

INVESTIGATIONS ON BRUCELLOSIS IN A LARGE DAIRY BUFFALO HERD

(With 9 Tables)

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دراسات عن مرض البروسيلا فى مزرعة جاموس حلاب

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أجريت دراسات عن مرض البروسيلا فى إحدى مزارع الجاموس الكبرى وذلك حينما حدث بها حالات متعددة من الاجهاض المتأخر. تم فحص الحيوانات بأختبارات مصلية مختلفة ووجد أنه لا يوجد اختبار كاف بمفرده للتشخيص حيث أن مستويات أضداد البروسيلا فى الحيوانات المجهضة كانت ما بين ١٠/١ الى ١٦٠/١. أظهر تقصى المرض أن معدلات الحدوث الكلية فى الإناث تبعا للاختبارات المصلية ١٩.٩% ولاختبار اللبن ١٢.٣% ووجد أن ٢٥% من الذكور تفاعلت مصليا ضد المرض. توصى الدراسة بأضافة استخدام اختبار اللبن الى جانب الاختبارات المصلية لتعزيز التشخيص.

SUMMARY

An investigation was carried out to find the seroprevalence of brucellosis in buffaloes in a large commercial dairy farm which had an alarming rate of abortions. Many abortions occurred in buffaloes at 7-9 months of pregnancy. The animals were subjected to various serological tests for brucellosis and it was found that no single test correctly identified all the animals. Aborted buffaloes showed titres against B.abortus antigen from 1/10 to 1/1/60. The overall incidence of brucellosis in females as revealed by blood tests was 19.9% and by milk ring test 12.3%. The incidence in examined males was 25%. The importance of carrying out milk ring tests for correct evaluation of brucellosis infection in buffaloes was stressed out.

Key words: Brucellosis- Dairy buffalo.

INTRODUCTION

Brucellosis is one of the most important diseases of farm animals including buffaloes. It can disrupt the whole breeding and production programme with added hazards of being an important zoonotic disease. Its prevalence has increased in recent years, especially in countries which have promoted change from traditional low density to intensive methods of dairy farming. The infection is widespread in many buffalo-breeding countries. The incidence of brucellosis varies from a few sporadic cases to more than 25% in closed herds (Hamada *et al.*, 1963; El-Ahwal *et al.*, 1968; Pal and Jain, 1986, Sharma *et al.* 1988).

In developing countries, dairies are coming up mostly around large cities. Brucellosis became obvious on this farm under study few months after buying no-tested buffaloes in mid 1992. Interesting observations have been highlighted here.

MATERIAL and METHODS

Dairy herd. There had been an alarming incidence of abortions took place in buffaloes during 7 to 9 months of pregnancy on a large organized dairy farm located at Giza Governorate.

Sampling:

I- Male animals. A total of 18 blood samples collected from 13 young and 3 adult bulls. In addition 2 samples of testicular exudate aspirated with a sterile syringe from a case of excessively enlarged right testis of young bull were taken.

II- Female animals. A total of 295 blood samples and 282 individual samples of milk were collected from 232 lactating buffaloes. Also 19 bulk samples of milk from buffaloes were included. The bulk samples contained the pooled milk of 15-20 animals at different stages of lactations. All milk samples were collected without adding any preservative. Further 44 blood samples were collected from 43 dry buffalo-cows. Three samples of foetal fluids secured after abortion were also examined for the presence of brucella.

Serological examination:

a- Buffered brucella antigen tests: The Rose Bengal plate test (RBPT) and the buffered acidified plate antigen (BAPA) test were performed as described by Alton *et al.* (1988).

- b- The serum agglutination test (SAT) was performed as described by Alton *et al* (1988) and all reacting samples were tested to their end-point. Serum samples were screened at first by the BAPA, RBPT and SAT using standardised *B.abortus* antigens (Serum & Vaccine Res. Inst., Abbasia, Cairo). Postive sera were further subjected to the EDTA modified SAT (Nielsen *et al*, 1979).
- c- Milk ring testes (MRT) were carried out according to the methods recommended by Morgan *et al*. (1978) on bulk milk samples (BMR test) and individual milk samples (IMR test). Haematoxylin-stained *B.abortus* antigen suspension supplied by Ser. & Vacc. Res. Inst. was used. Tests were read after 3 hours incubation at 37°C. Readings of \pm and above were considered reactions to the MRT.

Bacteriological examinations:

Were made using a brucella agar medium containing antibiotics and incubated at 37°C in an atmosphere of 10% CO₂ and in air for 6 days and then examined for brucella colonies. Suspected brucella colonies were identified and typed according to conventional methods (Alton *et al*, 1988).

RESULTS

Male animals

Out of 16 males tested, only 4 (25%) were found to be sero-reactors for brucella infection (Table 1). Low antibody titres were detected in the sera of 3 breeding bulls. However, There was one young bull exhibiting both serological and clinical evidence of brucella infection. He showed orchitis, periorchitis, and epididymitis. Upon slaughter, the right testis and spermatic cord were several times the size of the left. The involved testis was very hard in consistency with fluctuant areas due to gross abscess formation. A thick, creamy, flaky pus was obtained by examination of puncture samples from the testis but all cultural attempts to isolate brucella were unsuccessful.

DISCUSSION

Brucellosis is widely regarded as an extremely insidious disease, demanding the most exhaustive care in diagnosis. Many cases are more difficult to diagnose than others, and a few are extremely difficult to pick up, and could easily be missed altogether. (Robertson, 1971). Diagnosis of brucellosis in the buffalo herd under study was confirmed in the earlier cases of abortion by isolation of *B.abortus* from foetal fluids of buffalo-cow No. 24

by direct culture. This female animal showed very low serological titres early in pregnancy, and then it aborted at seven month gestation with a serum titre of 1/80. It is recognised that such animals can be infected early in pregnancy and yet show no rise in serum titre until near or at parturition (Kerr & Rankin, 1964). This heifer has been the only confirmed case of brucella abortion since the start of testing, no brucella organisms have been isolated from the other abortions which have occurred during the period of study.

In the course of the study 59 animals have encountered in the herd reacting to the SAT in low and in high dilutions. In analysing the trend of the agglutination titer in reacting animals, it was found that most of those reacting in a high titre showed either no decrease or an increase in agglutination titre. Of those that showed agglutinins in their blood during the study, 19 were slaughtered. However, in analysing the results, according to the symptoms exhibited by animals, it was found that some females were seronegative, especially when clinical signs (chiefly abortion) first appear. Also, some buffalo-cows that were chosen for serologic retests because of previous serologic reactions were test-negative when specimens were collected. Only one male animal with orchitis was detected during this study. Failure to isolate brucella organisms from this infected animal may be due to the erroneous treatment with Terramycin/L.A. at 20 mg/kg bodyweight prior to obtaining cultures. Oxytetracycline exert a primarily bacteriostatic effect on brucella organisms (Ariza *et al.* 1985).

Nature of Abortus Bang Ring test (ABR) reaction in farm animals has been studied by a few workers and there are controversial opinions regarding the formation of ring or sediment. Both ring and sediment types of reaction have been recorded in ABR test by using milk samples of cow (Ogonowski, 1955) and buffalo (Shalash, 1957). In the present study both types of reactions were recorded in buffalo milk samples.

Kenyon *et al.* (1966) demonstrated, under oil-immersion lens, that antigen particles yet adsorbed over the fat globules, thus giving conclusive reason to suggest the role of fat globules in the ring reaction type milk sample and indicating that the fat globules of the sediment milk do not participate in the antigen antibody clumps.

It was further recorded by Tanwani (1970) that the nature of reaction from the individual lactating animals remains the same (ring or sediment) when tested repeatedly on different occasions, suggesting that the particular type of reaction is due to the nature of surface of fat globules, which has some characteristic consistency.

However, Soni (1979) studied the relationship of ABR results with fat globule size in buffaloes and found in milk samples with large fat globules (av. 6.6 ± 0.9 μ m diam.) a ring was observed, but in samples with medium fat globules (av. 4.4 ± 1.1 μ m diam.) a button and ring occurred together, and with small fat globules (diam. 2.8 ± 0.6 μ m) only a button was seen. The MRT sometimes gives earlier diagnosis with pregnant animals (Table 3 Nos. 195 & 229).

Correlation between milk ring test and blood tests were obtained in 31 (55.3%) of lactating buffaloes. It was observed that sera of animals showing weakly positive or doubtful reactions gave erratic results to MRT test. On the other hand animal with a serum titre of 1/80 or more were invariably positive to this test.

Among MRT positive samples 54.9% showed ring reaction, 22.4% sediment and 22.4% both ring and sediment.

The presence of milk of early lactation may produce false positive reaction (Morgan *et al.* 1978) and subsequent routine tests allowed them to be reclassified as negatives (Table 7). After considerable culling, slaughtering of reactors (to SAT) and introduction of new stock had taken place, the results for the examination of bulk milk samples shown in Table (9) were obtained. Only 3 out of 19 pooled can-samples, were positive to the MRT.

A review of the present results shows that the current officially approved serological method (SAT) alone could not be used in diagnosis. Practical field experience testifies to the need for other tests in order to establish a diagnosis in certain cases.

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Table (1) Reacting males

Bull No.	Sample	RBPT	BAPA	SAT	EDTA
1 (adult breeding)	Blood	+	+	2/10	-
2 (adult breeding)	Blood	+	+	4/10	-
2 (young breeding)	Blood	-	-	2/10	2/20
4 (9/6/93) (young fattening)	Blood	+	+	1/1280	3/640
	Testicular exudate	-	-	3/2560	ND
(9/7/93)	Blood	+	+	4/640	4/320
	Testicular exudate	-	-	3/5120	ND
(28/7/93)	Blood	+	+	3/640	1/320
	Testicular exudate	+	+	3/1280	ND.

ND = Not done

Female animals

The variations in blood titres and other observations are noted in Tables (2-to 7) .

Table (2) Low -titred MRT negative reactors .

Animal No.	RBPT	BAPA	SAT	EDTA
26	+	+	4/10	1/10
38	+	+	4/20	3/10
68	-	+	4/10	1/10
76	-	+	4/10	2/20
107	+	+	3/20	4/20
112	+	+	4/10	1/10
117	+	+	3/10	-
120	+	+	1/20	4/10
126	+	+	2/40	1/20
138	-	-	4/10	-
162	+	+	1/40	3/20
201	+	+	4/10	-
213	-	+	4/20	4/20
223	+	+	4/10	-
228	+	+	2/10	1/20
240	+	+	4/10	2/10
243	+	+	3/10	1/10
401	+	+	4/20	3/20
402	+	+	3/10	-
411	+	+	2/10	1/10
412	+	+	3/10	-
419	+	+	3/40	4/20

Table (3) Low -titled MRT positive reactors .

Animal No.	Date	RBPT	BAPA	SAT	EDTA	MRT
6	23/6/93	-	+	1/10	-	Ring
	12/8/93	-	+	1/10	-	Ring
24*	23/6/93	+	+	3/20	3/10	±
	11/7/93	+	+	1/80	4/80	Ring
49	23/6/93	+	+	4/10	2/20	Ring & Sediment
	12/8/93	+	+	3/10	1/20	Ring & Sediment
50	23/6/93	+	+	4/10	3/10	Ring
	22/10/93	-	-	-	-	Sediment
81	23/6/93	+	+	4/10	4/10	Ring & Sediment
	5/8/93	+	+	3/40	1/40	Dry
	31/10/93	+	+	4/10	1/80	Dry
89	23/6/93	+	+	4/40	4/20	Ring & Sediment
	19/11/93	+	+	-	ND	Ring
108	19/6/93	-	-	4/10	1/20	Ring
	23/7/93	+	+	3/10	3/10	Ring
111*	23/6/93	+	+	4/20	3/10	Sediment
	12/8/93	+	+	2/40	4/20	Sediment
141*	25/8/93	+	+	3/20	1/20	±
	7/9/93	+	+	4/20	4/20	Ring
151	1/8/93	+	+	3/20	3/10	Ring
	4/8/93	+	+	2/10	1/10	Ring
155	25/6/93	+	+	4/20	4/20	Sediment
	8/10/93	+	+	4/20	1/40	Ring
171	1/8/93	+	+	4/10	1/10	Ring
172	23/6/93	+	+	4/20	4/10	Sediment
188	23/6/93	+	+	3/10	3/20	Ring & Sediment
195*	2/8/93	-	-	3/10	-	Sediment
202	23/7/93	+	+	1/20	2/20	Ring & Sediment
229*	21/6/93	-	+	3/10	1/10	±
	3/7/93	-	+	2/10	1/10	Ring
245	11/7/93	+	+	3/20	4/20	Ring
408	17/10/93	+	+	3/40	2/40	Ring
423	23/7/93	+	+	3/10	1/10	±

* Abortions took place in buffalo -cow Nos 24,111,141,195 & 229 during 7-to 9 months of pregnancy . B. abortus biovar 3 was isolated from the foetal fluids of No.24.

Table (4) High-titled MRT positive reactors

Animal No.	Date	RBPT	BAPA	SAT	EDTA	MRT
45	23/6/93	+	+	4/80	3/40	Ring
	Slaughtered 14/7/93	+	+	4/80	3/40	Ring
114	23/7/93	+	+	3/640	2/640	Ring
	Slaughtered 8/10/93	+	+	2/640	2/640	Ring
160	25/6/93	+	+	3/80	4/80	Ring & Sediment
185	20/6/93	+	+	4/80	1/80	Ring
	Slaughtered 13/7/93	+	+	4/80	4/80	ND
199*	19/6/93	+	+	2/160	1/80	Ring
	Slaughtered 23/7/93	ND	ND	ND	ND	Ring & Sediment
410	30/6/93	+	+	4/320	2/640	Ring
	Slaughtered 23/7/93	ND	ND	ND	ND	Ring
418	23/7/93	+	+	4/160	1/320	Ring & Sediment
	Slaughtered 31/10/93	+	+	3/20	4/20	ND

* Aborted on day 17/6/93 but brucella organisms could not be isolated .

Table (5) Dry reactors

Animal No	RBPT	BAPA	SAT	EDTA
73	-	+	4/10	3/10
90	-	+	4/10	4/10
168	+	+	4/10	4/10
176	+	+	4/10	-
191	+	+	3/40	3/20
409 (20-6-93)	+	+	4/80	2/80
Slaughtered (23-7-93)	+	+	4/80	2/40

Table (6) Serologically Neg. *buffaloes that were truly Pos. to the MRT .

Animal No.	Date	MRT
58	23/6/93 20/10/93	Ring & Sediment Ring
60	23/6/93	Ring & Sediment
62	6/10/93 13/10/93	Ring Ring
69	23/6/93 17/9/93	Sediment Sediment
405	23/7/93	Sediment
413	25/6/93	Sediment
425	23/7/93	Ring

Table (7) Serologically Neg .

buffaloes that were falsely Positive* to the MRT .

Animal No.	Date	MRT
99	25-6-93 23-7-93	± -
101	23-6-93 23-7-93	Sediment -
109	23-6-93 23-7-93	Sediment -
110	25-6-93 23-7-93	± -
258	23-7-93 16-8-93	Ring -

*False Positive reactions may be due to the presence of colostrum

Table (8) Summarized results of SAT & MRT on individual lactating animals.

SAT	No. of Animals	MRT				
		Neg	±	Pos.		
				R	S	Both R&S
Neg.	31	14	2	6	4	5
Pos.	25	8	2	8	3	4
Total	56	22	4	14	7	9

The collective results of tests on individual lactating animals are summarized in table (8) .

Using the SAT as the criterion , and considering only the animals which were - ve or + ve to that test, it can be said that milk test agreed with the blood test in 31 (55.3%) of 56 cases.

Table (9) positive bulk milk samples.

Can- sample No	BMRT
3	Ring & Sediment
14	Ring & Sediment
18	Sediment

