

Modification Of Milk Ring Test On Buffalo's Milk As A Screening Test For Brucellosis

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ABSTRACT

In this study a modified method have been developed for Milk Ring Test (MRT) on buffalo's milk to overcome some problems accompanied the routine test as the appearance of disc (sediment), thin ring or both (disc and ring) when the positive samples react with the antigen, beside that the routine reaction takes more time (about 3 hours incubation) for its appearance. The new modified method was applied on 140 buffalo's milk samples in dairy buffaloes farm having breeding troubles suggestive for brucellosis. The disease status was confirmed by serological and bacteriological examinations. This modified test proved to have more relative agreement with the serological tests on blood samples than the routine test also the strange reaction that accompanied the routine test disappeared and the result is obtained during one hours incubation only. One brucella strain have been isolated from a sample have sediment reaction to routine MRT and a clear ring by modified MRT, this isolate was proven by its identification that it is a *Brucella melitensis* biotype 3. This study has been advised the application of the modified MRT on buffalo's milk as a screening test for diagnosis of brucellosis in buffaloes.

INTRODUCTION

Brucellosis is a widespread disease, particularly among dairy cattle (1, 2). Also, it is an important zoonotic disease that could be transmitted from infected animals to man through drinking of raw milk causing undulant fever (3).

In cattle and buffaloes, brucellosis lead to great losses in calves through abortion, reduction in milk yields and reduced fertility (4).

Examination of milk by serological methods for brucellosis have been suggested as a good substitute for blood tests in detection of brucella infection in dairy animals (5, 6) as it easy performed, simple and more cheaper (7).

There are many of specific screening tests for detecting antibodies of brucella in milk as MRT which depend on the presence of brucella agglutinins in the milk (8). Milk ring test is an efficient complementary test and has close correlation with the results of blood serological tests for detecting of bovine brucellosis (9).

However, The behavior of MRT on buffalo's milk differ than that on cow's milk as the shape

of the reaction of the positive buffalo's milk to MRT (routine method) take variant shapes (10). This study was aimed to illustrate the different types of MRT reactions on buffalo's milk and explaining its nature and carry out a modification on the proper MRT in order to overcome the different behavior and shape of reaction of buffalo's milk to the routine MRT.

MATERIAL AND METHODS

Samples

A total number of 140 individual samples of blood and milk were collected from a herd of dairy buffaloes raised in a private farm at Sharkia Governorate, these samples were used for serological and bacteriological examination. The herd was suffering from reproductive problems such as abortion, stillbirth and retained placenta.

The milk samples were aseptically collected and examined using Schalm test (11) to exclude mastitis and to avoid other factors that may affect the results of MRT.

Brucella Antigens

Most of the used antigens, including Rose Bengal Plate Test (RBPT), Standard Tube

Agglutinating Test (TAT), Rivanol Test (Riv. T), Milk Ring Test (MRT) antigens were supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo.

Antigen for Complement Fixation Test (CFT)

The USDA: STT Concentrate (4.5% *B. abortus* biotype 1 strain 1119-3 cells in phenol saline, (6.8) final pH was kindly offered by National Veterinary Services Laboratories, Ames Iowa, USA.

Serological tests

Rose Bengal Plate Test (RBPT), Tube agglutination Test (TAT), Rivanol Test (Riv. T) and Complement Fixation Test (CFT) using warm fixation, carried out (12).

Bacteriological examination

Isolation and identification of brucella organisms from milk samples were carried out by inoculating the Albimia agar plate containing antibiotics (Oxide Co., Egypt) with sediment-cream mixture of milk. The plates were incubated at 10% carbon dioxide tension (12).

Milk Ring Test (MRT)

It was carried out according to the recommended method (13) on individual milk sample -Haematoxyline- stained *B. abortus* antigen suspension was used. Test was read after 3 hours incubation at 37°C.

Modified MRT

One drop (0.03ml) of fresh negative brucella cow's fat was added to each 1ml of skimmed buffalo's milk sample in a Wesserman tubes and the test was run as the routine test procedure.

RESULTS AND DISCUSSION

The state of brucella infection in this study was confirmed in the examined buffaloes herd by the results of serological and bacteriological examinations (Table 1).

Milk Ring Test was designed and standardized for testing brucella agglutinins in cow's milk with its low fat content (2-3%) (14) (Fig. 1). Yet when applied on whole buffalo's milk with its high fat content (7-9%) the reaction picture of positive cases differ than that of cow's milk and appeared as agglutinating disc (sediment) at the bottom of the tube

accompanied by variable degree of column decolorization with or without thin ring formation underneath the fat layer (10,15-18), this reaction was slower than that of cow's milk as it need at least 2 hours incubation to appear (10,19).

In this study three types of reactions to MRT (3 hours incubation) on buffalo's milk have been recorded, ring, sediment or both (Fig. 2). This finding was recorded in earlier (10, 15,19) and recent (18) studies. The nature of MRT reaction phenomena in buffaloes has been studied by few workers and there are controversial opinion regarding the formation of ring or sediment . It has been showed that 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 according to the type of milk sample and indicated that the fat globules of the sediment milk do not participate in the antigen antibody clumps (20). It was further recorded that the nature of reaction from individual lactating animals remain the same (ring or sediment) when tested repeatedly on different occasions suggesting that the particular type of reaction is due to the nature of the fat globule surface which has some characteristic consistency (21). However, the relationship of MRT results and fat globules size in buffaloes milk samples with large fat globules (av 6.6 ± 0.9 mu. m. in diameter) a ring was observed, but in samples with medium fat globules size (av 4.4 ± 1.1 mu. m. in diameter) a button and ring occurred together, and with small fat globules (av 2.8 ± 0.6 mu. m. in diameter) only a button was seen (22). *Patterson and Deyoe* (23) attributed the variation in MRT reaction to buffalo's milk to the size of fat globules and fat content of milk. So, the modification of the routine MRT in this study is based on the addition of fresh negative cow's fat to the tested skimmed buffalo's milk samples which share in the adsorption of antigen-antibody complex on the surface of its fat globules forming the coloured ring cream layer at the top (Figs. 3 & 4). This reaction of the modified test takes place in one hour incubation at 37°C with no other undesirable reactions (sediment) and the test is

adjusted completely as in the test proper on cow's milk (Fig. 1).

On carrying the routine MRT on the tested 140 buffalo's milk samples in this study, the total positive reactor percent were (9.3%) while by modified MRT were (10.7%) (Table 2). In the routine MRT there were 4 samples reacted positively with ring appearance and 7 samples were reacted with both ring and sediment, while 2 samples reacted positively with sediment (disc) appearance, while in modified MRT all positive samples were reacted clearly with ring appearance only (Table 2). Also, the number of subspecies cases were lowered in the modified MRT (2 cases) than that in routine MRT (4 cases) (Table 2).

In this study, the percent of brucellosis with blood serological tests were 13.6%, 10.7%, 10% and 11.4% by RBPT, TAT, Riv. T and CFT respectively, meanwhile, the routine MRT gave lower rate of brucella positive reactors 9.3% (Table 1) this might be due to the fact that MRT

depends upon the presence of IgA produced in the mammary gland (24).

The agreement between serological tests and modified MRT (Table 4) showed a better agreement (93.8%; 89.3%, 91.3% and 92.6%) than that of the routine MRT (Table 3) which was 90.7%, 87.9%, 89.3% and 91.4% to the serological tests, BBPT, TAT, Riv.T and CFT respectively.

The bacteriological examination of milk samples revealed the isolation of one strain of *Brucella melitensis* biotype 3 is from a sample of milk that gave sediment reaction to routine MRT, and clear ring by modified MRT. *B. melitensis* biotype 3 from buffaloes was in agreement with the finding proven by (25) which considered *B. melitensis* biotype 3 is the indigenous strain among farm animals in Egypt.

This study proven that the modified MRT can be considered as a good aid and more efficient in diagnosis of brucellosis in lactating buffaloes than the routine MRT.

Table 1. Results of different serological tests

| No. of samples | RBPT | | TAT | | Riv. T | | CFT | |
|----------------|------|-----|------|-----|--------|-----|------|-----|
| | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve |
| 140 | 19 | 121 | 15 | 125 | 14 | 126 | 16 | 124 |
| % | 13.6 | | 10.7 | | 10.0 | | 11.4 | |

Table 2. Results of routine MRT and modified MRT on buffalo's milk samples

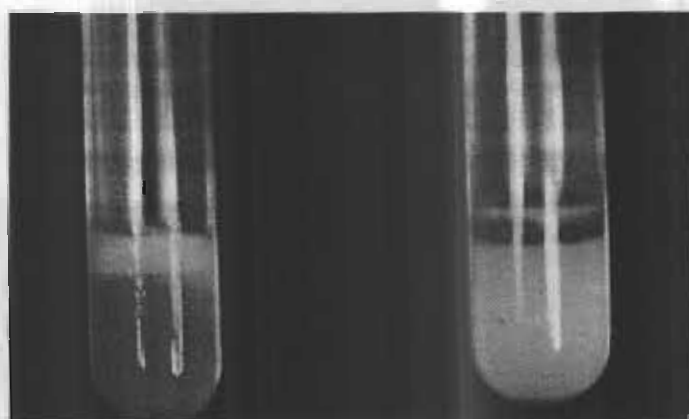
| Test | No. of examined samples | Type of reaction | | | | Total reactors | |
|--------------|-------------------------|------------------|-------------------|-----------------|----------|----------------|------|
| | | Ring | Ring and sediment | Sediment (disc) | ± clumps | +ve | % |
| Routine MRT | 140 | 4 | 7 | 2 | 4 | 13 | 9.3 |
| Modified MRT | 140 | 15 | - | - | 2 | 15 | 10.7 |

Table 3. Agreement between different serological tests and routine MRT on buffalo's milk samples

| Item | No. of Ex. samples | MRT & RBPT | MRT & TAT | MRT & Riv. T | MRT & CFT |
|--------------------|--------------------|------------|-----------|--------------|-----------|
| Both test positive | 140 | 11 | 8 | 9 | 10 |
| Both test negative | 140 | 116 | 115 | 116 | 118 |
| Total | | 127 | 123 | 125 | 128 |
| Agreement % | | 90.7 | 87.9 | 89.3 | 91.4 |

Table 4. Agreement between different serological tests and modified MRT

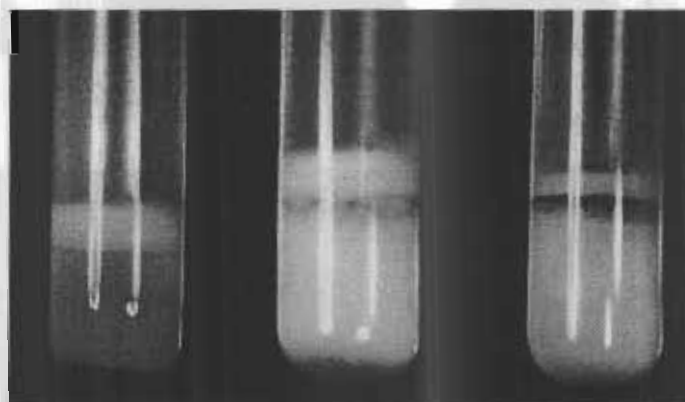
| Item | No. of Ex. samples | MRT & RBPT | MRT & TAT | MRT & Riv. T | MRT & CFT |
|--------------------|--------------------|------------|-----------|--------------|-----------|
| Both test positive | 140 | 12 | 9 | 10 | 11 |
| Both test negative | 140 | 119 | 116 | 118 | 119 |
| Total | | 131 | 125 | 128 | 130 |
| Agreement % | | 93.8 | 89.3 | 91.4 | 92.6 |



Negative

Positive

Fig. 1. Positive and negative reaction in MRT for cow's milk



Sediment

Ring &
sediment

Ring

Fig. 2. Different types of reaction of routine MRT in buffalo's milk after 3 hours incubation

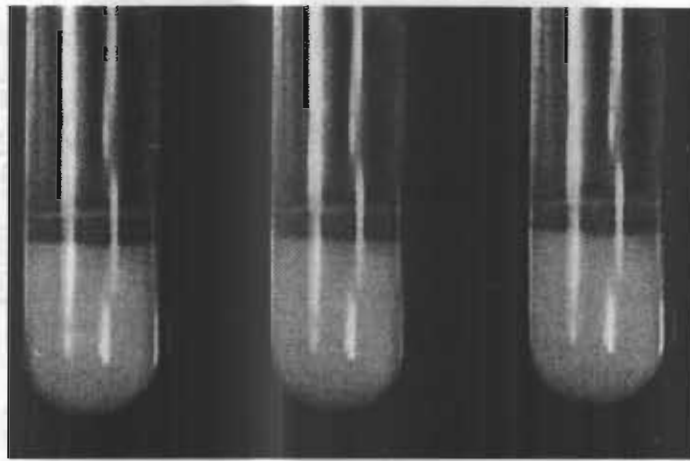


Fig. 3. Positive reaction in modified MRT in buffalo's milk

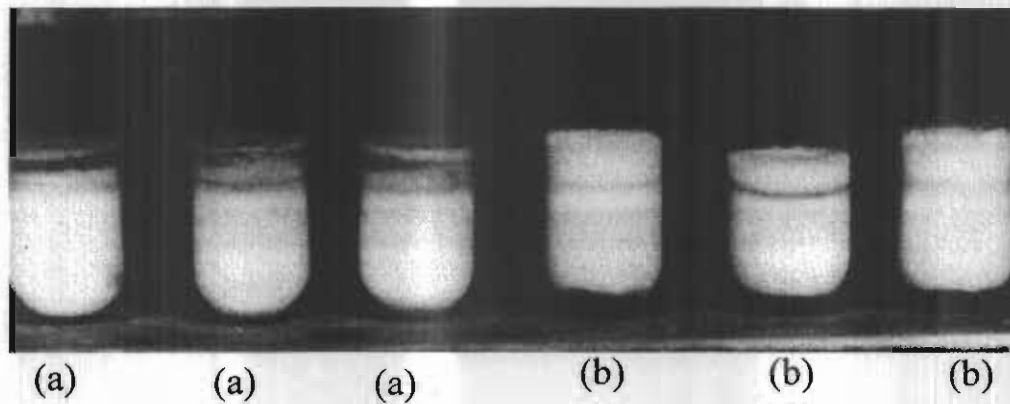
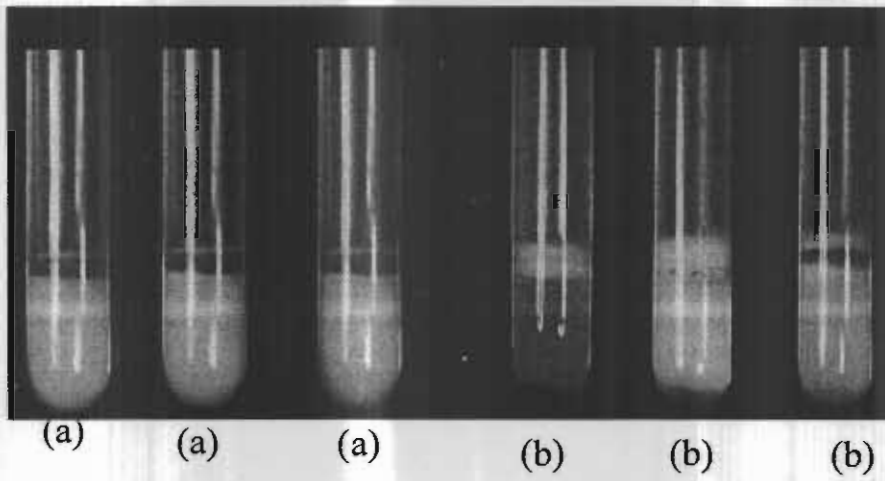


Fig. 4. Positive reaction in modified (left "a") and routine (right "b") MRT in buffalo's milk

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المخلص العربي

تطوير اختبار اللبن الحلقى علي ألبان الجاموس كاختبار مسحي للبروسيلة

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***قسم الأمراض المعدية - كلية الطب البيطري - جامعة القاهرة

في هذه الدراسة تم تطوير وتعديل لاختبار الحلقى علي ألبان في الجاموس للتغلب علي بعض المشاكل التي تصاحب الاختبار الروتيني مثل وجود راسب أو حلقة رقيقة أو كلاهما بالعينات التي تتفاعل إيجابياً لهذا الاختبار بالإضافة إلي أن الاختبار الروتيني يأخذ وقتاً أطول (حوالي ٣ ساعات في الحضانة) للظهور. تم تجريب هذا الاختبار المعدل علي ١٤٠ عينة لبن جاموسي من مزرعة ألبان جاموس كبيرة تعاني من مشاكل تناسلية مثل الإجهاض في الثلث الأخير من الحمل واحتباس مشيمة. وأعطى هذا الاختبار توافقاً أعلى نسبياً مع الاختبارات السيرولوجية علي الدم (٩٣,٨% ، ٨٩,٣% ، ٩١,٢% ، ٩٢,٢% لاختبار الـ روزينجال والتلبد الحلقى الأنبوبي ، الريفانول واختبار مثبت المكمل علي التوالي). من الاختبار الروتيني (٩٠,٧% ، ٨٧,٩% ، ٨٩,٣% ، ٩١,٤%) لنفس الاختبارات السيرولوجية السابقة علي التوالي.

كما اختفت التفاعلات الغريبة التي كانت تصاحب الاختبار الروتيني مثل الراسب والحلقة الرقيقة تحت طبقة الدهن ، كما أن وقت التفاعل قل إلي ساعة واحدة بالحضانة.

عزلت معزولة واحدة من ميكروب البروسيلة من عينة لبن أعطت في التفاعل للاختبار الحلقى للبن تفاعلاً غريباً (راسب) بينما أعطت حلقة واضحة بالاختبار المعدل وصنف علي أنه بروسيلا ميليتينيز النوع الثالث.

أوصت هذه الدراسة بتطبيق الاختبار المعدل لاختبار التلبد الحلقى للبن علي لبن الجاموس الذي يراد فحصه لتشخيص مرض البروسيلة حيث أن نتائجها كانت أكثر دقة وأقل في الوقت.