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VIABILITY OF BRUCELLA MELITENSIS IN BUTTER AND ICE CREAM

(With 2 Tables)

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مدى قدرة ميكروب البروسيلا مليتنسيس على المعيشه في الزبد والجيلاتى

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نظرا لزيادة معدلات الاصابه بمرض الحمى المتموجه بين الادميين وامكانية نقل العدوى عن طريق الالبان الخام ومنتجاتها لذا تم دراسة مدى قدرة عترتين من ميكروب البروسيلا مليتنسيس علي المعيشه فى الزبد والجيلاتى.

وقد اظهرت النتائج ان العترة الحقليه الثالثه كانت حية لمدة ٤٢٫٢٦ يوما فى الزبد الفلاحى والمستورد على التوالى بينما العترة القياسية ظلت حية لمدة ٣٨٫٢٠ يوما فى الزبد الفلاحى والمستورد على التوالى. بالنسبة للجيلاتى الذى تم حفظه عند درجة حرارة ٢٢ تحت الصفر كانت العترة الحقلية الثالثة حية لمدة ١٤ اسبوع فى نوعى الجيلاتى سواء كانت العدوى قبل او بعد التصنيع وبينما كانت العترة القياسية حية لمدة ٨ اسابيع فقط فى نوعى الجيلاتى. وقد نوقشت خطورة الميكروب على الصحة العامة وكذلك الاشتراطات الصحية الواجب مراعاتها عند انتاج وتصنيع منتجات الالبان.

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SUMMARY

The study was conducted to determine the survival time of the local field *Brucella melitensis* biovar 3 and *Br. melitensis* 16M (standard strain) in butter held at 4°C and ice cream stored at subfreezing temperature (-22°C). Local field *Br. melitensis* biovar 3 survived for 26 and 42 days in farm (cooking) and table imported butters, respectively. While, *Br. melitensis* 16 M strain died after 20 and 38 days in both types of butters, respectively. In both types of ice cream (pre-and post-manufacture infected) stored at subfreezing temperature, the local field *Br. melitensis* biovar 3 and 16 M strains were found viable for 14 and 8 weeks, respectively. The public health significance of the organism was discussed.

Keywords: Br. melitensis, butter, ice cream.

INTRODUCTION

Brucellosis has increasingly been recognized as a major foodborne disease in human beings *(BARTON, 1989).* Nearly all foodborne transmission of brucellosis occurs by consumption of milk and milk products. It was recorded that two-thirds of all human brucellosis cases (USA) arose through consumption of raw milk or raw milk products, *(WYNNS, 1944).* Therefore, the epidemiological evidence overwhelmingly incriminates milk and milk products as the primary source of foodborne brucellosis. The flora of raw cream and consequently butter manufactured from raw cream is expected to have the same flora of raw milk. Farm butter is rarely infected, being usually made from milk which has been firstly soured, and thus lactic acid kills most brucellae. Cold storage of butter and addition of salt (3%) may selectively predispose the multiplication of the organism. Literature about the incidence of *Br.melitensis* in butter is insufficient, however, *GROSSO and BERGAMINI (1959)* isolated brucellae from 1.8% of the 142 commercial butter samples. Research work carried out on *Br. melitensis* in butter revealed different survival periods. *GARGANI and GUERRA (1956)* reported that the survival of *Br. abortus and Br. melitensis* in butter is varied from one to six weeks depending on the initial infection. *NERI and ODASSO (1957)* found that brucellae survived at 4°C for 23 days in butter made from artificially infected milk. *BARTON (1989)* stated that *Br. melitensis* survived for 42 days in butter kept at 4-8°C.

Ice cream is one of the most highly nutritive refreshing and appetizing dairy products especially during hot weather. All the ingredients used in the manufacture of ice cream contain harmless bacteria often in very large numbers. Once again the infection of ice cream with brucella organisms depends largely on the contamination of the dairy ingredients. A reduction in the microbial content may occur during freezing of the mix, due to the disintegration of the microbial cells by the abrasive moving ice crystals during freezing *(FOLEY and SHEURING, 1966)*. Data about the role of butter and ice cream in transmission of brucella organisms to human beings is not available, so the aim of this investigation was to determine the survival time of *Br. melitensis* in butter and ice cream and their roles in transmission of the disease to consumers.

MATERIAL and METHODS

Strains of Br. melitensis

The local field *Br. melitensis* biovar 3, the prevalent biovar among animals and man in Egypt, and *Br. melitensis 16* M (standard strain) were chosen for the experiment, as they are more virulent and pathogenic than other *Brucella* species in man and animals *(HAMDY, 1992)*. The biotypes were obtained from *Dept. of Brucellosis,* Animal Health Research Institute (AHRI), Dokki, Giza.

Preparation of Brucella cultures

The chosen strains were subcultured on brucella agar, purified and then identified. A two-day old culture was harvested using sterile peptone saline. A viable count was carried out according to the technique adopted by *ALTON* et al. *(1975)*.

Preparation and inoculation of butter

Farm (cooking) and table imported, brucella free, butter samples were aseptically obtained in sterile covered wide mouth jars. Artificial infection of the samples were made by inoculation of melted butter (40°C for 10 min) to insure proper distribution of the organism in the samples, with saline suspension of the chosen strains to give an intial inocula of 6.5×10^6 CFU/g, and then thoroughly mixed. Initial inocula of *Br. melitensis* strains were determined at the time of inoculation. Infected butter samples were stored in refrigerator at 4°C, and examined periodically for the presence of *Br. melitensis,* as well as acidity and salt percentage.

Manufacture and inoculation of ice cream

A- Pre-manufacturing infection

Ice cream was manufactured according to the manufacturer instruction. 50 ml of brucella free raw whole buffaloes milk infected with saline suspension of the chosen *Br. melitensis* strains was added to the dry mix powder (250g) containing all the ingredients necessary to make ice cream. Then well mixed in sterile home ice cream machine for 5 min to give an initial inocula of 6.5×10^6 CFU/g. Initial inocula of the infected samples were determined at the time of infection. The infected samples were aseptically transferred into sterile covered wide-mouth jars, and then the mixture stored at subfreezing temperature ($-22^{\circ}C$), the traditional storage temperature, and examined periodically for the presence of the inoculated *Br. melitensis* strains.

B-post-manufacturing infection

This may occur by some dairy producers to increase the volume of ice cream. The infection of ice cream was carried out thourgh mixing of the infected raw whole buffaloes milk containing *Br. melitensis* strain with freshly prepared ice cream in sterile home ice cream machine for 5 min, to give an initial inocula of 6.5×10^6 CFU/g. Then the mixture was stored at subfreezing temperature ($-22^{\circ}C$), and tested periodically for the presence of the viable brucella organisms.

Bacteriological examination

Isolation of *Br. melitensis* was carried out by culturing butter and ice cream on brucella agar media containing crystal violet and different concentration of antibiotics using the technique adopted by *ALTON* et al. *(1975)*.

Sodium chloride percentage in butter

The salt content of butter was measured with the test described by *ATHERTON and NEWLANDER (1977)*.

Acidity percentage in butter

The titratable acidity (Lactic acid %) was determined according to *LING (1963)*.

RESULTS

All results obtained are recorded in Tables 1 and 2.

DISCUSSION

The period elapsing between the date of infection and the

last time the organisms could be detected was taken as the survival period of the organism in the product. Results recorded in Table (1) illustrate the viability of *Br.melitensis* strains, as well as the salt and acidity percentages in farm and imported butter samples stored at refrigerator temperature (4°C). The results indicate that the local field *Br. melitensis* biovar 3 strain survived for 26 and 42 days in farm and imported butters, respectively. While, *Br. melitensis* 16 M strain died after 20 and 38 days in both types of butter, respectively.

The results of the viability of *Br. melitensis in butter are in a great concordance with those recorded by GARGANI and GUERRA (1956), NERI and ODASSO (1957) and BARTON (1989).* The obtained results show a lower survival time of both *Br. melitensis* strains under experiment in farm butter than in imported butter. This finding may be attributed to the higher acidity of the farm butter, where it is usually made from raw milk which has been well soured first and the resulting lactic acid kills brucellae. The results of salt content of butter reported in the same Table show that sodium chloride percentages are nearly similar in both types of butter and varied from 3.1 to 3.46 %. So, the obtained results encourage us to suggest that the salt content of butter has little or no bactericidal effect on the organisms under investigation. Results of acidity percentages reveal that the farm butter was higher in acidity than imported butter, this finding may substantiate the short survival period of the tested *Br. melitensis* strain in farm butter.

Results recorded in Table (2) indicate the longevity of *Br. melitensis* strains in pre-manufacturing and post-manufacturing ice creams held at subfreezing temperature (-22°C). The local field *Br. melitensis* biovar 3 strain was present viable for 14 weeks in both types of ice creams, whereas 16 M strain was found to survive for 9 weeks in both types of ice cream.

No available literature about the survival of *Br. melitensis* in ice cream kept at subfreezing temperature. However, *BARTON (1989)* studied the survival of *Br. abortus* in ice cream stored at 0°C, and showed that the organism survived for 30 days. The lower survival period recorded by *BARTON (1989)* was attributed to the fact that high freezing temperatures are more lethal, where death of brucella cells was much greater at- 4°C to- 10°C than at-15°C to-30°C *(KUZDAS and MORESE, 1953 and CHRISTOPHERSEN, 1973).*

Temperature is a physical factor known to influence the growth and death of bacterial cells. *MITCHELL et al. (1951)*

reported that bacteriostasis may occur by adjusting the temperature of growing cells, in which cell division is more or less completly arrested, and there is a loss of viability as well. The kind of microorganisms and its state, the freezing rate, the intensity of freezing temperature, the time of frozen storage, the composition of the food and repeated fluctuations of temperature during freezing are the main factors that may dictate why some microorganisms die, some are injured and some are damaged *(FRAZIER and WESTHOFF, 1978).* Lethal effects of subfreezing temperature are thought to be the results of denaturation or flocculation of essential cell proteins or enzymes possibly as a result of the *increased concentration of solutes in the unfrozen water or perhaps in part due to physical damage by ice crystals (FRAZIER and WESTHOFF, 1978).*

The presence of *Br. melitensis* viable in ice cream for long periods is not surprizing. Hence, it has been recorded that the composition of the food influences the rate of death of different microorganisms during freezing and storage. Since, sugar, salt, proteins, colloids, fat and other substances may be protective, whereas high moisture and low pH may hasten the killing of these microorganisms *(FRAZIER and WESTHOFF, 1978).* In addition, the results reveal that the local field *Br. melitensis* biovar 3 strain survived longer than 16 M strain in both types of butter and ice cream, a finding which may be due mainly to that the local field strain is accommodated to the environmental conditions due to its several passages in animals and man, as it is the prevalent strain among animals and humans in Egypt, where it is isolated by higher incidence *(AWAD et al., 1975 and HAMDY, 1992).*

It is also evident that there is no significance difference in the survivability of *Br. melitensis* either the local or the standard strain in pre-and post-manufactured ice cream. This may be attributed in a large extent to the fact that the way in which ice cream is manufactured does not affect the organism.

On conclusion, the survival time of *Br. melitensis* in butter and ice cream may play an important role in transmission of brucellosis to human beings, and constitutes a public health significance. Since, ice cream as well as butter are consumed fresh and this may if contaminated be a source of human brucellosis. longer survival periods of *Br. melitensis* tested strains in this study increase the proportion of human risk. Recognition of the fact that brucellosis may be spread through contaminated butter and ice cream should serve to emphasize the importance of proper pasteurization of milk and/or cream prior

to butter and ice cream manufacture, as the time-temperature combination required for pasteurization is enough to inactivate the organism in dairy products. Local field *Br. melitensis* biovar 3 strain is more active than 16 M strain.

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Table (1) : Viability of ***Br. melitensis*** strains and percentages of salt and acidity in butter stored at refrigerator (4 °C) .

Storage Period (day)	Butter: Farm butter Surv. local	Surv. 16 M	Salt %	Acid %	Imported butter Surv. local	Surv. 16 M	Salt %	Acid %
1	+	+	3.27	0.057	+	+	3.1	0.030
3	+	+			+	+		
5	+	+			+	+		
7	+	+	3.31	0.074	+	+	3.15	0.034
9	+	+			+	+		
12	+	+			+	+		
14	+	+			+	+		
16	+	+	3.37	0.081	+	+	3.17	0.037
18	+	+			+	+		
20	+	+			+	+		
22	+	-	3.41	0.089	+	+	3.21	0.041
24	+	-			+	+		
26	+	-			+	+		
28	-	-	3.46	0.097	+	+	3.25	0.048
30	-	-			+	+		
32	-	-			+	+		
34	-	-			+	+	3.29	0.055
36	-	-			+	+		
38	-	-			+	+		
40	-	-			+	-		
42	-	-			+	-	3.32	0.063
44	-	-			-	-		
46	-	-			-	-		

Table (2) : Viability of the local field ***Br. melitensis*** biovar 3, and 16 strains in ice cream stored at - 22 °C.

Storage periods (Weeks)	Ice cream			
	Pre-manufacturing infection		Post-manufacturing infection	
	Survival		Survival	
	Local	16 M	Local	16 M
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+
8	+	+	+	+
9	+	-	+	-
10	+	-	+	-
11	+	-	+	-
12	+	-	+	-
13	+	-	+	-
14	+	-	+	-
15	-	-	-	-
16	-	-	-	-