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DIAGNOSIS OF BRUCELLOSIS IN LOW TITRED BUFFALOES

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SUMMARY

A total of 452 serum samples collected from non-vaccinated buffaloes were subjected to serological tests by using Rose Bengal Plate Agglutination Test (RBPT), Slow Tube Agglutination Test (SAT) and indirect ELISA (iELISA) detected 12.83%, 11.28% and 19.25% positive samples for brucellosis respectively. The relative sensitivity of RBPT and SAT was found 62.07% and 55.17%, respectively, considering iELISA as a gold standard test while the specificity was found 98.90% in RBPT and 99.18% in SAT; the overall agreement of RBPT and SAT with iELISA was 91.81% and 90.71%, respectively. Twenty one isolates out of 61 *B.melitensis* biovar 3 were isolated from buffaloes serologically positive to iELISA but negative to SAT of low titre ranged from 1:10 to 1:40. Therefore, iELISA was found to be a better serological test as compared with RBPT and SAT and it could be advocated for screening of brucellosis among buffaloes as will as the suspicious and /or the latent infectious cases.

INTRODUCTION

Brucellosis is a highly contagious, zoonotic and economically important bacterial disease of animals worldwide (OIE, 2000). The disease is caused by various species of the genus *Brucella*, which are facultative, intracellular bacteria capable of surviving and multiplying inside the cells of mononuclear phagocytic system (Jarvis *et al.*, 2002). The disease causes significant economic losses including abortion, loss in milk production, low fertility rates and cost of replacement of animals (McDermott and Arimi, 2002).

In water buffaloes (*Bubalus bubalis*), latent infections and prolonged incubation of *Brucella* organisms limit the water buffalo that occupy an economically important place in the livestock industry in many parts of the world (as Egypt). Only a few water buffalos that become infected develop clinical signs of the disease (spontaneous abortion), (Ibrahim

et al., 2002 and Borriello *et al.*, 2006). However, many infected buffaloes shed *Brucella* organisms in the milk (Ibrahim *et al.*, 2006). In Egypt eradication programs involving the slaughter of infected animals have been carried out for more than 20 to 30 years. However, latent infections, prolonged incubation of the pathogen, incomplete protection provided by vaccines, and difficulties in distinguishing serologically between vaccinated and naturally infected animals have limited the efficacy of eradication programs. Remarkably, even in water buffalo herds heavily infected with *Brucella* organisms, about 20% of the subjects remain negative by the conventional serological tests and presumably noninfected all the time (Borriello *et al.*, 2006).

Early detection, control and elimination of reactors are important considerations for the control of brucellosis. Brucellosis is diagnosed by classical serological techniques as agglutination, precipitation and complement fixation but these techniques have several drawbacks such as poor performance and lack of standardization (OIE, 2000). At present, application of the ELISA technique is considered as a better test in early detection of infection than complement fixation test (Rojas and Alonso, 1995). Indirect enzyme linked immunosorbent assays (iELISAs) have been developed and used in various countries for sero-diagnosis of brucellosis in cattle, and other animals (Romero *et al.*, 1995; Dajer *et al.*, 1998; Molnar *et al.*, 1998 and Omer *et al.*, 2001), however, such kind of work in buffaloes is limited (Guarino *et al.*, 2001).

So, the aim of the present study was to use a rapid and accurate test for confirmation of brucellosis among buffaloes with special regards to the suspicious and / or the latent infectious cases.

MATERIALS AND METHODS

Test sera:

A total of 452 serum samples were collected from non-vaccinated buffaloes (156 of them were having a history of various gynecological disorders like abortion, retention of placenta, endometritis, metritis, infertility and repeat breeding).

I-Serological tests:

I-1- Conventional tests:

The tests used were the Rose Bengal Plate Test (RBPT) and the Slow Tube agglutination test (SAT) as described by Alton *et al.* (1988). In the RBPT any degree of agglutination was considered to be positive. For the SAT, visible agglutination at the dilution of 1/40++ or more was considered to be positive.

I-2- Indirect ELISA:

An ELISA kit (SERELISA) provided by the SYNBIOTICS EUROPE SAS CORPORATION, FRANCE which contained all the necessary reagents was used. The test

was performed according to the manual which is accompanied with the kit.

Statistical Analysis:

The efficacy of the two conventional serological tests (RBPT and SAT) was compared with the gold standard test (iELISA) by testing serum samples of buffaloes. The iELISA was taken as the gold standard test because this is the most reliable test for the diagnosis of brucellosis as Hobbs (1985) and Nielsen *et al.* (1996).

II-Bacteriological Examination:

A total of 156 samples (98 Milk samples, 19 aborted foeti, 25 Retained placentas and 14 uterine swabs) were collected under sterile conditions from buffaloes having a history of various gynecological disorders. The suspected isolates were identified according to MacMillan (1990).

RESULTS

Out of 452 serum samples collected from buffaloes, 58 (12.83%) and 51 (11.28%) were found positive by RBPT and SAT respectively. While, iELISA gave more positive samples 87 (19.25%) as shown in Table (1).

Table (2) showed that 48 serum samples were positive in all tests at SAT end titre of 1:40++ to 1:320. On the other hand, 39 and only 6 serum samples were positive for iELISA and RBPT respectively at low titred SAT of 1:10++ to 1:40+. However, only 4 serum samples were positive by RBPT but negative by iELISA.

In buffaloes, iELISA was compared with RBPT and SAT for sensitivity and specificity. A total of 452 sera were tested by iELISA and compared with RBPT and SAT, Cross tabulation of RBPT and SAT with iELISA, considering iELISA as a gold standard test were statically analyzed as Hobbs (1985) and Nielsen *et al.* (1996) as shown in Tables (3 and 4).

Out of 156 samples collected from buffaloes with different gynecological disorders, 61 *Brucella* isolates were identified as *B.melitensis* biovar 3 and the correlation of *B.melitensis* with SAT end titre is recorded in Table (5).

Table 1: Detection of *Brucella* antibodies by RBPT, SAT and iELISA in buffaloes.

No. of	RBPT		SAT		iELISA	
Examined sera	+ve	%	+ve	%	+ve	%
452	58	12.83	51	11.28	87	19.25

Table 2: Correlation of SAT end titre with RBPT and iELISA in buffaloes.

SAT end titre		iELISA Positive	RBPT Positive
Low titre SAT	1: 10	11	1
	1:20	22	3
	1:40	6	2
Total		39	6
Positive SAT	1:40	18	22
	1:80	14	14
	1:160	12	12
	1:320	4	4
Total		48	52
Total		87	58

Table 3: Comparison of sensitivity and specificity of RBPT with iELISA results in buffaloes.

Test	Result	iELISA Positive	iELISA Negative	Total	Sensitivity (%)	Specificity (%)	Over all agreement (%)
RBPT	Positive	54	4	58	62.07	98.90	91.81
	Negative	33	361	394			
	Total	87	365	452			

Table 4: Comparison of sensitivity and specificity of SAT with iELISA results in buffaloes.

Test	Result	iELISA Positive	iELISA Negative	Total	Sensitivity (%)	Specificity (%)	Over all agreement (%)
SAT	Positive	48	3	51	55.17	99.18	90.71
	Negative	39	362	401			
	Total	87	365	452			

Table 5: Correlation of *B.melitensis* isolation with SAT end titre in buffaloes.

Type of samples	No. of samples	SAT end titre 1:10++	1:20++	1:40+	1:40++	1:80	1:160	1:320	Total
Milk	98	1	3	1	4	7	4	-	20
Aborted foeti	19	-	-	3	8	3	2	2	18
Retained placenta	25	3	3	2	1	3	2	2	16
Uterine Swabs	14	2	3	-	1	1	-	-	7
Total	156	6	9	6	14	14	8	4	61

DISCUSSION

The water buffalo (*Bubalus bubalis*) occupies an economically important place in the livestock industry in many parts of the world. One of these is Egypt. Brucellosis causes serious economic losses and is relevant also as a zoonosis (Boschiroli *et al.*, 2001). The diagnosis of brucellosis can be based on cultural isolation, serological tests and biotechnological techniques. Cultural

isolation is time consuming, cumbersome and requires specialized laboratory personnel.

From the aforementioned results, iELISA identify more positive samples in buffaloes (19.25%) than RBPT (12.83%) and SAT (11.28%) [Table, 1]. The discrepancy of this result is regarded to that RBPT is qualitative and SAT although it is quantitative, is mostly sensitive to IgM antibodies; while IgG antibodies are the most prevalent isotype with immune response to *Brucella* infection (Rose and Amerault,1964 and Lamb *et al.*,1979). Morever, this result supported by Chatterjee *et al.* (1984) who found 19.6 percent prevalence in buffaloes. Similarly, these results coincide with those previously reported by Rao *et al.* , 1999 ; Chakraborty *et al.*, 2000 ; Barbuddhe *et al.*, 2003; Sarumathi *et al.*,2003 ; Chand and Sharma,2004 ; Bhattacharya *et al.*, 2005 and Brahmabhatt *et al.*,2009. However, lower seroprevalences were reported by Isloor *et al.* (1998), 1.8 %; Bhattacharya *et al.* (2005), 11.94 % and Agarwal *et al.* (2007), 4.6 %, while the prevalence found in the present study was lower than that observed by Chauhan *et al.* (2000), 38.9 % in North Gujarat region of India.

The seroprevalences determined by various tests differed from one another, however, 48 serum samples were positive in all tests at SAT end titre of 1:40++ to 1:320; while 39 and only 6 serum samples were positive for iELISA and RBPT respectively at low titre SAT of 1:10++ to 1:40+. This could be due to variation in the numbers of false positives and false negatives detected by various tests (Table, 2). Similar findings were reported by Rao *et al.* (1999), and Singh *et al.* (2004). Also, iELISA is a sensitive test which can detect low concentrated antibody and test poor quality serum (Hobbs, 1985).

The application of multiple serological assays currently available for the detection of *Brucella* antibodies in various species of animals indicates that no single test can detect all infected animals and therefore, combination of serological tests should include more sensitive tests designed to reduce the number of false negative reactions which contribute to the persistence of infection as a herd problem in buffaloes. In the present investigation, iELISA in conjunction with RBPT and SAT were employed to compare their efficacy. The sensitivity of RBPT and SAT was 62.07% and 55.17%, respectively, considering iELISA as a gold standard test while the specificity was 98.90% in RBPT and 99.18% in SAT. Thus, RBPT was found to be more sensitive than that of SAT, while SAT was found to be more specific than RBPT. The overall agreement of RBPT and SAT with iELISA was 91.81% and 90.71%, respectively. Hence, iELISA was found to be a better serological test as compared with RBPT and SAT and it could be advocated for screening of animals (Table,3 and 4). Similar

results were obtained by Brahmabhatt *et al.* (2009) , they revealed sensitivity of RBPT (64.58%) much higher than SAT (56.25%), while specificity of both tests was (99.50%) when compared with iELISA as a gold standard. Also, Singh *et al.* (2004), they Reported sensitivity of RBPT (88.46%) much higher than SAT (46.15%), while specificity of SAT (98.31%) was found slightly higher than RBPT (97.75%) considering iELISA as a gold standard. However, in contrast to the present study, Chakraborty *et al.* (2000) found higher sensitivity (88.61%) and specificity (98.59%) of SAT over RBPT with sensitivity (56.96%) and specificity (96.77%). Paweska (2002) suggested that ELISA could replace not only the currently used confirmatory CFT, but also other two routine screening tests, namely the RBPT and SAT. Chand and Sharma (2004) advocated the use of ELISA in comparison to RBPT and SAT for assessing the situation of brucellosis in cattle to have better results because chances of non detection of an infected animal in ELISA are minimum.

Sixty One *B.melitensis* biovar 3 were isolated in this investigation (Table, 5), of them 21 isolates were isolated from buffaloes serologically positive to iELISA but negative to SAT of titre ranged from 1:10++ to 1:40+. These findings supports Uzal *et al.* (1995) who reported that iELISA became valuable tool for the diagnosis of bovine brucellosis, where little epidemiological information is available about this disease and where large numbers of sera should be tested to obtain such information.

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تشخيص مرض البروسيلا فى الجاموس ذات العيارية المنخفضة

محمد محمد بسيونى

فى هذه الدراسة تمت مقارنة نتائج اختبار الاليزا الغير مباشر بنتائج الاختبارات السيرولوجية التقليدية للكشف عن وجود أجسام مضادة لميكروب البروسيلا فى عدد ٤٥٢ جاموس غير محصن منهم ١٥٦ حيوان يعانى من تخلفات تناسلية مختلفة . وقد أشارت النتائج الى أن اختبار الاليزا الغير مباشر يعطى أعلي نسبة ايجابية (١٩.٢٥%) للأجسام المضادة للبروسيلا، بينما اختبارات الروز بنجال والتلزن الأنبوبى البطئ قد أعطت ايجابية بنسبة ١٢.٨٣% و١١.٢٨% على التوالى. وقد تم عزل ميكروب البروسلا مليتنسس من ٦١ حيوان منهم ٢١ ذات عيارية منخفضة باختبار التلزن الأنبوبى البطئ بالرغم من ايجابيتهم باختبار الاليزا الغير مباشر. ومن هذه الدراسة يمكن أن نستخلص أن: استخدام اختبار الاليزا الغير مباشر في الجاموس الغير محصن من أفضل الاختبارات للكشف عن مرض البروسيلا خصوصا في حالات الاشتباه أو الاصابة الكامنة بالبروسيلا.