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Characterization of Brucella organisms isolated from camels and some associated risk factors of brucellosis in Aswan governorate, Egypt

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Abstract

In this study, isolation, identification and molecular characterization of Brucella isolates detected from camels from different localities in Aswan governorate were performed. Ten Brucella isolates were recovered in this study, these included two from lymph nodes, three from udder tissues, one from testicular tissues, two from hygromas and two from milk secretions of the udder of slaughtered camels. Typing of these isolates revealed six Brucella abortus isolates and four Brucella melitensis. Brucella abortus was recovered from three udder tissues, one testicular tissue and two hygromas while Brucella melitensis was detected from two lymph nodes and two milk samples collected from the udder of slaughtered she camels. In the current study, the real-time PCR assured the identification of Brucella DNAs in the 10 bacteriologically identified Brucella isolates as six B. abortus and four B. melitensis. PCR results in this research confirmed the results of bacteriological examinations and cleared the power of PCR testing at detection and characterization of Brucellae. Therefore, it can be applied to complement the results gained from phenotypic tests. Different risk factors of Brucella infection in camels were investigated and discussed.

Keywords | *Brucellae*, Camel, PCR, *Brucella* DNAs

Introduction

Brucellosis remains as an important worldwide disease that seriously affects domestic animals and is highly transmissible to humans, **(Pappas et al., 2006).** The disease has been classified by the World Health Organization (WHO) as a "neglected disease" and remains enzootic in numerous of the developing countries **(WHO, FAO report, 2006)**.

Wernery (2014) stated that brucellosis is a contagious disease occasioned by Gram-negative bacteria that are non-motile and nonspore-forming of the genus Brucella. Truly the genus Brucella is divided into ten species and subdivided into biovars depends on biochemical reactions and

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agglutination with mono-specific sera.

Infection with *B. abortus* or *B. melitensis* has been reported before in the one-humped camel (*Camelus dromedarius*) and also the two-humped camel (*C. bactrianus*), as well as in the South American camelids and it was suggested that there is relation to contact with large and small ruminants infected with *B. abortus* or *B. melitensis* **WOAH Teresterial manual, (2022).**

Although the genus of Brucella exihibit greater than 95% homology depend on DNA-DNA hybridization studies, variations that appeared in host preference and biochemical properties gave rise to the division of the genus into the six classical Brucella species as reported by **Osterman and Moriyón (2006).** Camel brucellosis has been reported when camels came into contact with cattle, sheep and goats infected with *B. abortus* or *B. melitensis* as reported by **Radwan et al. (1992) and OIE (2019)**. The primary hosts of *B. melitensis* are sheep and goats, while *B. abortus* originally infected cattle (**Rossetti et al., 2022).**

Dromedary camels are always reared together with small ruminants and sometimes with cattle sharing the same watering points and pastures, therefore a higher prevalence of camel brucellosis is expected **(Teshome et al., 2003).** Consequently, a close contact between diseased and susceptible camels in a flock encourages the diffusion of disease.

The disease continues to occur all over the world with only few countries that are formally free of the disease and some cases as yet happened in people in enzootic countries **(Corbel and Beeching, 2004).** Several countries, including Australia, the United Kingdom and Japan, as well as parts of the United States of America and few countries in Europe have prospered in discarding brucellosis through strong health control measures, (**Wernery, 2016).**

During the last few years, the disease , camel brucellosis has been a subject for many projects in many countries of the world mostly those raising racing camels such as the Arabian Gulf countries as well as other countries where camels comprise an important part of their livestock in many Asian and African countries **(Yasmin and Remya, 2011).** Transmission of brucellosis relies on the Brucella species being prevalent in other animals sharing their husbandry as well as on habitat **(Musa et al., 2008)**.

The disease constitutes a significant public health issue in several regions of the world (**Pal, 2007; Hadush and Pal, 2013**). Moreover, **(Radwan et al., 1995**) in Saudi Arabia diagnosed the disease in 30% of the camel milkers and handlers and isolate the same *Brucella melitensis* biovars from aborted sheep and goats sharing the selfsame premises.

Von Hieber (2010) reported that Brucellae have been isolated from vaginal swabs, camel milk, fetal stomach fluid, aborted fetuses, fetal membranes, lymph nodes, testes and hygromas. **Wernery et al. (2007)**, inspected the localization of Brucella organisms in serologically positive lactating dromedaries which lambed to healthy calves and found that Brucellae were hidden in internal lymph nodes. This clearly reveals that there are significant epidemiological variations in dromedaries which abort and chronically infected animals which do not abort.

The locomotion of animals for grazing and watering during the dry season is an important factor share to spread of brucellosis, as gathering of camels around a watering point may increase the contact between infected and non-infected animals as suggested by **Waktole et al. (2022).**

In Darfur region of Sudan, introducing of camels into cattle, sheep and goat grazing regions led to high happening of disease level as reported by **Musa and Shigidi** (**2001).**

Therefore, it was important to characterize Brucella isolates from both local and imported camels in Aswan governorate.

Materials and methods

1. Animals and clinical samples: Different clinical samples of camels were collected for bacteriological examination as following.
   - Milk samples Of 48 lactating she camels at Shalateen region, Aswan, Draw, and Edfu were subjected for bacteriological examination of their milk samples.
   - A total of 32 slaughtered dromedary camels were employed for bacteriological examination of their tissues specimens for isolation of Brucella organisms, these include: Udder tissues and secretions (n=8), different lymph nodes (retropharyngeal, prescapular, prefemoral, internal iliac and supramammary) (n= 32) ofseropositive slaughtered animals, fluids from hygromas (n= 5) and testicular tissues (n= 6) were collected at Aswan, Kom-ombo and El Basatine abattoirs.

2. Identification of Brucella isolates: was carried out contributing to the recommendations of the FAO/WHO Expert Committee on Brucellosis **(Alton et al., 1988)** and the Guidelines of the **OIE (2019).**

3. Molecular identification of Brucella isolates.

   DNA was taken away 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,

Table (1): Master Mix preparation.

Component	Volume/reaction
2x QuantiTect Probe RT-PCR Master Mix	12.5 µl
Forward primer (50 pmol)	0.5 µl
Reverse primer (50 pmol)	0.5 µl
Probe (30 pmol)	0.125 µl
RNase Free Water	6.375 µl
Template DNA	5 Ml

The DNA that extracted 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 carried out utilizing primers and probe sets (Metabion, Germany).

Table (2): Real time PCR.

Stage	Temperature	Time	Cycles
Primary denaturation	95 ºC	5 min.	1

Amplification a)Secondary denaturation	95 °C	15 sec.	40
b)Annealing and extension	57 °C	1 min. (optics on)	

Results and discussion

Table (3): Clinical abnormalities in camels

Clinical abnormalities	Number of examined	Number
Hygromas	1782	9 (0.51%)
Orchitis	1734	5 (0.288%)

Table (4): Result of isolation and distribution of Brucellae from camels.

Samples	No.	Locality	Brucella isolation
Lymph nodes	32	Aswan and Kom ombo abattoir	2
Udder tissues	8		3
Testicular tissues	6		1
Hygromas	5		2
Milk from udder of slaughtered she camels	9		2
Milk from lactating she camels	48	• Aswan 8 • Draw 16 • Edfu 2 • Shalateen 22	0
Total	108		10

Table (5): Results of identification and typing of Brucella organisms.

Brucella isolation	***B. abortus 1***	***B.melitenisis 3***
10	**6** (3 from udder tissues, onetesticular tissues and 2 from hygromas)	**4** (2 lymph nodes and 2from milk)

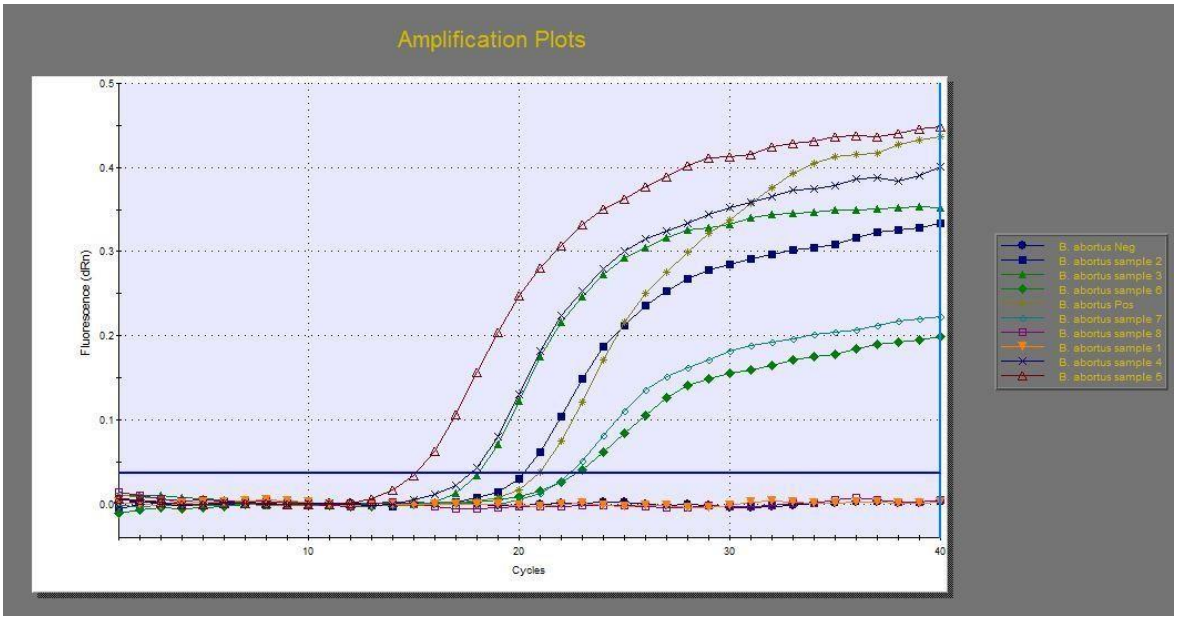

Figure (1): Amplification Plot of *Brucella abortus.*

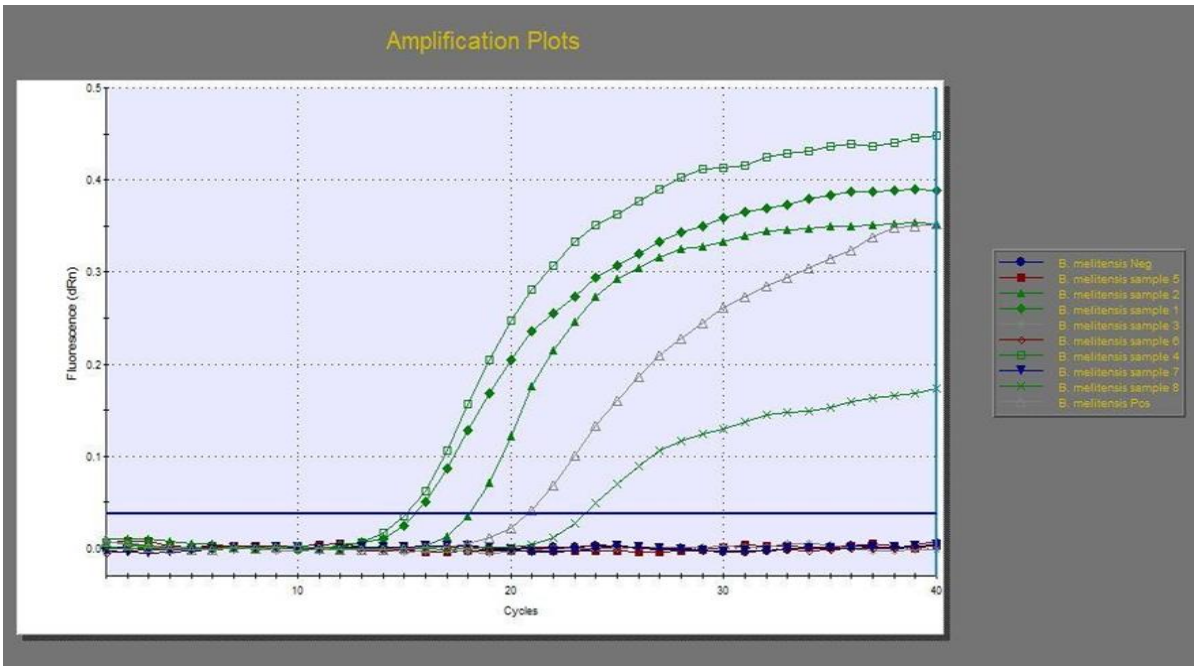

Figure (2): Amplification Plot of *Brucella melitensis.*

From the clinical aspect of view, it is important to mention there that among the seropositive dromedary camels diagnosed in this study, nine (0.51%) cases showed hygromas and 5 (0.288%) cases showed orchitis in seropositive males. No hygroma or orchitis was observed in sero-negative camels. Such lesions may be attributed to the 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 symptomatology of this disease is regarding to the specific tropism of the pathogen for the reproductive tract of males and females resulting in abortion, orchitis, epididymitis and infertility and in chronic cases the organism centralize in joints or intervertebral discs as mentioned by **Barbier et al. (2017) and Sarma and Singh (2022).** Such localization present to be the result of the particular affinity of the organism for erythritol in high concentrations at these sites and acts as growth stimulant for Brucella and appears to encourage the extracellular growth of Brucella as discussed by **Keppie et al. (1965) and (Ocholi et al. (2004)**.

History of abortion and breeding troubles were reported in this study from all investigated camel populations associated with breeding troubles such as increased inter-calving period, and infertility in females.

Furthermore, **Benfodil et al.** (**2022)** reported higher brucellosis seroprevalence in camels raised in herds with a history of abortion and Brucella infected females. Infected camels shed contaminated materials into the environment such as aborted fetuses, placenta, vaginal secretions, urine, milk and suppurative products as reported by **Anses (2014).**

Really, being in South of Egypt; Aswan governorate itself is an important risk factor of camel brucellosis sero-positivity. This is attributed to localization of high numbers of camels that are legally imported from enzootic countries like Sudan where camels are kept in their motherland with no surveillance system and without any control measures. This is in addition to camels that are continuously introduced illegally across the desert with final destination in Aswan governorate. Moreover these camels are exposed more frequently to close contact with Brucella infected cattle or small ruminants that have been proved in a previous study in different localities in Aswan governorate by **Hosein et al. (2024).** Moreover, **Reem (2023)** found that ruminants in different

localities in Aswan governorate were infected with both *Brucella abortus* and *Brucella melitensis* for which camels are highly susceptible. Moreover, cattle were considered as a possible origin of infection for camels as reported by **Musa et al. (2007).**

As shown in this study sharing of camels the same habitat and pastures with cattle or small ruminants highlighted the findings of **Hadush et al. (2013)** who reported that camel flocks with close contact in grazing land with cattle were 3.6 times and camel flocks with close contact in grazing land with small ruminants were 2.3 times more at hazard to be brucellosis seropositive than those with no contact. According to such findings, it can be concluded that keeping dromedaries with cattle or small ruminants might increase brucellosis prevalence, also while keeping camels unaccompanied by these animals is a serious step in the control of brucellosis in camels providing controlling of their movement.

In addition, another serious risk factor is the continuous locomotion of animals especially for grazing and watering that could be a causal factor to the distribution of brucellosis due to the chance of gathering camels around a watering point with other infected camels or ruminants leading to the increase of contact and exposure to infection as discussed by **Waktole et al. (2022).**

From the epizootiological point of view, the results obtained in this study highlight the role of camels in the infection chain of animal and human brucellosis. By other words, camels might perform as reservoir host for Brucella infection and infect other susceptible livestock like large and small ruminants kept with them and consequently spread the disease. Therefore, maintenance of Brucella organisms in the camel inhabitance could not be expected. In addition, brucellosis may be transmitted from camels to humans, mainly throughout consumption of row milk as the traditional habits of consumers of camel products in many nomadic settings believe that fermented and raw camel's milk has a curative effect on health as reported by **Racloz et al. (2013).**

In this research, isolation, identification and molecular characterization of Brucella isolates detected from camels from different localities in Aswan governorate were performed. The biological typing in this study relied on biochemical characteristics, agglutination with monospecific sera and phage typing according to the standard protocol of the **OIE** (**2019)**. Ten Brucella isolates were recovered in this study, **(Tables 3 and 4)**, these included two from lymph nodes, three from udder tissues, one from testicular tissues, two from hygromas and two from milk secretions of the udder of slaughtered camels. Typing of these isolates revealed six *Brucella abortus* isolates and four *Brucella melitensis* isolates, **Table (5)** and **Figures (1, 2)**. *Brucella abortus* was isolated from three udder tissues, one testicular tissue and 2 hygromas. *Brucella melitensis* was detected from two lymph nodes and two milk samples collected from the udder of slaughtered she camels. These types of Brucellae are circulating among animals as they were previously and repeatedly isolated from different animals species in Egypt including cattle, buffaloes, sheep, goats, dogs, cats and camels by **Hosein (1987); Menshawy et al. (2014); Wareth et al. (2015); Wareth et al. (2020) and Hosein et al. (2024)**. Epidemiologically it is serious to refer that isolation of *B. melitensis* from camels in this study and from cattle, buffaloes, sheep, and goats in previous studies suggests the capability of dromedary camels to create and everlasting as animal reservoirs in Egypt. This creates a potential dangerous infection chain with wide spread of infection in animals as well as humans due to the wide host range of susceptibility. Cross-species transmission of *B. melitensis* to other animal species including camels and other large ruminants were repeatedly recorded from Egypt **(Hosein et al., 2016; khan, 2020; Wareth et al., 2020).** Circulation of *B. melitensis*; the most virulent pathogen for human brucellosis in camels intensifies the hazard for human infection. It is considered as the most dangerous and virulent type among the different Brucella species affecting humans as reported by the **WHO, and FAO report (2006)**. Consuming of raw milk and dairy products of infected camels was reported to be related with brucellosis in people as reported

by **Ben-Shimol et al. (2012); Garcell et al. (2016) and Hosein et al. (2016).**

Brucella infected camels may result in environmental contamination as a result of shedding large number of Brucella organisms due to abortions or parturition of Brucella infected she camels **(Abdel-Hamid et al., 2020).**

In the current study, the real-time PCR assured the identification of Brucella DNAs out of the 10 bacteriologically identified Brucella isolates as six *B. abortus* and four *B. melitensis*.

PCR results in this research confirmed the results of bacteriological examinations and showed the power of PCR testing for detection and characterization of Brucellae. Therefore, it can be utilized to complement the results given out from phenotypic tests as reported by **(Bricker, 2002).** Detection of *B. abortus* and *B. melitensis* DNA in Aswan governorate in camels was predictable as previous reports clarified the enzootic nature of *B. melitensis* and *B. abortus* in these regions **(Hamdy, et al., 2017).**

Conclusion

The results of this study confirm the prevalence of *Brucella abortus* and *Brucella melitensis* in Aswan governorate in camels, these Brucella species were related with infection in buffaloes, cattle, sheep, and goats in previous studies in the same areas of the current study. Detection of Brucellae from clinical samples of camels indicates the possible role of camels as animal reservoir as some of these camels were apparently healthy; in addition, this highlights the public health significance of the disease.

Conflict of Interest

The authors have stated no conflict of interest.

Funding:

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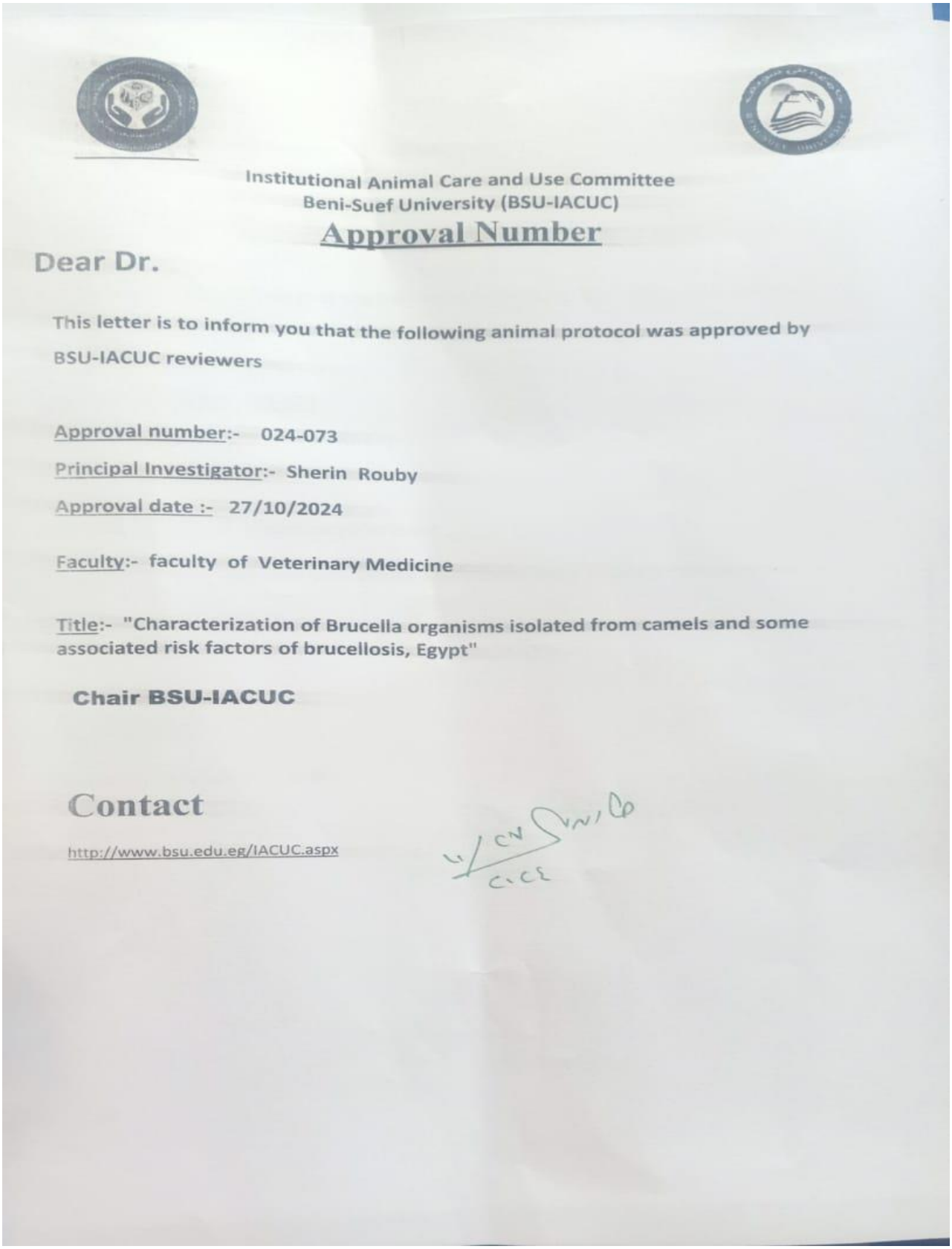

Institutional Animal Care and Use Committee
Beni-Suef University (BSU-IACUC)

Approval Number

Dear Dr.

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

Approval number:- 024-073

Principal Investigator:- Sherin Rouby

Approval date :- 27/10/2024

Faculty:- faculty of Veterinary Medicine

Title:- "Characterization of Brucella organisms isolated from camels and some associated risk factors of brucellosis, Egypt"

Chair BSU-IACUC

Contact

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