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Incidence of Brucella Organisms in Egyptian Milk

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Abstract: The aim of the present study was to detect Brucella in milk produced from apparently healthy animals in Egypt. Therefore, two hundreds bulk milk samples were collected from 4 cities at El-Gharbia and Kafr El-Sheikh Governorates at summer and winter seasons and examined by cultural method and using milk ring test as screen test. The isolated organisms from cultural method were identified biochemically, biotyping using media contained basic fuchsin & thionin with different concentrations and serotyping using monospecific anti *Brucella abortus* and *Brucella melitensis* sera. 12 samples collected in summer seasons from two cities proved to be contained *Brucella* antigen with Milk Ring Test while after culturing on specific media all positive MRT samples showed no colonies characteristic to Brucella organisms. On the other hand, in winter season, 17 samples gave positive results with MRT and 5 of them gave the characteristic features of Brucella organisms after culturing on Brucella specific media. All isolated cultures were identified biochemically, serologically and biotyping using thionin and basic fuchsine dyes.

Key words: Milk Ring Test · Culture · Serotyping · Biochemical Tests

INTRODUCTION

Raw Milk is defined as a natural biological fluid secreted from healthy udder, free from colostrum, pathogenic microorganisms, toxins and with low bacterial counts [1]. It provides the consumers with macronutrients as lipids, proteins, lactose and micronutrients as vitamins and minerals mainly calcium which play a significant role in both nutrition and health protection [2,3]. On the other hand, nutritional properties of raw milk, neutral PH and high water activity serve an ideal medium for growth of various microorganisms mainly spoilage and pathogenic microorganisms [4].

Brucellosis is a classic example of zoonotic milk borne disease, it caused by *Brucella* spp which causing systemic disease and can also localize in the mammary gland tissue and associated lymph nodes as well [5, 6] and shed in large numbers in the milk [7, 8]. So, consumption of raw milk and raw milk products are considered as a potential threat to public health [9]. It infects wide variety of animals as cows, goats, sheep, camels, pigs, swine and dogs then localize in the reproductive organs of host animals, causing abortions, foetal death, genital infections and sterility [10, 11]. In Egypt Brucella organisms were isolated from animals and humans, the main isolates were *Brucella melitensis biovar3* and *Brucella abortus biovar1* [9, 12]. Cows and buffaloes milk are the most common milk consumed in Egypt, so the risk for human infection is mainly confined to peoples who consume unpasteurized milk from Bulk milk tank [13,14] and immune compromised persons, including the elderly, pregnant women, infants and young children as well [15].

The conventional bacteriological examination of *Brucella* infection and the routine identification and differentiation which based on culture and phenotypic traits are time consuming and associated with a high risk of laboratory acquired infections and require biosafety level 3 laboratories [16-18]. Serological testes are usually

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a screening test of high sensitivity, followed by a confirmatory test of high specificity [19]. It was realized as it is inexpensive, simple and could be rapid but there is a problem that it gives false result with vaccinated animals as it gives similar result results from infection [20]. The milk ring test (MRT) is the first line of screening test for brucellosis particularly in single cow herds. The test can be applied to monitor the dairy herds at regular intervals.

This study aimed to detect the incidence of Brucella organisms in Egyptian Milk

MATERIALS AND METHODS

Milk Samples: A total of 200 samples of raw bulk milk tank (100 each from El-Santa city, El-Gharbia governorate and Sedi Salem city, Kafr El-Sheikh governorate) were collected from collection centers allover a year from February 2016 to February 2017.

The samples were collected in sterile falcon tubes and were transferred as soon as possible in an ice box at 4±1°C to the laboratory with a minimum of delay to be examined for Brucella microorganisms. Each sample (30ml) was mixed by inverting the falcon tube three to four times and then divided into 2 subsamples; the first 10 ml for milk ring test (MRT) and the second 20 ml for bacteriological examination. Milk samples used for MRT and bacteriological examination were stored at 4°C [21].

Milk Ring Test (MRT): One ml of well mixed milk was added to a Wassermann's tube. Then a drop of Haematoxylin stained milk ring test antigen (0.03 ml) was added. The contents of the tube were gently mixed by inverting the tube several times with avoiding of foam formation, then the tubes were incubated at 37 °C for one hour [21].

Bacteriological Examination: Isolation, identification and biotyping of *Brucella* organisms were carried out according to the recommendation of the FAO/WHO, Expert Committee on brucellosis [21].

Biotyping: Trypticase soy agar was used with final dilutions 1/25000, 1/50000 and 1/100000 of basic fuchsin (Catalogue no. DF 0191 – 13.4, Difco Laboratories, Detroit, Mich. USA) as well as thionin, finally diluted 1/2500 and 1/50000 (Catalogue no. T 7029, Sigma chemical Company, MO 63178, USA) [21].

Thionin: The stock solution was prepared in distilled water, sterilized and added to melted basal medium to obtain (A)1/25000, (B)1/50000 and (C)1/100000 final concentration of dye medium.

Basic Fuchsin Dye: Dye medium of basic fuchsin was prepared to obtain final concentrations of (A) 1/50,000 and (B) 1/100,000.

Serotyping [21]: Monospecific anti *Brucella abortus* (A) and *Brucella melitensis* (M) sera were obtained from central veterinary laboratory, New HAW, Weybridge, England.

RESULTS

Colonies on *Brucella* agar media appeared round with smooth margins, round edges, translucent and of golden color (Pale honey-colored).

Microscopic examination showed that the *Brucella* organisms in films appear as Gram negative coccobacilli.

Table 1: Incidence of *Brucella* spp. in milk samples collected at summer season.

	Test				
	MRT			Culture	
	Number of samples = 100				
City	Total	(+ve)	%*	No.	%*
El-Santa city	50	6	12	0	0
Sidi_salem city	50	6	12	0	0
Total	100	12	12	0	0

* Percent to total sample

Table 2: Incidence of *Brucella* spp. in milk samples collected at winter season.

	Test			
	Number of samples = 100			
	MRT		Culture	
City	No.	%*	No.	%*
El-Santa city	8	4	2	1.0
Sidi_salem city	9	4	3	1.5
Total	17	8	5	2.5

* Percent to total sample

Table 3: Biochemical and serological tests used for identification of *Brucella* species

					Agglutination with monospecific antisera			Bacteriostatic dyes				
								Thionin			Fuchsin	
Species	No. of isolates	CO_2 test	H_2S test	Urease test	A	M	Catalase test	A	B	C	A	B
Brucella melitensis	5	-	-	+1hr.	-	+	+	-	+	+	+	+
Brucella abortus	1	+	+	+2hrs.	+	-	+	-	+	+	+	+

A: *Brucella abortus* antisera

M *Brucella melitensis* antisera

DISCUSSIONS

Brucellosis was reported in almost all domestic animals particularly cattle, sheep and goats worldwide. In Egypt brucellosis, has been reported in buffaloes, equines, camels and swine and *Brucella melitensis* biovar 3 was the most commonly isolated strain from animals in Egypt, Jordan, Iran, Israel, Tunisia and Turkey. The milk ring test (MRT) is the first line of screening test for diagnosis of brucellosis in herd. While in milk it is adapted to test milk for antibody to Brucella spp., immunoglobulins attached to fat globules [21].

From the study prevailed in this article, 200 samples of milk were screened for Brucella antibodies as well as with culturing during summer and winter seasons (100 each). In table (1), 12 samples were positive for MRT, 6 from El-Santa city and 6 from Sidi_Salem city during summer season in contrary no samples showed Brucella organisms growth after culturing on specific media. Ilhan *et al.* [22] agree with our results as he Examined 102 milk sample in different regions in turkey and showed that 8 samples was positive by culture, 24 samples were positive by PCR and 28 sample were positive by MRT. So the agreement between MRT and culture in summer season in our results was 0% and disagree with the agreement percentages recorded by Ilhan *et al.* [22] who found it 12.7%. In winter samples at both cities, 17 samples showed MRT positive reactions, 8 samples from El-Santa city and 9 samples of Sidi-Salem city, among of positive MRT samples 2 samples from El-Santa city and 3 samples from Sidi_Salem city showed growth on specific Brucella medium (Table 2). The isolated colonies appeared as Gram-negative coccobacilli with Gram's stain the agreement percentage between MRT and culture method was 29.4 which is slightly higher than the percentages (28.57%) reported by Ilhan *et al*. [22].The chemical, serotyping and using bacteriostatic dyes of the isolated strains revealed presence of both Brucella melitensis and Brucella abortus (Table 3). The results agree with Montasser *et al.* [23] who reported that out of 22 isolates from six governorates in Delta region (El-Menofyia, El-Kalyubia, El-Gharbia, El-Behera, El-Dakahlia and Damietta) 21 isolates typed as *Brucella melitensis biovar 3* which proved to be the prevalent strain among cattle as the causative agent of brucellosis in Egypt and one isolate typed as *Brucella abortus biovar 1* recovered from El-Dakahlia governorate and Selim *et al.* [24] who concluded that the predominant strain of *Brucella* species among ruminants in Kafr El-Sheikh governorate is *Brucella melitensis biovar 3*. Due to high economic impact of brucellosis in dairy animals, OIE, 2009 set a guide lines for control and eradication of brucellosis which are based on the prevalence of the disease.

CONCLUSIONS

I can conclude that the brucella organisms are still contaminate milk in Egypt and the percentage of incidence is higher in winter than summer seasons and the milk ring test gave higher results than culture method.

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