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BEHAVIOUR OF MILK RING TEST ON MILKS OF SOME FARM ANIMALS WITH SPECIAL REFERENCE TO A MODIFIED SERUM RING TEST

(With 6 Tables)

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سلوك الاختبار الحلقي للألبان عند تطبيقه على ألبان بعض حيوانات المزرعة مع إشارة خاصة للاختبار الحلقي لمصل الدم المطور

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أجرى هذا البحث لدراسة السلوك الحقيقى للاختبار الحلقى للألبان عند تطبيقه علـــى ألبـــان بعض حيوانات المزرعة الخالية من البروسيلا وذلك بعد إضافة مصل دم إيجابي للبروســيلا لهذه الألبان. أثبتت النتائج أن لكل نوع من الألبان المختبرة له سلوكه الخاص لهذا الاختبـــار كما اتضح أن مستويات أضداد البروسيلا لاختبار ألبان الحيوانات المختلفـــة ليـــس لـــه دور واضح و محدد فى سلوك هذا الاختبار إذ أن النتائج أثبتت أن الاختبار الحلقى يسلك ســـلوكا يكاد يكون متشابها مع المصل الإيجابي من فصيلة مختلفة المضاف علـــى ألبـــان الأبقـــار والجاموس والماعز والأغنام . تم تطبيق الاختبار الحلقى لمصل الدم المطـــور علـــى ٢٥٠ عينة مصل تم فحصها باختبار تلازن المصل بالأنابيب أثبتت النتائج أن هناك اتفاق جيد بيـــن قراءات اختبار تلازن المصل بالأنابيب والاختبار الحلقى لمصل الدم المطور لـــذا توصـــى الدراسة بإضافة هذا الاختبار إلي جانب الاختبارات المصلية الأخـــرى لتعزيـــز تشـــخيص البروسيلا.

SUMMARY

An investigation was carried out to find the real behaviour of milk ring test (MRT) on milks of some dairy animals "free from brucellosis" to which brucella positive sera was added. The results show that each kind of milk had its own typical mode of behaviour with the MRT. On the

other hand, the brucella agglutinins of the different animal species had no distinct role in the behaviour of MRT because the results show that MRT behaves nearly similar behaviour with the different brucella positive sera added to cow's, buffalo's, sheep's and goat's milk. 250 animals were subjected to the tube agglutination test (TAT). A modified serum ring test (MSRT) on the afore mentioned 250 samples of blood sera (90 cattle, 70 buffaloes, 50 goats and 40 sheep). The results of MSRT were in good agreement with those of TAT. The importance of carrying out MSRT besides the other serological tests for correct evaluation of brucellosis in farm animals was stressed out.

Key words: *Milk ring test, modified serum ring test.*

INTRODUCTION

Since the milk ring test was first introduced by Fleischhauer (1937), it has received increasing attention for detecting brucellosis and it is now commonly used as screening test in dairy animals.

The milk ring test (MRT) is valuable for locating infected dairy herds with minimum efforts and expenses, thereby eliminates the needs for numerous blood testing in herds free from brucellosis.

Nature of MRT reaction in farm animals has been studied by some workers where it is noticed that there are controversial opinions regarding the formation of ring or sediment. Both ring and sediment types of reaction have been recorded in MRT by using milk samples of cow (Ogonowski, 1955), buffalo (Shalash, 1957 and Ayoub et al., 1965) sheep and goat (Ayoub et al., 1966). The simplicity and efficiency of the MRT drew attention of some investigators to make use of it as a sera-diagnostic method, for rapid detection of brucella agglutinins in blood sera (Fleischhauer, 1953 and Roushdy and Moursy, 1964).

In the view of the above results reported by these investigators in different countries and unfortunately the lack of literature concerning these points in Assiut Governorate, it is thought to study the nature of MRT in she-donkey, she camel, mare and human milks. Therefore, this work was planned to study the real behaviour of the MRT on milks of some dairy animals free from brucellosis to which brucella positive sera was added. Also to evaluate the modified serum ring test (MSRT), for the examination of blood sera of cows, buffaloes, sheep and goats using

brucella negative cow's milk as a diluent for the blood sera under examination.

MATERIAL and METHODS

I- Samples:

(a) Brucella negative milk samples were collected from cow, buffaloe, sheep, goat, donkey, camel and horse free from brucellosis as well as, human milk sample was obtained voluntarily from volunteer woman free from brucellosis.

(b) Brucella suspicious and positive sera having titers ranging from 1/10-1/320 of cow, buffaloe, sheep, goat, horse, donkey, camel and human obtained from brucella unit Animal Health Research - Assiut Lab.

(c) A total number of 250 serum samples of 90 cattle, 70 buffaloes, 50 goats and 40 sheep were collected from governmental and private sector farms in Assiut.

II- Experiments:

(a) Milk ring test (MRT) was performed according to the method recommended by Alton et al. (1988). The antigen used in this work was Haematoxylin stained ***B.abortus*** antigen supplied by Vet. Sera & Vaccine Research Institute Abbasia, Cairo.

(b) Tube agglutination test (TAT) according to the method described by Alton, et al., (1988), using tube agglutination test antigen obtained from Vet. Sera & Vaccines Research Institute Abbasia, Cairo.

(c) Modified serum ring test (MSRT). It was carried out according to the method by Roushdy and Moursy (1964).

RESULTS

The obtained results were recorded in Tables 1- 6.

DISCUSSION

It was generally observed that the reaction of negative cow's milk to which was added positive serum of cow or other species (buffalo, goat and sheep) showed a thick blue coloured ring (2-4 mm) placed through out the fat layer with decolourization of milk column in variable degrees without any formation of agglutination in the bottom of the tubes (Table 1). The behaviour of the reaction was suggested to be due to the

adsorption affinity of cow's milk fat. Kenyon et al. (1966) demonstrated under oil-immerssion 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 samples and indicating that the fat globules of the sediment milk do not participate in the antigen antibody clumps (Ahmed and Abd El-Aal, 1996). Furthermore, Tanwani (1970) suggested that the particular type of reaction is due to the nature of surface of fat globules, which has some characteristic consistency.

The behaviour of the reaction in buffaloe's milk started by the formation of stained clumps which descend to near the bottom. Later a part of such clump tends to float to form thin ring below the fat layer, while the rest dropped to the bottom as an agglutination disc with variable degrees of decolourization of milk column (Table 2). Ahmed and Abd El-Aal (1996) found that among 31 MRT buffaloe's milk positive samples 54.9% showed ring reaction, 22.4% sediment and 22.4 both ring and sediment. The behaviour of the reaction in buffaloe's milk suggested to be due to the attracting power supposed to be owned by buffaloe's fat and also probably to gravity of the antigen antibody compound formed (Ayoub et al., 1965). The variation in milk ring test reaction have been attributed in milk ring test reaction have been attributed to size and disparity in size of fat globules (Patterson and Deyoe, 1977). However, Soni (1979) studied the relation ship of MRT results with fat globules size in buffaloes and found that in milk samples with large fat globules (av. 6.6 ±09 mum. diam.) a ring was observed, but in samples with medium fat globules (av. 4.4 ± 1.1 mum. diam.) a sediment and ring occurred together, while in samples with small fat globules (diam 2.8 ± 0.6 mums. diam.) only a sediment was seen.

The behaviour of MRT in cow's milk is completed within one hour (Table 1), while in case of buffaloe's milk it is completed at the end of three hours (Table 2). An effect probably due to the slow creaming ability of buffaloe's milk.These findings substantiate what has been reported by Roushdy and Moursy (1963) who found that the positive MRT reaction in buffaloe's milk developed somewhat slower than in milk from cows having the same blood serum titer.

The nature of ring phenomenon in the MRT consists of two phases, the first phase is marked by loss of ability of particles to remain in suspension which may be due to the specific antibody antigen reaction, while the second phase is characterized by the adsorption of the

antigen antibody compound onto the surface of the fat globules (Hajdu, 1964).

It was generally observed that the intensity of colour after the addition of antigen in buffaloe's milk column was lighter than that of cow's milk either before or after incubation. While the decolourization of milk column in both milks simulate each other after over night refrigeration.

In both goat's and sheep's whole milks the MRT reaction usually begins with the formation of a complete agglutinating disc or not well formed agglutinating particles in the bottom of the tubes of goats and sheep milk respectively, associated with gradual decolourization of milk column. Later some of the agglutinating particles may ascent to form a thin complete or incomplete ring under the fat layer of milk containing high or low concentration of the positive serum titers, while in very low concentration such ring formation usually did not occur. Milk of both sheep and goats gave its behaviour in 2 hours (Tables 2,3 & 4).

As regards cow's, buffaloe's, sheep's and goat's milk, our findings concerning the behaviour of MRT reaction after positive serum was added in decreasing amounts, showed full agreement with those reported by Nada (1979); Nada (1982); Bastawrous (1987) and Ahmed and Abd El-Aala, (1996).

The results obtained in this work showed that there was similarity in general behaviour of MRT between she camel's, she donkey's, mare's and human's milk with slight difference in that the discs were not well formed in case of mare's milk even after two hours at 37°C. The disc formation is completed in she camel's, she donkey's and human milk in one hour while the behaviour in case of mare's milk is completed at the end of three hours (table,5). This behaviour which might be ascribed to the nature of mare's milk has not yet been as far as our knowledge fully interpreted by investigators.

Information derived from the results reported in Tables (1, 2, 3 and 4) reveal that the MRT behaves nearly similar behaviour with the different brucella positive sera added to cow, sheep, goat and buffaloe's milks. From the results achieved, one can easily conclude that brucella agglutinins of the different species have no distinct role in the behaviour of MRT. This conclusion substantiates what have been reported by Ismail (1976).

From the results obtained in Table (6), it is evident that 73, 59, 45 and 31 serum samples of cows, buffaloes, goats and sheep respectively having a negative blood serum titer by using TAT, did not respond positively to the modified serum ring test (MSRT) at any of the serum dilutions used indicating that the TAT and MSRT are 100% specific. Cow's milk was preferred to buffaloe's milk as a diluent for blood serum under examination due to the fast creaming ability of cow's milk and thus, the fast development of MRT reaction. The beginning of ring formation in tubes incubated at 37°C could be detected after 8-15 minutes (Roushdy and Moursy 1963).

The correlation between negative, suspicious and positive TAT and MSRT was obtained in Table (6). It was observed that sera of animals showing negative reactions to TAT gave negative MSRT, while the suspicious and weakly positive reactions to TAT gave erratic results to MSRT. On the other hand, animals with a serum titer of 1/80 or more were invaribly positive to MSRT.

As far as we know, little available literatures dealing with the MSRT, and therefore it was hard to discuss the aforementioned results but generally these findings agree to certain extent with those reported by Roushdy and Moursy (1964).

In conclusion, each kind of milk had its own typical mode of behaviour with MRT. The MSRT is a time safer and simple to perform test for diagnosis of brucellosis in dairy farm animals with other confirmatory tests. A positive MSRT reaction at a serum dilution of 1/40, could be considered indicative of infection as compared with TAT. While animals, whose blood sera show a doubtful results at this dilution showed be retested after one month.

In spite of the facts mentioned in this work. We are still on the intension that further investigations including the practical utilization of the test as a confirmatory test in parallel with other serological tests for individual animal of all species of dairy farm animals.

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Table (1): MRT in cow's milk bearing brucella positive sera of the same species and other species.

Time	Reaction character	Cow's milk free from brucellosis bearing brucella-positive sera				control
		cow's positive serum	buffaloe positive serum	goat positive serum	sheep positive serum	
At the end of one hour	Ring	blue ring throughout fat layer	blue ring throughout fat layer	blue ring throughout fat layer	blue ring throughout fat layer	fat layer was completely white
	Column	distinct decolouriza-tion,in the tubes had high titer sera, partially in the tubes had low titer sera	distinct decolouriza-tion,in the tubes had high titer sera, partially in thr tubes had low titer sera	distinct decolouriza-tion, in the tubes had high titer sera, partially in thr tubes had low titer sera	distinct decolouriza-tion, in the tubes had high titer sera, partially in thr tubes had low titer sera	blue coloured column
	Disc	Not formed	Not formed	Not formed	Not formed	absent

Table (2): MRT in buffaloe's milk bearing brucella positive sera of the same species and other species.

Time	Reaction character	buffaloe's milk free from brucellosis bearing brucella-positive sera				control
		Buffaloe's positive serum	cow positive serum	goat positive serum	sheep positive serum	
At the end of one hour	Ring	Not formed	fat layer was white	Not formed	Not formed	No changes
	Column	- Decolourization in tubes with high serum titer with zone of clump 2.5 cm from bottom -partial decolouriz-ation in tubes with low serum titer	partial decolourization	distinct decolourization	partial decolourization	No changes
	Disc	blue agglutination discs	thin agglutination discs	thin agglutination discs	thin agglutination discs	No changes

Table (2):continued

Time	Reaction character	buffaloe's milk free from brucellosis bearing brucella-positive sera				control
		Buffaloe's positive serum	cow positive serum	goat positive serum	sheep positive serum	
At the end of two hours	Ring	fat layer was completely white	not formed	thin ring under the fat layer	thin ring under the fat layer	no changes
	Column	decolourization become more distinct and clumps float to beneath fat layer	partial decolourization	distinct decolourization	partial decolourization	no changes
	Disc	blue agglutination discs	thin agglutination discs	distinct agglutination blue discs	thin agglutination blue discs	no changes
At the end of three hours	Ring	thin blue ring	thin ring	moderate ring	thin ring	slight sedimentation after three hours
	Column	distinct decolourization	partial decolourization	distinct decolourization	partial decolourization	
	Disc	obvious blue disc	thin aggkutination blue disc	distinct agglutination blue disc	thin aggkutination blue disc	

Table (3): MRT in goat's milk bearing brucella positive sera of the same species and other species

Time	Reaction character	goat's milk free from brucellosis bearing brucella positive sera				control
		goat positive sera	cow positive sera	buffaloe positive sera	sheep positive sera	
At the end	Ring	Absent	Absent	Absent	Absent	fat layer was white
of one hour	Column	partial decolourization	Slight decolourization	slight decolourization	slight decolourization	No change
	discs	Dense agglutination disc	small agglutination disc	small agglutination disc	small agglutination disc	Absent
At the end	Ring	Thin incomplete blue ring under the fat layer	Absent	Absent	Absent	Fat layer was white
of tow hours	Column	more decolouraziation	Slight decolourization	Slight decolourization	Slight decolourization	No changes
	discs	more dense agglutination blue disc	small agglutination disc	small agglutination disc	small agglutination disc	Absent

Table (4): MRT in sheep's milk bearing brucella positive sera of the same species and other species

Time	Reaction character	Sheep's milk free from brucellosis bearing brucella positive sera				control
		sheep positive sera	cow positive sera	buffaloe positive sera	goat positive sera	
At the end of one hour	Ring	fat layer was white	thin layer of blue ring under fat layer	moderate ring under the fat layer	moderate ring under the fat layer	absent
	Column	gradual decolourization of milk column	slight decolourization	partial decolourization	partial decolourization	No change
	discs	absent	absent	absent	absent	absent
At the end of tow hours	Ring	thin complete ring under the fat layer	thin layer of blue ring under the fat.	dense ring under fat layer	dense ring under fat layer	absent
	Column	slight decolourization	slight decolourization	moderate decolourization	moderate decolourization	No change
	discs	agglutinating particles	absent	fine agglutinating particles	fine agglutinating particles	absent

Table (5): Real behaviour of MRT in donkey, horse, camel and human milks bearing brucella positive sera of the same species.

Time	reaction character	Normal milk obtained from she donkey, mare, she camel and human bearing brucella-positive serum of the same species.				Control
		donky's milk	horse's milk	camel's milk	human's milk	
At the end of one hour	Ring	Absent	Absent	Absent	Absent	White
	Column	Distinct decolourization	Slight decolourization	Distinct decolourization	Distinct decolourization	blue
	Disc	obvious blue disc	Absent	blue agglutination disc	blue agglutination disc	Absent
At the end of 2 hours	Ring	Similar as at the end of one hour	Absent	Similar as the end of one hour	Similar as the end of one hour	White
	Column		partial decolourization			Blue
	Disc		Fine agglutinating particles			absent
At the end of three hours	Ring	Not done	Absent	Not done	Not done	white
	Column		Distinct decolouraization			Blue
	Disc		small agglutination disc			Absent

Table (6): Results of serum ring test on serial dilutions of blood sera of known titer using milk as a diluent

Blood serum titer	Species	Response to serum ring test						
		Rate of serum dilution using cow's negative milk						
		No. of samples						
		1/10	1/20	1/40	1/80	1/160	1/320	Total
0 (-ve)	C	73 (-)	-	-	-	-	-	208
	B	59 (-)	-	-	-	-	-	
	Sh	31 (-)	-	-	-	-	-	
	Go	45 (-)	-	-	-	-	-	
1/10	C	6	-	-	-	-	-	12
		3 (+), 3(-)						
	B	2 (-)	-	-	-	-	-	
	Sh	3	-	-	-	-	-	
		1(+), 2 (-)						
	Go	1 (-)	-	-	-	-	-	
1/20	C	4 (+)	2 (+)	-	-	-	-	7
	B	2 (+)	1 (+)	-	-		-	
	Sh	1 (+)	1 (+)	-	-	-	-	
	Go	- -	-	-	-	-	-	
1/40	C	2 (++)	1(++), 1(+)	-	-	-	-	7
	B	3 (++)	2 (++), 1(+)	1+	-	-	-	
	Sh	1 (++)	1+	-	-	-	-	
	Go	1 (++)	1++	1++	-	-	-	

Table (6) : continued

Blood serum titer	Species	Response to serum ring test						
		Rate of serum dilution using cow's negative milk						
		No. of samples						
		1/10	1/20	1/40	1/80	1/160	1/320	Total
1/80	C	3 +++	3 (+++)	3(+++)	3(++)	-	-	7
	B	1++	1 +++	1+++	1+++	-	-	
	Sh	1+++	1+++	1+++	1++	-	-	
	Go	2+++	2 +++	2+++	1+++, 1++	-	-	
1/160	C	1+++	1(+++)	1(+++)	1 ++	1++	-	5
	B	1+++	1(+++)	1(+++)	1++	1++	-	
	Sh	2+++	2(+++)	2(+++)	1+++, 1++	2++	-	
	Go	1+++	1+++	1+++	1++	1++	-	
1/320	C	1+++	1+++	1+++	1++	1++	1(+)	4
	B	2+++	2+++	2+++	2++	1++ , 1+	2(+)	
	Sh	1+++	1+++	1+++	1++	1++	1(+)	
	Go	-	-	-				
Total								250

C = cow

B = buffalo

Sh = sheep

Go = goat