^2 =$
an		0		0		0		0	0.514
(n=1									p=
00)									0.9158
									NS

Table (2): Molecular characterization of seropositive samples of camels and human as tested by multiplex PCR in Matrouh Province

Brucella species	Camels (n=10)		Humans (n=17)	
	Positive	%	Positive	%
B. abortus only	7	70	4	23.53
B. melitensis only	1	10	8	47.1
B. abortus and B. melitensis	1	10	1	5.88
Total PCR results	9	90.0	13	76.47



Fig. (1): PCR products of bcsp31 gene (223 bp) specific for genus Brucella, IS711element downstream of BMEI1162 gene (279 bp) specific for B. melitensis, and IS711 element downstream of the alkB gene (495 bp) specific for B. abortus isolated from the whole blood samples of camels and human in Matrouh Province.

L: 50 bp molecular weight DNA ladder with a size range of 50-1500 bp

Lane  $1\square 9$ : positive for bcsp 31KDa gene specific for genus Brucella isolated from whole blood samples of camels and human.

Lane 1, 2, 3 and 7: Positive for BMEI1162 gene specific for B. melitensis strains isolated from whole blood samples of human.

Lane 4: Positive for BMEI1162 gene specific for B. melitensis strains isolated from whole blood samples of camel.

Lane 5: Positive for both BMEI1162 gene specific for B. melitensis strains and alkB gene specific for B. abortus strains isolated from the whole blood samples of camel. (Mixed infection)

Lane 6: Positive for both BMEI1162 gene specific for B. melitensis strains and alkB gene specific for B. abortus strains isolated from the whole blood samples of man. (Mixed infection)

Lane 8: Positive for alkB gene specific for B. abortus strains isolated from the whole blood samples of camel.

Lane 9: Positive for alkB gene specific for B. abortus strains isolated from the whole blood samples of camel.



Fig. (2): PCR products of bcsp31 gene (223 bp) specific for genus Brucella, IS711element downstream of BMEI1162 gene (279 bp) specific for B. melitensis, and IS711 element downstream of the alkB gene (495 bp) specific for B. abortus isolated from the whole blood samples of human in Matrouh province.

L: 50 bp molecular weight DNA ladder with a size range of 50-1500 bp

Lane  $1\overline{\ }9$ : positive for bcsp 31KDa gene specific for genus Brucella isolated from whole blood samples of human.

Lane  $1 \Box 5$ : Positive for BMEI1162 gene specific for B. melitensis strains isolated from whole blood samples of human.

Lane 6: Positive for both BMEI1162 gene specific for B. melitensis strains and alkB gene specific for B. abortus strains isolated from the whole blood samples of human. (Mixed infection)

Lane 7: Positive for bcsp gene specific for genus Brucella isolated from whole blood samples of human.

Lane 8 and 9: Positive for alkB gene specific for B. abortus strains isolated from the whole blood samples of human

Test	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	AUC
	(95% CI)	(93% CI)	(93% CI)		
BAPAT	88.4	100	10.17	89.83	0.942
	(77.89-	(99.1-100)	(7.92-	(87.1-	(0.898-
	94.51)		12.94)	92.1)	0.986)
CFT	81.16	100	9.33	90.67	0.906
	(69.58-	(99.1 – 100)	(7.18-	(87.98-	(0.851-
	89.2)		12.02)	92.8)	0.961)
PCR	75.36	100	8.67	91.33	0.877
	(63.26-	(99.1 - 100)	(6.61-	(88.72-	(0.815-
	84.60)		11.28)	93.4)	0.938)
PPV	: Positive	NPV: Positive predictable AUC: area		a under the	
predic	table value	value		curve	

Table (3): Diagnostic accuracy of BAPAT, CFT, and PCR in comparing with RBPT as a gold standard technique

Table (4): Risk factors associated with prevalence of brucellosis in camels according to results of RBPT

Sex	No. of samples	Positive	%	
Males	21	0	0.0	
Females	79	10	12.6	
Total	100	10	10.0	
Chi <sup>2</sup> value	$X^2 = 2.954$		p=0.086 NS	
Age groups (Years)	No.	Positive	%	
1- < 7	16	1	6.25	
7- < 14	50	5	10.00	
≥14	34	4	11.76	
Chi <sup>2</sup> value	X <sup>2</sup> =0.368 p=0.832NS			
Season of the year	No.	Positive	%	
Summer	0	0	0.0	
Autumn	75	7	9.33	
Winter	25	3	12.0	
Chi <sup>2</sup> value	$X^2 = 0.148$	p=0.700NS		
Locality	No.	Positive	%	
Marsa Matrouh	50	4	8.0	
El-Dabaa	50	6	12.0	
Chi <sup>2</sup> value	$X^2 = 0.444$	p=0.505NS		

NS= non-significant at P > 0.05

Table (5): Risk factors associated with prevalence of brucellosis in human according to results of RBPT

Gender	No.	Positive	%	Chi <sup>2</sup>
Males	66	13	19.70	X <sup>2</sup> =1.001
Females	34	4	11.76	p=0.317 NS
Total	100	17	17.0	
				~~ v?
Age groups (Years)	No.	Positive	%	Chi <sup>2</sup>
< 20	16	3	18.75	
20 - < 40	45	9	20	$X^2 = 0.829$
40 - < 60	30	4	13.33	p=0.843 NS
> 60	9	1	11.11	p=0.845 NS
Total	100	17	17.0	
Season	No.	Positive	%	Chi2
Summer	40	6	15	
Autumn	27	3	11.11	$X^2 = 2.004$
Winter	33	8	24.24	p=0.367 NS
Total	100	17	17.0	
Locality	No.	Positive	%	Chi <sup>2</sup>
Marsa Matrouh	31	6	19.35	$X^2 = 1.859$
El-Dabaa	16	2	12.5	x = 1.859 p=0.868 NS
El-Hamam	15	2	13.33	p=0.808 NS
Al-Negela	13	2	15.38	
Sidi-Barrani	10	1	10.0	
Sallum	15	4	26.67	
Total	100	17	17.0	

# 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. Diagnosis of brucellosis depends on direct diagnosis through isolation and identification of the causative microorganisms from infected animals showing abortion, stillbirth and retained placenta or indirect diagnosis through the using of serological tests.

The presented data in Table (1) showed that the overall prevalence of brucellosis in camels by RBPT, BAPAT, CFT, and PCR were 10, 10, 9 and 9%, respectively while they were 17, 15, 14 and 13% for human samples with non-significant statistical association between them. The result obtained by RBPT (10 %) was higher than that recorded by Hosein et al., (2016) (4.17%), Ebrahim et al., (2017) (2.2%), Bayasgalan et al., (2018) (2.3%) and Alrawahi et al., (2019) (0.4 %). On contrary, It was lower than that recorded by Al-Majali et al., (2008) (12.1%) and Dawood, (2008) (15.8%).

The recorded prevalence of human brucellosis according to the results of RBPT (17%) was near to that obtained by Nossair and Haggag, (2016) (14.67) and Tumwine et al., (2015) (17.0%). On the other hand, it was extremely higher than that recorded by Assenga et al., (2015) (0.6%); Elmonir et al., (2016) (1.25%) and it was higher than that recorded by Awah-Ndukum et al., (2018) (5.6%), Abdelbaset et al., (2018) (9.44%) and Ramadan et al., (2019) (6.3%); on contrary, it was lower than that recorded by Zolzaya et al., (2014) (27.3%) and Diab et al., (2018) (24.3%). Nevertheless, it was very low when compared with the result recorded by Madut et al., (2018) (33.3%) and Kairu-Wanyoike et al., (2019) (35.81%). This variation in the 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 (Alton et al., 1988).

Serological evidence of brucellosis in camels may throw the light upon the dangerous role in the continuous spreading of brucellosis to other livestock as well as a human being throughout the year in Matrouh Province so strict control measures must be followed to avoid risks attributed to rearing of camels. Although seroprevalence in the study area was not so high; nevertheless, appropriate brucellosis control and prevention methods should be implemented to circumvent the future potential for economic losses and the public health hazard of the disease.

The obtained results as shown in Table (2) clarified that the highest infection rate with B. abortus was observed in camels (70%). On the other hand, the highest infection rate with B. melitensis was observed in human (47.1%) followed by B. abortus (23.53) and finally the mixed infection with the two species (5.88%). This finding may be attributed to presence of large flocks of sheep and goat within Matrouh Province that could be considered a potential source of infection to both camels and human. Concerning camels, the finding agreed with that obtained by Patel et al., (2017) who observed that out of 15 genus specific positive samples, 12 samples amplified specific gene (IS711) of B. abortus within blood samples of cattle and camels and 3 samples amplified specific gene (omp31) of B. melitensis and Gwida et al., (2016) who found that 36.96 % of the tested samples gave positive by PCR where the only species identified was B. abortus. Concerning human, the finding disagreed with that obtained by Saddique et al., (2019) who found that Brucella genes were detected in 26 (5.8%) cases and all of them identified as B. abortus only and Rahman et al., (2020) who found that B. abortus specific gene was amplified from all of the four RBT positive human serum samples tested and no B. melitensis gene could be amplified from human blood samples.

The results obtained in Fig. (1 and 2) showed that amplification of target gene of Brucella genus (bcsp31 gene) yielding an amplicon size of 223 bp as examined by Zerva et al., (2001), while amplification of target gene of B. abortus (alkB gene) yielding an amplicon size of 495 as examined by Song et al., (2019), finally amplification of target gene of B. melitensis (BMEI1162 gene) yielding an amplicon size of 279 bp as examined by Mutnal et al., (2007). It was clear that PCR assay was a highly specific and low sensitive diagnostic method for the detection of Brucella in animals' blood samples. Similarly, Probert et al., (2004), Gwida et al., (2016), Saddique et al., (2019) and Saeed et al., (2019) used the same primer pairs for detection of Brucella by using bcsp31 gene specific for B. melitensis, and IS711 element downstream of the alkB gene specific for B. abortus.

The obtained results as shown in Table (3) showed that the sensitivity of BAPAT, CFT, and PCR in the diagnosis of brucellosis in camels and human was 88.4, 81.16, and 75.36 %, respectively, while the specificity of all tests was 100 % as compared with that of the RBPT as a gold standard. These results were nearly similar to that obtained by Hosein et al., (2017) who found that the relative sensitivity and the relative specificity of BAPAT, RBPT, and CFT were (98.04% and 76.92%), (94.33% and 85.71%), and (93.46% and 88.23%), respectively.

The effects of some risk factors including; sex, age, season of the year and locality associated with prevalence of brucellosis in camels according to results of RBPT were presented in Table (4).

Sex-based seroprevalence of brucellosis in camels revealed that the prevalence in females was 12.66% while all samples of males were found to be negative. Chi-square analysis of the obtained result showed a non-significant relationship (Chi2 value =2.95, P > 0.05) between sex and the prevalence of brucellosis in camels. This result was in agreement with Al-Majali et al., (2008) and Alrawahi et al., (2019) who found that sex-related seroprevalence of brucellosis was higher in females than males. The higher rate of infection in the females may be due to infection within the female reproductive tract providing a potential reservoir for the organism to propagate.

Age-based seroprevalence of brucellosis camels revealed that the highest seroprevalence was observed in the age group ( $\geq$ 14 years) (11.76%) followed by the age group (7- <14 years) (10.0%) and finally, the age group (1- <7 years) (6.25%) and statistical analysis showed non-significant association (Chi2 value = 0.368, P > 0.05) between age and the prevalence of brucellosis. This result was in harmony with Dawood, (2008) who noticed that about 64.8% of the positive camels were adult over than 4 years old and the remaining 35.2% were young ranging from 6 months to 4 years old and Alrawahi et al., (2019) who noticed that seroprevalence was higher in adults (>4 years of age) as compared with young ( $\leq$ 4 years of age) camels.

The higher rate of infection in the older camels will be due to their advanced age, as the organism may remain latent or chronic for an unspecified period before manifesting as clini–cal disease. Alternatively, the aged animals have a greater chance of becoming infected and of coming into contact with other animals. On the other words, the susceptibility of animals is increased after sexual maturity because sex hormones and erythritol stimulate the growth of Brucella organisms. Younger animals tend to be more resistant to Brucella infections; however, latent infections can occur in these animals (Gul et al., 2013).

Concerning the season of the year in Matrouh Province, it was noticed that the highest seroprevalence of brucellosis in camels was in winter (12.5%) followed by autumn (9.33%). Statistical analysis showed a non-significant association between the seroprevalence of brucellosis and season. These results were in agreement with Megersa et al., (2011) and Diab et al., (2018) who found that the higher prevalence was observed in the winter season. On contrary, it disagreed was Haggag et al., (2016) who recorded that the highest seasonal incidence occurring in the synthesis season due to the effect of moderate atmospheric temperature that permits the survival of Brucella organisms in the environment. The increased prevalence during the winter season in Matrouh Province because it is rainy season that increase the droplet infection between animals as one of the common routes of the disease transmission.

Seroprevalence of brucellosis in camels according to locality clarified that the highest prevalence was observed in El-Dabaa (12 %) followed by Marsa Matrouh (8 %). Statistical analysis showed a non-significant association between the seroprevalence of brucellosis in camels and locality. These results disagreed with that obtained by Diab et al., (2018) who recorded a significant (P<0.01) relationship between locality and prevalence of brucellosis in Matrouh Province.

It was clear that the prevalence of brucellosis was higher in El-Dabaa that are nomadic areas in Matrouh Province with a high population of sheep and goats that may be considered the source of infection to other livestock. Also, the social pattern of this area may explain the lack of awareness about the disease and its control strategy so great efforts are needed to be done by the official and governmental authorities to involve the population in any control strategy.

The effects of some risk factors including; sex, age, season of the year and locality associated with prevalence of brucellosis in humans according to results of RBPT were presented in Table (5).

Gender based seroprevalence of brucellosis among examined humans depending on the results of RBPT showed that males' prevalence (19.70%) was higher than that in females (11.76%) and statistical analysis showed a non-significant association at (P > 0.05) between gender and the prevalence of brucellosis. The higher prevalence in males agreed with

Chegeni et al., (2014) (males, 54.3% and females, 45.7%), Ghoneim et al., (2014) (males, 23.3% and females, 17.5%); Nossair and Haggag, (2016) (males, 16.67% and females, 12.12%) and Tumwine et al., (2015) and Rafiemanesh et al., (2019) who recorded that the prevalence was highest among males (20.5% and 60.5%, respectively). Also, Elmonir 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 Troy et al., (2005); Zolzaya et al., (2014) and Saddique et al., (2019) who found that more women than men were seropositive and Abdelbaset et al., (2018) who found that gender of the tested humans differed significantly in acquiring the infection.

The effect of age on the prevalence of human brucellosis pointed out that Chi-square analysis of the obtained result showed a non-significant relationship at (P > 0.05) between the different age groups. The highest prevalence was observed in the age group (20 - < 40 years) (20%) followed by the age group (< 20 years) (18.75%) then the age group (40 - < 60 years) (13.33%) and lastly the age group (> 60 years) (11.11%). The increased prevalence in the age group (20 - < 40 years) may be attributed to this age group represents the most active age of work. This result was nearly similar to that obtained by Nossair and Haggag, (2016) where 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 a non-significant association between different age groups and prevalence of human brucellosis.

It was in agreement with Salem et al., (2016), Ramadan et al., (2019) and Alkahtani et al., (2020) who reported that the higher prevalence of Brucella infection was recorded among the age group 21-45 years than younger or/and older ones and Nasinyama et al., (2014) who found that there was no association between seropositivity with age. In contrast, it disagreed with the results obtained by Tumwine et al., (2015) and Saddique et al., (2019) who noticed that the elderly - above 60 years (22.2 %) was the highest age group.

Concerning localities in Matrouh Province, the highest prevalence was noticed in Sallum (26.67%) followed by Marsa Matrouh (19.35%), Al-Negela (15.38%), El-Hamam (13.33%), El-Dabaa (12.5%), and finally Sidi-Barrani (10%). Statistical analysis (Chi2 value= 1.859) showed that there was a non-significant association at (P > 0.05) between the seroprevalence of brucellosis among examined human beings concerning the place of residence in Matrouh Province. This agreed with Diab et al., (2018) who found no significant association between place of residence and the prevalence with a higher percentage of infection found in the Siwa region (27.6%). In contrast, it disagreed with those recorded by Nossair and Haggag, (2016) where there was a significant association at (P < 0.0001) between the seroprevalence of brucellosis among human beings concerning the place of residence.

The seasonal distribution clarified increased rate in winter (24.24%) followed by summer (15%) and finally autumn (11.11%). Chi-square analysis of the obtained result showed a non-significant relationship between the season and the prevalence of human brucellosis. This finding agreed with that of camel brucellosis in the current work confirming the role of camels in the transmission of brucellosis in the region under investigation. Additionally, this result agreed with that obtained by Diab et al., (2018) who noticed that the highest infection rate occurred during the winter season (43.1%). On contrary, this result disagreed with Rafiemanesh et al., (2019) who discovered that the majority of brucellosis cases occurred in spring and Alkahtani et al., (2020) who found a higher prevalence in the summer season with a significant relationship between season of the year and the prevalence of human brucellosis.

## 5. Conclusion

The recorded results in the current study throw the light upon the role of camels in Matrouh Province, West Egypt in the epidemiology of brucellosis. Under the conditions in this study and according to the data obtained, it is concluded that brucellosis is still remaining a problem in farm animals in Matrouh Province.

## Conflict of interest statement

No conflicts of interest.

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