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## MOLECULAR DIAGNOSIS OF BRUCELLOSIS IN CAMELS BY CONVENTIONAL POLYMERASE CHAIN REACTION IN WASIT PROVINCE, IRAQ

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**Key words:** Molecular, Serology, RBPT, ELISA, Camels, Brucellosis, Iraq

**Abstract**– Adopting a cross-sectional study design the current research aimed to determine the occurrence of the seroepidemiological infection brucellosis in camels in waist province of Iraq from November 2016 to April 2017. Brucellosis is one of the most common diseases among animals and human. Camel brucellosis represents a major public health concern, which affects social and economic development in developing countries. Blood samples were collected randomly from animals in the herds. 237 samples from animals test by Rose Bengal Plate Test (RBPT) and indirect enzyme linked immunosorbent assay (ELISA) kits. Total two hundred thirty seven 237 samples were collected from 6 different herd the sample were taken from camels 209 females and 28 males, all samples were examined by (RBPT), the prevalence of brucellosis depend of agglutination tests to determine the positive results out of 237 collected 51 sample were positive for RBPT and 186 sample negative results, while among 51 samples positive by RBPT confirmed by ELISA 39 sample were positive and 198 sample were negative, This positive sample divided in to 6 livestock groups, from 1 to 6 groups (5) 17.25%, (4) 11.10%, (8) 20%, (7) 17.5%, (10) 13.4%, (5) 18.5%, respectively with final percentage 16.29% while the negative result percentage 83.71%, high seroprevalence was recorded in moderate age (24) 10.12 % and the older than 10 years age (9) 3.79% while the less percentage in group 1 under 5 years of age (9) 2.53 %, while the seroprevalence recorded higher percentage in females (1) 97.43 % and less recorded in male (1) 2.57%. While the molecular detection of 2.95% for *Brucella abortus* and 2.1% for *Brucella melitensis* respectively. Camels are highly susceptible to brucellosis caused by *Brucella melitensis* and *Brucella abortus*. Clinical signs alone are not sufficient for brucellosis diagnosis. Difficulties can arise in diagnosis of camel brucellosis, especially as this disease provokes only few clinical signs in clinical course.

### INTRODUCTION

All domestic animals and man infected with Brucellosis including camels, it considered as a serious zoonotic disease. It is more important as one of the major world problem for public health (Radostits *et al.*, 2007). In Africa and Asia brucellosis was recorded Brucellosis in camel spreading from different countries (Wernery and Kaaden, 2002). Brucellosis is a higher rate widespread disease in camel when large animals kept in a farm in producing areasthat heavy production system. (Abbas and Agab, 2002).

The spreading of varying number of susceptible camels, virulence of the bacteria, bad of veterinary service, all this related with differences in the prevalence brucellosis in camelin different countries, lack of consciousabout the infectionof

camels by brucellosis and continued move of infected camels into herd that susceptible to brucellosis (Radostits *et al.*, 2007).

Infected camels' brucellosis that able to transmission from camel to persons include exposed group(herdsmen, dairymen, veterinary clinicians, slaughter men) because direct transmission from of animal with high risk of being worker of husbandry this hazard acquired of special worry for public health (Jelastopulu *et al.*, 2008).

*B. abortus* and *B. melitensis* more common species infected camels. Can causing a chronic disease with survive and persist in infected cells may be throughout of life time (Jelastopulu *et al.*, 2008; Cooper, 1991; Musa *et al.*, 2008). Animals in livestock (Cattle, goat, sheep, camels) consider the source and may be infected and transmit brucellosis to human especially Pastoralists in endemic areas of

infection (Skalsky *et al.*, 2008).

It is human health hazard worldwide because zoonotic disease and major cause of heavy economic losses recognized poses dangerous in livestock industry (Ochol *et al.*, 2005). The main sources of infection exhaustion of contaminated foods by bacteria (Annapurna *et al.*, 2012).

The common pathogens causing disease in the susceptible animals in the same or in other livestock affecting animal species. (Abbas and Agab, 2002). The few clinical signs was appeared in camel brucellosis, so can difficult in diagnosis comprised of disease provokes in clinically course of infected cattle (Mousa *et al.*, 1987).

The most conditions of brucellosis fetal death and retention of placenta due to placentitis, uterine infections, delayed maturity and infertility in females orchitis, epididymitis in males, it also caused arthritis and hygroma (Musa *et al.*, 2008; Tibary *et al.*, 2006; Ahmad and Nemat, 2007). Camels take up infection via contaminated feed or water inter through the alimentary tract or via contaminated dust or droplets through the respiratory system or via semen through the genital system (Kudi *et al.*, 1997). The infection spread among camels and other farm animals via direct contact with uterine secretions, fetuses, blood and placenta, while in human via consumption, milk and milk products or contaminated raw animal products it's the main sources of infection (Gwida *et al.*, 2012; Almuneef *et al.*, 2004; WHO, 2005; Corbel, 2006). Other route of transmission occurs via skin penetration or via conjunctiva or inhalation and udder contamination during milking (Radostits *et al.*, 2007). Prevalence of brucellosis lead to occurrence of Malta fever in humans and animals, controlled and eradicated of this disease should be to lessen in consideration avoid economic losses and health risks (Megid *et al.*, 2010). The major obstacles in eradication program of brucellosis difficult controlled movement of camel from *Brucella* free herds to from infected herd's area Camel milk having inhibitory effect on bacteria lead to storage life comparison to cow milk (Radostits *et al.*, 2007; Farah *et al.*, 2004).

Epidemiologically risk factors, poor managements, herding with other ruminants, large herd size, active abortions and single person milking more animals. Survival bacteria in the environment may effected in the epidemiology of the brucellosis (Wernery and Kaaden, 2002; Abou-Eisha 2000).

The polymerase chain reaction (PCR) is a fast,

sensitive, and relatively but cheap method and some study useful to detection of DNA from tissues and body fluids contaminated with *Brucella*, while non-viable or a low number of *Brucella*. There are few publications on using PCR in the detection of camel brucellosis (Ghorban *et al.*, 2013).

The aim of the present study was to determine the seroprevalence and molecular detection of camel's brucellosis in the Wasit province of Iraq.

## MATERIALS AND METHODS

The study conducted on 237 camels (209 females & 28 males) of dromedary breeding presenting in eastern region of Wasit province, Iraq during the November 2016 to April 2017. The animals were fed on natural pasture. Particularly in the area which lies between (32° 29' 38.86''N 45° 48' 51.7''E. Almost the population lives in rural areas in close contact with livestock. Information from examined camel was collected including its location, health status, sex, age, and herd size, no history vaccination of selected camels females and male against brucellosis.

The age of animals range between 1 to 15 years. Blood sample (10 mL) were collected from jugular vein in sterile tube without anti-coagulant, labeled, coagulated and centrifugation at 300 r.p.m. for 10 min. serum was separated and put in eppendorf tube. Sample storage at -20 for further analysis.

All samples were collected under sterile hygienic conditions. From each animal, 10 mL of whole blood (with anticoagulant) was aseptically taken and immediately divided into aliquots in tubes containing EDTA and used for PCR. All samples were kept on ice and transported to laboratory. Blood samples were kept frozen (-20 °C) until analysis.

Rose Bengal Plate Test (RBPT) was done by using RBPT antigen (Institute Pourquier, 3409 Montpellier Cedex 5, France) sera was kept in a refrigerator in 4°C before test. 40 µL from serum was mixed with 40 µL from RBPT reagent and the mixture were rotating for 4 minutes clear agglutination showed positive results.

The positive samples with RBPT were further confirmed by EUROIMMUN Anti-*Brucella* ELISA Camel (IgG) Anti-*Brucella* ELISA Camel (IgG), also used for the evaluation of IgG antibodies with ELISA set. Depend on the manufacturer's instructions antibody levels were evaluated by following on the set at the laboratory.

Analyze the data was done by Social sciences (SPSS) version 12.0 All data were using computer and statistically significant a *p*-value less than 0.05.

### DNA extraction from blood samples

#### DNA isolation

DNA from the 237 blood samples was extracted using the DNA extraction kit™ KAPA Express Extract KK7100 (50 rxns) according to the instructions of the manufacturer. Transfer DNA containing supernatant to a fresh tube. And may be diluted in TE buffer for long term storage at -20. (KAPA BIOSYSTEMS). DNA evaluation by Nano drop DNA is required with an A260/A280 quotient of 1.6 or greater.

#### Primers

After studying the genes and primers which were used so far for detection of *Brucella* and based on the sensitivity and specificity of the primers, suitable primers were selected for simultaneous detection and differentiation of the species of *B. abortus* and *B. melitensis* as follow: were used to amplify a 245 bp for the detection of *Brucella* spp. (Khamesipour *et al.*, 2013) were used to amplify a 494 bp for *Brucella abortus* (Mirnejad *et al.*, 2012) to amplify a 734 bp for *Brucella melitensis* (Mousa, *et al.*, 1987) Table 1.

#### PCR PreMixAccuPower® Bioneer/ Korea

**Master Mix component.** 25 µL reaction Top DNA polymerase1U, each: dNTP (dATP, dCTP, dGTP, dTTP), 250 mM Tris-HCl (pH 9.0), 10mM, KCl 30mM, MgCl 21.5mM and stabilizer and tracking dye, 0.4 mM of each primer, and 3 µL template DNA.

The amplification was performed in a DNA thermal cycler at a (95°C \* 5 min) + 35 (94 °C \* 45 s + 64.9 °C \* 1 min + 72°C \* 1 min) + (72°C \* 7 min) with a final hold at 4°C in a DNA thermal cycler. Distilled water instead of DNA was used as negative control in each PCR. Positive controls with genomic DNA of *Brucella* to detect any amplicon contamination or

failure with used a ladder (KAPA BIOSYSTEMS). KAPA BIOSYSTEMSUSA). A ladder (KAPA BIOSYSTEMSUSA), size of amplicons KAPA Universal ladder contain (100 ng/µL) 1x1 mL KAPA loading dye (60x) x1.5 mL contain eighteen DAN segment.

#### Gel visualization

Mix 10 µL of PCR product with 2 µL of 6× loading buffer; load on a 1% agarose gel prepared with 1× TBE containing 0.5 mg/mL ethidium bromide. Run at 80 V constant until bromophenol blue reaches 1/2 of the gel. Inspect under a UV source. A single band should be visible in the sample.

## RESULTS

In this study we used rose bangle test agglutination test (RBPT) and ELISA test, but also ELISA test used a conformance diagnosis in camels that RBPT positive, after serological detection the positive samples test by molecular method PCR.

A total of 237 serum sample were collected from 6 livestock of camel herds with no previous history of vaccination against brucellosis from all herds.

The data of the collection sample from 6 different herd 237 sample with 209 females and 28 males and three groups of age 1 to 5 years (31) 25.74% and moderate age (140) 48.95% and old age (66) 27.85% Table 2.

In addition, 51 positive out of 237 of collection of serum samples by RBPT samples and 186 negative results while among 51 samples positive by RBPT confirmed by ELISA 39 sample positive and 198 negative, This positive sample divided in to 6 livestock groups, from 1 to 6 groups (5) 17.25%, (4) 11.10%, (8) 20%, (7) 17.5%, (10) 13.4%, (5) 18.5%, respectively with final percentage 16.29%, (Table 2) while the negative result percentage 83.71%, the percentage of infection increased with increased number of herd size.

High seroprevalence was recorded in moderate age (24) 10.12 % and the older than 10 years age (9) 3.79% while the less percentage in group 1 under 5

**Table 1.** Primers for amplification of *brucella* gene (sequence (5-3) size bp.

<i>Brucella</i> spp. (Khamesipour <i>et al.</i> , 2013)	Bru-F	5' CTATTATCCGATTGGTGGTCTG 3'	245 bp
	Bru-R	5' GGTAAGCGTCGCCAGAAGG 3'	
<i>B. abortus</i> (Mirnejad <i>et al.</i> , 2012)	Ba-F	5' GACGAACGGAATTTTCCAATCCC 3'	494 bp
	Ba-R	5' TGCCGATCACTTAAGGGCCTTCAT 3'	
<i>B. melitensis</i> (Mirnejad <i>et al.</i> , 2012)	Bm-F	5' AAATCGCGTCCTTGCTGGTCTGA3'	734 bp
	Bm-R	5' TGCCGATCACTTAAGGGCCTTCAT 3'	

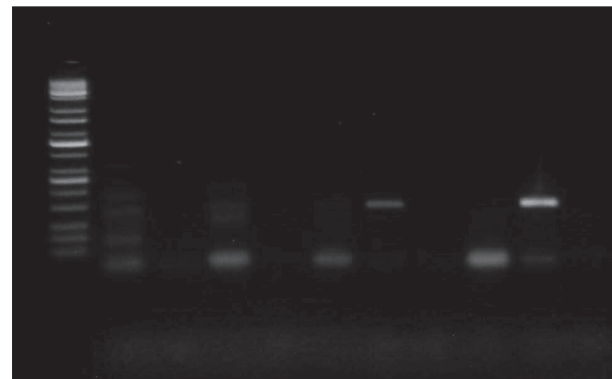
**Table 2.** The seropositive both RBBT and ELISA brucellosis in different herd groups

Farm	Less 5 years	5 - 10 years	More 10 years	Total	RBBT	Percentage	ELISA	Percentage
Farm 1	3	22	4	29	6	20.68%	5	17.25%
Farm 2	9	19	8	36	7	19.44%	4	11.10%
Farm 3	3	17	10	30	8	26.66%	8	20.66%
Farm 4	5	22	13	40	10	25%	7	17.5%
Farm 5	8	40	27	75	14	18.66%	10	13.4
Farm 6	3	20	4	27	6	22.22%	5	18.5%
Total	31	140	66	237	51	21.51%	39	16.45%
					Chi=10.612	P=0.060		

years of age (9) 2.53%, (Table 3). The seroprevalence recorded in was also higher in females 50 (21.1%) in RBBT and (16.03%) in ELISA (Table 3).

In a study when used application of ELISA for diagnosis of *Brucella*, sensitivity and specificity of IgG ELISA was detected as with significant differences  $p = 0.015$  for animals in different livestock herd. Table 4

In this study, were tested 237 blood sample 12 out of 237 positive (5.06%) for *Brucella* spp which had 245 bp band size (Figure 1). The positive samples of *B. abortus* 7 out of 237 (2.95%) which had 494 bp band size (Figure 3). While the positive of *B. melitensis* 5 out of 237 (2.1%) which had 734 bp band size (Figure 3).

**Fig. 1.** Gel electrophoresis (1% agarose, 7 v/cm<sup>2</sup>, 1. hrs) of PCR positive products for (Bru) gene was appeared in 245 bp, L1:- 100bp DNA

## DISCUSSION

Brucellosis in livestock animal's cause's massive economic, losses includes premature birth, death of feta's, abortion, decreased milk production,

infertility adding the zoonotic effects of the disease in camels to human (Pappas *et al.*, 2006).

In the world there are some reports on the detection of camel brucellosis, this is the first time *B. abortus* and *B. melitensis* used PCR to apply in the

**Table 3.** The seropositive both brucellosis depend on age and sex in different herd groups

No (237)	Less 5 years	5 - 10 years	More 10 years	All	Female	Male	Percentage
RBBT/ +	8 (3.75%)	32 (13.5%)	11 (4.64%)	51	50 (21.1%)	1	21.52%
ELISA/ +	6 (2.53%)	24 (10.12%)	9 (3.79%)	39	38 (16.03%)	1	16.45%
PCR/ <i>B. abortus</i> +/	5 (2.1%)	2 (0.85%)	7	7	/	2.95%	5.1%
PCR/ <i>B. melitensis</i> +	/	3 (0.89%)	2 (0.85%)	5	5	/	2.11%

Chi-square value is 18.29 with 6 d.f. Probability level  $P = 0.006$   
d.f.  $P =$  Probability level  $P = 0.957$

Chi-square value is 0.31 with 3

**Table 4.** Sensitivity and specificity of the seropositive both brucellosis in different herd groups

Test	RBBT test		ELISA		PCR/ <i>B. abortus</i>		PCR/ <i>B. melitensis</i>	
	Ve+	Ne-	Ve+	Ne-	Ve+	Ne-	Ve+	Ne-
Brucellosis	51	186	39	198	7	230	5	232
%	21.52	78.48	16.45	83.55	2.95	97.05	2.1	97.9

Chi-square value is 70.09 with 3 d.f. Probability level  $P < 0.001$



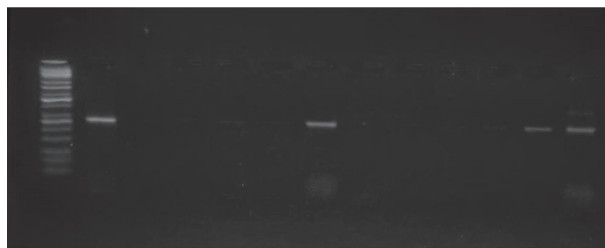


Fig. 2. Gel electrophoresis (1% agarose, 7 v/cm<sup>2</sup>, 1. hrs) of PCR positive products for (Ba) gene was appeared in 494 bp, L1:- 100bp DNA

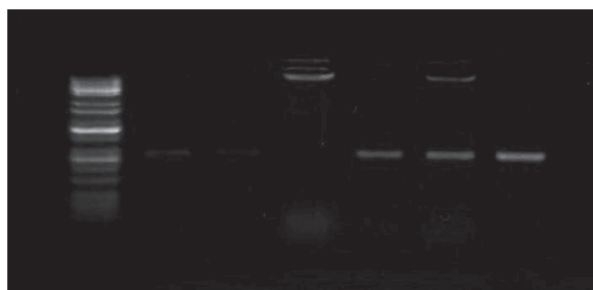


Fig. 2. Gel electrophoresis (1% agarose, 7 v/cm<sup>2</sup>, 1. hrs) of PCR positive products for (Bm) gene was appeared in 734 bp, L1:- 100bp DNA

diagnosis of camel brucellosis and has obtained suitable results with good specificity.

In future, an appropriate PCR technique could be used to confirmatory diagnosis for the identification and differentiation of camels' brucellosis with the lowest risk of zoonotic to laboratory personnel (Ghorban *et al.*, 2013).

The most infective agents in camels are *B. abortus* and *B. melitensis*, and are a transmission between Camels and other species due to that leads their habitat on the husbandry system, (Musa *et al.*, 2008; Agab *et al.*, 1994), during calving or abortion occurs contamination with other animals (Wernery and Kaaden, 2002; Abou-Eisha 2000).

Several researchers evaluated specificity and sensitivity of different serological tests (iELISA), (cELISA), RBPT, CFT, (SAT) and (2ME) for the diagnosis brucellosis of camel (Azwai *et al.*, 2001; Abdel Moghney, 2004; Alshaikh *et al.*, 2007).

In this study the positive sample in 6 groups, from 1 to 6 groups were (5) 17.25%, (4) 11.10%, (8) 20%, (7) 17.5%, (10) 13.4%, (5) 18.5%, respectively with final percentage 16.29% while the negative result percentage 83.71%. Therefore, this results were in agreement with true seroprevalence brucellosis of camel in Jordan in the south province with used the RBPT and CFT is 15.8% (Dawood, 2008). The survey of brucellosis in Al-Hodeida in

Yemen confirmed the presence of *Brucella* spp. the prevalence rate showing a significant in camels with (11%) (Al-Garadi *et al.*, 2015). Results of serological test in Al Mudawwara location of brucella in camels in Saudi Arabia, positive cases (17%) were recorded (Agab *et al.*, 1994).

While some study recoded high percentage of prevalence of infection one of this study in eastern Sudan reported 16.5–32.3% from the 948 camels in different herds, while in seven herdsexamined with 416 camels in western Sudan prevalence rate found a 23.3% (Musa, 1995).

The reported of higher prevalence of brucellosis (23.8%) from camel kept mixed with ruminant species in western Sudan (Musa *et al.*, 2008). However, seroprevalence was high relatively of infection in camel recorded in Sudan 30.5% (Omer *et al.*, 2007).

Another study recoded low percentage of infection, in Abu Dhabi Emirate the prevalence of brucellosis of camels that confirmed by c-ELISA (4.4%), (Mohammed *et al.*, 2013) while in Egypt 7.3% (EL-Boshy *et al.*, 2009). The study of brucellosis in Eastern Ethiopia revealed 2.43% of camel brucellosis (Tilahun *et al.*, 2013). To study brucellosis in 3413 camels raised in areas of Sudan 72 (7.3%) out of 993 males and in 196 (8.1%) out of 2420 females (Musa and Shigidi 2001). This seroprevalence is same the previous reports of 2.8% in Ethiopia (Teshome *et al.*, 2003) while 1.8% Southern Ethiopia (Megersa *et al.*, 2005) and from Eritrea Ethiopia, with recorded 3.1% (Omer *et al.*, 2000) and some study in the Somalia was revealed 0.3 to 1.9%, (Baumann and Zessin, 1992) and in other study was recorded 3.1% (Gahanem *et al.*, 2009).

The differences in prevalence of camel brucellosis in different countries might be due management, size of herd, virulence of bacteria, presence of susceptible animals in the region, bad of veterinary nursing, and movement of infected camels into different area contain a susceptible camel herd (Radostits *et al.*, 2007).

The management of different species of animals with different animals such as cattle, sheep and goats with camels lead to spread of infection to camels with possible source of infection. (Musa *et al.*, 2008; Teshome *et al.*, 2003; Musa, *et al.*, 2008; Teshome, *et al.*, 2003) the stray dogs and foxes may spread the infection by deliver the aborted material on the pasture on the wide area of the pasture (Teshome *et al.*, 2003). Camels may take up bacteria contaminated feed or water or via the respiratory

system and dust or droplets or via infected semen (Kudi *et al.*, 1997).

The prevalence and spreading of brucellosis is increasing in many developing countries despite the advances made in redaction and control, due to various hygienic, socio economic development, and policy governmental (Pappas *et al.*, 2006).

To our data, although some reports on the detection of camel brucellosis in other parts of the world, this is the first time used PCR has been applied in the diagnosis of camel brucellosis (*B. abortus* and *B. melitensis*) and has obtained suitable results with good specificity (Ghorban *et al.*, 2013).

### CONCLUSION

The present study showed seroprevalence of camel brucellosis was a moderate percentage the incidence in wasit province of infection associated with herd size, a widely extended grazing, and situation of vaccination, susceptibility to infection by virulence strain and delay diagnosis of infection, however, bad control and management to abortion and still birth. Further studies required about camels brucellosis. Although seroprevalence of camel brucellosis increased with the susceptibility of animals watering points in the river, and the seropositive animals may serve as foci of spreading of infection, with increased public health risk. Camel's brucellosis caused by *Brucella melitensis* and *Brucella abortus*. Only few clinical signs can appeared of camel brucellosis in contrast to its clinical signs when infected cattle. The commonly used serological test because difficulties in diagnosis after that finally can be conformance by used molecular and bacteriological cultures.

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