Molecular detection of *Brucella canis* in Blood of Dogs

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Abstract

The aims of this study was to evaluate a PCR for detecting *Brucella canis* in the blood of dogs, using a primer pair designed for *Brucella* spp.

A study was conducted on 150 blood sample collected from dogs suspected to Veterinary Hospital in Baghdad / Aden Square. All blood samples (150) were tested by PCR technique using a common primer of the 23S ribosomal RNA (23s RNA) gene and specific primer for *brucella canis* (B0548). The genomic DNA was extracted and PCR was applied. Our study recorded 5.3% of brucellosis in common primer and 3.3% in specific primer for *brucella canis* in dog in Baghdad city, the sequences of *Brucella canis* in dog in different isolates in our study recorded 99% compatibility recording to National Center Biotechnology Information (NCBI). Following correspondence from National Center for Biotechnology Information, the 23S ribosomal RNA gene was registered, given an agreement number, and became a resource for Iraq and Middle East, as well as the rest of the world. As more type strains are published, this set will grow, and it can be download from NCBI at: https://www.ncbi.nlm.nih.gov/nuccore/.

From this study we can conclude that, the percentage of Brucellosis in dogs in Baghdad city is 5.3% and 3.3% in a common and specific primer, respectively and the molecular method (PCR), is a good idea for confirmation of diagnosis of *Brucella canis* infection in dog.

Keywords: *Brucella canis, dogs, Iraq*

Introduction

Brucellosis is a disease of animals and humans caused by Gram negative, facultative intracellular bacteria of the genus *Brucella.* Of the 12 currently recognized species, four are considered zoonotic pathogens: *Brucella melitensis, Brucella suis, Brucella abortus, and Brucella canis* in decreasing order of pathogenicity. Canine brucellosis, caused by Brucella canis, is a worldwide disease of dogs that primarily results in reproductive disease and may be transmitted to humans[1,2] . Canine brucellosis were first recognized in 1966[3,4].

In bitches, this manifests as abortion and stillbirths and in males, predominant symptoms include prostatitis and epididymitis[5] . Like all *Brucella* species, *Brucella canis* invades via the conjunctival, oronasal, or venereal route and distributes to organs of the reticuloendothelial system, resulting in a chronic, persistent infection[6]. Clinical signs may not become apparent in infected dogs for months to years after infection, making it difficult to implement control measures and avoid spread of disease to other dogs and humans[7].

Materials and Methods

Methods

Clinical examination

Clinical examination was carried out to all animals prior to sample collection, which includes pulse rate, rectal temperature and respiratory rate, The case history was taken which include appetite and other signs, while age and Sex was recorded for each animal.

Animals and Data collection:

The number of sick cases of domestic and police dogs admitted to the Baghdad veterinary hospital were 150 animals in different Ages, sex and breed with variable cases, the study period extend from November 2019 to July 2020 by two visits weekly.

Blood sample collection:

Two milliliter of Whole blood samples with EDTA anticoagulants tubes were collected from 150 dog from cephalic vein. All sample was stored in deep freeze until used for DNA extraction. All samples were transferred in a cooling box to department of internal and preventive veterinary medicine/ college of veterinary medicine/ university of Baghdad.

Results and Discussion

Molecular identification of *Brucella Canis* in dogs

Brucella canis is a Gram-negative organism infecting, mainly, the genital organs of both sexes and resulted in several reproductive problems[8].

All blood samples (150) were tested by PCR technique using a specific primer of the 23S ribosomal RNA (23s RNA) gene. The genomic DNA extraction was given concentrated DNA range of 1.5 µl of genomic DNA were used for each PCR reaction, with purity reached at 260/280 nm and read by a Nanodrop.

Table (1) has shown that the percentage of Brucella Canis in dogs by PCR in Baghdad Governorate. This table show that from 150 samples, 8 (5.3%) Positives and 142 (94.7%) Negatives which found locally in Iraq in common primer, while 5 (3.3%) positive and 145 (96.7%) negative as shown in table (2)

Table (1): percentage of *Brucella Canis* in dogs by PCR (common primer)

No.	Number of samples	Positive (%)	Negative (%)
1.	150	8 (5.3%)	142 (94.7%)

Table (2): percentage of *Brucella Canis* in dogs by PCR (specific primer)

No.	Number of samples	Positive (%)	Negative (%)
1.	150	5 (3.3%)	145 (96.7%)

In recent years, several studies were carried out to evaluation of PCR in diagnosis a specific DNA for *B. canis* in depending on different samples[9,10]. Aras and Uçan, [11] demonstrated that PCR technique had a detectable effectiveness equally for bacteriological culture in diagnosis of brucellosis with extra advantages including the fastness, speediness in performance, absence of riskiness with the highly sensitivity and specificity.

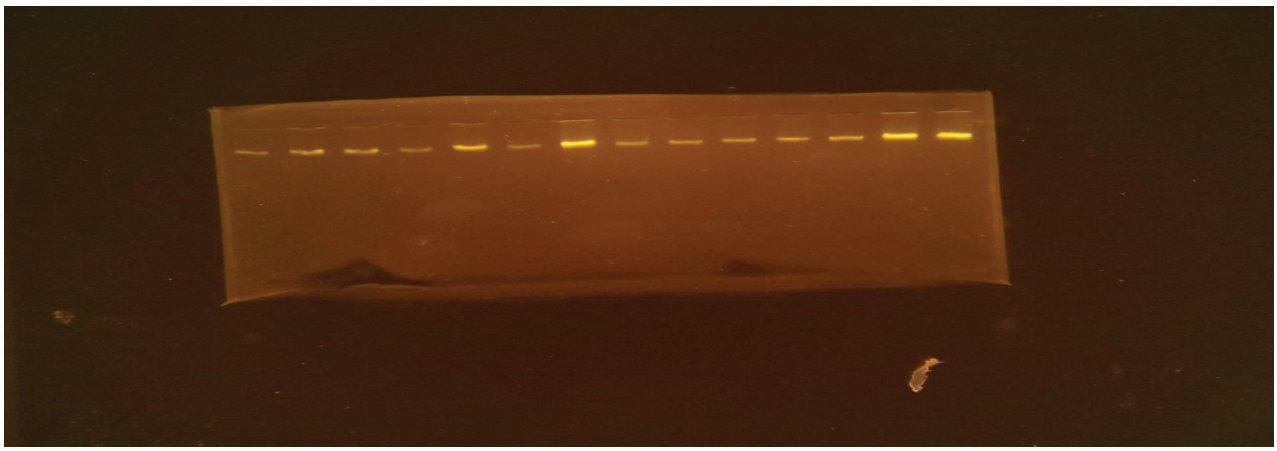
The current results showed that the prevalence of B. canis was 5.3%, this result was slightly agreed with a result of Alfattli [8] who found that the prevalence of B.

canis in Iraq by using PCR was 3.05%. Also, Tamimi and Wali [12] showed that prevalence of canine Brucellosis in Iraq was 2.7%.

The present result was also in agreement with results of Kang et al. [13] who reported that the prevalence of B. canis in dogs was 8.5%.

DNA Extraction:

The wizard genomic DNA purification Kit (Promega/ USA) is used to DNA isolation from the blood in a short time. All isolates showed bands, which indicated the genomic DNA on Agarose Gel Electrophoresis. As seen in figure (1).



Figure(1): Agarose gel electrophoresis of genomic DNA on 1%agarose gel, (5 Volt/30 minute).

***23S rRNA* Genetic Polymorphism.**

One and half µl of genomic DNA were used for each PCR reaction. A conventional PCR protocol was used to analyze simultaneously the presence of *23S ribosomal RNA (23s RNA) gene* of *Brucella Canis*. The presence of the *23s RNA* gene was identified by 214 bp, as shown in figure (4.2).

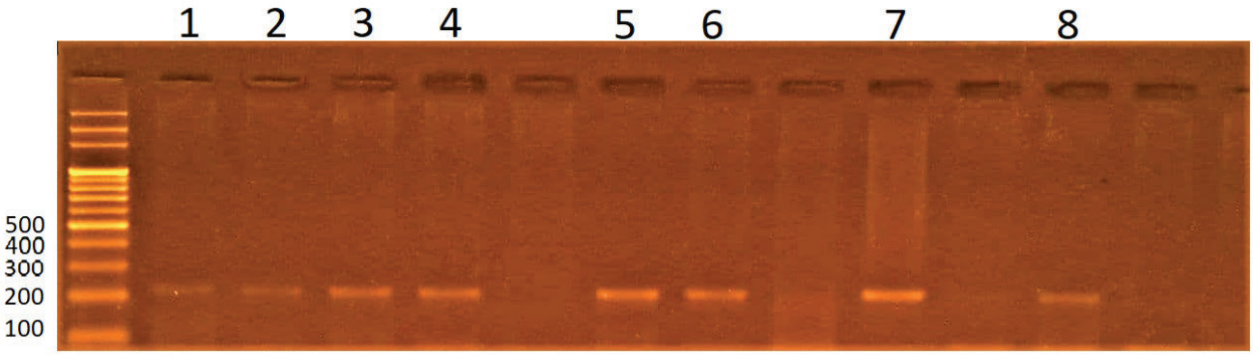


Figure (2):- Agarose gel electrophoresis for *23S ribosomal RNA* gene (214bp) of *Brucella Canis*. Bands were fractionated by electrophoresis on a 2% agarose gel (2 h., 5 volt/cm2, 1X TBE) and visualized under U.V light after staining with Ethidium bromide stain. Lane M: DNA ladder (100bp).

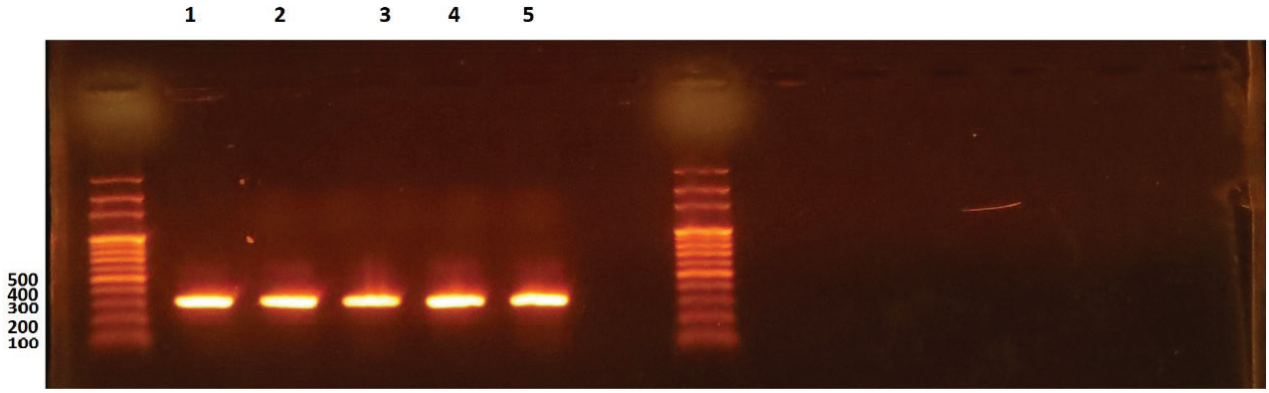


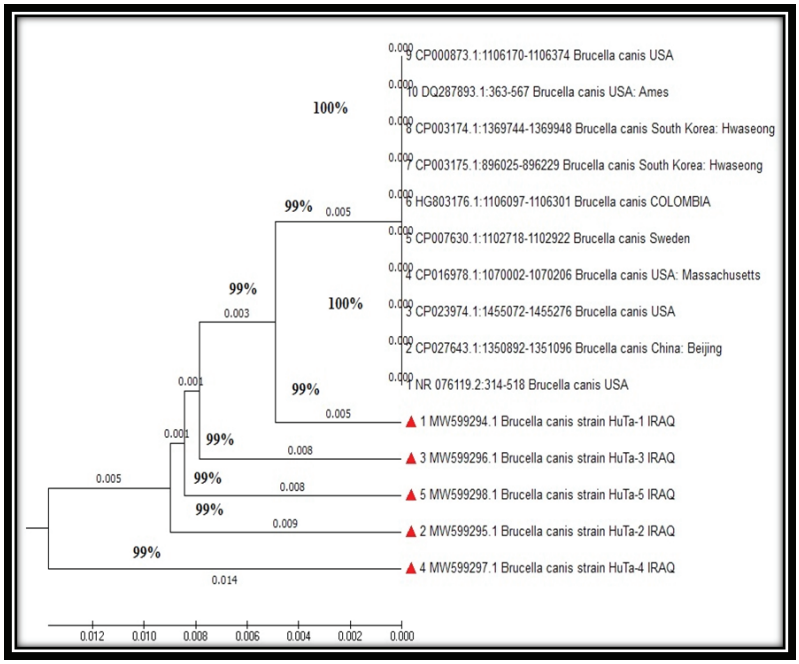
Figure (3):- Agarose gel electrophoresis for *B0548 ribosomal RNA* gene (300bp) of *Brucella Canis*. Bands were fractionated by electrophoresis on a 2% agarose gel (2 h., 5 volt/cm2, 1X TBE) and visualized under U.V light after staining with Ethidium bromide stain. Lane M: DNA ladder (100bp).

Keid *et al*. (2007) using of 23S ribosomal RNA gene for detection of *B. canis* by using PCR and they found that the percentage was 30.5%.

It has been found that polymerase chain reaction is positive for *B. canis* DNA indicated by only amplification of 214 bp product using of 23S ribosomal RNA gene (ARAS *et al*., 2015).

Phylogenetic tree structuring of Brucella Canis

When comparison between Brucella Canis isolated from dogs. recorded in the National Center Biotechnology Information (NCBI) and isolated from different source have under sequence (ID: NR_076119.2, MW599295.1, CP023974.1, CP016978.1, CP007630.1, HG803176.1, CP003175.1, CP003174.1, CP000873.1, DQ287893.1) respectively with source of isolation and showed compatibility the highest identity (99%). As seen in figure (4).



Figure(4.): Neighbor-joining tree of Brucella Canis 23S rRNA gene.

Submission of local Iraq isolate in NCBI.

The 23S ribosomal RNA gene were registered after the correspondence of the National Center for Biotechnology Information and obtained accession number and became a reference to Iraq and the Middle East and the world. Ongoing work will add to this set as more type strains are published and it is available for download at NCBI: https://www.ncbi.nlm.nih.gov/nuccore/ MW599294.1, MW599295.1, MW599296.1, MW599297.1, MW599298.1 of Brucella Canis.

Conclusions

1. The prevalence of Brucellosis in dog in Baghdad city must be taken into consideration because the samples were collected randomly.

2. Molecular method (PCR), is a good idea for confirmation of diagnosis of Brucellosis in dog.

3. 99% compatibility in the sequences of *Brucella canis* in dog in different isolates in our study recording to the National Center Biotechnology Information (NCBI).

4. Our results about 23S ribosomal RNA gene will adds as more strain types are published and it becomes available for downloading at NCBI.

Ethical Clearance:: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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