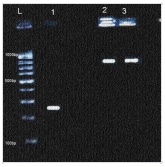

Jun 29, 2021

Brucella species detection from blood and milk samples

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dx.doi.org/10.17504/protocols.io.bskpncvn

Brucella sp detection

Rania Baleela

DISCLAIMER

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ABSTRACT

Brucellosis is a widespread zoonotic disease which is characterized by

reduced fertility and abortion in several species of animals and in human.

Camels are highly susceptible to *Brucella abortu*, and *Brucella melitensis* but they are not the primary hosts.

Consumption of *Brucella*infected milk and meat from camels is a serious public health issue though. Therefore rapid detection and diagnosis of

Brucellosis is crucial for any control program.

In this study, a sensitive and specific PCR assay was adapted for the detection and identification of *Brucellaspp*. in100 camels. Almost half of the collected samples were positive for *Brucellaspp*. (i. e. 41%). 44% of the samples 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 camels were male adults.Most of

the positive samples were collected at the slaughterhouse in Nyala. This

finding is alarming as Sudanese consumers are known for eating raw

camels' liver. Therefore the main recommendation ofthis study is toadopt this sensitive and species-specific PCR protocol for routine

detection of brucellosis in animals to be slaughtered in the future .

In the mean time this protocol can be used as a confirmatory test for the

detection ofbrucellosis in live camels as well as for other animals to be slaughtered because it is the protocol with the lowest risk for laboratory

personnel.

DOI

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EXTERNAL LINK

http://onlinejournals.uofk.edu/index.php/SJS/article/view/1395

PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Zienab A.A. Abdallah, Rania M. H. Baleela (2017). Detection and molecular identification of Brucella species infecting Camels in Darfur, Sudan. Sudan Journal of Science, volume 9 issue 2

IMAGE ATTRIBUTION

Zienab A.A. Abdallah

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Jun 29, 2021

PROTOCOL INTEGER ID

47471

MATERIALS TEXT

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mso-level-tab-stop:none; mso-level-number-position:right; text-indent:-9.0pt;} ol {margin-bottom:0cm;} ul {margin-bottom:0cm;} -->

Blood, Filter paper or milk samples

$NaHCO_3$

$NH4Cl_6$.

Distilled water

Tris–HCl

SDS

EDTA

Boric acid

Phenol

Chloroform

Isomyalcohol

Ethanol

iNtRONMaxime™PCR premix

Primers

Agarose

Ethidium bromide

Falcon tubes

Centrifuge

pH meter

-20°C Refrigerator

Incubator.

NanoDrop™ 3300 Fluorospectrometer

Fluorospectrometer

NanoDrop™ 3300 **nd-3300**

Thermal cycler

T100 PCR thermal cycler

BIO-RAD **https://www.bio-rad.com/pt-br/product/t100-thermal**

specific PCR product molecular size for each Brucella species

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DNA extraction: The phenol / chloroform method was used to extract genomic DNA.

1 Wash 1ml of blood with Red Blood Cells lysis buffer (NaHCO3 0.0841g, NH4Cl6.15135g 1000ml distilled water) twice centrifuge at 60000 rpm for 5min

2 Add 1ml lysis buffer and leave at 60°C overnight.

3 Add 500µl of Phenol/Chloroform/Isomyalcohol (25:24:1) to each sample then centrifuge for 5mn at 10000rpm ,

4 Transfer supernatant to another tube and add 500µl of Chloroform/Isomyalcohol (24:1)

5 Centrifuge at 100000rpm for 5min.

6 Add equal volume of 100 % ice cold ethanol and leave at -20°C overnight.

7 Centrifuge precipitated DNA at 14000 rpm for 10 min and wash with 70% ethanol two times.

8 Discard the supernatant and leave the tubes up- side-down to allow precipitated DNA to dry for 40min inside an incubator.

9 Dissolve the extracted DNA in 100µl double distilled water;

10 Quantify the concentration and store at -20°C.

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11 Same protocol is used to extract DNA from filter paper samples: add three 1cm pieces to 500µl lysis buffer (15.76gTris–HCl (pH 8), 10% SDS, and 1.8612gEDTA in 1000ml distilled water) leave overnight at 60 °C.

12 Same protocol is used to extract DNA from milk samples except for the use of RBCS lysis buffer.

Detection of Brucella spp: For a total volume of 25 µl:

13 5 µl iNtRONMaxime™ PCR premix

14 2 µl each primer (step 15),

15 2 µl template DNA

16 Brucella spp. Detection primers (adopted from Khamesipouretal., 2014)

B. abortus
Forward primer: GACGAACGGAATTTTTCCAATCCC
Reveres primer:TGCCGATCACTTAAGGGCCTTCAT
Expected molecular size 494bp
B. melitensis
Forward primer: AAATCGCGTCCTTGCTGGTCTGA
Reveres primer: TGCCGATCACTTAAGGGCCTTCAT
Expected molecular size 743bp

Conditions

17 Preheat lid to110°C,

18 95°C for 5 min;

19 35 cycle: 95°C for min, 65 °C for 1min, 72°C for ,1 min extension at 72°C for 7 min,

20 Final hold at 4°C.

21 **Visualization**:
2% (w/v) agarose gel containing 0.5× TBE buffer (743 mMTris–HCl (pH 8), 87 mM boric acid, and 5.3mM Na2EDTA), stained with ethidium bromide.

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