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Serological diagnosis and molecular detection of *Brucella* species in Camels in West Kurdufan state, Sudan

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Abstract:
The one humped camels (*Camelus dromedarius*) possess an economic importance in the Sudan as well as, in several other countries all over the world .Brucellosis is a worldwide zoonotic disease that is recognized as a major cause of heavy economic losses to the livestock industry and poses serious human health hazard.The aim of this study was to detect *brucella* in the camels blood . A total of 500 blood samples (serum and whole blood) were collected from Alnehood, Elkhwai and Ghubaish localities in West Kurdufan state and tested by Rose Bengal plate test (RBPT), Serum Agglutination Test(SAT) and polymerase chain reaction (PCR). Ninteen samples (3.8%), seventeen samples (3.4%) were found seropositive by RBPT and (SAT) respectively however, none of the sample was found positive by PCR. Despite the molecular method used in this study was highly sensitive and specific none of the sample was found positive for *Brucella* by PCR whereas nineteen and seventeen samples were positive by using Rose Bengal and Serum Agglutination tests SAT test respectively. The obtained results suggest that control and eradication programs for *Brucella spp*. infection seem to be necessary in camels.

Keywords: |PCR, Brucellosis, Camels.

Introduction:

The one humped camel (*Camelus dromedarius*) is an important livestock species in Sudan, Sudan represent the second largest camel population in the world, estimated at nearly 4, 700,000 head of camels (M A R F – 2012), which constitute a major source of income for their owners and consequently the national economy. They are concentrated in three main regions: theEastern and Western Regions Darfur and Kurdufan , the Butana plains andthe Red Sea hills.

Brucellosis is one of the most important bacterial zoonoses with a global distribution (Teshale *et al* .,2006;Young, 1995;Lopes R*et al.,* 2010; Angara and Ali 2014). The disease represent an occupational hazrd for veterinarians, farm workers, abattoir workers as well as laboratory workers (Madkour, 1992).It is an infectious disease, almost invariably transmitted by direct or indirect contact with infected animals or their products (Teshale *et al*., 2006). The disease is caused by*Brucella* species are small, nonmotile,

aerobic, facultative intracellular, Gram-negative coccobacilli bacteria which belong to the genus Brucella which includes *Brucella melitensis* and *B. ovis* as well as many other species (Lopes*et al.,* 2010).

The disease in dromedary camels can be caused by *B. abortus, B. melitensis* and *B. ovis* (Seifert, 1996). Different studies showed that *B. abortus* and *B. melitensis* are the most frequent isolates (Radwan *et al.,* 1992; Gameel *et al*., 1993; Agab *et al.*, 1994;Abou-Eisha, 2000 and Hamdy and Amin, 2002).

Recently progress has been made in applying new molecular and genetic diagnostic methods to improve the diagnosis of brucellosis and nucleic acid amplification techniques might circumvent the diagnostic window being presented before production of specific antibodies (Bricker BJ,2002;Ghorbani *et al*., 2013; Gwida *et al* ,.2011).

Nucleic acid amplification methods, such as polymerase chain reaction (PCR), are rapid, sensitive, and highly specific and can counteract limitations of conventional detection methods (Elfaki *et al* .,2005; Wareth *et al* .,2015). PCR considered an additional means for detection of the presence of *Brucella* DNA in a sample , furthermore can provide a complementary identification and typing method based on specific genomic sequences.(OIE2018)

Biotyping and molecular characterization provide valuable epidemiological information to know the sources of infection in outbreak scenarios and the strain diversity in endemic regions (Kattar *et al*., 2008; Álvarez *et al*., 2011).

Six species are recognized within the genus *Brucella* and classified according to the differences in host preference and in pahogenicity to *Brucella abortus* , *Brucella melitensis* , *Brucella suis* , *Brucella ovis* , *Brucella canis*, and *Brucella neotomae* (Alton*et al*.,1988; Corbel *et al*., 1984).

However their overall characteristics are not as similarly to those of any of the six recognized *Brucella* species(Jahanas *et al*., 1997;Clavareau *et al*., 1998; Cloeckaert *et al.*, 2001).

Species identification and sub-typing of *Brucella* isolates are very important for epidemiologic surveillance (to know the species and/or biovar diversity) and investigation of outbreaks (to know the source of infection) in *Brucella*-endemic regions (Al Dahouk *et al.*, 2007; Marianelli *et al.*, 2007) The objective of this study was to determining the prevalence and potential risk factors of *Brucella* spp in the camels in West Kurdufan State by using serological and PCR methods and identification of *brucella* species in blood of camels

Material and methods:

Study Area:

The study was carried out in three localities of West Kurdufan State in Sudan namely, Alnehood, Elkhwai and Ghubaish localities.

West Kurdufan State located within latitudes 27°-29°N, and longitudes 14°-20°E. The state borders North Kordofan, South Kordofan, East Darfur, North Darfur and South Darfur. Its area is 14400 square kilometers extending from low rainfall savanna to high rainfall and hill catena and its vegetation varies greatly (Alshareef, 1994; IFAD, 2003;)

Study Population and design:

Apparently healthy camels(500 animals)with no history of vaccination against brucellosis were randomly selected in three localities. Multistage random sampling was designed

based on, locality, herd and animal. Selection between localities, herds and individual animals based on simple random sampling.

Sample Size:

The sample size of the study animals was determined by using the formula given for simple random sampling method. (Thrusfield, 2007).

The expected prevalence in the present study was estimated as 50%. This was based on a previous study with prevalence rates ranging from 1.4 to 89.5% (Musa and Shigidi., 2001**)**.

Sample collection:

In the period between September 2018 and April 2019, approximately a total number of 500 blood samples were collected aseptically from apparently healthy camels *(Camelus dromedaries)*from Alnehood (245 animals), Elkhwai (43 animals) and Ghubaish(212 animals) in West kurdufan state ,each sample divided to two tubes ,the first one plain tube with serum clot activator for serological and second one EDITA tubes for molecular examination.

About 7 to 10 ml of blood was collected aseptically from the jugular vein of camle,the samples in plain tubes were kept in an upright position at 25 ºC for about 2 hours, sera were separated by centrifuging at 6000 x g for 10 min. The separated serum was collected in a screw capped plastic vials and transported to the laboratory of the Veterinary Research Institute (VRI)in El-Obeid where they were stored at -20ºC till used. Whole Blood samples were kept in the refrigerator (2–8 °C) and one day later Convenient amount ofwhole blood droped in a screw capped plastic vials and transported to the central Veterinary laboratory for molecular examination.

A questionnaire was completed Information of each herd and each camel sampled was obtained by by interviewing the herdsman or owner to identify possible location, age, sex(male and female), whether reared individuallyor contact with other ruminant species (yes and no), contact with other camel herds (yes and no).

Serological test:

RBPT: (Rose Bengal Plate Test):-

This test was carried out at the laboratory of the Veterinary Research Institute (VRI), El-Obeid, Sudan.The test procedure was conducted as recommended by Alton *et al*., (1975). Thirty µl of RBPT antigen were dispensed to each circle on the plate and 30 µl of the antigen was added after the antigen bottle was shaken to ensure homogenous suspension, the antigen and test serum were mixed thoroughly by wooden a spreader. The plate was shacked gently for 4 minutes and the degree of agglutination reactions were noted immediately after 4 minutes and recorded as 0 (no agglutinations) in negative reactions, + (weak fine agglutinations using magnifying glass) , + + (visible fine agglutination) , + + + (clumping and some clearing)and + + ++ (coarse clumping and clearing) in case of positive reactions.

Molecular Test

All (500) whole blood samples were tested by PCR for detection of brucellosis. For DNA extraction method (Unver.,*et al* 2006). Multiblex PCR used for the detection of *B. abortus*, the following set of primers was used with an expected product size of 494bp: forward 5'GACGAACGGAATTTTTCCAATCCC-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'
According to Khamesipour *et al*., (2014).

Statistical analysis

Statistical analysis was performed using 'Statistical package for the social sciences' (SPSS), version 26.0 software for windows (SPSS Inc., Chicago, IL, USA).by using the Chi square test.

Result:

Out of 500 camel blood samples examined In this study,19(3.8%) ,17 (3.4%)were Positive by using RBPT, (SAT) respectively .Whereas none of them was found positive by PCR.

This study carried out in three localities in the West kurdufan State- sudan namely , Alnehood, Elkhwai and Ghubaish localities.The seroprevalence of the disease was higher in Elkhwai (16.2%,N=7), Alnehood (3.4%,n=9),Ghubaish (1.5%,n=3). There was statistical significance between three localities (p.value=0.000).

Out of 19 positive samples(4% n=14) were females and(3.6 % ,n=5) were males .Depend on the age factor camels divided to three age groups :young group were considered under 5 years ,medium(5-10year) and old age group(over 10 year) old,the higher percentage of positive samples found in medium age group camles(4.1%,n=15) ,and lower percentage found in old age group (2.6%,n=1),whereas young group was(3.2 %,n=3). There was no statistical significance between age groups (p.value=0.843).

Out Of the 500 camels sampled(32.6 % , n=163) kept with other ruminants(sheep and goats) ,whereas (67.4 % , n=337) kept without other ruminants. Camels with other ruminants showed seroprevalence (5.6%,n=9) % higher than that in camels kept alone (3 % ,n=10). There was statistical significance between the two categories (p.value.0.011) , and (30.4 % ,n=152) out of total camels tested were in contact with other camel herds, while the remain of camel herdsrepresent (69.6 % ,n=348)of camels tested .The positive samples in the first group (in contact) was(7.3%,n=11) and in the second group (not in contact) was(2.3 % ,n=8). These finding showed statistical significance between the two categories (p.value.0.000) .

In the present study three of risk factors showed $P<0.05$ in analysis by Chi-square (contact with other camels herds P=0.000, localities P=0.000 and contact with other ruminant species P=0.011),furthermore the three variables were analyzed by using the logistic model and revealed statistical significant with the occurrence of the disease ($p<0.05$) .

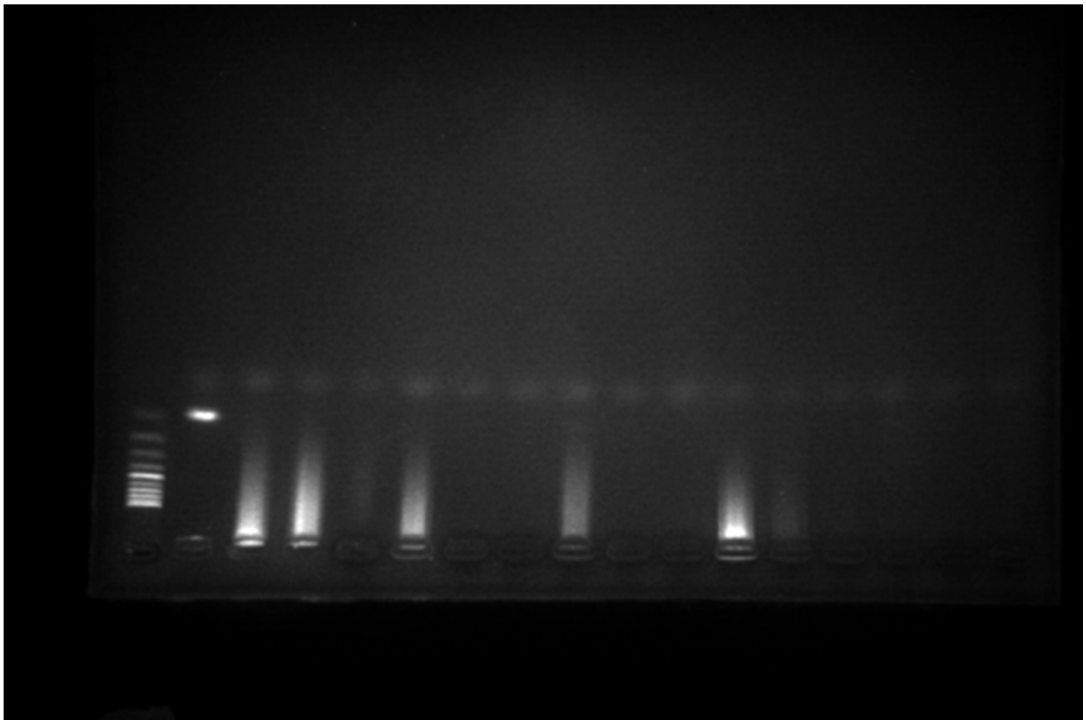

Figure 1. Ethidium bromide-stained agarose gel electrophoresis of PCR products (100 bp) for the detection of *Brucella* spp. in camel samples after PCR amplification.
Lane 1: 100 bp DNA ladder (Fermentas, Germany); lane2: positive control ;lanes 3to 16 negative sample; lane 17: Negative control.

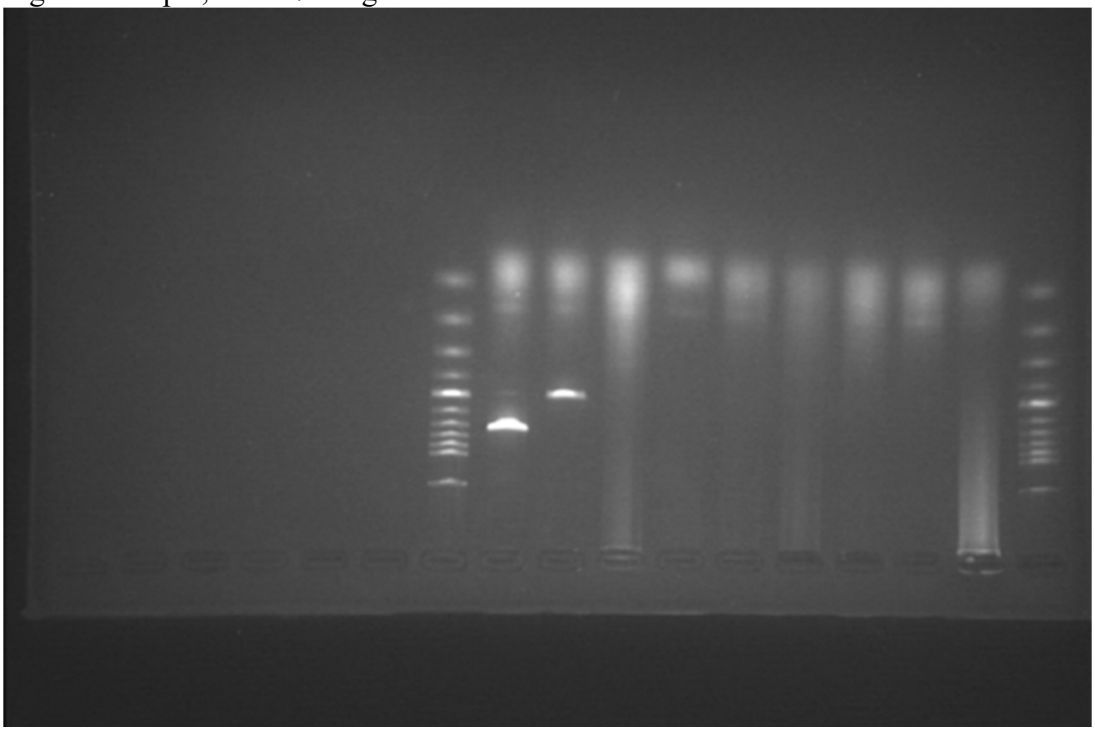

Figure 2. Ethidium bromide-stained agarose gel electrophoresis of PCR products (100 bp

) for detection of *B. abortus and B. melitensis* in camel samples after PCR amplification. Lane 1 and 11 : 100 bp DNA ladder (Fermentas, Germany); lanes 2 and 3 positive control (lane 2: *B. melitensis* in camel; lane 3: *B. abortus* in camel); lane 4: Negative control; lanes 5 to 10 negative samples

Discussion:

In this study the result show 19 samples out of 500 camels sera were positive for brucellosis whereas no positive samples detected by PCR from blood of 500 camels . This result in accordance of finding of UllahS*et al.,*(2015) which detect 0.00% positive sample from seropositive camles blood by using PCR ,this may attributed to present of antibodies in healthy camels or oscillating of immunoglobulin titers(Gwida *et al*., 2011). It is noteworthy to mention that all serologically positive camels were clinically normal at the time of sampling.

Also our findings in agree with (Abdelgawad *et al*., 2017) who found five camels where positive for serological tests (BAPAT ,RBPT and CFT) but negative for PCR. different result were obtained by (Abdallah & Baleela, 2017) who detected 41 out of 100 camels were positive for Brucella spp by PCR.

Kaushik.,*et al* 2006 found Only 18 samples were positive by PCR compared to 62 by RBPT and 41 by i-ELISA and attributed the Wide variation in samples detected to many factors. PCR detects DNA, which may be in low quantity in blood samples even though antibody titer is quite high.

In this study and based on the results of RBPT, the prevalence of Brucellosisof examined camels was 3.8 %. This was in accordance with those recorded by Ghanem*et al*.,(2009);Graber (1968)and Jawad (1984).

The prevalence of brucellosis in camels kept with other ruminants (cattle, sheep and goats)was in significant statistics (P=0.011).Camels reared with other ruminants showed seroprevalence of (6.7% ,n=12) higher than that in camels kept alone which was (2.2 % ,n=7). This findings agreed with that reported by Teshome *et al*., (2003);Al-Majali *et al*., (2008);Mohammed *et al*., (2011) andMohamed*et al* .,(2015).

The present result is in accordance with that recorded by Radwan *et al*., (1995). Frequent isolation of *Brucella melitensis* from camels further suggests the role of small ruminants in the occurrence of camel brucellosis.Also Abbas *et al*., (2002) and Al-Majali *et al*., (2008) suggested the role of small ruminants for dissemination of infection with *Brucella.*

Camels within herds contact other camels in pasture and point of water showed prevelance of the disease (9.2%) higher than individual in herds not in contact(1.2%)with statistically significant difference P=0.000.Spread of the disease is due to movement of infected animals to disease free herds get the infection from infected herd at water points where a number of herds come together .

Conclusion:

The appearance of *Brucella* antibodies in blood of camels indicated the present of disease within the camels herds in West Kurdufan state despite there was no molecular detection for *Brucella* ,so More studies need to be carried in camel brucellosis in addition to control program.

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