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SEROPREVALENCE STUDY ON BRUCELLOSIS IN NATIVE AND IMPORTED CAMELS

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ABSTRACT:

Seroprevalence study was carried out on 470 camels (123 native camels in contact with other farm animals in Assiut and El- Wadi El Gadid Governorates and 347 imported camels from Sudan, through Daraw, Aswan Governorate) these camels with different ages and sexes were examined for detection of brucella antibodies. A high incidence of seroreactive cases was observed among native camels (2.43%, 12.19%, 4.87% and 2.43%) while it was (0.57%; 0.86%; 0.29 % and 0.29%) among imported camels using Rose Bengal plate Test, Buffered Acidified plate Antigen Test, Tube Agglutination Test and Rivanol test, respectively. Quantitatively, four serum samples were true seropositive - without any clinical signs - with a titer ranged from 1/25 to 1/400 using Rivanol test, while 3 serum samples were seroreactors with a titer of 1/40, 1/80 and 1/320 using TAT. Three (1.07%) of positive camels were adult over 4 years old and the remaining 1(0.52%) was young under 4 years old. It can be concluded that brucella seroprevelence in native camels was significantly different ($P < 0.05$) than in imported ones, however no significant differences could be recorded based on the sex or the age. The study proved that Rose Bengal plate Test followed by Tube Agglutination Test agreed with Rivanol test in the rapid diagnosis of brucellosis in camels.

Abbreviation:

(TAT) = Tube Agglutination Test.
(ELISA) = Enzyme linked Immune Sorbent Assay.
(RBPT) = Rose Bengal plate Test.
(BAPAT) = Buffered Acidified plate Antigen Test.
(PCR) = Polymerase chain reaction.

INTRODUCTION:

Camels have a great economic importance among farm animals in Egypt as well as other countries all over the world, they are considered as one of the main sources of meat, milk and hides. In Arab countries camels are estimated to be 11.918 million (FAO, 1989), while in Egypt camels reach up to 102327 (General Organization for Veterinary Service, 1998).

Wilson *et al.* (1989) and Wernery & Kaaden (1995) suggested that camels are less susceptible to diseases than other livestock, while Wilson (1984) and Higgins (1986) concluded that they are more susceptible than other animals to certain disease like brucellosis,

since camels may be carriers, or susceptible to infectious diseases, however Gwida *et al.* (2011a) suggested that camels are highly susceptible to brucellosis which caused by *Brucella melitensis* and *Brucella abortus*.

B. melitensis and *B. abortus* are widespread in Africa and USSR respectively (Wernery and Kaaden 1995). The incidence of brucellosis in camel populations appear to be related to breeding and husbandry practices (McGrane and Higgins, 1986), and depends upon the infection rate in the primary hosts being in contact with them (Cooper, 1991). In which larger herd size was identified as a risk factor for brucellosis in camels, also contact of camel herds with small ruminants was incriminated in the transmission of brucellosis to camels (Radwan *et al.*, 1992 & 1995; Abbas *et al.*, 2000; Abbas and Agab, 2002 and Al–Majali *et al.*, 2008) which concluded that generally the epidemiology of camel brucellosis is complicated by importation of live animals and by uncontrolled movement of animals across national borders, describing two patterns of camel brucellosis, the first is the low prevalence (2-5%) in camels kept extensively and the second is the high prevalence (8-15%) in camels kept intensively.

In Egypt at different localities the disease had been reported in camels with different prevalence rates (1.0-23.3%), (Salem *et al.*, 1990; Abou- Eisha, 2000; Abdel Moghney, 2004; Al Gaabary & Mourad, 2004; Fahmy & Zaki, 2006; El-Naggar *et al.*, 2006 and El- Boshy *et al.*, 2009). In Sudan camel brucellosis had been reported with seroprevalence of 37.5% (Omer *et al.*, 2010).

Three main camel populations were found in Egypt; local breed in country- side contact with other farm animals, imported camel breeds through El-Arbayn road and finally farm camels which are new camel categories kept for production.

Egypt import large number of camels from Sudan, so the aim of this work is directed to determination the prevalence of the disease in the imported camels from Sudan and native camels that in contact with other animals and in turn to evaluate the efficiency of some serological tests, like RBPT, BAPAT, TAT and Rivanol test for diagnosis of camel brucellosis.

MATERIALS AND METHODS:

I-Materials:

1-Samples :

Animal involved in the serosurvey for detection of brucella antibodies were 470 (423♂ & 47♀) dromedary camels {347 serum samples of camels imported from Sudan through Daraw, Aswan Governorate and 123 serum samples from native camels, incontact with other farm animals, at Assiut and El–Wadi El Gadid Governorates}. Two hundred and eighty camels were over 4 years old and the rest 190 camels were less than 4 years.

2-Antigens:

Rose Bengal, Buffered acidified, Tube agglutination and Rivanol antigens were supplied by Sera and Vaccine Research Institute, Abbassya, Egypt.

II-Methods:

A-Two qualitative tests:

1-Rose Bengal plate test (RBPT) was carried out according to Alton *et al.* (1988).

2-Buffered acidified plate antigen test (BAPAT), was carried out according to Anon (1984).

B-Two quantitative tests:

1-Tube agglutination test (TAT) was performed by heat inactivation technique according to Nielsen (2002) and the Central Veterinary Laboratories, Weybridge, England (Alton *et al.*, 1988) using 0.5% phenol saline as diluents.

2-Rivanol test (RIV. Test) was carried out according to the method described by Alton *et al.* (1988).

The agreement % of RBPT, BAPAT and TAT with Rivanol test was calculated According to Sayour, (1995) as:

$$\text{Agreement \%} = \frac{\text{Both tests positive+both tests negative}}{\text{(Total cases examined)}} \times 100$$

Statistical data analysis was done using Chi-square by SPSS, 2005 program (Statistical Package for Social Sciences for Windows Release 14.0.0.).

RESULTS:

The obtained results were recorded in Tables (1- 5).

Table 1: Seroreactive animals for brucellosis among imported and Native camels using RBPT, BAPT, TAT and Rivanol tests

Localities		Total No	Qualitative tests				Quantitative tests			
			Rose Bengal Plate Test		Buffered Acidified Plate Test		Tube agglutination Test		Rivanol Test	
			+ve	%	+ve	%	+ve	%	+ve	%
Native Camels*	El-wadi El-Gadid	113	3	2.65	15	13.27	6	5.3	3	2.65
	Assiut	10	0	0	0	0	0	0	0	0
	Total	123	3	2.43	15	12.19	6	4.87	3	2.43
Imported camels*		347	2	0.57	3	0.86	1	0.29	1	0.29
Total		470	5	1.06	18	3.83	7	1.48	4	0.85

* Significant statistical variations $\chi^2 = 4.98$ $p < 0.05$

Table 2 : Different titres of seroreactive camels for TAT and Rivanol tests

Animal Type	Tube Agglutination Test							Rivanol Test					
	Total reactors	1/10	1/20	1/40	1/80	1/160	1/320	Total reactors	1/25	1/50	1/100	1/200	1/400
Imported camels	1	0	0	1	0	0	0	1	0	0	0	0	1
Native camels	6	4	0	0	1	0	1	3	1	0	0	0	2
Total	7	4 57.14%	0	1 14.28%	1 14.28%	0	1 14.28%	4	1 25%	0	0	0	3 75%

Table 3 : Agreement % of other serotests with Rivanol test

Test	RBPT	BAPAT	TAT
Agreement %	99.79	97.02	99.36

Table 4 : Results of brucella seroreactive camels in relation to sex

Sex	No.	Positive reactors							
		RBPT		BAPAT		TAT		Rivanol test	
		No.	%	No.	%	No.	%	No.	%
♂	423	3	0.71	14	3.31	5	1.18	3	0.71
♀	47	2	4.26	4	8.51	2	4.26	1	2.13
Total	470	5	1.06	18	3.83	7	1.48	4	0.85

Table 5 : Results of brucella seroreactive camels in relation to age

Group	No.	Positive reactors							
		RBPT		BAPA		TAT		Rivanol test	
		No.	%	No.	%	No.	%	No.	%
< 4 years	190	2	1.05	7	3.68	3	1.58	1	0.52
> 4 years	280	3	1.07	11	3.92	4	1.43	3	1.07
Total	470	5	1.06	18	3.83	7	1.48	4	0.85

DISCUSSION:

Brucellosis of camels and other livestock is considered one of the great public health and widespread zoonosis in the world (Radostits *et al.*, 2007).

In the present study, Table (1) showing that among 470 imported and native camel serum samples examined for detection of brucella specific antibodies, the prevalence of seroreactive was 1.06%, 3.83%, 1.48% and 0.85% by RBPT, BAPT (qualitative serotests) and heat inactivation TAT and Rivanol tests (quantitative serotests), respectively. Since the TAT may be associated with blocking or incomplete antibodies or due to the molecular size of brucella antibodies in camels, using heat inactivation technique to avoid these phenomena (Nielsen, 2002).

Using TAT as a serological diagnostic test in camel brucellosis, Egyptian camels showed incidences scored as very high (23.3; 14 & 11.5 %) or high (5.6 & 6.66%) or low (1%) recorded by Salem *et al.* (1990); El–Sawalhy *et al.* (1996); Nada and Ahmed (1993); Nada (1990); Fayed *et al.* (1982) and Abou- Eisha (2000), respectively. Sudanese camels – using the same test - revealed prevalence of 31.5% (Agab, 1993) or very high as 70.6%(Gwida *et al.*, 2011 b), while Somalian camels showed 1.9 & 3.9% (Baumann and Zessin, 1992 and Ghanem *et al.*, 2009), and racing camels in United Arab Emirates showed 0.76% as positive reactors (Afzal and Sakkir, 1994).

By using RBPT, Egyptian camels showed prevalence percentages of 5.6; 5.3 & 4.4% (Fayed *et al.*, 1982; Nada, 1990 and El– Sawalhy *et al.*, 1996), while Sudanese camel- through a study (Gwida *et al.*, 2011 b)- showed a very high seroprevalence as 70.7%. Using BAPAT, positive reactor results were obtained in an Egyptian camels study as 7% (El–Sawalhy *et al.*, 1996), or as 10.6%through a Sudanese imported camels one (El-Naggar *et al.*, 2006).

In the present obtained results, the prevalence of brucella seroreactive imported camels were (0.57%, 0.86%, 0.29% and 0.29%) by RBPT, BAPT, TAT and Rivanol tests, respectively, while in native camels they were 2.43%, 12.19%, 4.87% and 2.43% using the same tests, respectively, Table (1). Through a previous study by Fahmy and Zaki (2006) the prevalence of brucella seroreactive was 0.9.5, 10.6, 8.5 and 9.515% for RBPT, BAPAT, TAT and Rivanol tests, respectively in imported camels (Sudanese camels), while in camels incontact with other animals (native camels) was 8.30%, 9.40%, 6.94 and 8.30%, respectively using the same previous tests. El-Naggar *et al.* (2006) used ELISA test concluded that the prevalence for local camels was 9.47% and for imported camels was very high as 25.8%. Ascertained the suggestion of Al-Gaabary and Mourad (2004) that the Seroprevalence of brucella among imported camels for slaughtering was higher than that in contact animals.

The wide variances may be attributed to that Egyptian native camels live in contact with some farm animals such as incriminated small ruminants with high risk infection than imported ones (Barsoum *et al.,* 1995; Abou Zaid, 1998; Abbas *et al.,* 2000 and Al-Majali *et al.,* 2008), and the imported camels were either kept with other infected farm animals or imported from an already infected focus (Gwida *et al.*, 2011 b).

In Egypt, Barsoum *et al,* (1995) detected a high percentage of positive brucellosis reactors in camels which in contact with other farm animals than that of other camels kept in closed farms or imported for slaughtering without contact with other farm animals. All the brucella isolates that recovered form camels at central region of Saudi Arabia appeared to be *B. melitensis* infect of sheep and goats (Radwan *et al.,* 1992 and Al–Dubaib, 2007). The high incidence of brucellosis raises especially in intensive breeding farm than camels grazing in the desert (Ghoneim and Amjad, 1993), or among the camel herds of agrapastralists than the herd of nomadosts (Agab, 1993). Moreover the varying husbandry and management practice, the number of susceptible camels, existence of reactor animals in the region, as well as the uncontrolled movement of humans and animals across the national borders play an important role in disease transmission and pathogen dissemination (Abbas and Agab, 2002). Generally, regional variation and diagnostic technique used are the main factors in these differences (Gwida *et al.,* 2011 b).

In the present study Table (2), using of quantitave tests showed that by TAT, the total seropositive reactors were 7 as follow; 4 (57.14%), 1 (14.28%), 1 (14.28%) and 1 (14.28%) of samples showing titres of 1/10, 1/40, 1/80 and 1/320, respectively. The high titre 1/320 indicated that this sample was from a camel having a high titre of blood serum agglutinins (El-Gibaly *et al.,* 1991). While by using Rivanol test, the positive results were 4 as follow; 1 (25%) and 3 (75%) of samples in titres of 1/25 and 1/400, respectively. The higher titres

1/400 indicted that these samples may be come from chronically infected camels (late stage of infection) as the Rivanol test determines only the agglutinating activity of the IgG isotype produced later in infection (FAO/WHO, 1986 and Alton *et al.*, 1988). The true positive samples using TAT was 3, with a titres of 1/40, 1/80 and 1/320, while they were 4 samples using Rivanol; 3 with titres of 1/25 and one 1/400.

The variation between different serotests in camels may reflect the serological differences in immunoglobulin classes active in each test (Mona *et al.*,1995; and Atwa, 1997), as the high percent of seroreactive camels (3.83%) using BAPAT may be attributed to the lower final antigen concentration 3% after addition of serum (Alton *et al.*, 1988). Moreover, the final pH of the test (4.0±0.04) after the addition of serum will permit the detection of both immunoglobulins (IgG & IgM) more than both other two tests which detect them. Since the IgG which is the specific brucella antibodies (Abo-El- Hassan *et al.*, 1991 and El–Sawalhy *et al.*, 1996), RBPT that revealed (1.06%) seroreactive cases with lower pH (3.65) inhabiting more IgM and enhance the agglutination of IgG (El-Sawalhy *et al.*, 1996) and TAT which revealed (1.48%) seroreactive cases.

Rivanol test revealed four seropositive reactors which were the true positive in the present study (Table 2). As it is one of the specific serological test (Sayour, 1995), it precipitates all IgM molecules- the non specific immunoglobulins which impair the results making cross reactions of other pathogens- and detects only the specific IgG1 and IgG2 reactions (Qureshi *et al.*,1999). The test was used in brucella diagnosis in cattle (Dajer *et al.*, 1999), sheep (Celebi, and Atabay, 2009), goat (El-Razik *et al.*, 2007), equine (Acosta-González *et al.*, 2006) or even in wildlife; as in elk (Schumaker *et al.*, 2010), white-tailed deer (Qureshi *et al.*,1999), and rather than in humans (Ciftçi *et al.*, 2005 and Acosta-González *et al.*, 2006). The test does not yield any false positive results (Schumaker *et al.*, 2010).

As mentioned above and for the recommendation of Acosta-González *et al.* (2006) to confirm the results of RBPT with Rivanol test, the present study considered the Rivanol test as a reference test to study the efficacy of other serological tests in rapid diagnosis of camel brucellosis. In the present work the agreement percentages were 99.79, 97.02, 99.36 % for RBPT, BAPAT and TAT, respectively (Table 3), concluding that - on the basis of specific Rivanol test–it is recommended to use of RBPT as qualitative and TAT as quantitative methods. The agreement of RBPT with Rivanol was reported (Ciftçi, *et al.*, 2005) and recommended by Gwida *et al.* (2011 b) in combining with real time PCR to screen camels for brucellosis.

In the present study Table (4), sex parameter study showed that female camels showed seropositive reactors more than males. Female camels are at a higher risk of contracting the infection than males since the presence of erythritol-a brucella growth supporting substances-occurs in higher

concentration in placenta and fetal fluids of pregnant females than in the seminal vesicles and tests of males (keppie *et al.*, 1965). In relation to age, Table (5) is showing that camels over 4-years old has a higher incidence than those under 4-years old, where the progressive increase of antibodies positive reaction in older camels could possibly be due to the increase chance for exposure to infection (Majid and Goraish, 2000).

It can be concluded that brucella seroprevelence in native camels was significantly different ($P<0.05$) than in imported ones, however statistically; no significant differences could be recorded based on the sex or the age. Otherwise, the study proved that RBPT followed by TAT agreed with Rivanol test in the rapid diagnosis of brucellosis in camels. So that camel populations must be put in consideration beside other farm animals during controlling the disease.

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دراسة سيرولوجية استبيانية على البروسيلا في الجمال المحلية والمستوردة

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أجريت الدراسة على عدد ٤٧٠ جمل (١٢٣ من الجمال المحلية تعيش ملاصقة لبعض حيوانات المزرعة، بمحافظتي أسيوط والوادي الجديد، ٣٤٧ من الجمال المستوردة من السودان، دراو- أسوان) من كلا الجنسين ومختلف الأعمار، وذلك بغرض استبيان نسبة تواجد البروسيلا في الإبل. أظهرت النتائج أن نسبة الحالات الإيجابية في الجمال المحلية (٢,٤٣٪، ١٢,١٩٪، ٤,٨٧٪، ٢,٤٣٪)، بينما في الجمال المستوردة كانت (٠,٥٧٪، ٠,٨٦٪، ٠,٢٩٪، ٠,٢٩٪)، وذلك باختبارات الروزبنجال، واختبار المحمض المخمد الشريحي، واختبار التلازن الأنبوبي البطيء واختبار الريفانول على التوالي. معياريا أظهرت ٤ عينات مصل الدم نتائج ايجابية – وبدون أية أعراض إكلينيكية – بعياريه تتراوح بين ١/٢٥ إلى ١/٤٠٠ باختبار الريفانول في حين أظهرت ٣ عينات فقط منها نتائج متجاوبة بعياريه ١/٤٠، ١/٨٠، ١/٣٢٠ باختبار التلازن الانبوبى البطئ. وكانت نسبة الإصابة في الجمال الأكبر من ٤ سنوات ١,٠٧٪ بينما في الجمال الأصغر من ٤ سنوات كانت ٠,٥٢٪.

أسفرت الدراسة أن الإصابة البروسيلا في الجمال المحلية تختلف معنوياً ($P<0.05$) منها فى المستوردة، ولم تسجل أية اختلافات معنوية بين المجموعات على أساس الجنس أو العمر. كما أثبتت الدراسة أن اختبار الروزبنجال يليه التلازن الأنبوبي البطيء الأكثر توافقاً مع الريفانول فى التشخيص السريع للبروسيلا في الجمال.