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PREVALENCE OF CAMEL BRUCELLOSIS AT AL-SHALATEEN AREA

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ABSTRACT

Sera of 801 apparently healthy dromedary camels were collected from Al-Shalateen quarantine and tested by BAPAT, RBPT and there results were confirmed by CFT. The results revealed that the prevalence of camel brucellosis was 12.9%, 11.6% and 11.5% for BAPAT, RBPT and CFT respectively. Also results revealed that seroprevalence was higher in camels younger than 2 years (immature) (13.3%) than mature camels (2-4 years old) (10.6%) and camels at breeding age (older than 4 years) (10.8%). In addition, prevalence on females (19.1%) were higher than males (7.1%). Moreover, Brucella melitensis biovar 3 was isolated from stomach content of aborted camel fetus. Statistical analysis of these results revealed that the apparent prevalence (AP) was estimated as (11.5%) while true prevalence (TP) was estimated as (13.6%) (95%CI: 11.2-16%). There is No significant difference were detected between different age groups while a highly significant difference were detected between season and gender in the frequency of +ve and –ve samples in different tests.

INTRODUCTION

Brucellosis in farm animals can cause severe persistent reproductive failure like abortion mainly at late pregnancy (stormy abortion on cattle), stillbirth, placentitis retained placenta in female and orchitis and epididymiyis in male **(Radostitis, 2007).** While in camels the diseases provoke little manifestation if compared to cattle or even asymptomatic with abortion as the most prevalent signs of the disease in camels so it may silently affect the reproductive performance of camels **(Gwida et al., 2012).** However information about economic losses due to camel brucellosis is scarce.

Although camels are not the primary host of brucella, *B. abortus* and *B. melitensis* was isolated from milk, aborted fetus, lymph nodes and vaginal swabs **(Radwan et al., 1992; Gameel et al., 1993; Agab et al., 1994; Abou-Eisha, 2000; Hamdy and Amin, 2002).** So transmission of the disease will depends on brucella species being prevalent in contact animals **(Musa et al., 2008).** The zoonotic properties of brucellosis either through contentious contact to camels or through consumption of raw milk were recorded **(Al-Juboori and baker, 2012).**

The present study aimed to:

1. Investigate the seroprevalence of camel brucellosis in Al-Shalateen using BAPAT, RBPT and CFT as confirmatory test.
2. Isolation of brucella species from serologically positive cases.

MATERIAL AND METHODS

1. Sampling:

1.1. collection of blood serum sample (Alton et al., 1988):

Blood samples were collected without anticoagulant by vein puncture from jugular vein of 801 camels. About 10 ml blood were aseptically collected in sterile silicon-coated vacuum tubes ,which kept in a slant position in the shade for about 2 hours for complete clotting and then identified and transferred on ice packs to the laboratory with avoiding shaking as far as possible.

Blood samples were kept overnight at 4° C to allow for separation of serum then centrifuged at 3000 r.p.m. for 10 minutes to obtain amber clear serum. Sera were kept at -20° C each in 2 aliquots in sterile bijou bottles till examined.

1.2. collection of tissue specimen for bacteriological examination:

1.2.1. Stomach fluid of aborted camel calf:

Stomach content collected carefully from aborted fetus by heating the outer surface of the abomasum by heated spatula then introduction of sterile syringe from the sterile point to obtain some of the stomach content for bacteriological isolation and identification.

1.2.2. Tissues:

supramammary lymph node, retropharyngeal lymph node and inguinal lymph node were collected immediately after slaughtering with its surrounding fats and corresponding heart blood, placed in sterile plastic bags.

Collected tissue specimen and Stomach fluid were collected labeled, identified and transferred quickly with ice to the laboratory and then stored at –20°C until examination for bacteriological isolation and identification.

2. Serological examination:

***2.*1. Rose Bengal Plate Test (RBPT):** according to **Alton, et al (1988)**.

***2.*2. Buffered Acidified Plate Antigen Test (BAPAT):** according to **Alton et al. (1988)**.

***2.*3. Complement Fixation Test:** according to **Alton *et al.* (1988)**

3. statistical analysis:

All the following analyses were performed using IBM SPSS Statistics, Version 21, IBM Corporation, 2012, under the environment of Windows® 8.1, Microsoft Corporation.

Estimation of the True Prevalence: It was estimated according to Rogan and Gladen (1978) from the following equation:

True prevalence = apparent prevalence + combined specificity of RBPT and CFT- 1 / combined sensitivity of RBPT and CFT + combined specificity of RBPT and CFT - 1

4. bacteriological examination and Identification:

Bacteriological examination was carried out in Brucellosis Research Departement, Animal Health Research, Institute, Doki, Giza, Egypt. It was performed according to the recommendation of **FAO/WHO Expert committee on Brucellosis (1986)** cited in **Alton, et al. (1988).**

RESULTS

1. Clinical examination of camels under investigation:

In the present study, all 801 camels were clinically normal at the time of sampling and there is no history of abortion, orchitis or vaccination against brucellosis, except one she-camel which aborted at its sevens month of gestation. The aborted fetus was red in color and appeared to be undeveloped as it was without hide and small in size. On necropsy, organs were undeveloped and congested surrounded with serosangounus fluid tinged red. On thorathic cavity the lung showed fibrinous pleurisy. The placenta was smooth but leathery and congested and unlike what stated on cattle, it appears to descend normally without retention.

2.Seroprevalence of brucellosis among camels in Al-Shalateen Quarantine:

2.2. Statistical analysis:

Statistical analysis of these results revealed that the apparent prevalence (AP) was estimated as (11.5% by CFT) while true prevalence (TP) was estimated as (13.6%) (95%CI: 11.2-16%). A high significant difference was recorded among different seasons of the year being high in Spring and Autumn. There is No significant difference were detected between different age groups while A highly significant difference were detected between male and female in the frequency of +ve and –ve samples in different tests.

2. Isolation, identification and typing of brucella organisms from seropositive and aborted she camel:

Our attempts to isolate brucella species from five lymph nodes were failed. However, we managed to isolate *brucella melitensis biovar 3* from stomch content of one aborted fetus.

Table (1): Seroprevealance of camel brucellosis in relation to seasons.

Season	Total No. of animals	Total No. of samples	Positive samples					
			BAPAT		RBPT		CFT	
			No.	%	No.	%	No.	%
Spring 2014	1445	145	18	12.4	17	11.7	17	11.7
Summer 2014	498	49	2	4.1	2	4.1	2	4.1
Autumn 2014	2317	233	40	17.2	36	15.5	36	15.5
Winter 2015	957	96	3	3.13	3	3.13	3	3.13
Spring 2015	2781	278	40	14.4	35	12.6	34	12.2
Total	7998	801	103	12.9	93	11.6	92	11.5

P<0.05(significant differences between different seasons by all tests 0.003, 0.010 and 0.010 for BAPAT, RBPT and CFT respectively)

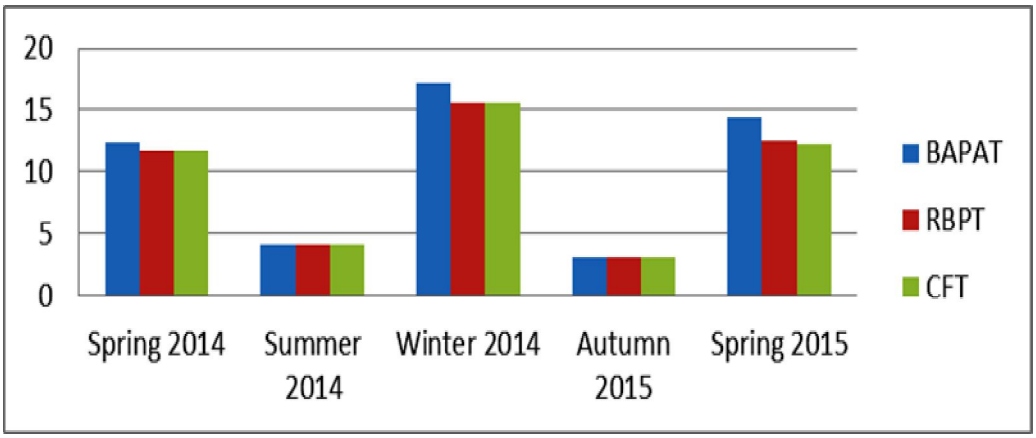

Fig. (1): Seroprevealance of camel brucellosis in relation to seasons.

Table (2): Seroprevealance of camel brucellosis in relation to age.

	No. of samples	positive samples					
		BAPA		RBPT		CFT	
		No.	%	No.	%	No.	%
Immature (1- 2 years old)	248	42	16.9	33	13.3	33	13.3
Mature (2-4 years old)	294	33	11.2	32	10.8	31	10.6
Breeding age (≥ 4 years old)	259	28	10.8	28	10.8	28	10.8
Total	801	103	12.9	93	11.6	92	11.5

P.>0.05(None significant differences between different age groups by all tests) .061, 0.575and 0.522for BAPAT, RBPT and CFT respectively)

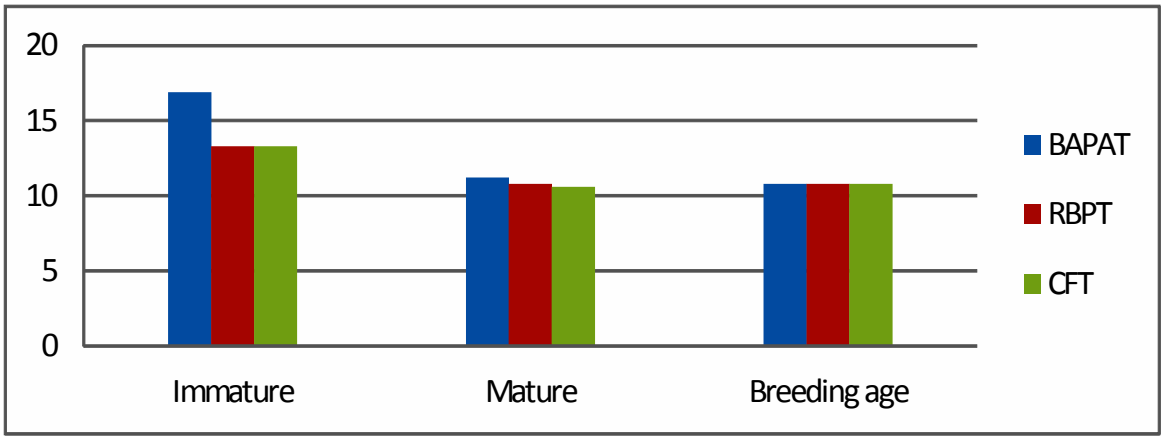

Fig.(2): Seroprevealance of camel brucellosis in relation to age.

Table (3): Seroprevealance of camel brucellosis in relation to sex according to CFT results.

Sex	Age	No. of samples	Positive samples						Total No. of samples	Total positive samples	
			BAPAT		RBPT		CFT			No.	%
			No.	%	No.	%	No.	%			
Male	Immature	168	16	9.5	10	5.95	10	6	507	36	7.1
	Mature	177	10	5.6	9	5.1	9	5.1			
	Breeding age	162	17	10.5	17	10.5	17	10.5			
Female	Immature	80	26	32.5	23	28.8	23	28.8	294	56	19.3
	Mature	117	23	19.7	23	19.7	22	18.8			
	Breeding age	97	11	11.3	11	11.3	11	11.3			

P<0.05(significant differences between the two sexes by all tests) 0.000, 0.000and 0.000for BAPAT, RBPT and CFT respectively)

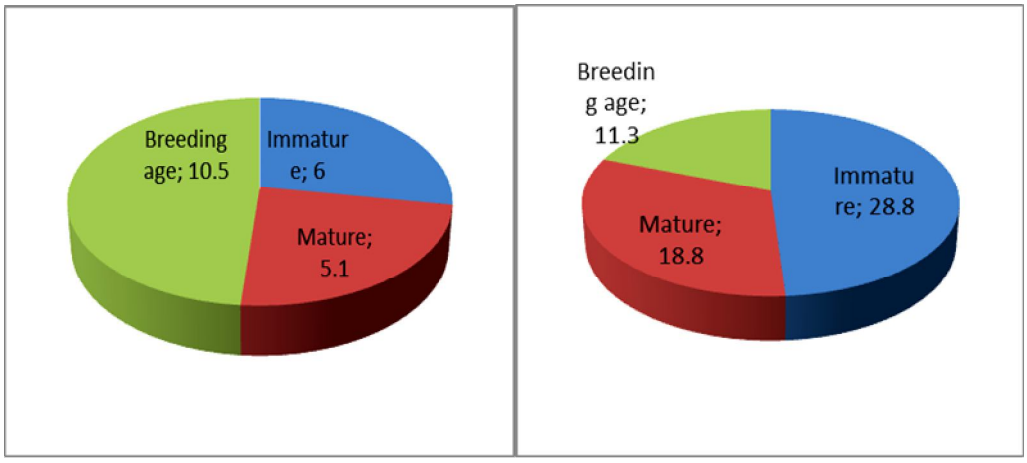

Fig. (3): Serological diagnosis of camel brucellosis according to sex

DISCUSSION

Lately in Egypt, there were increasing on the demand of animal protein (meat), so the government take the way to import different sources of animal protein (frozen meat, feedlot, camels…etc) to compensate the nutritional gap.

In the present study, blood serum samples of 801 dromedary camels were collected for serological investigation at the period between 2014 and 2015 from Al-Shalateen Quarantine, Red Sea Governorate. All serum samples were subjected to BAPAT and RBPT as screening test **(Morgan et al., 1969; Hunter and Allen, 1972; Farina, 1985).** Moreover we used CFT as confirmatory test for the positive serum samples according to **OIE (2012).**

The obtained results summarized in **Table (1)** and **Figure (1)** revealed that the prevalence of brucellosis among examined 801 camels were 103(12.9%), 93(11.6%) and 92(11.6%) for BAPAT, RBPT and CFT respectively. Statistical analysis of results revealed that the apparent prevalence (AP) was estimated as (11.5%) while true prevalence (TP) was estimated as (13.6%) (95%CI: 11.2-16%). High prevalence appears to be due to the fact that these camels were imported from Sudan where high prevalence were recorded **(Omer et al., 2007** (12.3%, 15.5% and 30.5% during 2004, 2005 and 2006 respectively)**; Musa et al., 2008** (23.8% and 9.7%)**; Omer et al., 2010** (37.5%)**)** due to insufficient preventive measures and the lack of adequate control programs as well as uncontrolled animal transportation across "open" borders. Chi square analysis for comparison between occurrence of brucella infection by different tests and seasons revealed that there were high significant differences among different seasons ($P \leq 0.05$) being high in Spring and Autumn enhancing the fact saying that camels were seasonal breeders. **Abdel-Raouf and El-Naggar (1964**) reported that rutting season on males occurs in spring in Egypt. While **Shalash (1965)** reported also that breeding season of female being prevalent mainly on the period between December to May. Breeding season also reported from March to August in Sudan **(Musa and Abusineina, 1978)** and from April to May in Somalia **(Mares, 1954).**

In Egypt the prevalence of camel brucellosis has been reported by different authors at different localities by different tests. Our results were higher than that recorded by **Abdel Moghney (2004)** (9.26), **Al-Gaabary and Mourad (2004)** (6.75), **El-Boshy et al. (2009)** (7.35%), While these results were quietly in agreement with those of **Hamada et al. (1963)** (10.29%), **Ahmed and Nada (1993)** (11.6), **El-Sawally et al. (1996)** (11.3%),and this results were lower than that recorded by **Nada (1984)** (23.1%) and **Salem et al. (1990)** (13.9%).The differences in seroprevalence observed in this study, as opposed to those recorded by previous researchers, might also be due to differences in herd size, camel origin, tests used, agro ecological and management conditions, and the presence or absence of infectious foci, such as *Brucella-infected herds*, which could spread the disease among contact herds.

The RBPT detect 93 (11.6%) reactors lower than BAPAT which detects 103(12.9%) reactors, this variation on the incidence of positive reactors may be attributed to the difference in the acidity of their antigen as reported by **Davis (1971)** and **Corbel (1973)** the acidic PH of the RBPT antigen (3.65± 0.05) inhibits more amount of IgM fraction **(Alton et al., 1988).**The test is an excellent screening test but may be oversensitive for diagnosis in individual animal particularly

vaccinated animals **(Wolrld Health Organisation, 2006).**

MacMillan (1990) considered that although IgM could be measured by the CFT, IgG_1 was the main immunoglobulin measured with a possible cause that IgM is denatured during the test procedure**. Curtain (1971)** and **Cho and Ingram (1972)** showed that CFT only measured IgG1 and that IgG2 and IgA do not fix complement. **Plackett and Alton (1975)** has showen that results from the CFT may be adversely affected by IgG2 interference (prozone effect) and by anti-complementary activity. **Al-Dahouk et al. (2003)** considered that the CFT should be used only as a confirmatory test and he noted that in practical terms sensitivity and specificity could vary widely.

In the present study, all 801 camels were clinically normal at the time of sampling and according to the owners, none had previously shown clinical signs of brucellosis. Prevalence of brucellosis in apparently healthy camels in the present study indicates that many infected camels might be silent carriers for brucellosis and their products may pose a serious health problem for consumers. This finding was in harmony with reports by **Abu Damir *et al.*, (1989) and Bekele (2004). Abu Damir *et al.*, (1989)** mentioned that non pregnant camels experimentally infected with *B. abortus* had no clinical manifestations and only negligible pathological changes were found. On the contrary, individual cases of abortion, fetal death, mummification, delayed sexual maturity, infertility, stillbirth, mastitis, orchitis and joint disease might be encountered in naturally infected camels with *B. abortus* **(Higgins, 1986; Obeid *et al.*, 1996; Musa and Shigidi, 2001)**.

Prevalence of brucella infection among 801 examined camels according to their age was summarized in **Table (2)** and **Figure (2).** Out of 248 examined young camels less than 2 years old 42 (16.9%) and 33 (13.3%) camel were positive for BAPAT and RBPT respectively and about 33 (13.3%) samples were confirmed as positive reactor for CFT. Also out of 294 examined mature camels between two and four years old 33 (11.2%) and 32(10.8%) were positive for BAPAT and RBPT respectively and about 31 (10.6%) samples were confirmed as positive reactor for CFT. In addition, out of 259 examined mature camels at the breeding age more than four years old 28 (10.8%) and 28(10.8%) were positive for BAPAT and RBPT respectivily about 28 (10.8%) samples were confirmed as positive reactor for CFT. Chi square analysis for comparison between occurrence of brucella infection by different tests and different age groups revealed that there are no significant difference were detected between different age groups ($p>0.05$) suggesting that all ages of camels were susceptible to brucellosis and so brucellosis can started early in life probably through sucking and persisted into adulthood and this confirmed by highly significant infection rate in she-camels in this study. Also younger animals may be infected through transmission of the disease from adults to young animal during the long journey from Sudan until reaching Al-Shalateen quarantined through contact with other herds around source of water. Our results was supported by **Higgins et al. (1986)** who reported that young camels under (11) month were resistant to brucellosis and the infection was contracted mentioned that brucellosis is a disease of adult mature animals and younger animals tend to be more resistant to disease because sex hormones and erythritol tend to increase by age and sexual maturity.

Prevalence of brucella infection among 801 examined camels according to their sex was summarized in **Table(3)** and **Figure (3).** Out of 507examined male 36 (7.1%) camel were positive for CFT. While, out of 294 examined she-camel 56 (19.3%) were positive

for CFT. Chi square analysis for comparison between occurrence of brucella infection by different tests and sexes revealed that there are a high difference between male and females being higher in females than males. This results may be associated with erythritol (a sugar alcohol synthesized in the ungulates placenta stimulates the growth of virulent strains of *brucella species*) **(smith et al., 1962).** Also relaxation of immunity in females associated to lactation, pregnancy and other reproductive stress may also contribute to higher prevalence in female camels **(Gyles and Prescott, 2004).** These results agreed with **Bekele (2004)** and **Hadush et al. (2013)** from Ethiopia, **Yagoub et al. *(*1990)** and **Agab et al. (1994)** from Sudan, and **Ajogi and Adamu (1998)** and **Junaidu et al. (2006)** from Nigeria. On the other hand, others results shows equal distribution between both sexes **(Abu-Damir et al., 1989; Abbas et al., 1987).**

In the present study, lymph node from five seropositive camels at Al-Shalateen Abattoir and stomach content of one aborted fetus were subjected to isolation and identification.

Our trial to isolate the organism from the stomach content of aborted fetus has been successful and the morphological, cultural, biochemical and serological identification of the isolated brucella strain revealed isolation of ***Brucella melitinsis biovar 3.*** The ***Brucella melitinsis biovar 3*** was previously identified and considered as the prevalent type in Egypt in different animals as recorded by **(Sayour, 2004; Hoda et al., 2006; Khoudair and Sarfenaze, 2007; El-Diasty 2009; Rehab, 2011; Abdel Hamid, 2012; Menshawy, 2013; Affi et al., 2015).** Originally Brucella melitensis affects mainly sheep and goat. Such inter-species transmission situation may be the outcome of close contact between (sheep and goats) and camels **(Musa et al., 2008)** who suggested transmission of brucellosis to camels from in contact animals. That may explain the occurrence of this biotype in camels in our study also it may reveals the possibility of transmission of the disease to camels from sheep and goats.

Our attempts of isolation from lymph nodes failed. The isolation may fails if the number of viable brucella organisms in a test samples is low or contaminated with other bacteria which may prevent Brucella growth **(Seleem et al., 2010).** The specificity of serological tests cannot usually be determined by bacteriological isolation because some animals that yield negative culture results are in fact infected **(Alton et al., 1975; Poster et al., 2010)**.

From this study we concluded that high seroprevealance in camels imported from Sudan and imported she-camels is major source of infection and contamination of environment. Serological set of BAPAT , RBPT and CFT are recommended for brucellosis diagnosis. *Brucella melitensis biovar 3* was isolated from the stomach content of aborted camel.

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