SVU- International Journal of Veterinary Sciences, 6(3): 33-47, 2023
Print ISSN: 2535-1826 **Online ISSN: 2535-1877**

Research Article Open Access

Seroprevalence and Molecular Detection of Brucella Species among Camels at Aswan governorate

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Abstract

Brucellosis is considered one of the highly contagious disease in animals and is zoonotic disease for human causing undulant fever. So, the objective of this study is to detect the seroprevalence of brucella among camel and detect the brucella species by PCR. A total of 200 serum samples were collected from camel in Aswan governorate and were subjected for mRBPT, CFT. The seropositive sample of mRBPT and some negative serum samples were subjected for PCR to detect Brucella species. the obtained results in this study show that 6% of serum samples were positive by mRBPT. while only 4 % of serum samples were positive by CFT. 9 out of 12 mRBPT positive serum sample were positive by PCR and the Brucella meltiness were detected in 6 out of 9 serum samples and the Brucella suis were detected in 3 serum samples out of 9 positive PCR serum samples.in conclusion detection of brucellosis in camel causing highly economic losses and causing public health hazards on human being.

Keywords: Brucellosis, PCR, Complement Fixation

DOI: 10.21608/svu.2023.208647.1271 Received: May 03, 2023 Accepted: July 24, 2023 Published: August 04, 2023
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Citation Shaban et al., Seroprevalence and Molecular Detection of Brucella Species among Camels at Aswan governorate. SVU-IJVS 2023, 6(3): 33-47.

Competing interest: The authors have declared that no competing interest exists.

Introduction

The lethal zoonotic disease brucellosis affects humans as well as all domestic animals, including camels. It is regarded as one of the world's most serious public health problems. **(Radostits et al.,2007).** A contagious illness called brucellosis is caused by the Brucella bacteria. Brucellae are aerobic, facultative intracellular coccobacilli that can penetrate epithelial cells, placental trophoblasts, dendritic cells, and macrophages. They are Gram-negative coccobacilli that are do not produce spores and are non-motile. **(Seifert 1992).** Farm animals that contract brucellosis can develop severe, persistent reproductive failure, including stillbirth, abortion, retained placenta, orchitis, and epididymitis in females and males, respectively. **(Radostitis., 2007).** Abortion is one of the most prevalent symptoms of disease in camels, however even if camel diseases are less symptomatic than those in cattle or even asymptomatic, they may nevertheless have an impact on camel reproductive performance. **(Gwida et al., 2012).** According to reports, some seropositive camels exhibited no clinical symptoms **(El-Diasty et al., 2022).** Due to the lack of clinical signs, camel brucellosis cannot be effectively diagnosed clinically until the third trimester of pregnancy, when abortion occurs **(Gwida et al., 2011; Salisu et al., 2017; Bayasgalan et al., 2018**). The illness, often referred to as "undulant fever" or "Malta fever" in humans, is a serious public health concern. Over 500,000 new instances of human brucellosis are recorded worldwide each year, making it one of the most prevalent zoonotic diseases **(FAO /WHO, 1986).** Researchers looked at how brucellosis spreads through either direct contact with camels or consumption of raw milk **(Al-Juboori and baker, 2012).** A high number of human brucellosis cases have been caused by the excessive consumption of raw milk and meat, which has resulted in a drought and transportation issues for rural areas. (**Schwartz and Dioli,1992**).

Cattle, sheep, goats, camels, and other species can transmit diseases to each other (**Ghanem et al., 2009).** Camels are susceptible to both *Brucella abortus* and *Brucella melitensis* despite not being known as the major hosts of Brucella microorganisms. (**Cooper, 1991; Seifert, 1996; Abbas and Agab, 2002; Gwida et al., 2012)**.

The bacteria concentrate in the placenta of pregnant camels and are most prevalent (up to 10^{13}) in abortion material such as the fetal gut, vaginal discharge, and colostrum. (**Millar and Stack, 2012**).

Camels are affected by any of the three major *Brucella* species, *Brucella abortus*, *Brucella melitensis* and *Brucella suis* (**Rutter and Mack, 1963 and Higgins, 1986**).So, transmission of the disease will depend on brucella species being prevalent in contact animals **(Musa et al., 2008).**

The ability to isolate the pathogen or identify the antibodies by a battery of serological tests with variable degrees of sensitivity and specificity is essential for the diagnosis of brucellosis in various animal species. The gold standard of diagnosis is, however, direct detection of this pathogen using bacterial isolation from contaminated materials, however this is challenging, less sensitive, tedious, and time-consuming because it takes many days to weeks for this pathogen to be properly identified **(Alton et al., 1988; OIE, 2016).** The reference standard tests for the diagnosis of brucellosis are considered bacterial culture and Brucella isolation **Hamdy and Amin,**

2002; Nielsen and Yu, 2010 However, Brucella is a fastidious bacteria that is challenging to cultivate and is frequently contaminated with other microbes, which reduces the sensitivity of the culture (**Matope et al., 2011)**. Furthermore, most developing countries lack the sophisticated laboratory infrastructure, technological know-how, and funds necessary for Brucella culture. (**Arif et al., 2018**).

Because they are affordable to execute in low-income countries, serological tests are therefore frequently employed to diagnose brucellosis **(Gwida et al., 2011; Pfukenyi et al., 2020).** Currently, a number of serological tests, including the Rose Bengal Test (RBT), Buffered Plate Antigen Test (BPAT), Complement Fixation Test (CFT), Slow Agglutination Test (SAT), indirect ELISA (i-ELISA), and competitive ELISA (c-ELISA), have been used to diagnose Brucella infection in camels **(Gwida et al., 2011; Hamdy et al., 2017).** Diagnostic qualities of serological tests for camels have seldom been reviewed, and most published research used standard test evaluation, which requires patients' genuine illness status to be known. (**Gwida et al., 2011; Elbehiry, 2014; Hamdy et al., 2017).**

Given that no test is perfect, all brucellosis serological assays have a degree of diagnostic error that varies with disease prevalence. (**Alton et al., 1975**).

The mRBT is a straightforward brucellosis diagnostic procedure that is commonly used for the serological diagnosis of sheep and goat brucellosis. The RBT is a low-cost, quick, easy, and efficient screening test that is used to screen individual animals and herds, as well as humans. This test was proven to be effective in diagnosing acute human brucellosis and is still used to diagnose chronic cases. Normally, results are obtained in a matter of minutes **(Khan et al., 2017; Teng et al., 2017).** Although quick and effective for screening, this test is not trustworthy for vaccinated animals because of its high sensitivity (**Smirnova et al., 2013).** To improve RBT sensitivity in small ruminants and reduce conflicts with CFT results, three volumes of serum and one volume of antigen, or approximately 75µl of serum and 25µ l of antigen, are employed, and the test is known as the modified m RBT. **(Blasco et al., 1994; Ferreira et al., 2003**).

The most used test for identifying antibodies in various animal species, including camels, is the complement fixation test. Because complement fixing antibodies persist in the blood for a longer period of time than SAT antibodies, CFT is thought to be the most sensitive and accurate test for brucellosis (**Tserendash and Shumilov, 1970; Waghela et al., 1978).** The CFT, which has a high level of specificity, is commonly used to establish Brucella infection in a variety of animal species, including camels. The confirmatory test CFT has been and continues to be used extensively in control and eradication procedures.

As a brucellosis diagnostic tool, polymerase chain reaction (PCR) technology is progressing and will soon be able to take the place of actual bacterial isolation. It is quick, safe, and economical. The PCR offers an additional method for finding and identifying Brucella species, including the real-time format **(Bricker and Halling, 1994; Bricker et al., 2002; Yu and Nielsen, 2010**).

So, the objective of this study is seroprevalence and molecular detection of brucella species from camel.

Material and methods

Population research

The study's source population includes camels imported from Sudan and quarantined at the Abu Simbel quarantine, which is located in Abu Simbel city, which is part of the Aswan governorate. It is located on the western bank of Lake Nasser, some 230 kilometers southwest of Aswan, near the Sudanese border, and samples were gathered from camels that dwell in Aswan (Daraw), both male and female. These imported camels are quarantined in the Abu-Simple area before being transported to slaughterhouses or animal markets in other Egyptian governorates. As indicated in table 1, we inquire about origin, herd size, sex, age, health state, and history of abortion, whether raised singly, with other animals, or in a camel herd.

Table (1): data information of camels used in this study

Total number of collected samples	Period of sample collection		Sex		Age	Health Status	History Of Abortion	Rearing	Origin
	From	To	Female	Male					
200	Jan 2021	feb 2022	30	170	From 4 To 15 years	All apparently health	No information about abortion	Females reared with different animals	Sudan

Sample collection

After the camel's owner has handled it properly and we have put on protective clothing to ensure our safety, Blood sera were taken from the jugular veins of (200) dromedaries without the use of an anticoagulant. About 10 ml of jugular-vein blood was aseptically collected in sterile silicon-coated vacuum tubes and kept in a slant position in the shade for about 2 hours for complete clotting before being labeled, identified, and transferred to the laboratory on ice packs as much as possible to avoid shaking. Samples were stored at 4° C overnight to allow serum separation, then centrifuged at 3000 r.p.m. for 10 minutes to extract amber clear serum. Sera were stored at -20° C in two aliquots in sterile bijou bottles until analyzed.

Serological examination of camel serum for diagnosis of brucellosis

Modified Rose Bengal Test (mRBT)

This test was carried out according to the protocol given by (**Blasco et al., 1994**), using 75 µL of sera and 25 µL of antigen mixed. Any agglutination that appeared within 4 minutes of shaking the plates was reported as a positive reaction.

Complement Fixation Test (CFT)

CFT was carried out exactly as described by **(Alton et al. 1988**). The CFT identifies anti-Brucella antibodies that can activate the complement system. The complement system is made up of a complicated set of proteins that, when activated by an antigen-antibody combination, react sequentially to produce cell lysis **(Hill, 1966)**. There are various

CFT versions in use, however this test is most easily performed in a micro titer format. Despite its complexity, the Complement fixation test (CFT) is still the confirmatory test for brucellosis used by various reference laboratories (**Sayour et al., 2015).**

PCR

DNA extracts of (16) Brucella serum samples (12 positives modified rose bangal plate test and 4 negatives for modified rose bangal plate test were examined by conventional PCR according to **(Bricker ,1994)**

DNA extraction.

The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used to extract DNA from serum samples, with changes from the manufacturer's recommendations. In brief, 200 µl of serum sample suspension was treated with 10µl of proteinase K and 200 µl of lysis solution for 10 minutes at 56ºC. Following incubation, the lysate was treated with 200 µl of 100% ethanol. The sample was then washed and centrifuged according to the manufacturer's instructions. The nucleic acid was eluted with 100 µl of the kit's elution buffer. Photometric analysis was used to assess the purity and concentration of DNA using a Nano Drop ND-1000 UV-Vis spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA).

PCR amplification

PCR amplification was performed using published primers, and gene identification was performed using a DNA thermal cycler (Thermo cycler, ASTEC, Japan). The primers used in this work are listed in the following table (2). the PCR assay was optimized in a 25µl reaction mixture containing 5 µl of DNA template, 12.5 µl of 2x master mix (Promega Go Taq Green Master Mix), 1 µl each of forward and reverse primers (10 pmol/l), and the remaining volume was made by adding nuclease free water. The following PCR temperature and time parameters were used: Primary denaturation at 94 ºC for 5 minutes, then 35 cycles of denaturation at 94 ˚C for 30 seconds, annealing at 55ºC for 30 seconds, and extension at 72 ºC for 30 seconds, followed by a final extension at 72 ºC for 10 minutes. PCR products were electrophoresed in 1.5% agarose gel and observed with a Gel Documentation unit (BIORAD, USA).

Table (2) : Oligonucleotide primers used in cPCR

Primers used were supplied from bio basic (Canada) and are listed in

Target gene	Target	Primers sequences	Amplified segment (bp)	Reference
1S711	*B. abortus*	1S711-specificPrimer TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT	498	Bricker and Halling, 199
		B. abortus-specific Primer GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC		
	B. melitensis	1S711-specificPrimer TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT	731	
		B. melitensis-specific Primer AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA		
	B. suis	1S711-specificPrimer	285	

		TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT		
		B. suis-specific Primer GCG-CGG-TTT-TCT-GAA-GGT-TCA-GG		

Results

During Jan 2021 to February 2022 a total of 200 apparently healthy dromedary camels (170 males and 30 females) were serologically examined using the modified Rose Bengal Plate Test (mRBPT), complement fixation test and PCR

The results of mRBTshowed that 12 out of 200 serum samples were positive, while complement fixation test showed that 8 out of 200 samples were positive as mentioned in table (3) and figure (1,2). The positive mRBPT sera in addition four negative sera were subjected for PCR for detection the species of Brucella. The results of PCR showed that 9 out of 12 were positive for Brucella species (6 *Brucella melitensis* and 3 *Brucella suis)*. While 4 samples negative mRBPT tested by PCR were Negative by PCR for any Brucella species as shown at table (4) and figure 2, 3, 5).

Table (3) : Seroprevalence of camel Brucellosis using modified Rose Bengal Plate test and complement fixation test

Number of blood serum samples	Positive for mRBPT	Positive for CFT
170 male	11(6.47%)	7(4.11 %)
30 female	1(3.33%)	1 (3.33%)
200 total	12(6%)	8 (4%)

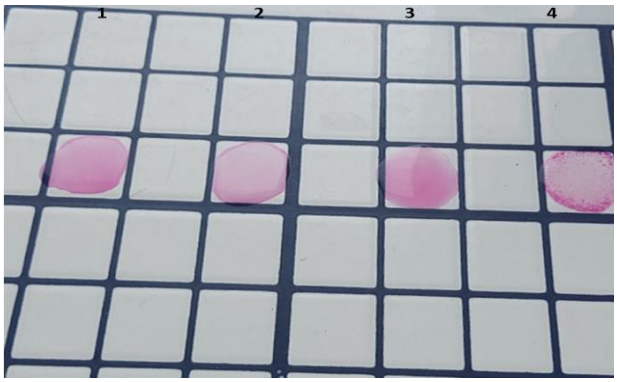

Fig. 1: The results of mRBPT

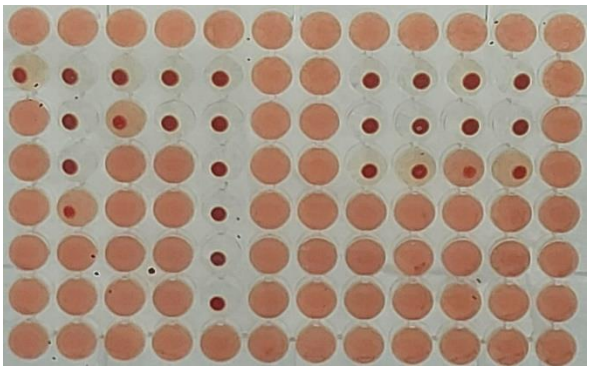

Fig. 2: The results of complement fixation test

Table (4): results of PCR of positive and negative rose bangle tested sera

No of tested sera samples for PCR	Results PCR (+ve)	
	Number	%
12 +ve mRBPT	9	75 %
4 - ve mRBPT	0	0%

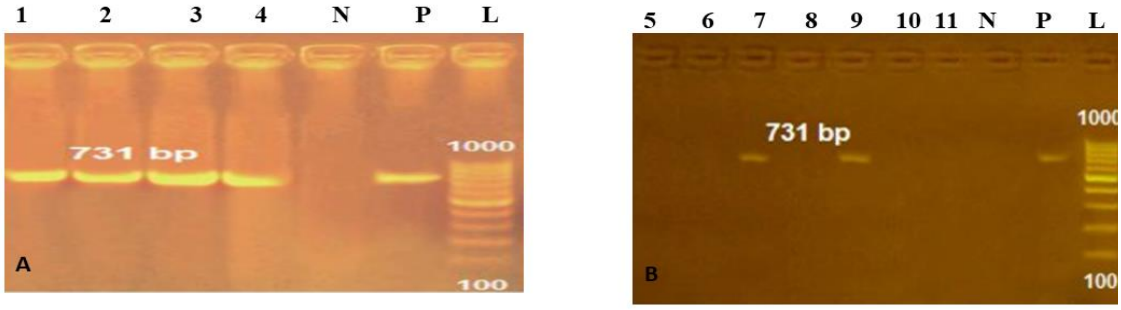

Fig. 3: Agarose gel electrophoresis of PCR of *B. melitensis*-specific Primer (731bp): Ladder: 100bp P: control positive, N: control negative Lane: 1-4 and 7,9 were positive and 5,6,8,10,11were negative.

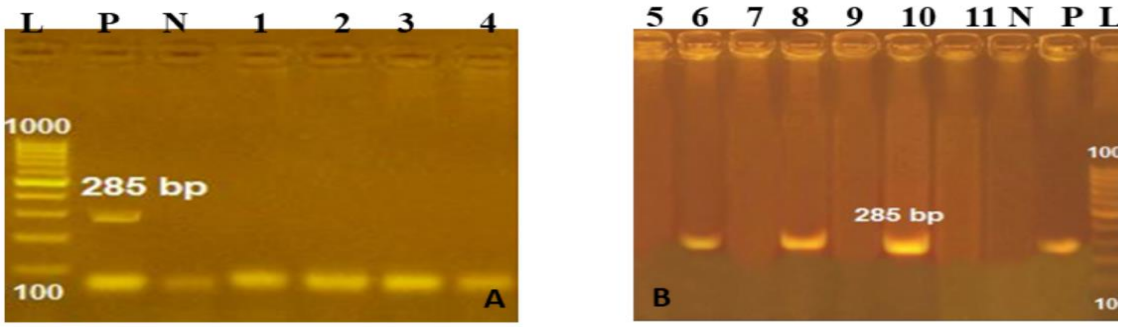

Fig. 4: Agarose gel electrophoresis of PCR of *B. suis*-specific Primer (285bp): Ladder: 100bp P: control positive, N: control negative Lane: 6,8,10 was positive and 1-5, 7,9,11 were negative

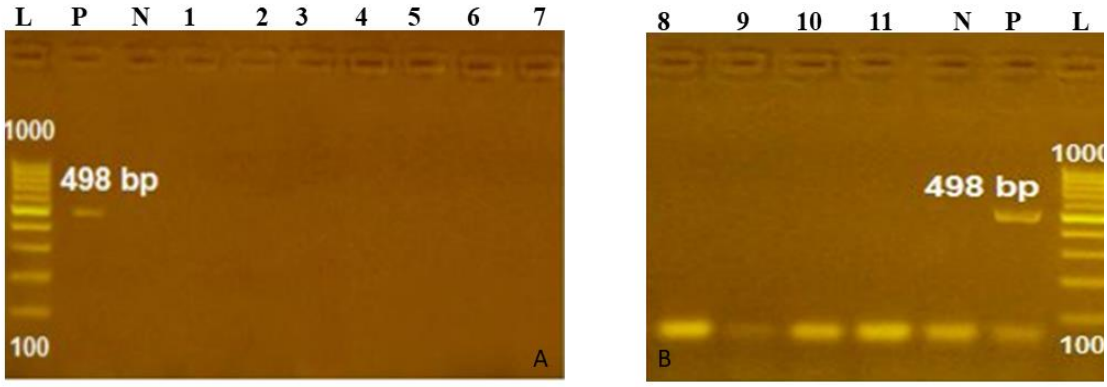

Fig. 5: Agarose gel electrophoresis of PCR of *B. abortus*-specific Primer (498bp) Ladder: 100bp P: control positive, N: control negative Lane: 1-11 were negative

Discussion

In most nations, Brucella infection in agricultural animals was seen as a serious problem. Early identification of Brucella infection in a herd or flock is thus essential for the effective management and eradication of one of the primary issues thought to be a contributing cause to infertility and sterility as well as the potential transmission of infection to humans (Wasseif, 1992).

Camels have been documented to have brucellosis in Saudi Arabia (Alshaikh et al., 2007), Kuwait (AL-Khalaf and EL-Khaladi, 1989), Jordan (Dawood, 2008), Yemen (AL-Shamahy, 1999), Iran (Ahmad and Nemat, 2007), Sudan (El-Ansary et al., 2001), Egypt (Abdel Moghney, 2004), Libya (EL-Boshy et al., 2009) and Somalia (Ghanem et al., 2009). Even in the United Arab Emirates, where camels compete in races, it has been reported (Moustafa et al., 1998).

Serological testing is still the gold standard for brucellosis diagnosis (Konstantinidis et al., 2007). For the purpose of brucellosis diagnosis, mRBPT was employed (Farina, 1985). A total of 200 dromedary camels from Aswan governorate were employed in the current investigation, and mRBPT, CFT, and PCR were used as screening and confirmatory assays for diagnosis of camel brucellosis and detection of spontaneously infected individuals.

All the camels in the investigation were apparently healthy at the time of sampling, and none had previously displayed any clinical symptoms of brucellosis, according to the owners. The findings of this work suggest that many sick camels may be silent brucellosis carriers, and their products may pose a major health risk to people. Our findings are corroborated by a study (Abu Damir et al., 1990).

In the current investigation, serum samples were subjected to a screening test (mRBT), which identified an overall prevalence of (6%). Using various assays in various locations, various publications have reported the seroprevalence of camel brucellosis in Egypt. The current results outperformed those obtained by (Hosein et al. ,2016) (4.17%) and (M. Yawoz et al. ,2012) (3.03%).

Our findings were less significant than those reported by (Abd El Tawab et al., 2018) (8.8%) , (El-Sawally et al., 1996) (11.3%), and (Sayed-Ahmed et al., 2017) (12.9%) The current findings are in agreement with those found in (Al-Gaabary and Mourad, 2004) (6.75%). According to (Ahmed et al.,2021), Aswan's mRBT findings were less favourable than those of Qena (18.75%), Sohage (15.8), Giza (24.4%), and El Fayoum (23.9%). The variations in seroprevalence noted by the previous researchers could be explained by variations in the size of the herd, the origin of the camels, the tests used, the management practises, seasonal variations, and the presence or absence of infectious foci, such as Brucella-infected herds, which could spread the disease between contact herds . Also, we performed CFT as a confirmatory test for the serum samples (OIE, 2012). In the current investigation, 8 (4%) of 200 serum samples and all eight were serum samples that tested positive by mRBT. 12 serum samples that tested positive by mRBT were evaluated by two confirmatory tests (CFT, PCR), and the results showed that there was sero reaction in (66.7 %) and (75%) of the samples, respectively. PCR and CFT both were recorded the highest reactions. Our findings for CFT were higher to those found by (Abdel Moghney 2004) (10.3%), (Sayed-Ahmed et al., 2017) (11.5%) .in line with

this disparity in the results could be due to several factors, including the escape of some imported positive reactors during quarantine procedures, the absence of a national programme for the eradication of camel brucellosis, which would include regular testing and the slaughter of positive cases, and the lack of a camel vaccination programme based on Egyptian field strains, which was demonstrated with imported camels . Aswan areas are believed to be on Egypt's borders, and the bulk of camels slaughtered there originate from neighboring countries, which could be a route of infection transfer if those nations harbour the microorganisms (Abdel Moghney, 2004).

Our findings regarding mRBT and CFT disagree with those reported by (Shahzad A. et al. 2021) who noted that the males results of mRBT (8.1%), CFT (17.4%) %) these results lower than females which were mRBT (17.6%), CFT (34.9%) . than those in our study, it was noted that males' mRBT (6.4%) and CFT (4.7%) findings were greater than those of females, who had mRBT (3.3%) and CFT (3.3%).

(Shahzad A. et al. 2021) stated that females are more likely to contract Brucellosis than males are. Nonetheless, our study noted that males had a higher incidence of Brucellosis than females He noted that females are more likely than males to contract Brucellosis. On the other hand, our study noted that the prevalence of Brucellosis was higher in males than in females.

All of the camels in the investigation were healthy clinically at the time of sampling, and none had previously shown any clinical symptoms of brucellosis, according to the owners.The findings of this work suggest that many healthy camels may be silent brucellosis carriers, and their products may repesent amajor health risk to people .

In the present study, mRBPT-positive serum samples included Brucella DNA was found of all mRBPT positive serum by PCR (Table 4). The presence of 6 *B. melitensis* and *3 B. suis* were detected by conventional PCR. It was anticipated that *B. melitensis* would be found in camels raised in the Aswan governorate because previous findings had shown that the disease was already widespread in this area It was anticipated that *B. melitensis* would be found in camels raised in the Aswan governorate because previous findings had shown that the disease was already widespread in this area (Menshawy et al., 2014). *B. melitensis* DNA was found in camel milk from Giza and Aswan, according to investigations of a similar kind (Ibrahim et al., 2016) and (Hamdy 2002). Given that camels are typically raised in herds and that sheep and goats are raised in mobile flocks, it is possible that small ruminants are the source of *B. melitensis* for camels (Musa et al.,2008 and Abdel-Hamid., 2017).

According to (Shahzad et al. 2021), the detection of *B. suis* DNA from camel sera may represent a novel discovery From Abu Simble Quarantine, was only detected in 3 seropositive male animals. *B. suis* was previously isolated from cattle (Menshawy et al., 2014) and or its DNA was found in pigs in Egypt (Ibrahim 1996 and Khan et al., 2019), thus the discovery of *B. suis* in the current investigation is not unexpected. The domestic or wild pigs, such as the wild boars (Sus scrofa) of the nearby Eastern Desert, could be the source of *B. suis* in camels. Al-Bahr Al-Ahmar (the Red Sea), which shares a border with Sudan and has some pig farms to the west of Kassala state, is also likely to have *B. suis* imported from

Sudan. Due to the presence of domestic and wild pigs in both South Sudan and the Central African Republic, the unrestricted transboundary movement of Sudanese livestock to these two neighboring African nations may aid in the spread of *B. suis* (Gilbert 2010) With large camel populations, the countries in the Horn of Africa have small pig numbers comparable to Egypt rather than Uganda (Gilbert ,2010) .

The exact source of *B.suis* should be traced to stop further transmission as camels could have acquired the disease from a local source as previously mentioned (Al-Majali etal., 2007;Fatima 2016; Abebe et al.,2017 and Bayasgalan et al., 2018) whose reported that raising camels among other farm animals may be a significant risk factor for camel brucellosis .

Conclusion

The detection of *Brucella meltiness* in camel considered one of the most highly contagious microorganisms and causing public health effect on human as it is causing undulant fever and it take long time for treatment. So, this study give alarm for future control of brucellosis in camel.

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