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A Study of Brucellosis in Camels: Modified Milk Ring Test

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Abstract

Brucellosis is one of the most common zoonotic diseases present in Kazakhstan. It possesses huge economic threat infecting domestic livestock including camels. Countries practicing camel breeding often consume camel milk that raises the need in taking food safety measures and control of milk for contamination with *Brucella spp*. Widely known milk ring test used for diagnosis of cow milk is not suitable for diagnosis of camel milk because of physical and chemical properties of camel milk. Milk samples obtained from camels were subjected to serological tests and milk ring test. We developed modifications needed to standard milk ring test that will allow performing milk ring test on camel milk. Adding cow milk to seropositive camel milk samples allow to observe blue ring. In this paper, we discuss amount of milk and its fat percentage that needs to be added in order to get visually favorable results. Results show that proposed modifications will allow milk ring test to be used as screening test.

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Introduction

Milk is considered to be full of nutrients for a human (Baimukanov, 2009). However, it can be a source of infections that can transmit from an animal to humans (Baimukanov, 2009; Trilenko, 1951; Seidakhmetova, 2004). In Kazakhstan and some other regions involved in animal husbandry, brucellosis is often found among animals, which not only causes great economic damage, but also has social significance (Myrzabekov, 2014; Gwida et al., 2012). Human acquire brucellosis through a direct contact with an animal or ingestion of infected milk. In the Republic of Kazakhstan (Myrzabekov et al., 2010; Sprague et al.,2012), Middle and Near East (Rafieipour et al., 2007), as well as individual regions of the Russian Federation, the population often consumes milk of different kinds of animals, including camel milk. Contaminated milk is dangerous for people who consume the product (Sprague et al., 2012). Thus, controlling milks bio safety should be in place. Presently, there is milk ring test diagnostic tool which is used to evaluate cow's milk (Shimol et al., 2012).

There are some reports in the literature on the possibility of using a healthy cow's milk in a study of camel milk for brucellosis. However, the technique for setting up the reaction, the characteristic of the healthy cow's milk used (fat content, which plays a decisive role in

evaluating the reaction, the percentage of reaction components, exposure, etc.) are not described in those studies, and require certain research, since the milk of camels differs from the cow's milk in its physical and chemical characteristics (Muratov, 1953; Van Straten, 1997; Iskanderov, 2012; Wernery, 2016). In addition to that camels are susceptible to both *B. abortus* and and *B. melitensis*, and are not known to be primary hosts of Brucella (Trilenko, 1951; Gwida et al., 2012).

The need to develop an immunological test and a kit for diagnosing camel milk will ensure the detection of the disease in a lactating animal when it is infected by various *Brucella* species.

Materials and methods

The research related to animals use has been complied with institutional regulations and policies for the care and use of laboratory animals. The material for the study is milk samples obtained from camels, animals with a certain epidemiological background of brucellosis, diagnostic tools and reagents (colored antigen prepared by staining *Brucella* of various species, negative and positive milk for brucellosis).

The physicochemical features of camel milk, in particular, the fine dispersity of fat globules do not allow immune complex to appear as a blue ring test on top of the milk, therefore, the color ring does not form in samples even with the presence of *Brucella* antibodies. Instead of it we can observe aggregation of *Brucella* and precipitation in the form of agglutinate, which we call as sedimentary reaction. Two ml of a milk sample and 50µl of antigen mixtures are incubated at 37°C for 90 minutes and then the results can be observed. The degree of agglutinate expression is estimated by four-point system (Fig. 1).

Fig. 1: Sediment (agglutinate) at the bottom of the tubes when testing milk samples positive for brucellosis.

Table 1. Proposed test result keys for standard MRT on camel milk.

-	No agglutinate (milk is uniformly colored in bluish color, or the sediment forms smooth "button") - the result is negative
+	Agglutination is poorly expressed (the milk is also bluish in color) - the result is doubtful
++	Quite clear agglutination (milk is slightly colored in bluish color) - the result is positive
+++	Clearly expressed agglutination (milk is white) - the result is positive

As a basis for modified milk ring test (mMRT) we used basics of milk ring test used on cow milk samples. The essence of the MRT is that when the colored antigen is added to the milk the fat globules adsorb the "antigen + antibody" complex (if brucellosis antibodies are present in the milk) and, upon settling the product, the milk column in the test tube floats up and form a ring of blue color (the color of the antigen-stained with hematoxylin). In the absence of one of the components of the immune complex, the color antigen is evenly distributed throughout the entire mass of the milk that gradually acquires a bluish-violet color (Table 1).

In order to make mMRT work for diagnosis on camel milk, different dose of colored antigen, fat content and volume of cow's milk as complement, temperature and exposure of the reaction, evaluation of the degree of agglutination were tested.

Then we studied the possibility of using the phenomenon of the appearance of a colored ring in the case "antigen + antibody" complex. To achieve that we

had to create a large-dispersed medium in a tube. This was achieved by adding cow's milk containing larger fat globules to the camelmilk. Larger fat globules are capable of absorbing the immune complex (colored antigen + antibody) and lift it to the top of the milk column creating ring on the surface.

We worked out the ratio of individual components for greater demonstrativeness that allows visual manifestation of the ring reaction and the evaluation of its results.

Along mMRT we performed standard serological and bacteriological tests and then compared the results of it with mMRT results.

Guinea pigs were used for bioassays and tested then by rose-bengal test (RBT), agglutination test (AT) and complement fixation test (CFT). The indicated immunological reactions in the study of blood sera of bioassay animals and camels were carried out according to generally accepted methods using commercial antigens and, positive and negative sera as controls.

RBT test was performed on enameled plates using 30-50µl of serum placed on plate and then adding equal volume of antigen near serum spot. Antigen and serum were then mixed thoroughly and agitated for 4 minutes. Visible reaction with flakes was considered as a positive.

The agglutination test was performed in 4 dilutions of blood serum (bioassay guinea pigs from 1: 5 dilutions, camels - 1:25, 1:50, 1: 100, 1: 200, in a volume of 500µl.) Then we added 500 µl of the commercial single brucellosis corpuscular antigen in the titrated dose into each tube.

Prolonged complement fixation test was done using: a titrated slurry of inactivated *Brucella* (single brucellosis antigen) that were used as antigen, serum, complement and hemolysin in the titrated dose and a shear erythrocyte suspension.

Skin test in camels was carried out by intradermal injection of brucellizate in a dose of 200µl in the middle third of the neck, using a needleless injector.

Bacteriological methods included bacterioscopy (using Gram-stained-smear), bioassays (subcutaneous administration to guinea pigs), and isolation of pure culture (by cultivation on commercial erythritol agar). Obtaining a positive result in at least one of the tests was considered as an evidence of the detection of *Brucella.*

Prior to collection of milk samples udder was washed with warm water and nipples were treated with 70% alcohol. Last portions of milk were taken from each quarter of the udder into separate sterile test tubes.

Seedings from bacteriological material were carried out on erythritol agar with the addition of 0.02% yeast extract and 0.2% cystine. Then incubated in desiccators with a high content of carbon dioxide for 30 days with observation of growth in 5-7 days. Colonies with suspicion of *Brucella* were subjected to further studies such as SAT, Gram-stained-smears were examined under a microscope. At the same time, samples of milk in a volume of 1.5 ml were administered subcutaneously to guinea pigs (bioassay), which were then examined by serological tests (SAT, CFT). If resulting positive, it was believed that the presence of *Brucella* inmilk is proved, even with unsuccessful culture isolation.

Results and discussion

Brucellosis in camels can be diagnosed using serological (RBT, SAT, CFT) and bacteriological methods. In this study we tried to modify widely known milk ring test which is recommended by OIE to use in cattle only. Cow's milk and camel milk is different in its physicochemical properties, therefore we had to modify existing method to be able to use MRT on camel milk samples.

In case of brucellosis of camels the causative agent might localize in mammary gland therefore *Brucella* antibodies can be found in milk. Brucella antibodies in camel milk can be detected by milk ring test if using proportions and modifications we propose. We found that adding to 1.5 ml of camel milk sample 0.5 ml of cow's milk that has high fat dispersity and absorption properties and colored antigen in the volume of 50µl.

Our studies showed that adding at least 10% cow's milk to the camel milk sample will facilitate MRT. Adding 0.5 ml of cow's milk of 3.5% fat 1.5 ml to the camel milk sample will guarantee the appearance of a colored ring the column of milk in case of *Brucella* presence. A smaller volume of complementary cow's milk does not always make it possible to obtain a clearly expressed

result, but a larger volume of it helps to dilute the sample being examined, which lowers the diagnostic titers. Table 2 shows the evaluation scheme for camel milk.

The tubes were thoroughly mixed and incubated at 37-38°C for 30-45 minutes. The test could be performed at a room temperature but it prolongs the exposition to 1-1.5 hrs. Evaluation of the results was carried out according to the following scheme and expressed conditionally in crosses (Fig. 2). We run tests using cow's milk of different fat percentages. Most readable and sufficient results are observed when using milk with percentage of 1.25% to 5%. In order to have different milk fat percentages we titrated milk cream (40% of fat) (Table 3).

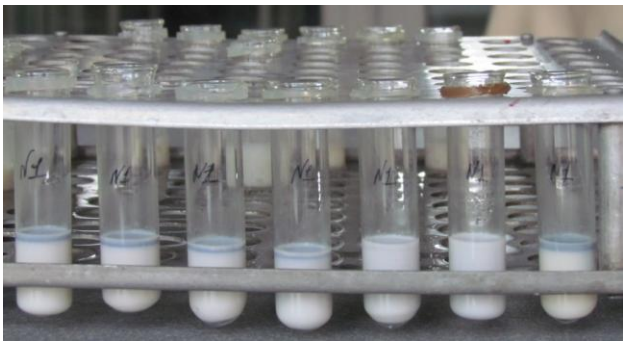

Fig. 2: mMRT on camel milk samples.

Table 2. Evaluation scheme.

#	Clearly expressed ring in the upper part of the milk column, the rest remains white
+++	Sufficiently pronounced ring, the bottom of the milk column has a slightly bluish color
++	Presence of a ring, the bottom of the milk column is blue
+	Weakly expressed ring, the milk column is blue
-	No ring, the milk column remains uniformly colored in blue

Table 3. mMRT results using milk containing a different amount of fat.

Samples	**Whole cream**	**1:1**	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**
Positive	-	-	+	#	#	#	+	-	-
Negative	-	-	-	-	-	-	-	-	-

Serological test results were compared to the results of mMRT.

Table 4. Comparative results of studies on brucellosis of blood serum and whole milk of camels.

Animal	**Udder quarters**	**mMRT**	**Results**		
			RBT	**SAT**	**CFT**
1	1	#	+++	1:400 +++	1:10+++
	2	–			
	3	#			
	4	#			
	5 (from all quarters)	#			
2	1	#	+++	1:400 #	1:10#
	2	#			
	3	#			
	4	#			
	5 (from all quarters)	#			
3	1	–	+	1:100 +++	1:10 –
	2	–			
	3	#			
	4	#			
	5 (from all quarters)	#			
4	1	#	#	1:400 +++	1:10+++
	2	–			
	3	#			
	4	#			
	5 (from all quarters)	#			

From the Table 4, it can be seen that the results might differ depending on the milk sample from different quarters of udder, however samples that are strongly positive in mMRT are also positive in serological tests. Comparing these test results we can observe a certain correlation between the results of mMRT and serological tests.

Table 5. Comparative evaluation of the results of allergic and serological studies of camels from zones with different epidemiological status

Samples	Number of animals	Epidemiological status of the zone	Results			
			Skin test		SAT + CFT	
			Positive+doubtful	negative	Positive+doubtful	negative
1	143	infected	8/5.59	135/94.4	7/4.9	136/95.1
2	20	non-infected	-	20	-	20

A percentage of 5.59 animals were tested positive by skin test however number of animals reacting positive to SAT and CFT – 4.9%. Almost 98.6% of cases have exact match (both negative and positive)in skin test and serological tests, but there were 2 cases reacting positively to a skin test but negative by serology.

Milk samples from 4 camels reacting positively to serological tests were subjected to bacteriological examination. Cultures were seed using milk samples obtained from each quarter of the udder. We compared those results with serological test results in Table 8.

Table 6. Degree of coincidence in the results of serological tests and skin test.

Skin test	Serological tests			Total
	Positive	Doubtful	Negative	
Positive	5	1	1	7
Doubtful	-	1	-	1
Negative	-	-	135	135
Total	5	2	136	143

Table 7. Results of serological tests on blood from infected areas after skin tests.

SAT + CFT	Positive	Doubtful	Negative	Total
Infected	7	1	135	143
Non-infected	-	-	20	163

Table 8. Results of bacteriological tests reacting positive to sedimentation reaction and mMRT.

Animal	Sedimentation test and mMRT	Results	
		Culture isolation	Bioassay
1	# (milk sample mixed from all quarters of the udder reacting positive)	Isolated	Positive
	– (milk sample mixed from all quarters of the udder reacting negative)	Not isolated	Negative
2	# (milk sample mixed from all quarters of the udder reacting positive)	Isolated	Positive
3	# (milk sample mixed from all quarters of the udder reacting positive)	Isolated	Positive
	– (milk sample mixed from all quarters of the udder reacting negative)	Not isolated	Negative
4	# (milk sample mixed from all quarters of the udder reacting positive)	Not isolated	Positive
	– (milk sample mixed from all quarters of the udder reacting negative)	Not isolated	Positive

From the data in Table 8, it can be seen that the *Brucella* culture is predominantly isolated from those quarters of the udder that react positive to sedimentation test and mMRT. Our studies showed that this method have high sensitivity and specificity suggesting to use this method as a diagnosis tool.

Experiments demonstrate that allergen used on negatively or doubtfully reacting animals are then resulted in a sample reacting positive that suggests performing serological test after 15-20 days since skin tests. So, brucellosis diagnosis should be based on a complex of different methods including bacteriological, serological test, therefore complementing each other.
Results also show that for camel brucellosis diagnosis, milk samples should be obtained from each quarter of the udder. Those samples reacting positive in mMRT are most likely to seed a culture.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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Author's contributions

AS and NI supervised the work and designed experiments. FB, AA, SS, RS performed samples collection and laboratory tests. NI, DC, FB analyzed obtained results. The manuscript was drafted by NI, reviewed and edited by DC. All authors read and approved the manuscript.

References

Baimukanov, D., 2009. Camel Breeding of 21st Century in Kazakhstan: Dedicated to 70th Birthday of Professor Baimukanov. 208p.

Gwida, M., El-Gohary, A., Melzer, F., Khan, I., Rösler, U., Neubauer, H., 2012. Brucellosis in camels. Res. Vet. Sci. 92(3), 351-355.

Iskanderov, M., 2015. Animal brucellosis in Russia: epizootological differences and improvement of specific prevention measures. Candidate thesis.

Muratov, S., 1953. Materials on evaluation of the milk ring test on bovine brucellosis. Veterinariya. 9, 9-14.

Myrzabekov, A., 2014. Epidemiological situation on brucellosis in Kazakhstan and its prevention measures. Available at: rb- 51.kz/uploads/mk.ppt

Myrzabekov, A., Mizambayeva, S., 2010. Data report of Sanitary Epidemiological Station.

Rafieipour, A., Ziaei, N., Bahonar, S., 2007. Brucellosis of camels in Iran. Univ. of Kerman.

Seidakhmetova, R., 2004. Brucellosis in Camel. Doctoral thesis.

Shimol, S., Dukhan, L., Belmaker, I., Bardenstein, S., Sibirsky, D., Barrett, C., Greenberg, D., 2012. Human brucellosis outbreak acquired through camel milk ingestion in southern Israel. Isr. Med. Assoc. J. 14(8), 475-478.

Sprague, L., Al-Dahouk, S., Neubauer, H., 2012. A review on camel brucellosis: A zoonosis sustained by ignorance and indifference. IPathog. Glob. Health. 106(3), 144-149.

Trilenko, P., 1951. Evaluation of milk ring test for brucellosis diagnosis. Veterinariya. 8, 50-52.

Van Straten, M., Bercovich, Z., Ur-Rahman, Z., 1997. The diagnosis of brucellosis in female camels (*Camelus dromedarius*) using the milk ring test and serum and milk ELISA- a pilot study. J. Camel Prac. Res. 165-168.

Wernery, U., 2016. Camelid brucellosis: A review. J. Bacteriol. Mycol. 3(1), 10-19.

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