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Article *in* Asian Journal of Microbiology, Biotechnology and Environmental Sciences · February 2019

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Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 21 (February Suppl.) : 2019 : S88-S94

ISSN-0972-3005

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

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(Recieved 8 September, 2018; accepted 27 October, 2018)

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 1under 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 *Brucellaabortus* and 2.1% for *Brucellamelitensis* respectively. Camels are highly susceptible to brucellosis caused by *Brucellamelitensis* and *Brucellaabortus*. 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

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

It is human health hazardin 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, epididymitisin 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 contaminateddust 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, bloodand placenta, while in human via consumption, milk and milk productsor contaminated raw animal products it's the main sources of infection (Gwida *et al.,* 2012; Almuneef *et al.,* 2004; WHO, 2005; Corbel, 2006). Other rout 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 bacterialead 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 andsingle 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 regain 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 (32º 29′ 38.86′′N 45º 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 statue, 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 eppendrof tube. Sample storage at -20 for farther 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 Pourquer, 3409 Montpellier Cedex 5, France) sera was kept in a refriageter in 4C °before test. 40 µL from serum was mixed with 40µL from RBPT reagentand the mixture were rotating for 4 minutes clear agglutination showed positive results.

The positive samples with RBPT were further confirmed byEUROIMMUN 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 *Brucellaabortus* (Mirnejad *et al.,* 2012) to amplify a 734 bp for Brucellamelitensis (Mousa, *et al*., 1987) Table 1.

PCR PreMix*AccuPower*® Bioneer/ Korea

Master Mix component. 25 µL reaction Top DNA polymerase1U, each: dNTP (dATP, dCTP, dGTP, dTTP), 250 mMTris-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 useda 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 1under 5

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

Brucella spp. (Khamesipour *et al.,* 2013)	Bru-F	5′ CTATTATCCGATTGGTGGTCTG 3′	245 bp
	Bru-R	5′ GGTAAAGCGTCGCCAGAAGG 3′	
B. abortus (Mirnejad *et al.,* 2012)	Ba-F	5′ GACGAACGGAATTTTTCCAATCCC 3′	494 bp
	Ba-R	5′ TGCCGATCACTTAAGGGCCTTCAT 3′	
B.melitensis (Mirnejad *et al.,* 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 tested237 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).

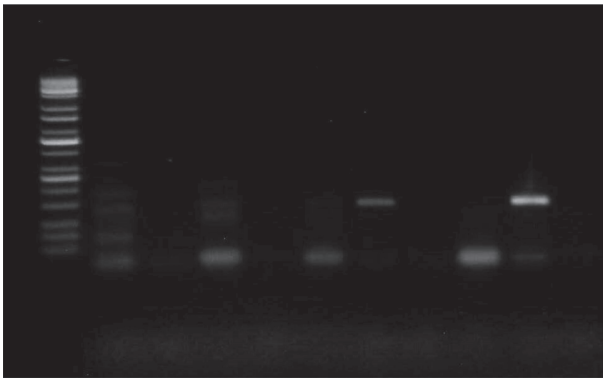

Fig. 1. Gel electrophoresis (1% agarose, 7 v/cm2, 1. hrs) of PCR positive products for (Bru) gene was appeared in 245 bp, L1:- 100bp DNA

DISCUSSION

Brucellosis in livestock animal'scause'smassive economic, losses includes premature birth, deathof 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/ B abortus +/	5 (2.1%)	2 (0.85%)	7	7	/	2.95%	5.1%
PCR/ B malteanses +	/	3 (0.89%)	2(0.85%)	5	5	/	2.11%

Chi-square value is 18.29 with 6 d.fProbability level P = 0.006 Chi-square value is 0.31 with 3 d.f.P=Probability level P = 0.957

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

Test	RBBT test		ELISA		PCR/ *B abortus*		PCR/ *B malteanses*	
	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

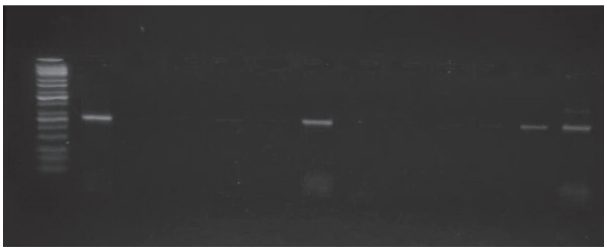

Fig. 2. Gel electrophoresis (1% agarose, 7 v/cm2, 1. hrs) of PCR positive products for (Ba) gene was appeared in 494 bp, L1:- 100bp DNA

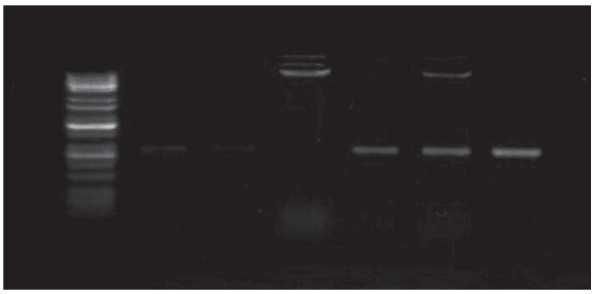

Fig. 2. Gel electrophoresis (1% agarose, 7 v/cm^2, 1. hrs) of PCR positive products for (Bm) gene was appeared in 734 bp, L1:- 100bp DNA

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 specify 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 reported16.5–32.3% from the 948 camels in different herds, while in seven herdsexaminedwith 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 infectionin 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 thatconfirmed 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 issame 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 reveled 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,susceptibilityto 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 *Brucellamelitensis* and *Brucellaabortus*. Only few clinical signs can appearedof camel brucellosis in contrast to its clinical signswhen infected cattle. The commonly used serological test because difficulties in diagnosis after that finally can be conformance by used molecular and bacteriological cultures.

REFERENCES

Abbas, B. and Agab, H. 2002. A review of camel brucellosis. *Preventive Veterinary Medicine*. 55 : 47–56.

Annapurna, S.A., Srikrishna, I. and Prabhudas, K. 2012. Seroprevalence Study of Human Brucellosis by Conventional Tests and Indigenous Indirect Enzyme-Linked Immunosorbent Assay. *The Scientific W. J.* pp: 5.

Ahmad, R. and Nemat, Z. 2007. Brucellosis of camels in Iran, Shahid Bahonar University of Kerman. Iran, Copyright 2007 Priory Lodge Education, priory.com.

Almuneef, M. A., Memish, Z.A., Balkhy, H.H., Alotaibi, B., Algoda, S. and Abbas, M. 2004. Importance of screening household members of acute brucellosis cases in endemic areas. *Epidemiol Infect*. 132 (3) : 533-540.

Abou-Eisha, A.M. 2000. Brucellosis in camels and its relation to public health. *Asuit. Vet. Med. J*. 44 (87) : 54-64.

Agab, H., Abbas, B., El-Jakack, H. and Mamon, I.E. 1994. Firist report on the isolation of Brucellaabortusbiovar 3 from camel (camelus dromedaries) in Sudan. *Revue. Elev. Med. Vet. Pays. Trop.* 47: 361-363.

Azwai, S.M., Carter, S.D., Woldehiwet, Z. and MacMillan, A. 2001. Camel brucellosis:evaluation of field sera by conventional serological tests and ELISA. *Journal ofCamel Practice and Research.* 8 : 185–193.

Abdel Moghney, F.R.A. 2004. A preliminary study on brucellosis on camels at Behira province. *Assuit University Bulletin Environmental Researche*. 7 : 39–43.

Alshaikh, A.A.M., Al-Haidary, A., Aljumaah, R.S., Al-Korashi, M.M., ElNabi, G.R.A. and Hussein, M.F. 2007. Camel brucellosis in Riyadh region, Saudai Arabia. *Journal of camel Practice and Research*. 14 : 113–117

Al-Garadi, M.A., Al-Hothi, A. and Al-sharma A. 2015. Bacteriological and serological study on brucellosis infection in camel (*Camelus dromedaries*), Al-Hodeida governorate, Yemen. *International Journal of Advanced Research*. 3 (1) : 786-791.

Baumann, M.P.O. and Zessin, K.H. 1992. Productivity and health of camels (*C. dromedrius*) in Somalia associations with *Trypanosomiasis* and *Brucellosis*. *Trop. Anim. Health Prod.* 24 : 145-156.

Cooper, C.W. 1991. The epidemiology of human brucellosis in a well defined urban population in Saudi Arabia. *Journal of Tropical Medicine and Hygiene.* 94 : 416– 422.

Corbel, M. J. 2006. Food and Agriculture Organization of the United Nations, World Health organization, World Organization for Animal Health. Brucellosis in humans and animals. Geneva: World Health Organization.

Dawood, H.A. 2008. Brucellosis in Camels (*Camelus dromedorius*) in the south province of Jordan. *Am. J. Agri. & Biol. Sci*. 3 (3): 623-626.

EL-Boshy, A.H., EL-Khodery, S. and Osman, S. 2009. Cytokine response and Clinicopathological findings in *Brucella*infected camels (*Camelusdromedarius*). *Vet. Med*. 54 : 25-32.

Farah, Z., Abdulkadir, O. and Abdurahman, S.H. 2004. *Milk and Meat from the Camel: Handbook on Products and Processing*. Hochshuleverlag AG ander ETH Zürich. 15–28.

Gwida, M.A., El-Gohary, A., Melzer F., Khan, I., Uwe Rösler and Neubauer, H. 2012. Brucellosis in Camels. *Res in Vet. Sci*. (92) : 351–355.

Ghorbani, A., Rabbani Khorasgani, M., Zarkesh-Esfahani, H., Sharifiyazdi, H., Dehghan Kashani, A. and Emami, H. 2013. Comparison of serology, culture and PCR for detection of brucellosis in slaughtered camels in Iran. *Comp Clin Pathol.* 22 : 913-917.

Gahanem, Y.B., El-khodery, S.A., Saad, A.A., Abdelkader, A.H., Haybe, A. and Muse, A. 2009. Seroprevalence of camel brucellosis (*Camelus dromedaries*) in

Somaliland. *Trop. Anim. Health Prod*. 41 : 1779-1786.

Jelastopulu, E., Bikas, C., Petropoulos, C. and Leotsinidis, M. 2008. Incidence of human brucellosis in a rural area in western Greece after the implementation of a vaccination programme against animal brucellosis. *BMC Public Health*. 8 : 241.

Khamesipour, F., Doosti, A. and Taheri, H. 2013. Molecular Detection of *Brucella* spp. in the Semen.

Kudi, A.C., Kalla, D.J.U., Kudi, M.C. and Kapio, G.I. 1997. Brucellosis in camels. Journal of Arid Environments 37, 413–417.

Musa, M.T., Eisa, M.Z., M El Sanousi, M., Abdel Wahab, E.M. and Perrett, L. 2008. Brucellosis in Camels (Camelusdromedarius) in Darfur, Western Sudan. *Journal of Comparative Pathology.* 138 : 151–155.

Mousa, A.M., Mousa. Elhag, K.M., Khogali, M. and Sugathan,T.N. 1987. Brucellosis in Kuwait. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 81 (6) : 1020–1021.

Mirnejad, R., Hosseini Doust, R., Kachuei, R., Mojtaba Mortazavi, S., Khoobdel, M., Ahamadi, A. 2012. Simultaneous detection and differentiates of *Brucellaabortus* and *Brucellamelitensis* bycombinatorial PCR. *Asian Pacifi c Journal of Tropical Medicine*. 24-28.

Megid, J., Antonio, L., Mathias and Carlos A. R 2010. Clinical Manifestations of Brucellosis in Domestic Animals and Humans. *The Open Veterinary Science Journal*. 4 : 119-126.

Musa, M.T. 1995. *Brucellosis in Darfur States: the magnitude of the problem and methods of diagnosis and control*. Ph.D. Thesis, University of Khartoum, pp. 73– 98.

Mohammed, M.A., Shigidy, M.T. and Al Juboori, A.Y. 2013. .Sero-Prevalence and Epidemiology of Brucellosis in Camels, Sheep and Goats in Abu Dhabi Emirate. *Int. J. Anim. Veter. Adv*. 5 (2) : 82-86.

Musa, M.T and Shigidi, M.T.A. 2001. Brucellosis in Camels in Intensive Animal Breeding Areas of Sudan. Implications in Abortion and Early-Life Infections. *Rev. Elev. Med. Vet*. 54 (1): 11-15.

Megersa, B., Molla, B. and Yigezu, L. 2005. Seroprevalence of brucellosis in camels (*Camelus dromedaries*) in Borena lowland, Southern Ethiopia. *Bull. Anim. Health. Prod. Afr*. 53: 252-257.

Ocholi, R.A., Kwaga, J.K.P. Ajogi, I. and Bale, J.O.O. 2005. Abortion due to Brucellaabortus in sheep in Nigeria. *Rev. Sci. Tech. Off. Int. Epiz*. 24 : 973-979.

Omer, M.M., Abdul-Aziz, A.A., Abusalab, M.A.S. and Ahmed, M.A. 2007. Survey of brucellosis among sheep, goats, camels and cattle in Kassala area, Eastern Sudan. *J. Anim. Vet. Adv.* 6 : 635-637.

Omer, M.K., Skjerve, E., Holsad, G., Woldehiwot, Z. and Macmillan, A.P. 2000. Prevalence of antibodies to *Brucella* species in cattle, sheep, goats, horses and camels in state of Eritrea. *Epidemiol. Infect.* 125 (2) :447-453.

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L. and Tsianos, E.V. 2006. The new global map of human brucellosis. *Lancet Infectious Diseases* 6 : 91–99.

Radostits, W., Gay, C.C., Hinchcliff, K.W., Constable, P.D. 2007. *Veterinary Medicine*, 10th ed. Elsevier Saunders, London, pp. 389-390.

Skalsky, K., Yahav, D., Bishara, J., Pitlic, S. and Leibovici, L. 2008. Treatment of human brucellosis: Systematic review and meta analysis of randomized controlled trials. *BMJ*. 336 : 678-679.

Tibary, A., Fite, C., Anouassi, A. and Sghiri, A. 2006. Infectious causes of reproductive loss in camelids. *Theriogenology*. 66 : 633–647.

Testis and Blood Samples of Cattle and Sheep. *Journal of Pure and Applied Microbiology*. 7 (Spl. Edn.) : 495-500.

Tilahun, B., Bekana, M., Belihu, K. and Zewdu, E. 2013. Camel brucellosis and management practices in Jijiga and Babile districts, Eastern Ethiopia. *J. Vet. Med. Anim. Health*. 5 (3) : 81-86,

Teshome, H., Molla, B. and Tibbo, M. 2003. A seroprevalence study of camel brucellosis in three camel rearing regions of Ethiopia. *Tropical Animal Health and Production*. 35 : 381–390.

Wernery, U. and Kaaden, O.R. 2002. *Infectious Diseases of Camelids*. London: Blackwell Science Inc., pp. 99 – 116.

WHO, 2005. Brucellosis in humans and animals. WHO guidance, Geneva.