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Epidemiology, sero-prevalence and clinical aspects of camel brucellosis in Aswan governorate

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Abstract

A total of 1782 camels were employed for serological diagnosis of Brucella infection in different localities at Aswan governorate. Blood sera were screened serially using modified Roe Bengal plate test (mRBPT 75), and positive samples were assured by complement fixation test (CFT). Positive results were considered in samples that gave positive results in both screening and confirmatory tests. Blood sera of 35 camel sera including 27 samples positive in both modified RBPT (mRBT) and complement fixation test (CFT) were subjected to modified rose Bengal test (mRBT), standard Rose Bengal Test 25 µ seruml, (sRBT), Tube agglutination test (TAT), Complement fixation test (CFT) and Real time PCR test (RT-PCR) as parallel testing. The real-time PCR targeting the DNA of Brucella was used for revelation of Brucella DNA in camel serum. Results of the RT-PCR on camel's blood sera revealed the presence of Brucella DNA in blood sera of 28 camels including (19 Brucella abortus DNA and 11 Brucella melitensis DNA) as 2 camel's sera showed mixed infection. Results of different serological assays in this study by parallel testing revealed 29 (82.86%), 22 (62.86 %), 27 (77.14 %), 30 (85.71%),using (mRBPT) 75 µ serum, s (RBT) 25 µ serum, CFT, and (TAT). mRBT revealed 100 %, 85.71%, 96.55% and 100% relative sensitivity, relative specificity, positive predictive value and negative predictive value for mRBT respectively. sRBT revealed 78.6%, 100%, 100 % and 53. 8% respectively. TAT showed 100%, 71.43%, 93.33%, and 100% respectively. CFT revealed 96.43%, 100%, 100 % and 87.50 % respectively.

Keywords | Brucellosis, Camel, RBPT, CFT, TAT, PCR.

Introduction

Camelus dromedaries; "the one-humped camel" plays a vital socio-economic function within the rural and agricultural system in both dry and semi dry regions of many Asian and African countries, **(Gwida et al., 2011).** The importance of camels is attributed to the need for camel meat, milk, leather and wool, in addition, the use of camels for transport, riding, and packing, **(Wilson, 1984);(Rollefson, 2000).**

Brucellosis is a communicable zoonotic disease caused by Gram negative coccobacilli , genus Brucella involving wide range of domestic and wild animals, **(Corbel, 1997); (Seimenis et al., 2006).**

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Camel brucellosis causes extensive losses due to abortion and infertility as reported by **Ocholi et al., (2005).** Moreover, **Gessese et al. (2014)** stated that camel brucellosis can largely induce huge loss of animal productivity through low fertility, late first calving age, long calving interval time as well as decrease of milk yield in camels. Lameness, swollen lymph nodes and history of abortion are considered the important criteria suggesting camel brucellosis as reported by **Abdirahman (2020).** Moreover, one of the great public health problems all over the world, that brucellosis causes a dangerous sickness in humans especially those contact with infected animals and those devour infected animal products as reported by **Marei et al. (2011) and Sayour et al. (2015).**

The real status and epidemiology of camel brucellosis in several countries including Egypt are not well recognized, although the Office International des Epizootics (OIE) has considered brucellosis as one of the most diffused diseases in the world as reported by **Schelling et al., (2003**). In Egypt, camel brucellosis was reported by several researchers with variable prevalences; 10.3% (**Hamada et al., 1963),** 2% (**El-Nahas 1964),** 8.3% **(Fayed et al., 1982),** 7.9% **(Nada 1990),** 10.7% **(Barsoum et al., 1995)** and 4.17% **(Hosein et al., 2016).**

Mousa, (1987) reported that brucellosis in camels show fewer clinical abnormalities and antibody levels than those reported in cattle due to a prorated resistance of camels to brucellosis. Moreover, **(Gwida, et al., 2012)** revealed that the spread of brucellosis among dromedary camels can silently jeopardize their reproduction, where the disease is less symptomatic as compared to cattle.

Serology is the most widely used methods of diagnosis of brucellosis. Limitations of serotesting include the likelihood of creating false positive results which are commonly clarified as a result of cross reactivity with several types of Gram-negative bacteria especially Yersinia enterocolitica O:9 and Pasteurella multocida **(Kagunya and Waiyaki, 1978; Emmerzaal et al., 2002; Kaltungo et al., 2014; OIE, 2019)** for which camels are highly susceptible.

The current investigation aims to clarify the role of camels imported from African countries through Abo simple quarantine and other different illegal routes in spreading of the disease. In addition, it was important to evaluate the sensitivity and specificity of some commonly used available serological tests utilized for diagnosis of camel brucellosis.

Material and methods

1. Animals and samples| In this study, blood sera were collected from a total of 1782 imported and local camels at Aswan governorate for serological diagnosis of brucellosis.
2. Oligonucleotide primers and probes used in Real time PCR.

Target gene	Primer sequence (5'-3')	Reference
B. abortus IS711	GCGGCTTTTCTATCACGGTATTC CATGCGCTATGATCTGGTTACG FAM- CGCTCATGCTCGCCAGACTTCAATG- TAMRA	**Probert et al., 2004**
B. melitensis IS711	AACAAGCGGCACCCCTAAAA CATGCGCTATGATCTGGTTACG FAM- CAGGAGTGTTTCGGCTCAGAATAATCC ACA-TAMRA	

3. **Serological examination**

a. **Rose Bengal test (RBT)** was carried out according to **OIE (2019).** An amount of 25 µl of blood serum were placed on a white enamel plate for standard rose Bengal test (sRBT), while 75 µl of serum sample were used for modified rose Bengal test (mRBT) according to **Blasco et al., (1994) and OIE (2019).**

b. **Complement fixation test (CFT) was** done according to **(Alton et al., 1988)**.

c. **Tube agglutination test (TAT)** was carried out according to the method used by the Central Veterinary Laboratory, Weybridge, England and as described by **Alton et al., (1988).**

4. **Taqman real time PCR:** DNA was extracted from serum samples and Brucella identified colonies by QIAcube according to QIAamp DNA mini kit instructions. Reference strains of *B. abortus* S 544 (ATCC 23448) and *B. melitensis* 16 M (ATCC 23456) were used as positive controls. Reference strain of *Escherichia coli* (ATCC 25922) and serum form non infected animals were used as negative extraction control in each cycle. The extracted DNA from the positive samples were examined with the Brucella IS711 species specific real-time PCRs for *B. abortus* and *B. melitensis* as described by **Bricker and Halling, (1994).** Species-specific *B. abortus*, and *B. melitensis* real-time PCRs were used for detection of Brucella DNA. PCR was performed using primers and probe sets (Metabion ,Germany).

5. Assessment of sensitivity and specificity using RT-PCR as the gold standard was carried out according to the methods described by the **WOAH Terrestrial Manual (2023)** as follow:

Sensitivity (Se): It is the capacity of the test to detect diseased animals, when compared with the gold standard test
Sensitivity= True positive TP / True positive TP + false negative FN x 100
Specificity (Sp): It is the capacity of the test to detect non-diseased animals, when compared with the gold standard test
Specificity = True negative TN / True negative TN + false positive FP x 100
Positive predictive value (PPV) = TP / (TP + FP) x 100
Negative predictive value (NPV) = TN/ (TN+FN) x 100

Results and **discussion**

Table (1): Sero-prevalence of Brucellosis in camels in Aswan governorate using serial testing.

Test	Examined animals	Positive	Negative
RBPT (Initial test)	1782	29 (1.6 %)	1753 (98.4 %)
CFT	29	27	2

(Confirmatory test)		(93.1 %)	(6.9 %)
Result of serial testing (Confirmed cases)		27/ 1782 = (1.5 %)	

Table (2): Sero-prevalence of Brucellosis in camels in Aswan governorate using serial testing in different localities.

Locality	Number of tested camels	Number of confirmed sero-positive cases
Abu Simbel	1020	20 (2.2 %)
Aswan*	220	2 (0.9 %)
Draw *	330	1 (0.3 %)
Edfu*	190	4 (2.1 %)
Halayeb and Shalateen *	22	0 (0 %)
Total	1782	27 (1.5 %)

*** Camels that were mixed with large and small ruminants included 762 camels. Out of them 7 (0.92%) were seropositive.**

Table (3): Sero-prevalence of confirmed Brucellosis in males and females in Aswan governorate using serial testing.

Test	Examined animals	Positive	Negative
Males	1734	24 (1.4 %)	1710 (98.6%)
Females	48	3 (6.25%)	45 (93.75 %)
Total	**1782**	**27 (1.5 %)**	**1755 (98.5%)**

Table (4) Clinical abnormalities in camels in different investigated areas in Aswan governorate

Clinical abnormalities	Number of examined	Number
Hygromas	1782	9 (0.51%)
Orchitis	1782	5 (0.28%)

Table (5): Results of the RT-PCR on camel's blood sera.

Samples	*Brucella abortus*	*Brucella melitensis*	*Negative*

35	**19**	**11**	**7**
	Total 28 camels were positive as 2 camels showed mixed infection		

Table (6): Collective results of several serological assays and RT-PCR for exposure of brucella infection using parallel testing method.

No. of examined camels	Test	Positive
35	**mRBT**	29 (82.86%)
	sRBT	22 (62.86 %)
	TAT	30 (85.71%)
	CFT	27 (77.14 %)
	RT-PCR	28 80 %)

Table (7): Positive predictive value, negative predictive value, Sensitivity and specificity of modified RBPT using RT-PCR as the gold standard.

Sero testing outcome of mRBT		Infection status as determined by the RT-PCR		Total	
		Positive	Negative		
Result	Positive	28 (TP)	1 (FP)	29 (TP + FP)	PPV TP / (TP + FP) 96.55%
	Negative	0 (FN)	6 (TN)	6 TN+FN	NPV TN / (TN + FN) 100%
Total results		28 TP+FN	7 TN+FP	35	
Sensitivity and specificity		Se=TP/(TP+FN) 100%	Sp=TN/(TN+FP) 85.71%	Total number of samples processed from both tests	

Table (8): Positive predictive value, negative predictive value, sensitivity and specificity of standard RBT using RTPCR as the gold standard.

Sero testing outcome of sRBT		Infection status as determined by the RTPCR		Total	
		Positive	Negative		
Result	Positive	(TP) 22	0 (FP)	22 (TP + FP)	PPV TP / (TP + FP) 100 %
	Negative	(FN) 6	7 (TN)	13 TN+FN	NPV TN / (TN + FN) 53. 8 %
Total results		28 TP+FN	7 TN+FP	35 Total number of samples processed from both tests	
Sensitivity and specificity		Se=TP/(TP+FN) 78.6%	Sp=TN/(TN+FP) 100%		

Table (9): Positive predictive value, negative predictive value, sensitivity and specificity of TAT using RTPCR as the gold standard.

Sero testing outcome of TAT		Infection status as determined by the RTPCR		Total	
		Positive	Negative		
Result	Positive	28 (TP)	2 (FP)	30 (TP + FP)	PPV TP / (TP + FP) 93.33%
	Negative	0 (FN)	5 (TN)	5 TN+FN	NPV TN / (TN + FN) 100%
Total results		28 TP+FN	7 TN+FP	35	
Sensitivity and specificity		Se=TP/(TP+FN) 100%	Sp=TN/(TN+FP) 71.43%	Total number of samples processed from both tests	

Table (10): Positive predictive value, negative predictive value, Sensitivity and specificity of CFT using RTPCR as the gold standard.

Sero testing Outcome of CFT		Infection status as determined by the RTPCR		Total	
		Positive	Negative		
Result	Positive	27 (TP)	0 (FP)	27 (TP + FP)	PPV TP / (TP + FP) 100 %
	Negative	1 (FN)	7 (TN)	8 TN+FN	NPV TN / (TN + FN) 87.50 %
Total results		28 TP+FN	7 TN+FP	35 Total number of samples existed from both tests	
Sensitivity and specificity		Se=TP/(TP+FN) 96.43%	Sp=TN/(TN+FP) 100%		

In this study, a total of 1782 camels were employed for serological diagnosis of Brucella infection in different localities at Aswan governorate. Blood sera were serially screened using mRBPT 75, and positive samples were confirmed by CFT. Positive results were considered in samples that gave positive results in both screening and confirmatory tests. A total of 29 (1.6 %) camel's sera were found positive for Brucella antibodies by mRBPT. Out of these 29 positive serum samples, CFT identified 27 (93.1 %) seropositive camels, (Table 1) using serial testing. Preliminary testing with RBT is usually proceeded as a sensitive rapid screening test; the test uses acidified antigens to decrease the binding of IgM antibodies and to increase the IgG1 binding **(OIE, 2019**). However, due to cross-reactivity with other gram-negative bacteria, furthermore serial testing with another tests such as complement fixation test (CFT), and competitive enzyme-linked immunosorbent are highly recommended **(OIE, 2019)**.

RBPT is commonly beholded to be a sensitive and CFT is recognized as the most reliable diagnostic test for confirmatory objectives keeping false positives to a negligible level, **(OIE, 2019).** Among the 27 confirmed seropositive camels, 20 (2.2 %) were imported camels at Abu Simbel quarantine, 2 (0.9 %) from Aswan, 1 (0.3 %) from Draw and 4 (2.1 %) from Edfu. These results clarified the wide distribution of brucellosis in dromedary camels in Aswan governorate. Interestingly, it is important to mention there that wide distribution of brucellosis has been reported in a recent study in sheep and goats in the same area by (**Hosein et al., 2024)**.

The sero-prevalence of disease of Brucellosis in males and females in Aswan governorate using serial testing was 24 (1.4 %) and 3 (6.25%) respectively, **Table (3).** The percentage of infection in females was more likely than males to have brucellosis although most of the examined camels in this study were males as they were imported camels. This result coincides with many previous studies: a study on the assembled prevalence of camel brucellosis in 20 countries: **(Tadesse, 2016**; **Alhussain et al., 2022**; **Dadar et al., 2022**).

From the clinical aspect of view, it is substantial to mention there that among the seropositive dromedary camels diagnosed in this study, 9 (0.51%) cases showed hygromas and 5 (0.288%) cases showed orchitis in seropositive males (**Table 4).** No hygroma or orchitis was observed in seronegative camels. Such lesions may be attributed to increased concentration and localization of Brucella organisms in these sites. This can be explained on the bases of the principals of the infection cycle of brucellosis where the clinical disease is linked to the specific tropism of the pathogen to the reproductive tract of males and females resulting in orchitis, epididymitis, infertility, abortion and in chronic cases the organism focused in joints or intervertebral discs as mentioned by **Barbier et al., (2017); Sarma and Singh (2022).** Such localization seems to be due to special affinity of the pathogen for erythritol where it is found in high concentrations at these sites and acts as growth stimulant for Brucella as well as appears to encourage extracellular growth of Brucella **(Keppie et al., 1965; Ocholi et al., 2004)**.

In this study, Blood sera of 35 camels including the 27 positive samples in both mRBPT and CFT in addition to other CFT negative 8 blood sera were subjected to parallel testing using mRBPT, sRBPT 25 µ serum, (RBT), TAT, CFT and RT-PCR test to investigate their diagnostic performances.

RT-PCR test in this study was employed to express the actual value of investigated cases as a gold stander and as a mean of direct diagnosis evaluating the diagnostic performance of the employed serological tests which express the predictive value.

Results of the RT-PCR on camel's blood sera revealed Brucella DNA in blood sera of 28 camels including (19 *Brucella abortus* DNA and 11 *Brucella melitensis* DNA) as 2 camel's sera showed mixed infection, **Table (5)**. Real-time PCR assay in this project, misused the polymorphism emerged from species-specific localization of the genetic element IS711 in the Brucella chromosome. Species-specific *B. abortus*, and *B. melitensis* real-time PCRs were utilized for detection of Brucella DNA. Detection of Brucella DNA can be explained on the bases of the persistence of Brucella DNA in camel's sera. Such persistence is believed to be attributed to the singular immunoglobulins that lacking of light chains **(Conrath et al., 2003).** During the bacteremic phases of Brucella infection, the heavy chain immunoglobulins in blood play a role in death of Brucella organisms with liberation of Brucella DNA in camel serum (**Castano and Solera, 2009; Takele et al., 2009)** that persist in infected camel sera for long periods. Since *B. abortus* is enzootic in Egypt, detection of *B. abortus* DNA in camel sera might indicate that *B. abortus* may be the prime spp. in camels in this area but more studies are still needed to ensure such opinion.

There were also two blood serum samples containing *B. abortus* and *B. melitensis* confirming of the possibility of repeated exposure of camels to infection. Similar results were reported by **(Hamdy et al., 2017).** Moreover, mixed Brucella species infections were reported in Bactrian camels in Russia by **(Solonitsyn, 1949)**

Results of different serological assays in this study by parallel testing revealed 29 (82.86%), 22 (62.86 %), 27 (77.14 %), 30 (85.71%),using (mRBPT) 75 µ serum, s (RBT) 25 µ serum, CFT, and (TAT), **(Table 6).**

For evaluation of different serological procedures, it is important to understand that the interpretation of a diagnostic test is known by its sensitivity and specificity, each involving the capacity of the test to appear the "true" disease situation , as discussed by **(Speybroeck et al.,**

2013).

Parallel testing of camel sera revealed that relative sensitivity, relative specificity, positive predictive value and negative predictive value of mRBT **(Table 7)** were estimated as, 100 %, 85.71%, 96.55% and 100% respectively. sRBT revealed 78.6%, 100%, 100% and 53. 8%, respectively **(Table 8)**. TAT showed 100%, 71.43%, 93.33%, and 100% respectively, **(Table 9)**. CFT revealed 96.43%, 100%, 100 % and 87.50%, **(Table 10)** respectively.

It is visible that the parallel testing raise the sensitivity of the screening tests. Accordingly, it is recommended in the circumstances of herds with suspected brucellosis pointing at accelerating the removal of the disease in infected areas.

mRBT75 using 75 µl of serum, detected the 29 (82.86%) positive samples with high sensitivity (100%) that can be attributed to increasing the amount of antibodies in blood sera (75 µl of serum), compared with 78.6% sensitivity of the sRBT using (25 µl of serum). Such modification was previously suggested by **Blasco et al., (1994); Ferreira et al., (2003)** to minimize the disagreements with the CFT results and to increase the sensitivity of sRBT in small ruminants. Moreover, The **WOAH Terrestrial Manual, (2022)** recommended the modifications adopted by **Blasco et al., (1994)** to minimize the discrepancies with the CFT and to increase the sensitivity of the test using (75 µl) and one volume of antigen (25 µl,).

The standard RBT (sRBT) using 25 µl of serum in this study revealed the least (78.6%) sensitivity. This reduces its efficiency as a screening test. Due to the lower sensitivity (78.6%) and the lower NPV 53. 8 % of the RBT in camels in this study it is not as a screening test for brucellosis supervision in camels, in spite of that it is a cheap and most easily handled test among the other assessed tests. Moreover, **Bayasgalan et al., (2018)** reported lower sensitivity of the test in camels as compared to other livestock species and they believed that it seems should not be recommended as a screening test for brucellosis supervision in camels. Such failure can be explained on immunological bases. The nature of camel's humoral immune response and the singular nature of their heavy chain antibodies might be an illustration for these puzzling results.

Tube agglutination test (TAT), revealed 100 % sensitivity. This may be due to the affinity of this test to both IgG and IgM fractions. IgM as a result of cross-responding antigens stimulates high level of agglutinating immune response which causes specificity problems in the TAT. Owing to this fact, the test in spite of being sensitive should not be used alone, but rather with other tests. Other limitations of the test include false positive and false negative results as reported by **Poiester et al. (2010)**. On the other hand TAT gave the lowest specificity 71.43% in this study, this may be due to low efficiency of detection of IgG1, resulting in low assay specificity as discussed by **Corbel, (1972).** It is substantial to mention that the utilize of TAT with detection of IgM antibody can be beneficial as the simultaneous existence of IgM and IgG indicates acute brucellosis and IgG alone is an sign of chronic brucellosis **(Godfroid, et al., 2010).**

Compared to other serological tests appointed in this study, the CFT evidenced to have the best result in the criteria of test validations, namely; specificity (100%), PPV (100 %).

CFT evidenced to have the highest rate of specificity 100 % and positive predictive value 100%, and revealed the least false positives, 0 cases (0%) **(Table 10).** This suggests that the mRBT positive results should be confirmed by CFT. **Al Dahouk et al., (2003a)** thought that CFT should be used only as a confirmatory test.

Importantly, the **WOAH Terrestrial Manual, (2022)** recommended the use of tests appointing acidified antigens such as BAPAT and the RBPT as screening tests, and CFT as the confirmatory reference test for international trade of animals. Although CFT was established to be the highest specific test in this project, it is a complex method to carry out necessitating good laboratory services and skilled staff. The incorporation of these serological tests, although more expensive,

time consuming, and require more specialized laboratories, except that will reduce false positive and false negative results.

Conclusion

The results of the present study suggest that camel brucellosis presents up to significant sero-prevalence in Aswan governorate and is likely enzootic in different localities of this governorate. Because of lower sensitivity of the standard RBT in camels as proved in this study compared with other serological tests, it is not recommend for screening of camel brucellosis. mRBT is recommended for screening combined with CFT as confirmatory test for serial testing. CFT ensured to have the highest rate of specificity 100 % and positive predictive value 100%, and revealed the least false positives, 0 cases (0%).

Confliction of interest:

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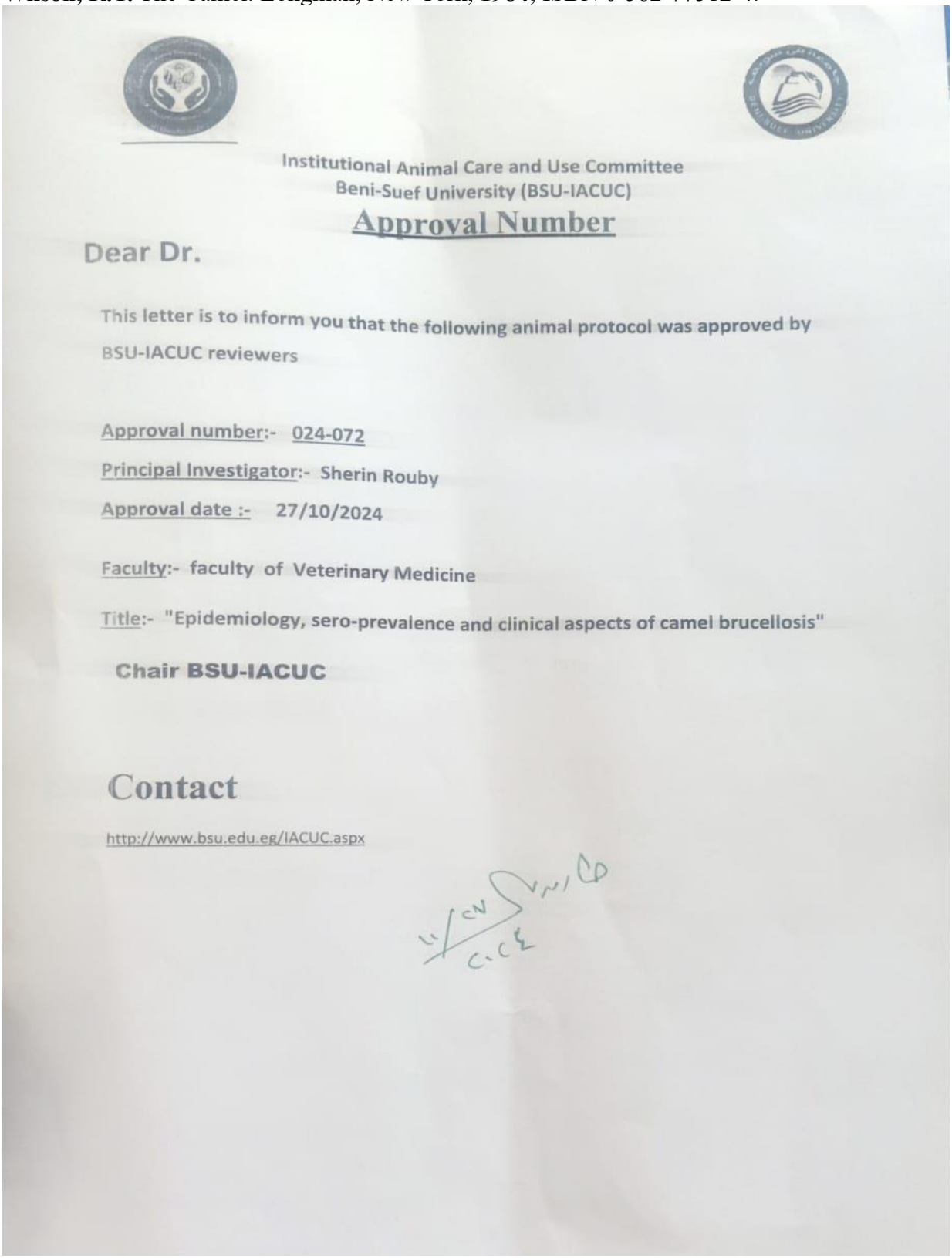

Institutional Animal Care and Use Committee
Beni-Suef University (BSU-IACUC)

Approval Number

Dear Dr.

This letter is to inform you that the following animal protocol was approved by BSU-IACUC reviewers

Approval number:- 024-072

Principal Investigator:- Sherin Rouby

Approval date :- 27/10/2024

Faculty:- faculty of Veterinary Medicine

Title:- "Epidemiology, sero-prevalence and clinical aspects of camel brucellosis"

Chair BSU-IACUC

Contact

http://www.bsu.edu.eg/IACUC.aspx