

Detection and molecular identification of *Brucella* species infecting Camels in Darfur, Sudan**Zienab A.A.Abdallah^{1*} and Rania M. H. Baleela²**

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Abstract

Brucellosis is a wide spread zoonotic disease which is characterized by reduced fertility and abortion in animals and in humans. Although not the primary hosts, camels are highly susceptible to *Brucella abortus* and *B. melitensis*. Camel meat and milk are consumed raw some times in Sudan, which may increase the prevalence of brucellosis.

The aim of this study was to screen camels from three districts of Nyala in South Darfour, Sudan for brucellosis. A sensitive specied-specific PCR assay was adopted for the detection and identification of *Brucella* spp. in 100 blood samples and 30 milk samples.

41 camels were positive for *Brucella* spp. Out of which, 44% were positive for *Brucella abortus*, 43% were positive for *B. melitensis*, and 22% were positive for a co-infection of *B. abortus* and *B. melitensis*. The majority of infected samples were collected at the slaughterhouse from camels to be consumed.

The finding of *Brucella* spp in camels' meat and milk is alarming as Sudanese consumers eat raw camels' liver and drink unpasteurized milk. Therefore the main recommendation of this study is to adopt a sensitive and species-specific PCR protocol for routine detection of brucellosis especially in animals to be slaughtered.

Keywords:

Brucella; species-specific primers; Brucellosis; Camels; Diagnosis; Darfur; Sudan

1. Introduction

Several pastoral groups and communities all over the world depend on camels for their

livelihood. The health status of camels has not yet received proper attention from

researchers and scientists though (WHO, 1997).

Old world camels are highly susceptible to *Brucella abortus* and *Brucella melitensis* but are not primary hosts. Thus, camel brucellosis depends on the *Brucella* species prevalent in other animal species sharing the same habitats, and on husbandry methods too. The main species affecting humans are *B. abortus* and *B. melitensis*, which cause brucellosis (Gwida, 2010). Consumption of *Brucella* infected milk and meat from camels has led to a high number of human brucellosis cases and is a serious public health issue. Reliable and sensitive diagnostic tools play a crucial role in the

control of brucellosis in livestock, wildlife and humans.

The aim of this study was to screen some Sudanese camel herds from Darfur for the presence of brucellosis and to identify which species are present.

2. Materials and Methods

Ethical clearance for this study was obtained from the Directorate of Animal Health & Epizootic Disease Control, Ministry of Animal Resources & Fisheries. A total of 100 blood samples and 30 milk samples were collected from apparently healthy camels at three locations in Nyala including Nyala Slaughterhouse, Soug Almawashey and an area near Nyala called Taysha (Figure 1).



Figure 1 sample collection. 1 Taysha area and . 2 soug Almawashey

Ten ml blood samples were collected in EDTA tube under hygienic sterile

conditions. Plain tubes were used to collect 7 ml of fresh milk from each animal. Samples

were kept on ice and transported to the laboratory. Blood and milk samples were stored at -20 °C for further investigation. Filter paper samples were stored at room temperature after being dry.

A modified phenol / chloroform method was used to extract genomic DNA. Briefly, 1ml of blood was washed with Red Blood Cells lysis buffer (NaHCO₃ 0.0841g, NH₄Cl 6.151g 1000ml distilled water) in falcon tube two times centrifuged in 60000 rpm for 5min then left in 1ml lysis buffer overnight at 60°C. for filter paper samples three 1cm pieces were add to 500µl lysis buffer (15.76g Tris-HCl (pH 8), 10% SDS, and 1.8612g EDTA in 1000ml distilled water) left overnight at 60 °C .500µl of Phenol/Chloroform/Isomyalcohol (25: 24: 1) was added to each sample then centrifuged for 5mn at 10000rpm , supernatant was then transferred to another tube and 500µl of Chloroform/Isomyalcohol (24: 1) was added and centrifuged at 10000rpm for 5min .Then 100 % ice cold ethanol was added to transferred supernatant and samples were left at -20°C overnight. Precipitated DNA was centrifuged at 14000 rpm for 10 min and washed with 70% ethanol two times. The supernatant was discarded and tubes were left up- side-down to allow precipitated DNA to dry for 40 min inside an incubator. Extracted DNA was dissolved in 100µl double distilled water;

concentration was quantified and stored for further investigation at -20°C. DNA from milk samples was extracted using the same protocol except for the use of RBCS lysis buffer.

For the detection of *B. abortus*, the following set of primers was used with an expected product size of 494bp: forward 5'-GACGAACGGAATTTTCCAATCCC-3' and reverse: 5'- TGCCGATCACTTAAGGGCCTTCAT-3'. For the detection of *B. melitensis* the following set of primers was used with an expected product size of 733bp: forward 5'-AAATCGCGTCCTTGCTGGTCTG-3' and reverse 5'-TGCCGATCACTTAAGGGCCTTCAT-3' (Khamesipour *et al.*, 2014). For a total volume of 25 µl, 2 µl primer, 2 µl template DNA were added to 5 microliter iNtRON Maxime™ PCR premix and 16 µl double distilled water.

Conditions were as follow: preheated to 110°C, 95°C for 5 min; 35 cycle were performed: 95°C for 1 min, 65 °C for 1min, 72°C for ,1 min extension at 72°C for 7 min, with a final hold at 4°C.

The product was then visualized using a 2% (w/v) agarose gel dissolved in 0.5× TBE buffer (743 mMTris-HCl (pH 8), 87 mM boric acid, and 5.3mM Na₂EDTA), stained with ethidium bromide.

3. Results:

Out of the 100 camels sampled here, 44% were females and 56% were males. 90% were adults and 10% were youngsters.

41% of the samples were positive for *Brucella sp.* Infected camels were basically adults (99%) and males (93%).

Out of the positive samples 44% were positive for *B. abortus*, 34% were positive for *B. melitensis* and 22% were positive for both species (Figure2).

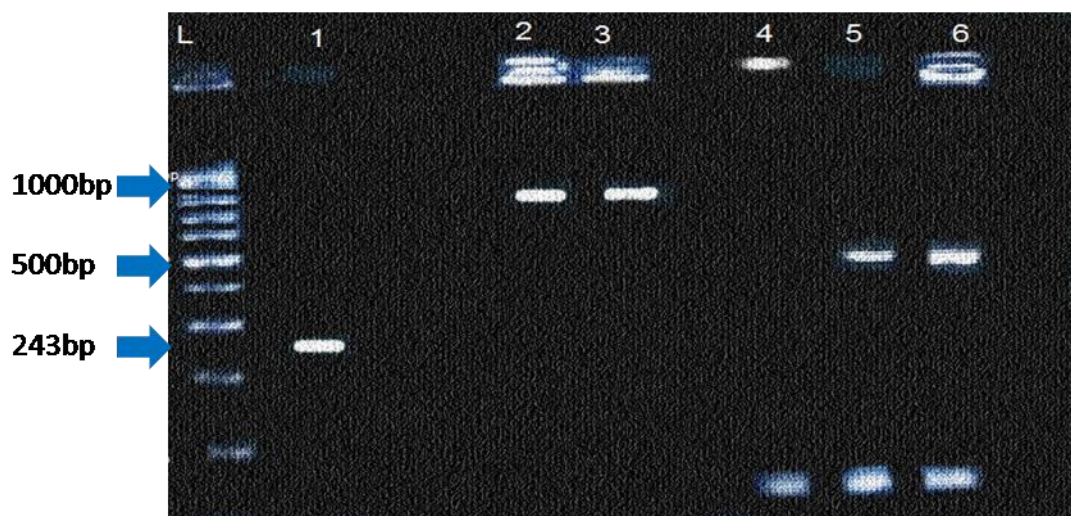


Figure-2 Detection of *B. melitensis* and *B. abortus* in camel. L: DNA ladder (1000bp), 1 unidentified *Brucella spp* in blood (product size 243bp), 2 and 3: *B. melitensis* in blood, 4 negative control 5: *B. abortus* in milk sample; 6: *B. abortus* in blood sample

68 % of infected camels samples were collected from the slaughterhouse, 24% from Soug Blaial and 8% were from Taaisha herd.

Out of the infected males, 52% were positive for, *B. abortus* and 43% were positive for *B. melitensis*. 5% of the positive male samples showed different band size from the expected (500bp and 243bp). From

30 milk samples 6% were positive for *B. abortus*.

4. Discussion

Almost half of the samples (i.e 41%) were found positive for either or both of the *Brucella spp.* This is alarming.

The rapid detection of *Brucella* is important because it infects humans causing different clinical features and at late diagnosis in chronic cases can drive serious

complications as well as being considered a bioterror organism.

Isolation of bacteria using classical methods (i.e. media cultures) is cheap but time consuming and hazardous for laboratory personnel. On the other hand, rapid diagnosis with molecular methods may be expensive in Sudan; but fast and is getting cheaper and is useful to differentiate between two bacterial species, especially slow-growing ones.

In this study a PCR assay was used to detect and differentiate between two *Brucella* species: *B. abortus* and *B. melitensis* in blood and milk samples collected from camels.

Camels are economically very important in Darfur and can acquire the infection from goats reared by the same herd owner.

In Sudan, herders among others drink camels' non-pastoralized milk and eat uncooked liver. Most of the brucellosis-positive camels investigated in this study were apparently clinically healthy. Most of the *Brucella* positive samples in this study were collected from the slaughterhouse which is serious and may lead to increase the prevalence of Brucellosis if infected camel meat is consumed.

Therefore, there is a great need for developing a routine test for *Brucella* detection that is accurate, fast and cheap. In this study, milk and blood samples proved to be useful for detection purposes.

However, the majority of positive samples were male camels making the use of blood samples more useful. The primers used here are sensitive and specific making them useful for routine detection and differentiation of *Brucella spp* even in the field as samples collected from herds outside Nyala were actually transported and typed in Khartoum.

The PCR detection of *Brucella* performed in this study prove to be more reliable than classical routine because it is specific and minimizes contamination. This observation is in agreement with that of Kumar *et al*, (2011), Huber *et al*. (2009) and Mirnejad *et al*. (2012). Mohammad Hasani *et al.*, (2016) used a Multiplex PCR to detect *Brucella spp*. and showed its advantages over classical methods too.

Conclusions

Up to our knowledge, this is the first study in Sudan to use molecular genotyping to detect and differentiate between the two *Brucella spp.*: *B. abortus* and *B. melitensis* infecting Sudanese camel.

The method used here was highly sensitive and specific. We therefore recommend the use of this method for routine detection of such an important pathogen.

Special attention should be given to animals to be slaughtered because the majority of

samples found infected in this study were collected at the slaughterhouse.

Ethics approval

This study was approved by the Directorate of Animal Health & Epizootic Disease Control, Ministry of Animal Resources & Fisheries.

Availability of data and material

The datasets during and analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' Contributions

ZAAA and RMHB conceived the research problem, ZAAA collected samples and carried out the laboratory work, ZAAA and RMHB wrote the manuscript. All authors read and approved the final manuscript

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