Kafrelsheikh Vet. Med. J. Vol. 9 No. 1 (2011) (229-247)

A SEROPREVALENCE STUDY OF BRUCELLOSIS IN GOATS AT KAFRELSHEIKH GOVERNORATE, EGYPT

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ABSTRACT

A random survey to study the seroprevalence of caprine brucellosis has been carried out during January to May 2011 in Matbool village of Kafrelsheikh Governorate, Egypt. A total number 276 sexually mature goats blood sera were collected from randomly sporadic 12 flocks of accessible unvaccinated goats to be analyzed. Rose Bengal Plate Test (RBPT) and indirect ELISA (I-ELISA) were used to screen all serum samples. On herd level, out of 12 flocks examined, 8 (66.66%) and 9 (75.0%) flocks were seropositive by RBPT and I-ELISA respectively. On animal level, overall seroprevalence of brucellosis among examined goats were 6.16% (n=17), and 7.97% (n=22) by RBPT and I-ELISA respectively. A non significant differences were found between the examined goats in relation to sex (p=0.8). As out of examined 112 male goats sera, 5.36% (n=6) and 7.14% (n=8) by RBPT and I-ELISA respectively, while for 164 female goats sera, 6.7% (n=11), and 8.54% (n=14) by RBPT and I-ELISA respectively. These results indicate high specificity of I-ELISA for brucella diagnosis in comparison to RBT.

Keywords: caprine, brucellosis, serology, prevalence, Rose Bengal test, ELISA.

INTRODUCTION

Brucellosis is a zoonosis that exists worldwide and is more or less endemic within most countries of Africa ***(Chukwu, 1985; Anonymous, 1986; Akakpo and Bornarel, 1987)***. For several decades it has been recognized as a significant public health problem in the Middle East and recent reports suggested that its incidence is increasing in both ruminants and humans ***(Benkirane, 2006; and Refai, 2002)*** and that currently applied control measures may not be capable of reducing the levels of infection in ruminants ***(Hegazy et al., 2009).***

Nearly all livestock animals are raised by traditional methods of husbandry in small herd/flocks with other species (sheep, cattle and/or camels) in small settlements with restricted movements except for grazing or water sources. There are six different species of *brucella*, where cattle, goats, sheep, pigs, buffaloes, camels, reindeer and, less frequently, other mammals are affected by brucellosis ***(Charters, 1980)***. Small ruminant brucellosis is mostly caused by *Brucella meltensis* ***(Omer et al., 2002)***. *Brucella ovis* is also an important cause of orchitis and epididymitis in sheep but it is not recognized as a cause of natural infection in goats ***(Smith and Sherman, 1996)***. In goats, excretion of the organisms from the vagina is prolonged and copious (2 to 3 months generally).

The spread of infection between flocks generally follows the movement or gathering of infected animals. Intermingling of flocks may occur under conditions of husbandry and also in static village flocks where animals are taken daily for grazing on common pastures.

Brucellosis has been reported in small ruminants from different parts of the world. Prevalence rates of 5.0% in goats in south provinces in Egypt ***(Montasser et al., 2011)***, 1.5 % in goats in Sudan ***(Abdala, 1966)*** ; 2.8% in goats in Kenya ***(Waghela, 1976)*** ; 5.29 % in goats in Somalia ***(Falade and Hussein, 1997)*** ; 3.8 % in goats in Eritrea ***(Omer et al., 2000)***; 4 % in goats eastern Sudan ***(El-Ansary et al., 2001)***; 4.75 % in goats in Nigeria ***(Shehu et al., 1999)***; 1.3 % in goats in Ethiopia ***(Ayele, 1991)***.

In order to be able to screen a large number of animals, the diagnostic tests should be inexpensive, easy to perform, rapid, highly sensitive and fairly specific, and suitable for screening individual animals ***(Nielsen, 2002).*** Tests currently used for the serological diagnosis of *brucella melitensis* infections in goats were initially developed for the diagnosis of *B. abortus* infections in cattle. The diagnosis can be directly by isolation of *Brucella*, or indirectly by the detection of immune response against its antigens. The rose bengal test (RB) are the most widely used test for the serological diagnosis of sheep brucellosis ***(Farina, 1985; and MacMillan, 1990)***. They are currently the official tests used in member states of the European Union (Council Directive 91/68/EEC).

However, recent results showed that the sensitivity of the classical RB antigen prepared with *B. abortus* biovar 1 (A-dominant) was adequate for diagnosing ovine and caprine populations infected with the M dominant *B. Melitensis* biovar 1 ***(Blasco et al., 1994b)***, and is internationally recommended for the screening of brucellosis in small

ruminants ***(Joint FAO/WHO, 1986); Garin-Bastuji and Blasco, 1997)***. The outer membrane of smooth *Brucella* is composed of phospholipids, proteins and lipopolysaccharide (smooth lipopolysaccharide, S-LPS). The S-LPS is the immunodominant antigen. Most serological tests, particularly those using whole-cell suspensions as antigen (such as RB, CF), as well as ELISA, have been developed to detect antibodies to this antigen ***(Díaz et al., 1968a)***. The large majority of EIAs in use for brucellosis diagnosis are indirect ELISAs (iELISA). ***(Gorrell et al., 1984); Rylatt et al., 1985); Sutherland et al., 1986); MacMillan, 1990); Greiser-Wilke et al., (1991); Nielsen et al., 1991); Marín et al., 1999); Nielsen et al., 2000)***. (iELISAs) have been developed using more or less purified S-LPS as the antigen and have been reported to be at least as sensitive and specific as the combination of both RB and CF tests for the diagnosis of brucellosis in ruminants ***(Alonso-Urmeneta et al., 1998)***.

Therefore, a combination of the two tests (RBPT and I-ELISA) shows a degree of sensitivity and specificity which appears sufficient to detect infected animals, and removal of those animals appears to contribute to disease control. The objective of this study was, therefore, to determine the prevalence of goat brucellosis in a village at kafrelsheikh governorate, Egypt.

MATERIAL AND METHODS

1- Study Area:

This study was conducted during the period from January to May 2011 in Matbool village, Kafrelsheikh Governorate, Egypt.

2- Study Animals:

A total number of unvaccinated 276 goats were examined in this study constituting 12 herd flocks (112 males and 164 females) of animals older than 6 months. (The number of heads per flock ranged between 18-31). Information of each herd sampled was obtained including its location, herd size, sex, age, health status, history of abortion, wheather reared individually or with other species.

3- Blood sample:

Blood samples were collected from unvaccinated 276 goats of both sexes. A 10 ml blood samples were collected using vacutainer tubes with a separate needle for each blood sample and each sample was allowed to clot and transferred onto ice box as quickly as possible to the laboratory of the central laboratory of Faculty of Veterinary Medicine, Kafrelsheikh University. The sera were separated by centrifugation at 2000 rpm for 10 min and each serum sample was aspirated in an eppendorf tube using a pasteur pipette, labeled and stored at -20° until testing

4-Serological tests:

All collected sera were screened for antibodies against *Brucella* by the Rose Bengal plate Test (RBPT), and the Indirect Enzyme Linked Immunosorbant Assay (I-ELISA) (SERELISA) as described by the manufacturers

4.1. Rose Bengal Plate Test (RBPT):

Rose bengal antigens were obtained from ***CZ Veterinaria, S.A. SPAIN (Batch number, 07014)***. Antigens stored between +2 and +8°C, and protected from light. Test procedure according to the manufacturer

as follows: A control serum was tested one day before the test to verify the test conditions. The sera and antigens were removed from the refrigerator and left at room temperature (+22°C ± 4°C) for at least 30 minutes before the test was performed. Briefly, 30µl of sera samples were dispensed onto the white 12 well ceramic plate, and 30µl of RBPT antigen using a micropipette was mixed with the sera using tooth picks and shaken for 4 min using an electric shaker in single direction before examined for agglutination. For interpretation of the results, both positive and negative controls were employed. Results of RBPT were interpreted as negative, doubtful and positive (no visible agglutination regarded as negative, slight agglutination as doubtful, clear visible agglutination and rim formation as positive).

Indirect Enzyme Linked Immunosorpent Assay (I-ELISA):

According to the procedure of the manufacturer **(Synbiotics Europe "SERELISA® Brucella OCB Ab Mono Indirect" 2, Rue A. Fleming 69007 LYON – FRANCE; Batch number, 8 SBRU30CB 08),** indirect ELISA was performed. Kit uses an indirect immunoenzymatic technique allowing the detection of *Brucella* lipopolysaccharide (LPS) antibodies in individual serum samples of *B. aborus* and *B. melitensis*. Serum and ELISA kit are allowed at room temperature for at least 30 minutes before use. The reaction is composed of the following steps: Each individual serum sample is placed in a well sensitized with the *Brucella* LPS. Antibodies present in the sample bind to the bacterial antigen coated to the wells. A 100 µl of the diluted negative control (N)

distributed in wells A1 and A2, and 100 µl of the diluted positive control (P) in wells B1 and B2. Dilute the samples at 1:10 in sample diluent (SD) in a dilution plate. Dilute again at 1:10 directly in sample diluent (SD). Distribute 100 µl in each well, cover the wells with adhesive film, mix by gentle shaking the plate manually and incubate of the plate for 1 hour (± 5 min) at + 37°C (± 3°C). Dilute the concentrated washing solution (W) 1:10 in distilled water. Carefully remove the adhesive film and wash 4 times (300ul/well). Dilute the concentrate (CJ) 1:200 in the conjugate diluent (CD). Add 100 µl of diluted conjugate to all wells and cover with a new piece of adhesive film. Incubate 30 minutes (± 5 min) at +37°C (± 3°C). Carefully remove the adhesive film and wash 4 times. Add 100 µl of buffered peroxidase substrate (PS) per well. Do not cover with adhesive film at this stage. Mix by gentle shaking the plate manually. Incubation of substrate for 30 min. ± 5 min at laboratory temperature (+20°C ± 5°C), shielded from light. Add 50 µl of stop solution (S) per well. Mix by gentle shaking the plate manually. Measure the optical density (OD) monochromatically at 450 nm and/or bichromatically at 630 nm. The results of each test run are valid if: OD P $\geq$ 0.5 and OD N $<$ 0.3 The presence or the absence of antibodies against LPS of *Brucella* is determined by comparing the Optical Densities (OD) to the threshold values obtained from the positive control. Positive threshold value in index = 0, Sample index = 0.50 x (sample OD – 0.6 x OD P). Any sample presenting an index $\leq$ 0 is considered as positive. Any sample presenting an index $<$ 0 is considered as negative.

RESULTS

Seroprevalence results:

On herd level, out of 12 flocks examined, 8 (66.66%) and 9 (75.0%) flocks were seropositive by RBPT and I-ELISA respectively (Table 1 and 2). On animal level, overall seroprevalence of brucellosis among examined 276 goats were 6.16% (n=17), and 7.97% (n=22) by RBPT and I-ELISA respectively. A non significant differences were found between the examined goats in relation to sex ($p<0.8$). As out of examined 112 male goats sera, 5.36% (n=6) and 7.14% (n=8) by RBPT and I-ELISA respectively, while for 164 female goats sera, 6.7% (n=11), and 8.54% (n=14) by RBPT and I-ELISA respectively (Table 3). The obtained data had been analyzed statistically using chi-square test according to **Snedecor and Cochran, (1980).**

Table (1): Seroprevalence of caprine brucellosis on herd level in Matbool village of Kafrelsheikh governorate.

District	Species herd No. Numbers tested				RBPT Positive				I-ELISA Positive			
					M.		F.		M.		F.	
	Goats	Total	males	No.	No.	%	No.	%	No.	%	No.	%
Matbool	1	18	7	11	0	0.0	0	0.0	0	0.0	0	0.0
	2	31	13	18	1	3.23	1	3.23	1	3.23	2	6.45
	3	26	12	14	1	3.85	2	7.69	1	3.85	3	11.54
	4	19	7	12	0	0.0	0	0.0	0	0.0	0	0.0
	5	22	9	13	0	0.0	0	0.0	1	4.55	0	0.0
	6	24	10	14	1	4.17	1	4.17	1	4.17	1	4.17
	7	26	11	15	1	3.85	2	7.69	1	3.85	2	7.69
	8	15	5	10	1	6.67	1	6.67	1	6.67	2	13.33
	9	11	3	8	0	0.0	0	0.0	0	0.0	0	0.0
	10	28	13	15	0	0.0	1	3.57	0	0.0	1	3.57
	11	27	11	16	1	3.7	1	3.7	1	3.7	1	3.7
	12	29	11	18	0	0.0	2	6.9	1	3.45	2	6.9
Total	12	276	112	164	6	5.36	11	6.7	8	7.14	14	8.54

Table (2): Seroprevalence of brucellosis among examined flocks and animals.

	No. of examined	RBPT		I-ELISA	
		+Ve	%	+Ve	%
Flocks	12	8	66.66	9	75.0
Animals	276	17	6.16	22	7.97

Table (3): Seroprevalence of brucellosis among examined goats in relation to sex.

Test Sex	RBPT			I-ELISA		
	No. of animals	+Ve	%	No. of animals	+Ve	%
Male	112	6	3.36	112	8	7.14
Female	164	11	6.7	164	14	8.54
Total	276	16	6.16	276	22	7.97

DISCUSSION

Brucellsosis for several decades has been recognized as a significant public health problem in the Middle East and recent reports suggested that its incidence is increasing in both ruminants and humans ***(Benkirane, 2006; and Refai, 2002)*** and that currently applied control measures may not be capable of reducing the levels of infection in ruminants ***(Hegazy et al., 2009).*** Reported brucellosis prevalence in small ruminants (sheep and goats) in Sub-Saharan Africa (SSA) varied from 2.4 % to 22.7 % ***(McDermott and Arimi, 2002).***

The present study showed that the prevalence of *Brucella* antibodies in goats was 6.16% using RBPT, and 7.97% by I-ELISA. ***Nada, (1982)*** found nearly similar results (4.7%). ***Kaoud et al., (2010)*** found higher results (14.5%). This difference between the prevalence of brucellosis determined by RBPT and that of I-ELISA may be attributed

to the fact that I-ELISA is more sensitive in detection of IgM as well as IgG immunoglobulin ***Stemshorn et al., (1985)***. I-ELISA has been found to be more sensitive and more specific test for detection of *Brucella* antibodies that provides relatively accurate results. In addition, I-ELISA has been found to detect antibodies in chronically infected animals, while RBPT detects antibodies only in acutely infected subjects ***(Alonso et al., 1995; S' Rtmadzhiev et al., 1998; Sting and Ortmann, 2000; Ongor et al., 2001; and Cruz et al., 2002)***.

In the present study, the LPS were used for the development of I-ELISA. The LPS were used because *Brucella* LPS been considered the most important antigen during immune response and are the target for many serological and immunological studies. In addition, a small quantity is required for the screening of a large number of samples. According to ***Guarino et al., (2001)***, the high percentage of positivity was due to the ability of this test to detect very low levels of antibodies present in the early stage of infection, while RBPT and SAT cannot detect it. This standardized I-ELISA could be a useful diagnostic test for detection of *Brucella* antibodies.

It is note worthy that no single test can identify all infected animals at all stages of the disease and therefore a combination of serological tests should be included to reduce the number of both false negative and false positive serological reactions ***(Morgan, 1971; and Cordes and Carter, 1979).***

The higher prevalence of *Brucella* infection detected in the present investigation could be favored by the husbandry practices in the regions and the absence disease monitoring and control policy. Goats were found

to be at higher risk than sheep, in part due to the greater susceptibility of goats to *Brucella* infection than sheep and partly it is due to the fact that sheep unlike goats do not excrete the *Brucella* organisms for longer periods of time. The variation in prevalence between sheep and goats could be supplemented by the fact that goats which are inherently more susceptible than sheep.

There were little significant differences of the seroprevalence between male and female goats studied as it was in 112 males sera 5.36% (n=6) by RBPT and 7.14% (n=8) by I-ELISA, while it was in 164 females sera 6.7% (n=11) by RBPT and 8.54% (n=14) by I-ELISA. It was reported that serological response of male animals to *Brucella* infection is limited as it was indicated by ***Crawford et al., (1990)***, and also, testing of infected male animals were usually observed to be non-reactors or showed low antibody titers. Similarly, one research finding showed that male cattle are more resistant than females ***(Nicoletti, 1980).***

However, the apparently higher seroprevalence figure in female animals compared to males in this study agrees with other works. ***(Asfaw et al., 1998); Bekele et al., 2000; and Tolosa, 2004).*** Further epidemiological studies and identification of the *Brucella* biotypes involved is recommended. The system must be able to detect early any change in incidence and prevalence. Vaccination of young animals can be combined with a test and slaughter policy in a long term action to control brucellosis in small ruminants ***(WHO/MZCP, 1998)***. It is usually accepted that a program of eliminating brucellosis by test and-slaughter policy is justified on economic grounds only when the prevalence of infected animals in an area is about 2% or less ***(Mustafa, and Nicoletti,***

(1993). The provision of information and education concerning the disease to farmers and local communities is essential. Climatic conditions may affect concentration patterns of herds in given areas. This prevalence according the number tested may be due to many factors such as lacking of vaccination against brucellosis and lack of effective program for eradication including periodical testing and slaughtering of reactors. The used I-Elisa indicate higher specificity and sensitivity in diagnosis of brucellosis of goats.

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الملخص العربى

فى هذه الدراسة، تم عمل لدراسة معدل إصابة الماعز بالبروسيلا سيرولوجيا خلال الفترة من يناير الى مايو عام 2011 بقرية متبول بمحافظة كفر الشيخ بمصرفى عدد 276 ماعز فى الاعمار اكبر من 6 شهور. تم تجميع عينات السيرم من عدد 12 قطيع غير محصنة. اجرى اختبارى الروز بنجال والاليزا الغير مباشرة للكشف عن الأجسام المضادة فى عينات السيرم. اظهرت النتائج، بفحص عدد 12 قطيع عدد 8 قطعان موجبة سيرولوجيا بنسبة 66.67٪ وعدد 9 قطعان موجبة سيرولوجيا باختبارى الروز بنجال و الاليزا الغير مباشرة على التوالى. على مستوى الحيوانات كانت النتائج للمسح السيرولوجى الكلية لسيرم الماعز المفحوصة وعددها 276 هو 6.16٪ (عدد=17) و7.97٪ (عدد=22) باختبارى الروز بنجال و الاليزا الغير مباشرة على التوالى. كانت نتائج المسح السيرولوجي حسب الجنس غير ذات اهمية معنوية . بالنسبة لعدد عينات السيرم المحوصة لعدد 112 ذكر الماعز، كانت النسبة 5.36٪ (عدد=6) ونسبة 7.14٪ (عدد=8) باختبارى الروز بنجال والاليزا الغير مباشرة على التوالى بينما كانت النسبة لعدد 164 عينة سيرم لانثى الماعز هو 6. 7٪ ' (عدد=11) ونسبة 8.54٪ (عدد=14) على التوالى. نستخلص من هذه النتائج أهمية اختبار الاليزا الغير مباشرة فى تشخيص مرض البروسيلا فى الماعز.