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SOME STUDIES ON BRUCELLOSIS IN WATER BUFFALOES DURING TIME OF ABORTION AT ASSIUT GOVERNORATE

(With 2 Table)

By

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بعض الدراسات عن مرض البروسيلا في الجاموس أثناء وقت الاجهاض بمحافظة أسيوط

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خلال شهر أغسطس الى شهر نوفمبر سنة ١٩٩٢ حدثت ظاهرة الاجهاض فى عدد ١٧ حاله من الجاموس كانوا فى مراحل مختلفه من الحمل فى مناطق مختلفه بمحافظة أسيوط. أوضحت دراسة الاعراض الاكلينيكيه للحالات المجهضه مدعمه بالفحوص البكتريولوجيه والسيرولوجيه أن سبب الاجهاض فى الجاموس هو ميكروب البروسيلا. الفحوص البكتريولوجيه للبن كشفت عن عزل عدد ١٠ من ميكروب البروسيلا صنفت جميعها الى نوع واحد من البروسيلا ميليتيزيس النوع الثالث. أوضحت الدراسة السيرولوجية والبكتريولوجية فى اللبن أن اختبار التلبد الحلقى فى لبن الجاموس اثناء وقت الاجهاض ذا حساسية عالية واختبار ثقة حيث أنه كشف عن ٩٤ر٥٢ ٪ من الحالات الايجابيه فى العزل البكتريولوجى. أوضحت الدراسة أن ميكروب البروسيلا يمثل ٨٢ر٥٨ ٪ من مسببات الاجهاض فى الجاموس طبقًا لعزل الميكروب من اللبن.

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SUMMARY

During august to november 1992, a phenomenon of abortion occured in 17 cases of buffaloes aged from 4 - 6 years old at different stages of pregnancy at different localities at Assiut Governorate. The clinical condition of each aborted buffalo supported by the results obtained from serological and bacteriological examinations ensured the cause of abortion to be due to Brucella infection. Bacteriological examinations revealed 10(58.82%) of local brucella isolates recovered from buffaloes' milk. Isolates were belonged to one strain of Brucella melitensis biotype 3. Sero-bacteriological examinations proved that the abortus Bang ring test was more specific, highly susceptible and reliable test in diagnosis of buffalo-brucellosis during time of abortion, where, 52.94% of positive brucella cultures were detected in bufalloes' milk. Brucella micro-organisms represented 58.82% of the causitive agents of abortion in buffaloes according to isolation of brucella organisms from buffaloes' milk.

INTRODUCTION

Brucellosis is still a serious disease of farm animals in many countries due to its economic and zoonotic importance. In Egypt. *SHAWKAT* et al. *(1976)* stated that the main cause of abortion in buffaloes was Brucella abortus. *SAYOUR* et al. *(1970). SALEM and HOSEIN (1990) and REFAI* et al. *(1990)* could isolate brucella micro-organisms from cows and bufaloes. *POLDING (1948)* made extensive survey of abortion and Brucellosis among cattle and buffaloes and observed that the infection was some what less prevalent in buffaloes and the rate of abortion was 5%. Various investigators including *AWAD* et al. *(1977) and ALI (1989)*, determined the incidence of Brucellosis among bufaloes by using serological tests and showed that the disease was prevalent in a lower rate in buffaloes than in cattle.

In this investigation, three principal items were aimed firstly, isolation and identification of brucella microorganisms to confirm the infection from milk of aborted buffaloes, secondl,. Biotyping of brucella strains isolated from milk and lastly establishing an association between positive brucella cultures and antibodies of brucellosis in serum and buffaloes' milk during time of abortion.

MATERIAL and METHODS

1- Samples:

a) Blood sera samples: 17 blood sera were collected from aborted buffaloes and were used for tube agglutination and Rose bengal plate tests according to method of *MORGAN (1967).*

b) Milk samples: Individual milk samples, 20 ml each, were collected aseptically, in sterile screw capped tubes, from each aborted buffalo. These samples were used for Abortus Bang Ring test and bacteriological examination according to methods of *ALTON and JONES (1967).*

2- Antigens:

a) Haematoxyline stained Brucella abortus antigen, was used for Abortus Bang Ring test. It was obtained from United States Department of Agriculture Animal and Plant Health Inspection Services. U.S.A. Serial No. 58401.

b) Standardised Brucella abortus antigen, was used for tube agglutination test and was obtained from Vet. Res. Lab. Dokki-Cairo.

c) Rose Bengal Antigen. It was applied for Rose Bengal plate test and was obtained from Merieux Institute. France.

3- Reagents:

1- Monospecific anti-sera of Brucella abortus (A).
2- Monospecific anti-sera of Brucella melitensis (m). These were obtained from Wellecome. London.
3- Brucella phage (Tb). It was obtained from Vet. Res. Lab., Dokki-Cairo.

4- Media:

Serum dextrose agar with actidione (10 mg\ml) bacitracine (200 U\ml) and polymxine. B. Sulphate (5000 U\ml).

Bacteriological examination:

Culture: Cream and sediment of each individual milk sample were cultured on serum dextrose agar plates with antibiotics. The cultures were incubated at 37°C in Candle - jar and examined daily for 15 days for the presence of brucella micro-organisms *(ALTON and JONES, 1967).*

Identification of Brucella organism:

Isolated brucella strains were identified according to methods of *ALTON and JONES (1967)* including Co2 riguirment. H2s production. inhibitory action of different dyes concentrations, reaction with monospecific sera and phage lysis.

Typing of isolated brucella organisms:

It was carried out according to the methods recomended by Committee of Toxonomy of Brucellae and the Combined Committee of FAO/WHO of Brucellosis (1967).

RESULTS

1- History and clinical findings:

During August to November 1992, a phenomenon of abortion occured in 17 cases of buffaloes at different stages of pregnancy aged from 4-6 years old in different localities at Assiut Governorate. One case of them aborted at the 9th month in previous year and aborted in the second year again at the 5th month of pregnancy. Aborted foeti were at full term and covered with brownish yellow mucopurulant exudate. In all cases of abortion, the placenta were always retained with presence of vaginal discharges and deprived appetite and irregularity of body temperature. The phenomenon of abortion was suspected to be due to brucella infection.

2- Serological examinations:

Carrying out the serological tests, namely tube serum agglutination and Rose Bengal plate tests on the 17 blood sera samples collected from aborted buffaloes, revealed that the sera were with low titres ranging from 1/10 to 1/20, four (23.53%) of doubtful cases and 6(35.29%) of negative cases produced positive brucella cultures in the buffaloes' milk (table 1). The Rose Bengal plate test failed to detect any the antibodies in the sera of aborted buffaloes and 10(58.82%) of negative cases were yielded positive brucella cultures in the buffaloes' milk (table 1).

By the Abortus Bang Ring test on 17 individual milk samles collected from aborted buffaloes 13(76.47%) were reacted positivelly and 9(52.94%) from them yielded brucella isolates. The remaining 4(23.53%) milk samples were negtive and one of them (5.88%) was culturally positive (table 1).

3- Bacteriological examination:

a) Examination of caltures: All individual milk samples were examined bacteriologically and showed that 10(58.82%) were positive brucella cultures and 7(41.18%) were negative. The isoaltes grew readily on serum dextrose agar media with antibiotics, producing colonies of typical Brucella species morphology from 4 to 7 days after incubation at 37°C in air. The isolates were gram negative coccobacilli microscopically. Supplementary carbon dioxid was not required for growth and hydrogen sulphide production was not produced. The recovered isolates grew well on serum dextrose agar media with basic

fuchsin 1/25000, 1/5000, 1/100.000 and did not grow on thionin 1/25000. The isolates were agglutinated strongly with monospecific antisera of Brucella melitensis (16M) and Brucella abortus (544) (table 2).

b) Biotyping of Brucella isolates: All Brucella isolates were typed to one strain of Brucella melitensis biotype (3) in the central Vet. Lab. Res., Dokki-Cairo according to the methods recommended by Committee of Toxonomy of Brucellae and the Combined Committe of FAO/WHO of Brucellosis (1967).

DISCUSSION

In this investigation, the main clinical symptoms of brucellosis in buffaloes were abortion at late stage of pregnancy, vaginal discharges, retained placenta and aborted foetus. The above mentioned clinical observations agreed with the results obtained by *EL-GIBALY* et al. *(1975) and EL-NAGGAR* et al. *(1980)* who recorded the same observations of brucellosis in friezian dairy farm in Egypt.

In the present study, 17 blood sera were collected from aborted buffaloes and were examined for brucellosis by using different serological tests. The results of tube serum agglutination test showed that the sera of aborted buffaloes were with low titres ranging from 1/10 to 1/20 and 4(23.53%) of doubtful cases and 6(35.29%) of negative cases were produced positive brucella cultures in the milk (table 1). This observation agreed with the results obtained by *EL-GIBALY* et al. *(1975); SALEM* et al. *(1987)* in Egypt and *NICOLETTI and MURASHI (1966)* in U.S.A. observed result could be attributed to lowered protein level in the serum and thus the infected animals became unable to develop the antibodies in their sera. The results of Rose Bengal plate test revealed that 10(58.82%) of negative cases yielded positive brucella cultures in the buffloes' milk (table 1). Our results agreed with the results obtained by *CORDS and CARTER (1979) and ZOWGHI* et al. *(1990).* In our opinion, the result is attributed to many factors such as (1) General Lack of humoral response to brucella microorganisms. (2) Presence of incomplete or "blocking" antibodies which belong to all immunoglobin classes with the exception of IgM. (3) Some animals have an elevated ratio of IgG_2 to IgG_1 antibody. By using Abortus Bang Ring test. the achived results indicated that 13(76.47%) of individual milk samples were reacted positively and 9(52.94%) from these were positive brucella cultures. This observation agreed with the results of many authors such as *EL-GIBALY* et al. *(1975) and CORDS and CARTER (1979)* who proved that the milk ring test was highly sensitive and reliable test in the diagnosis of brucella infection. This observation is attributed to localization of

brucella microorganisms in the udder and related lymph nods and that most infected animals excrete large numbers of organisms in the milk for the first few days after abortion. In this investigation, the milk ring test revealed that 4(23.53%) were negative and one of them (5.88%) was positive culturally, it is attributed to high fat content of the buffaloes' milk and irregularity in the excreetion of the agglutinins in the buffaloes' milk *(AWAD et al., 1977).*

The results of bacteriological studies showed that a total of 10(58.82%) local brucella isolates were recovered from 17 individual milk samples collected from aborted buffaloes. All brucella isolates were identified and biotyped to one strain of Brucella melitensis biotype 3 (table 2). Our results were in agreeent with the results obtained by *KAMEL and ABDEL-FATTAH (1962) and SAYOUR et al. (1970)* who isolated brucella melitensis from buffaloes' milk. The incidence of Brucella melitensis among buffaloes was due to rairing of sheep in the same pasteure of buffaloes and thus the infection was transmited from sheep to buffaloes.

Sero-bacteriological examinations cleared also that Abortus Bang Ring Test was more specific, highly susceptible and reliable test in diagnosis of brucellosis in buffaloes during time of abortion. The test detected 52.94% of positive brucella cultures and thus, it could be used as a reliable test with other serological tests for detection of brucellosis in buffaloes.

The present work cleared also that the brucella microorganisms represented 58.82% of the causitive agents of abortion in buffaloes according to isolation of brucella organisms from buffaloes' milk.

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Table (1)

* Number of Positive brucella cultures in buffaloe's milk

Test	Positive	Suspicious	Negative
ABRT	13 (9)*	(-)	4(1)*
	(52.94%)**	(-)	(5.88%)**
STAT	(-)	4 (4)*	13 (6)*
	(-)	(23.53%)**	(35.29%)**
RBPT	(-)	(-)	17(10)*
	(-)	(-)	(58.82%)**

ABRT = Abortus Bang Ring Test.
STAT = Standardised Tube Agglutination Test.
RBTT = Rose Bengal plate Test.
(-) = Negative reactor.
** = % of positive brucella culturer.

Table (2)

Different characters of Brucella melitensis biotype 3 isolated from buffaloe's milk

Test	Result
Co_2 . Requirment	- ve
H_2S production	- ve
Urease	+ ve
Catalase	+ ve
Growth on Dyes	
Basic Fuchsim	
1 : 25000	+ ve
1 : 50000	+ ve
1 : 100000	+ ve
Thionin:	
1 : 25000	- ve
1 : 50000	+ ve
1 : 100000	+ ve
Agglutination by monospecific Antisera	
Abortus (544)	+ ve
Melitensis (16M)	+ ve
Agglutination by Acriflavine	
1 : 1000	+ ve
Lysis by phage at RTD : (Tb)	Non-haemolytic