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Isolation of *Brucella Melitensis* and Evaluation of Serum Biochemical Changes in Camels: A Pilot Study to Prevent Human Brucellosis

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Abstract: Brucellosis is an infectious disease of livestock animals, which is reflected as one of the most severe public health issues worldwide. The main aim of this study is to isolate the *B.melitensis* and to examine the biochemical parameters in the serum of brucella infected and healthy camels. A total of 200 blood samples were collected from the camels of Shahaniya Governorate, Qatar. The Brucella isolation was done using different bacterial growth techniques. The status of blood metabolites and hepatic marker enzyme activities, kidney function markers, and mineral contents were estimated in the serum samples. Our findings revealed the probability of *B.melitensis* growth with 93-98%. The 34-50% of other species such as *S.paucimobilis*, *A.lwoffii*, *A.salmonicida*, and *O.ureolytica* were also noted in the serum of camels. In biochemical studies, the decreased status of glucose, total protein, albumin, and globulin in the serum of brucella-affected camels. The activities of AST, ALT, ALP, LDH, and GGT were up-regulated in the serum of brucella-infected camels. The levels BUN, creatinine, urea, creatine kinase, and bilirubin were also increased in the serum of brucella-affected camels. The calcium, magnesium, phosphorus, and iron were decreased in the brucella-infected camels. Here, we found the 93-98% probability of *B.melitensis* growth in the blood samples of camels. Furthermore, Brucella infection adversely altered the blood metabolites, liver function marker enzyme activities, kidney function markers, and mineral contents in the serum of camels.

Keywords: Brucellosis, Glucose, *Brucella Melitensis*, Aspartate Transaminase, Calcium, Urea, Dromedary Camels.

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1. INTRODUCTION

Brucellosis is a worldwide zoonotic disease that causes economic losses to animal breeders due to abortions, retained placenta and metritis in females, orchitis, and epididymitis in males, and infertility was reported in both sexes.[1,2] Brucellosis like other diseases causes an imbalance in the oxidants-antioxidant state and induces changes in organ functions and protein electrophoretic patterns of the infected animals.[3] Genetic markers of serum proteins can be used for the identification of animals naturally bearing susceptibility and/or resistance to brucellosis in selection programs.[4] Brucella infects humans by the utilization of raw milk and its products or through contact with the affected animals.[5,6] Brucellosis can cause serious economic losses particularly it decreases productivity and results in the abortion of infected animals. In most cases, the Brucellosis is asymptomatic in camels. The disease in camels is characterized by abortion and male infertility was observed.[7] Brucellosis in camels is often asymptomatic and the symptoms in affected camels are not described clearly yet. In the majority of cases, most of the affected camels are soundless carriers of brucellosis. The utilization of Brucella-affected camel milk products such as milk and meat can leads to high incidences of human brucellosis.[8,9] In humans, brucellosis is a devastating disease that lacks pathognomonic symptoms, being a main public health threat, and imposed economic crisis in many countries.[10,11] Brucellosis is a chronic bacterial disease, which affects numerous domestic and wild mammals with significant public health importance.[12] Like in domestic and wild mammals, this disease spread among people through the consumption of unpasteurized contaminated milk, raw liver,[13] and contact with infected tissues and discharge.[14] The epidemiological state of Brucellosis in the region has been described in several studies, but there are few reports of outbreaks of brucellosis acquired through camel milk in 15 members of an extended family in Israel.[15,16] Al-Shaar *et al.*[17] described an outbreak related to the consumption of raw cheese in Lebanon. The consumption of unpasteurized camel milk has been identified as a main source of human brucellosis in Qatar. An additional risk is associated with contact with infected animals, especially the assistance of parturition or the consumption of insufficiently cooked or raw meat.[18] *Brucella abortus* and *Brucella melitensis* are the etiological agents associated with camels and goats, which constitute the most important source of the disease in Qatar.[19,20] Among these, three Brucella spp., namely, *B. abortus*, *B. melitensis*, and *B. suis,* are known to have high zoonotic and economic importance.[21] Brucella organisms enter into the body of host through inhalation, ingestion, and mucous membrane or broken skin.[22] The well-identified Brucella species are named as per their preferences of the host, for example, *B. abortus* (infects cattle), *B. melitensis* (infects goats and sheep), *B. suis* (infects pigs), and *B. ovis* (infects sheep).[23,24] In several developing countries like the Middle East, the Mediterranean, and Indian subcontinent Brucellosis is an endemic disease and it produces severe economic losses in livestock production.[25,26] The analysis of blood components is considered a valuable strategy to determine the health status of the animal. Brucellosis has severe consequences on the health status of livestock animals because it mainly affects the vital organs of the body, which results in their damage and impaired functions as per the degree of infection.[27,28] The previous literature on the abnormality in the status of biochemical markers in the Brucellosis-affected camels is scarce. The boosted activities of serum marker enzymes are noteworthy indicators of the pathological degree of different tissues as it releases intracellular enzymes from the damaged tissues. Furthermore, changes in the globulin/albumin levels and their determination are believed to be reliable diagnostic markers in livestock animals.[29] The elevation in metabolic functions during the time of infection may lead to changes in the biochemical markers. The changes in the routine serum biochemical parameters are yet to be explored properly during the period of infection and other diseases.[30] The liver is a vital organ of the body, which is essential for the metabolism and maintenance of other body functions.[31] To determine the health and normal functions of the liver, some hepatic biomarker enzymes such as AST, ALT, ALP, and GGT were examined frequently. During the occurrence of hepatic damage, the enzymes within the hepatocytes are released into the bloodstream.[32] The albumin, globulin, and total protein are also the vital markers used to determine the liver function and health status. The urea, creatinine, and blood urea nitrogen (BUN) are excretory products, which is excreted from the blood by the kidneys. The evaluation of creatinine level in the serum is a valuable indicator used to determine the kidney functions.[33] The increased status of BUN in the serum generally shows that the functions of kidneys are impaired due to the infection or other diseases. These biochemical parameter is important in demonstrating the detrimental effect in various organs caused by Brucella infection. The total protein, albumin, glucose, globulin, BUN, AST, and ALT are imperative in determining the health status of livestock animals.[34] Therefore, determination of activities of serum biomarker enzymes and fractions in association with other strategies are helpful in determining the development of disease, which makes easier during the disease diagnosis. For the control of brucellosis, the several tests such as rose Bengal test (RBT), TAT, CFT, and ELISA are carried out. These assays relies on the identification of anti-brucella lipopolysccharide (LPS) antibodies. However, these tests cannot define if the antibodies resulting from natural infection or vaccination. It also can give false positive reactions with LPS of other Gram negative bacteria.[35] However these serological tests were to estimate the tendency of disease to increase or decrease and can't be considered as accurate prevalence. Furthermore, the literatures on the biochemical parameters in the blood of camels affected by Brucellosis are extremely poor.[36] Therefore, the current work was designed to examine the alterations in the biochemical parameters such as activities of liver function marker enzymes, renal function biomarkers, blood metabolites, and mineral contents in the camels diagnosed with brucellosis in comparison with the healthy controls to reflect the extent of the Brucellosis in the dromedary camels.

2. MATERIALS AND METHODS

1.1 Animals and samples

A total of 200 blood samples were collected from the Brucella infected and healthy dromedary camels, respectively near the Governorate of Shahaniya, Qatar. Blood samples were collected from the camels with utmost care to abate the unexpected stress, which may happen to the camels. The 10ml of blood was drawn from the jugular vein into the clean vacutainer tube and then labeled properly using code numbers. The samples were carefully collected on the 8ml of gel biotin tube and taken to the laboratory in ice box. Samples were then centrifuged at 6000rpm for 10min at 37°C (SL40F,

ThermoFisher Scientific, USA). Then serum was collected and utilized for the additional confirmation assays.

1.2 Identification of Brucellosis

All the collected camel serum was examined for the presence of Brucellosis by using various assays. The BAPAT assay was executed by the previous technique explained by the Alton et al.[37] The RBT was executed by the previous approach explained by the Morgan et al.[38] The competitive ELISA test was carried out using the assay kits as per the guidelines of the manufactures (Svanovir™ Brucella-Ab cELISA kit, Sweden). In those assays, 20 µl of reagent was mixed to 50 µl of serum for RBT and 30ul reagent of with 80ul serum for BAPAT serum using ELISA Kits (SVANOVIR® Brucella-Ab C-ELISA-Sweden). Manual ELISA was done and plates were read at 450nm using microplate reader (Thermoscientific, USA).

1.3 Culturing of Brucella on the microbial growth media

The samples were prepared using OXOID (Oxoid Ltd, Basingstoke Hants, UK) diagnostic reagents. The different bacterial growth mediums such as Tryptone soy agar (TSA), MacConky agar, and Blood agar was prepared. For this, 40g of respective growth medium (Himedia, USA) were dissolved in a 1000ml of distilled ware and then boiled to dissolve them properly. For sterilization, the prepared media was autoclaved at 121°C for 15 min using autoclave (SM511, Yamato Scientific Co Ltd.,). Then growth media was loaded on the petri dishes and cooled to room temperature on well-sanitized inoculation chamber. After that, the serum samples were inoculated on the surface of pre-labelled plates loaded with growth medium and then incubated at 37°C for 10 days in CO2 (5%) incubator (HERAcell VIOS 160i CO2 Incubator, Thermofisher Scientific, USA). Followed by the incubation, the growth of the microbes were observed as small yellowish smooth colony and used for the further identification tests with the help of VITEK 2 COMPACT- BioMérieux.

1.4 Culturing of Brucella on the selective media

The isolation of Brucella spp. were performed on the 20 seropositive serum samples using a Brucella selective supplement media. The selective growth media was prepared and enriched with Brucella agar (contains inactivated 5% horse serum along with 12,500 IU of bacitracin, 50mg of cyclohexamide, 2,500 IU of polymyxin B, 10mg of vancomycin, 50,000 IU of nystatin, and 2.5mg of nalidixic acid (Oxoid, UK)) and incubated at CO_2 (5%) incubator at 37°C for 10 days. After the 14 days, the plates were discarded if no growth was visible. For sub-culturing, the TSA and Blood agar base was utilized.

1.5 Determination of biochemical parameters

The level of total protein in the serum samples were analyzed by the previous procedure done by Doumas et al.[39] The albumin was examined by the previous technique.[40] The level of mineral contents such as calcium,[41] inorganic phosphorus,[42] magnesium,[43] and iron,[44] were analysed in the serum samples of healthy and Brucella infected camels. The activities of the biomarker enzymes such as AST and ALT were analyzed by the standard technique.[45] All the other biomarkers such as alkaline phosphatase (ALP), globulin, BUN, creatinine, LDH, GGT, bilirubin, urea, and glucose were analyzed by using the assay kits using the guidelines recommended by the manufacturer (BioMérieux, France and Spinreact, Spain).

3. STATISTICAL ANALYSIS

The values are depicted as mean±SD of triplicates, which is analyzed by the computer program SPSS (version 17.0) using one way ANOVA test, followed by Tukey's post hoc assay. The minimum level of significance was set at $P < 0.05$.

4. RESULTS AND DISCUSSION

Brucellosis is a highly infectious disease of livestock animals, which is one of the severe public health issues, particularly in developing countries.[46] In earlier days, the camels were believed as highly resistant to the infections and other diseases, which affects other livestock animals. However, the historic isolation of camel in desert areas apart from other animals may stand behind this finding. The camels were believed as more vulnerable than other livestock to several ailments including brucellosis.[47] The control of measurements of the brucellosis in livestock animals and humans mainly reliable on the accuracy and reliability of the strategies utilized to detect and identify the pathogens. Although worldwide the disease is distributed in population regardless of age and sex, studies conducted in ME countries have shown that children are strongly.[48-50] This finding could be related to cultural and nutritional issues that increase children's risk of exposure to the consumption of camel milk. The preferences of consuming raw camel milk due to changes in the taste and the health properties of milk after boiling constitute an important cultural factor related to this outbreak. Although, brucellosis diagnosis in camels is highly complicated process. The Brucellosis can mimic several other ailments. The clinical symptoms of camel brucellosis are frequently lacking and precise diagnostic strategies are not described yet. In our current work, all the camels were found normal during the collection of samples, none had demonstrated the symptoms of brucellosis. These observations revealed that the several affected camels may be soundless carriers of brucella and their products can cause serious health complications to the consumers. A previous report done by the Abu Damir et al.[51] were supported our observations, who highlighted that camels infected with *B. abortus* had no symptoms and only slight pathological alterations were occur. The findings of this study revealed the bacterial growth on the surface of growth medium. The OXOID signal blood culture system was revealed the positive growth indication for Brucella (Figure 1).

Fig 1: Blood culture in oxoid signal culture system

The positive growth indicator bottles were sub-cultured in MacConky agar and Blood Agar. The TSA, Blood agar, and Maconckey agar was revealed the growth of 50% of *Brucella melitensis* and 34-50% of other species such as *Sphingomonas paucimobilis*, *Acinetobacter lwoffii*, *Aeromonas salmonicida*, and *Oligella ureolytica* in the serum of camels (Table 1 and Figure 2). Colonies were presumptively identified as Brucella by morphology and Gram staining.

Table 1: Percentage of different isolates from the blood sample

S.No	Name of the species	Percentage (%)
1	*Brucella melitensis*	93-98%
2	*Acinetobacter lwoffii*	50%
3	*Sphingomonas paucimobilis*	50%
4	*Aeromonas salmonicida*	50%
5	*Oligella ureolytica*	50%

Fig 2: Bacterial growth on the different culture medium

Furthermore, the TSA agar was prepared without the blood revealed the 50% of *B.melitensis* growth, however TSA supplemented with blood also demonstrated the 50% *B.melitensis* growth. Therefore, the *B.melitensis* species were subcultured again with same TSA+Blood (Figure 3) and results revealed the 93-98% of probability of identification of *B.melitensis* in seropositive samples of camels with very good ID confidence in VITEK 2 COMPACT-Biomerieux using GN card [Lot num"241676103]. The analysis time was 7.78 hours analysis message was obtained as 'Important presumptive identification, Highly Pathogenic organism'.

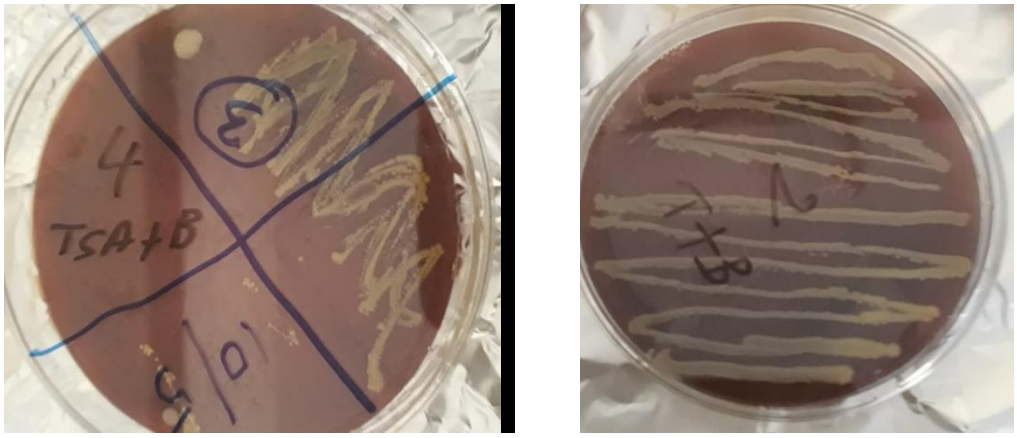
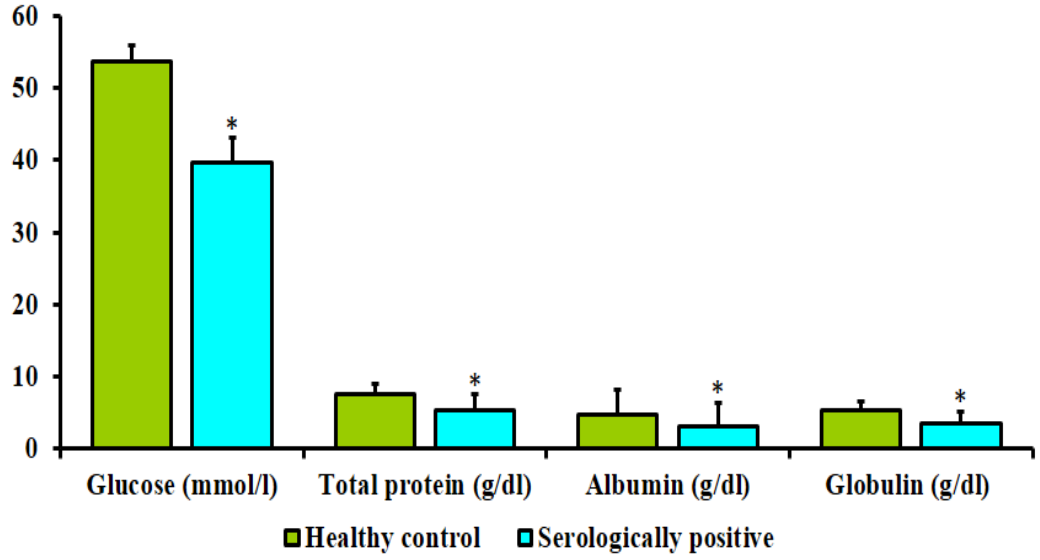


Fig 3: Brucella growth on the Tryptone soy agar with blood

The 6/11 of culture samples obtained from seropositive camels revealed a positive result *B.melitensis*. Sensitivity of the each test was confirmed by the repeated culture and sub-culture techniques and the suspected species was finally identified as *B.melitensis* by using VITEK 2 COMPACT- BioMérieux. The RT-PCR results also revealed a positive for Brucella species confirmed with IS711 pre-published primers and 1.5% agarose gel electrophoresis (data not shown and published earlier). Brucellosis plays a role in the remarkable disturbance of the biochemical parameters in the blood of the diseased camels and man; this reflects a disturbance in the general health condition of the host.[52] The references of serum biochemical parameters of brucellosis in camels are extremely poor. The determination of such biomarkers can provide consistent hint to elucidate the pathophysiology of the brucellosis in camels. This investigation was carried out to examine the alterations in the hepatic marker enzymes, renal biomarkers, blood metabolites, and mineral contents in the serum of camels infected with Brucella. Concentrations of normal blood components may give a insight to the health status of the livestock animals. The determination of several blood ingredients were utilized to diagnose the occurrence of diseases,[53] growth traits,[54] and milk production.[55] In the present research work, we have collected 200 blood samples from both healthy camels and brucella infected camels. The brucella infection was confirmed by using the several serological tests such as cELISA, BAPAT, RBT, and antigen suppression tests (data not shown here and published previously). Blood components was used to examine metabolic level of animals and to diagnose pathological status like certain transmittable diseases like brucellosis. Therefore, here we investigated the levels of blood metabolites such as glucose, total protein, albumin, and globulin in the serum of both healthy and brucella infected camels. Our results clearly demonstrated that the brucella infected camels exhibited the decreased concentrations of glucose, total protein, albumin, and globulin when compared with the healthy controls (Figure 4). These results indicates that the brucella infection can adversely alter the blood metabolite concentrations in the camels.

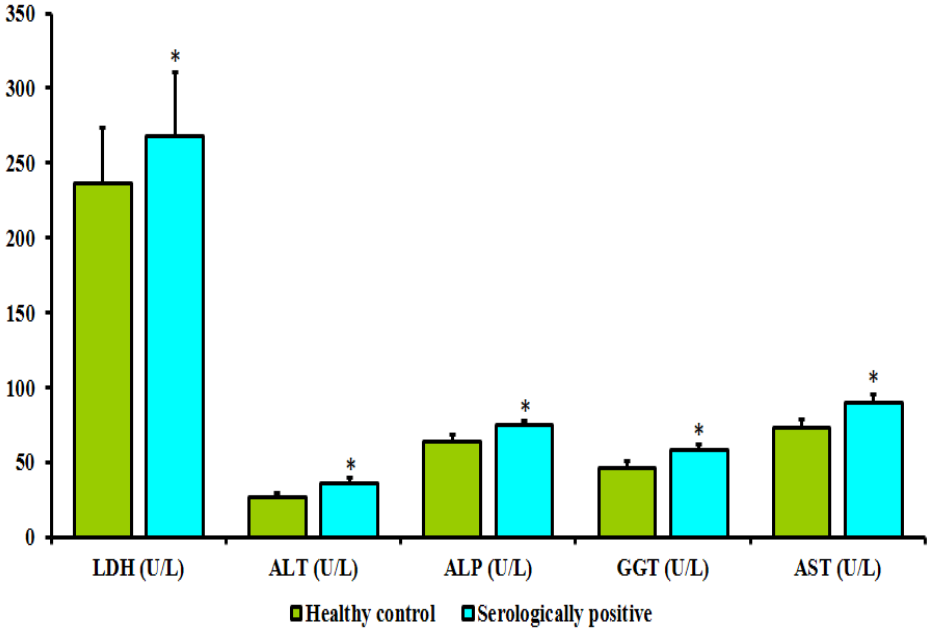


Each bar represents the mean±SD of three independent assays, which is examined by one-way ANOVA and Tukey's post hoc assays. Note: '*' indicates that values are significantly differ at p<0.05 from healthy control group.

Fig 4: The levels of glucose, total protein, albumin, and globulin in the serum of healthy control and brucella infected camels

The outcomes of the current study were matches with the earlier research study.[56,57] They also reported the altered blood metabolite concentrations in the serum globulin level in the Brucella affected cattle. The decreased status of glucose in the serum of camels, which observed here may be due to the

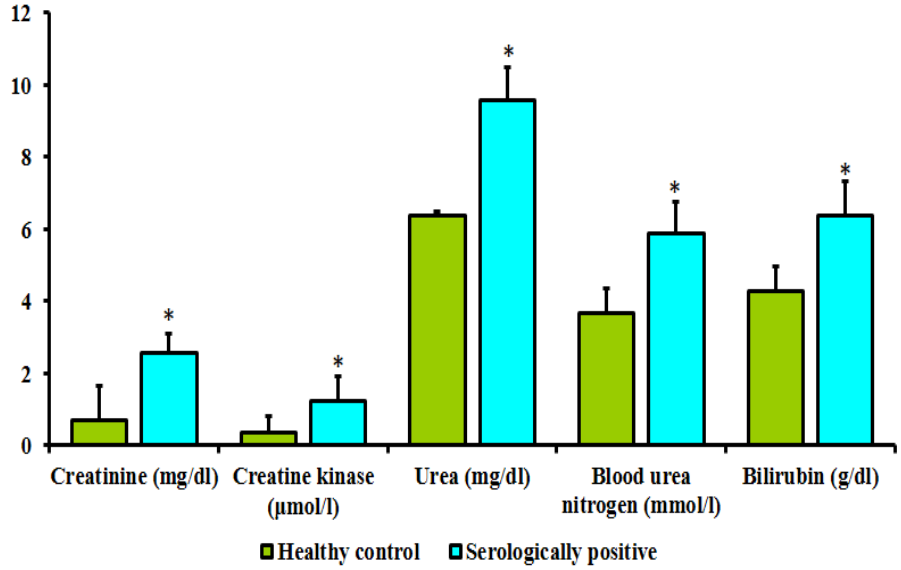
deficiency of energy and proteins in the diet due to the brucellosis infection.[58] Regarding the biochemical changes in the camels with brucellosis the available literature is poor. The liver is the most important organ and the disturbance in hepatic function which may be accompanied with brucellosis in camels have serious consequences for the productivity and reproductively of the diseased animals.[59] Brucellosis can impair liver metabolism.[60,61] In the current work, we have demonstrated that the hepatic marker enzyme activities such as AST, ALT, ALP, LDH, and GGT was found up-regulated in the serum of brucella affected camels when compared with the healthy control. The remarkable changes were noted on the activity of these marker enzymes when compared with the healthy controls. These outcomes evidenced that the brucella infection can adversely impair the liver functions by deregulating these marker enzyme activities (Figure 4). The increased activities of LDH and AST enzymes were already reported in the cattle positive for brucellosis. Generally increased LDH activity could be a useful indicator of hemolysis, muscle damage, cardiovascular, hepatocellular injury and uterine and placental pathology.[62] Furthermore, GGT is a membrane-bound enzyme, which represents the function of hepatic and renal tissues. The notable increase in the GGT activity are already highlighted in the several infectious disease conditions. The boosted activity of GGT is frequently believed as a reliable diagnostic marker than ALP during the diagnosis of several diseases in cattle.[63] The hepatic damages caused by the infections and other diseases are usually predicted by measuring the serum activities of the aminotransferases such as AST, ALP, and ALT.[64] Here, the boosted activities of AST, ALT, ALP, LDH, and GGT in brucella infected camels than healthy control was observed (Figure 5), as indicated by El-Boshy et al.[7] in Brucella affected camels. Changes in the concentration of creatinine, creatine kinase, BUN, urea, and bilirubin depend not only on the renal dysfunction. The raised status of BUN, urea, and creatinine in the serum of camels are represent the impairments in the kidney functions in response to the infections or other diseases.



Each bar represents the mean±SD of three independent assays, which is scrutinized by one-way ANOVA and Tukey's post hoc assays. Note: '' indicates that values are significantly differ at p<0.05 from healthy control group.*

Fig 5: The activities of liver function marker enzymes in the serum of healthy control and brucella infected camels

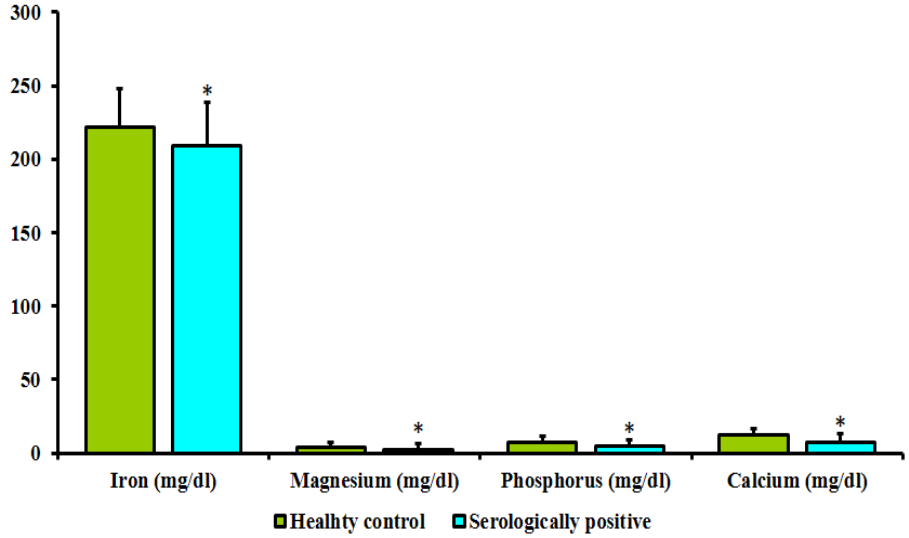
In this research work, we evaluated the levels of kidney function markers such as creatinine, creatine kinase, bilirubin, urea, and BUN in the serum of healthy and brucella infected camels. Our results demonstrated that the brucella infected camels displayed the increased levels of kidney function markers such as BUN, urea, creatinine, and creatine kinase in the serum when compared with the healthy control (Figure 6). These outcomes provide as evidence that the brucella infection can interfere with kidney function and increase the levels of BUN, urea, creatinine, and creatine kinase in the serum of camels.



Each bar represents the mean±SD of three independent assays, which is scrutinized by one-way ANOVA and Tukey's post hoc assays. Note: '' indicates that values are significantly differ at p<0.05 from healthy control group.*

Fig 6: Levels of BUN, creatinine, bilirubin, creatine kinase, and urea in the serum of healthy control and brucella infected camels

The amounts of creatinine generation mainly relies on the total content of creatine in the body through dietary uptake and creatine generation rate.[20] The camels has a strong mechanisms of recycling of urea, which can recycle nearly 90% of BUN. The nitrogen recycling in camels augments during the period of infection.[18] Camel have peculiar anatomical arragements in the renal system.[65,66] Here, our findings revealed that the levels of BUN, urea, creatinine, and creatine kinase were found increased in the serum of brucella affected camels than in healthy controls (Figure 6). Our present outcomes were supported by the earlier literature.[67] It is well known that all living organisms need trace elements (minerals) for survival and replication. As a defense mechanism, the body cells minimize mineral utilization by microbes to control its survival and replication.[68] mammalian cell has homeostasis mechanism by binding minerals in certain proteins keep them non toxic.[69] In accordance with this statement, here we examined the mineral contents such as iron, magnesium, phosphorus, and calcium in the serum of both healthy control and brucella infected camels. Our results exhibited that the levels of iron, magnesium, phosphorus, and calcium were found decreased in the serum of brucella affected camels than in normal healthy control (Figure 7). These results proved that the brucella infection can decrease the mineral contents in the serum of camels as a response to the infection.



Each bar represents the mean±SD of three independent assays, which is scrutinized by one-way ANOVA and Tukey's post hoc assays. Note: '' indicates that values are significantly differ at p<0.05 from healthy control group.*

Fig 7: The levels of mineral contents in the serum of healthy control and brucella infected camels

The infection prevention and control practices should include measures to address human and animal infections. For human infections, ongoing surveillance, including the education of the medical staff for the prompt diagnosis and treatment of patients and the education of the population about the disease and its prevention should be considered. For animal health, the serologic surveillance of brucellosis and the immunization of herds constitute essential measures.[70]

5. CONCLUSION

The results of this study provides a new insight into the cultural identification of Brucella in the dromedary camels of Qatar. For the first time, we revealed the presence of *B.melitensis* in the camels of Qatar using microbial culture techniques. However, the further molecular level identification tests such as 16sRNA sequencing still require in the future. We also found that the Brucellosis can adversely alter the blood metabolites, liver function marker enzyme activities, kidney function markers, and mineral contents in the serum of camels. The present study provides an insight that Brucellosis can cause both renal and hepatic toxicity to the camels, which further worsen the health status camels and may responsible for the major economic losses. Therefore it was concluded that the examination of biochemical parameters in the serum of camels can provide a key information to diagnose the extents of damages done by the Brucella organism.

5.1 Availability of data

All the associated data are available from the corresponding author based on the reasonable request.

6. ACKNOWLEDGEMENT

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7. CONFLICT OF INTEREST

Conflict of interest declared none.

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