The role of cow's raw milk in transmission of brucellosis

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Summary

A total of 150 individual samples of blood and raw milk of cows collected from El-Behera Governorate were examined for detection of brucellosis. The samples were tested for SAT. The milk samples were subjected to MRT and isolation of brucella organisms, while the whey was examined for WRBPT and WRiv.T. The results showed that MRT was found to be reliable and sensitive for diagnosis of brucellosis in milk, as it gave positive results in 8 % of samples, as compared with the results of SAT (10%) for serum and WRBPT (4.7%), and WRiv.T (4.0 %) for milk whey. Brucella organism could be recovered from one (0.7%) sample which identified as *Br. melitensis* biovar 3.

The results of experimental study reveal that *Br. melitensis* biovar 3 survived for 8 days in refrigerated raw milk (4 ±1°C) and 2 days in raw milk kept at room temperature (22 ±2°C). While, it also survived for 12 days in yoghurt cream and 8 days in yoghurt body. The organism cannot withstand pH4.7 in raw milk and 4.2 in yoghurt.

The public health significance, epizootiological importance as well as the necessary measures recommended to control brucellosis were discussed.

Introduction

Brucellosis is an important milkborne zoonotic disease that it could be, under natural conditions, transmitted from animals to man. This hazard practically spread allover the world, only 17 countries of the whole world have been declared free from animal brucellosis (OIE, 1985). Brucellosis is still reported in some people of these countries, where the disease is usually contracted during travel to endemic areas. The disease in man can be caused by any of the *Brucella* species, *abortus, melitensis* or *suis.* The disease due to *Br. melitensis* is more severe and virulent than that caused by the other *Brucella* species.

The infected animals (sheep, goat, cow, buffaloe and camel) excrete brucella organisms in their milk sporadically throughout the entire period of lactation in counts varied from a few to up to 15000 cells / ml milk (Robertson, 1961; Sdiwerifeger, 1963; Ismail, 1971; Awad et al., 1975 and El-Gibaly et al., 1981). The brucella content of milk depends on the stage of lactation, as usually, the largest number of brucellae are in the milk at the onset of lactation periods, but both the occurrence and numbers of brucella excreted at any time can vary (Elberg, 1986). Hence, raw milk and its products from infected dairy animals play a significant role in the transmission of the disease to man. It was recorded that two-thirds of brucellosis in human cases at California, USA, arose through consumption of raw dairy products (Wynns, 1944).

Milk ring test (MRT) and blood serological tests are mainly used for diagnosing the disease among cattle. The evaluation of these tests was carried out by many workers (Nicoletti and Muraschi, 1966 and Katz et al., 1976). They stated that neither single milk nor blood serological tests were sufficient to give conclusive diagnosis of positive cases.

Tests for detection of brucella antibodies in milk are considered the principal methods for detecting infected herds and for diagnosing brucellosis in an individual cattle, because it is difficult to recover brucella from infected animals. The ideal diagnostic test for brucellosis should be easy, simple, and rapid test that will detect infected animals as early as possible during the course of the disease. Furthermore, this test should not be influenced by presence of non-specific antibodies arising from other Gram-negative bacteria (Morgan, 1977). Morgan et al. (1978) and Alton et al. (1988) described more specific tests for detecting brucella antibodies in milk such as milk ring test (MRT), whey buffered acidified plate antigen test (WBAPAT), whey rose bengal plate test (WRBPT), whey Rivanol test (WRiv.T), whey ELISA (WELISA) and ELISA milk tests. They were divided classically into screening and confirmatory tests. The screening tests include the MRT, WRBPT and WBAPAT, while the cofimatory tests involve WRiv.T, WELISA and ELISA milk tests.

Therefore, the present work attempts to study the prevalence of brucellosis in cows milk, to select the best screening and confirmatory test or tests suitable for accurate diagnosis of brucellosis, and to study the viability of *Br. melitensis* biovar 3 in raw milk and yoghurt.

Material and Methods

A total of 150 individual samples of blood and cow's milk of different breeds were collected from different localities in El-Behera Governorate. The milk samples were examined to be free from subclinical mastitis using Schalm test (A.P.H.A., 1985), and lactation period of 1 to 5 months to avoid factors affecting false-positive results of MRT. Each milk sample was divided into two parts, the milk and the milk whey prepared according to Morgan et al. (1978). Each sample was subjected to different diagnostic tests as recorded in the Table.

Samples and diagnostic tests of brucellosis

Samples	Tests	References
- Serum	- SAT	-Alton and Jones (1967)
- Milk	-MRT -Isolation of brucellae	-Alton et al. (1988) -Alton et al.(1975)
- Whey	-WRBPT -WRiv.T	-Alton et al. (1988) -Nat. Vet. Serv. Lab. (1984)

The titre of MRT, WRBPT and WRiv.T of positive samples of milk and the milk whey were detected using double-serial dilutions of normal milk (non-mastitis, fresh and brucella-free milk), or its whey. Isolation of brucella organisms were carried out by inoculation of albini brucella agar containing antibiotics with the sediment-cream mixture of milk. Simultaneously, enrichment technique using tryptose broth with antibiotics was run parallel. The plates were incubated at 10% carbon dioxide tension. Cultured plates were examined for brucella growth at the 4^{th} day and daily up to 14^{th} day. Suspected brucella colonies were identified according to Alton et al. (1975).

Experimental technique

Preparation of culture

The isolated local field strain of *Br. melitensis* biovar 3, the prevalent biovar among animals and man in Egypt, was used for the experiment. A two-days old culture was suspended in a sterile saline to be used for inoculation of milk and yoghurt.

Viability of *Br. melitensis* in raw milk

Freshly drawn cow's milk, free from brucella, in a clean and sterile stoppered-bottle was inoculated with the prepared culture of the organism to produce an initial inocula of $6x10^4$ cells / ml. The control and the inoculated milk were distributed aseptically into two sterile stoppered-bottles (one litre capacity). The first was placed in refrigerator ($4 \pm 1°C$), while the second was kept at room temperature ($22 \pm 2°C$). The cream layer as well as milk were examined daily for presence of the organism according to Alton et al. (1975), and pH by using Jeway pH meter.

Viability of *Br. melitensis* in yoghurt

Two lots of yoghurt were prepared from milk free from brucella organisms for test and control. The milk were inoculated with *Br. melitensis* biovar 3 at 42°C immediately after the starter to provide an initial inocula of $6x10^4$ cells / ml. Addition of starter cultures was done according to Lampert (1975). The control and infected yoghurt after being prepared were kept in refrigerator ($4 \pm 1°C$). Fat layer and curd of yoghurt were examined daily for presence of *Br. melitensis* and pH value.

Results

Results of the study are presented in Tables 1, 2, 3 & 4

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Discussion

The incidence of brucellosis in dairy animals becomes high with the importation of cattle, this was due in fact to the open door policy where a marked increase in the numbers of intensive breeding farms was recorded following the importation of large numbers of foreign breeds of animals from different countries (Adawy, 1985).

Serological examination of blood serum using SAT showed a higher reactors percentage (10.0 %) than of the milk or milk whey (Table 1). These findings substantiate the results of Alton (1963); El-Gibaly et al. (1990); El-Gibaly et al. (1991) and Hosein and El-Kholy (1993). This result can be attributed to the high sensitivity of this test to detect both IgG and IgM fractions (Salem et al., 1987).

Examination of cow's milk by MRT reveal that 12 (8.0%) of 150 samples gave positive results (Table 1). More or less nearly similar results were recorded by Hamdy (1992), who found 10 % of cow's milk samples were positive. Higher incidence (38%) was recorded by Saaed and Salem (1980), 82.4% by El-Gibaly et al. (1990), 29.2% by El-Sheery (1993) and 66.6% by Hamdy (1997), while lower incidence (4 %) was recorded by Hosein and El-Kholy (1993). The lower reactors detected by MRT, in comparison with blood serological test, may be ascribed to the stage of infection, or to the irregularity in the filteration of the agglutinins from the blood to the milk (Lembke et al., 1950). Moreover, it may be due to the level of the agglutinins in the blood not enough to be excreted in the milk (Pat and Panigahi, 1965).

Boer (1981) considered MRT as unreliable for individual diagnostic test, while other investigators referred to the test as simple, accurate, time saving, highly sensitive, reliable and usefull for detecting brucella agglutinins in milk of individual cows or herds (Ferguson and Robertson, 1954; Nicoletti and Burch, 1969; Morgan et al., 1978; Salem et al., 1987; El-Gibaly et al., 1991 and Hamdy (1992). MRT is known for its sensitivity for IgA (Collin, 1976 and Sutra et al., 1986). It also gave some false-positive cases, as in late lactation period and shortly after parturition, milk from cows with hormonal disorders and those with lower clustering power (Bercovich and Moearman, 1979 and Corbel et al., 1984), which are avoided in this study.

Shifting to the results of the whey agglutination test, it was clear that the over all results revealed low incidence of brucellosis ranging from 4.0 % for WRiv.T to 4.7%. for WRBPT (Table 1). This finding substantiate what have been recorded by Morgan et al. (1978); El-Gibaly (1990) and Hamdy (1997), who found that the whey tests are less sensitive, but less influenced by non-specific factors than MRT. The lower sensitivity of whey tests may be attributed to that the defattening process may deprive milk whey from some immunoglobulins mainly IgA adsorbed to the fat surfaces (Sutra et al., 1986). The removal of the solid parts by rennin, the change in the pH of the whey by the addition of rennet and the changes in molecular weight of immunoglobulins are the other additional factors that may led to low sensitivity of the whey agglutination tests (Sutra et al., 1986 and Hamdy, 1997).

Regarding the sensitivity of the diagnostic tests, results in Table 2 indicate that cows having a high positive serum titres showed positive MRT reaction when their milk diluted up to 1/128 with negative milk, followed by WRBPT (dilution 1/16), and WRiv.T (dilution 1/4). It was also observed that agglutinins titres of milk and whey correspondingly increased with those of blood serum, this may be attributed to the fact that brucella agglutinins in milk originate from the blood stream (Martin and Frank, 1970). These findings are coincident with the results of El-Gibaly et al. (1990); El-Gibaly et al. (1991); Hamdy (1997) and Roepk and Stiles (1970). This result refers to the reliability and sensitivity of MRT in picking up the infected cases than injuring animals for collecting blood serum.

Brucella organisms could be recovered from one (0.7%) milk sample of SAT, MRT, WRBPT and WRiv.T positive, and this isolate was typed as *Br. melitensis* biovar 3, the more prevalent biovar among animals and man in Egypt (Hamdy, 1992). Isolation of *Br. melitensis* from cattle, as non-original host, was firstly recoverd in Malta by Shaw (1906), who found that two cows shedding *Br. melitensis* in their milk. Isolation of such organism from cows milk in Egypt was recorded by El-Gibaly (1969); El-Gibaly et al. (1975); Abdel-Aal (1985); El-Sheery (1993); Salem et al. (1987); Hamdy (1989); Hamdy (1992); Hamouda (1989) and Hosein and El-Kholy (1993) by variable incidence ranging from 0.9% to 1.6% . The low rate of recovery of brucella organisms from milk may be ascribed to that these organisms were secreted intermittently in milk (Elberg, 1986) .

Br. melitensis is endemic in the mediterranean countries. The concept that *Br. melitensis* infects only sheep and goats is nothing , but a hypothesis paradox. When *Br. melitensis* is endemic in sheep and goats, the disease can be easily transmitted to cattle and buffaloes leading to human infection (Verger, 1985). Recovering of *Br. melitensis* from cow's milk represents both epidemioloical and zoonotic importance, as such organism is most virulent and pathogenic than other *Brucella* species in man and animals (Elberg, 1986). Moreover, this strain does not lose its pathogenicity in cattle (Ivanov and Kolmakin , 1959 and Hamdy, 1989) .

Results presented in Table 3 show that *Br.melitensis* biovar 3 was survived for 8 days in refrigerated raw milk (4±1°C) and for 2 days in raw milk kept at room temprature (22± 2°C). Nearly similar survival periods were recorded by Awad et al . (1975), who found that the organism survived for 5-9 days in raw milk. Also, Hamdy (1992) recorded survival periods of 5 days in refrigerated raw milk and only one day in raw milk kept at room temperature. The difference in survival periods may be due to the bacterial population of raw milk, as well as the initial inocula used in the experiment. The survival periods of such organism in cream layer exeeds those in milk column. This finding run parallel to those recorded by Hamdy (1992). This might be due to that cream is usually more heavily infected than milk , as the organism tend to adhere to the surface of the fat globules forming a complex and the protective effect of the high fat content of cream layer (Champnyz, 1953). The killing effect of milk on the brucella organisms may be due to the acidity developed by the lactic acid bacteria .

Table 4 illustrates the viability of *Br. melitensis* in yoghurt, as the organism was viable longer in fat layer of yoghurt (12 days) than in yoghurt curd (8 days). This may be due to the that the organism tends to be carried up to the top by the fat globules protecting it from yoghurt acidity. Nearely similar results were reported by Hamdy (1992).

Storage temperature obviously affects the survival rate, as brucellae survived longer at refrigerated temperature. These findings are coincident tothose obtained by Nour et al. (1975); Abdel-Hakiem et al.(1994) and Hamdy and Abdel-Hakiem (1994). This may be due to the different degrees of acidity developed in raw milk or yoghurt stored at different storage temperatures.

It was found that the pH was determinative for the brucella organisms. When pH reached 4.7 in raw milk and 4.2 in yoghurt, it was impossible to recover *Br. melitensis* (Tables 3 & 4). As the storage period advanced, the acidity percentage increased and consequently affects the survival of the organism. This observation agree with that of Kudaz and Morse (1954); Ghoniem (1972); Hamdy (1992) and Hamdy and Abdel-Hakiem (1994).

It can be concluded that raw milk and its products may be considered a significant vehicle for transmission of brucellosis to man, bearing in mind that *Br. melitensis* is the most virulent biovar to man. MRT proved to be sensitive test and useful in

diagnosing of infected individual cows. As *Br. melitensis* can survived in raw milk and yoghurt, so the prevention of the disease in man depends mainly on the eradication of disease in animals as well as heat-treatment of milk to safeguard the consumers, as effecient pasteurization was enough to destroy brucella organisms in milk.

Table 1: Prevalence of brucellosis according to different diagnostic tests

No. of samples	TAT		MRT		WRBPT		WRiv.T		Culture	
	No.	%	No.	%	No.	%	No.	%	No.	%
150	15	10	12	8.0	7	4.7	6	4.0	1	0.7

Table 2: Sensitivity of diagnostic tests (blood serum, milk and milk whey) of brucella positive samples.

Positive samples	End titre at which positive reaction occurs			
	TAT	MRT	WRBPT	WRiv. T.
1	1/ 80	¼	-	-
2	1/80	½	-	-
3	1/ 320	1/128	1/16	1/4
4	1/320	1/64	1/4	1/2
5	1/160	1/8	-	-
6	1/160	1/16	-	-
7	1/80	1/2	-	-
8	1/80	1/4	-	-
9	1/320	1/64	1/2	-
10	1/80	1/16	-	-
11	1/80	1/2	-	-
12	1/320	1/128	1/8	1/2
13	1/40	-	-	-
14	1/80	-	-	-
15	1/40	-	-	-

Table 3: Survival of *Br. melitensis* biovar 3 in raw milk.

Survival periods (days)	At refrigerator (4 ±°1C)			At room temperature(22 ± 2°C)		
	Cream layer	Milk column	pH	Cream layer	Milk column	pH
1	+	+	5.9	+	+	5.1
2	+	+	5.6	+	-	4.8
3	+	+	5.5	-	-	-
4	+	+	5.4	-	-	-
5	+	+	5.4	-	-	-
6	+	-	5.3	-	-	-
7	+	-	5.1	-	-	-
8	+	-	4.9			
9	-	-	4.7			

Table 4: Survival periods (days) of *Br. melitensis* biovar 3 in yoghurt.

	Survival periods (days)											
	1	2	3	4	5	6	7	8	9	10	11	12
Fat layer	+	+	+	+	+	+	+	+	+	+	+	+
Curd	+	+	+	+	+	+	+	+	-	-	-	-
pH	4.7	4.7	4.6	4.6	4.5	4.5	4.5	4.4	4.3	4.2	4.2	4.2

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