

VIABILITY OF BRUCELLA MELITENSIS IN DAMIETTA CHEESE

(With 2 Tables)

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(Received at 6/2/1994)

مدى قدرة ميكروب البروسيلة ملينسيس على المعيشة في الجبن الدمياطى

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تزايدت فى الاونه الأخيره زيادة معدلات الاصابه بمرض الحمى المتموجه بين الادميين ، ونظراً لان الالبان الخام ومنتجاتها تمثل دوراً هاماً فى نقل العدوى ، لذا تم دراسة مدى قدرة العترة الحقلية الثالثه من ميكروب البروسيلة ملينسيس على المعيشه فى الجبن الدمياطى . وقد اظهرت النتائج ان الميكروب استمر حياً لمدة ١٥ ر ١٥ و ٨ يوماً فى الجبن الدمياطى المضاف اليه ٥ % ، و ٧ % ، ١٠ % ملح الطعام على التوالي والذى تم حفظه عند درجة حرارة الغرفة (٢٢ م) . بينما ظل الميكروب حياً لمدة ٢٥ ر ٢٥ و ٢٢ يوماً فى انواع الجبن الدمياطى الثالثه على التوالي الذى تم حفظه عند درجة حرارة الثلجه (٤ م) .

وقد نوقشت خطورة الميكروب على الصحة العامه وكذلك الاشتراطات الصحيه الواجب مراعاتها فى انتاج الالبان وتصنيعها .

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SUMMARY

The viability of the local field strain of *Brucella melitensis* biovar 3, the prevalent strain in man and animals in Egypt, was studied in Damietta cheese with different concentrations of salt and stored at room temperature (22°C) and in refrigerator (4°C). At room temperature the organism remained viable up to 15, 15 and 8 days in cheese containing 5, 7.5 and 10% sodium chloride, respectively. At refrigerated temperature, the organism survived for 25, 25 and 22 days in the three types of cheeses, respectively.

Keywords: Damietta cheese (Soft cheese).

INTRODUCTION

Brucellosis is an important zoonotic disease that it could be, under natural conditions, transmitted from animals to man. This hazard actually spread all over the world. Only 17 countries of the whole world have been declared free from animal brucellosis (OIE, 1985). Among the six species of the genus *Brucella*, *Br. melitensis* is the most virulent and pathogenic strain for both man and animals.

Brucella-infected sheep, goats, cows, buffaloes and camels excrete brucella organisms in their milk sporadically throughout almost their entire period of lactation. Hence, the consumption of untreated milk and milk products from infected animals exposes man to serious risk of infection by *Br. melitensis* and *Br. abortus*. The brucella content of milk depends on the stage of the disease in the animal concerning and the stage of lactation. Usually, the largest number of brucellae are in the milk at the onset of the lactation period, but both the occurrence and numbers of brucellae excreted at any time can vary (ELBERG, 1981).

Brucellosis can be transmitted from animal to man either directly or indirectly. Transmission of the disease through direct contact originally takes place through contact with infected animals and thus the disease is of occupational pattern as the infection is limited to those who involved in the field of animal husbandry. On the other hand, the indirect route of transmission has a wide range of victims, as the disease is mainly transmitted through the ingestion of milk or milk products without prior heat treatment, where brucellae are

usually found in the udder of the infected lactating animals and can be easily excreted in milk (MEYER, 1964).

Damietta cheese is one of the most popular varieties of cheeses in Egypt. It is traditionally prepared from raw milk without addition of lactic acid starter or colorant. Moreover, it may be consumed fresh or preserved in whey and it could if contaminated be a major source of foodborne brucellosis.

No available literature is accessible concerning the survivability of *Br. melitensis* in Damietta cheese and its role in spreading the disease among human consumers. Therefore, this research was planned to fulfil this gap.

MATERIAL and METHODS

Strains of *Brucella melitensis*:

The chosen strain was subcultured on brucella agar, purified and then identified. Two days-old culture was harvested using sterile saline. A viable count was made according to the method adopted by ALTON et al. (1975). The culture was added to milk before its manufacture to obtain a final concentration 6.8×10^6 CFU/ml milk.

Preparation of Damietta cheese:

Cheese was made from raw brucella free buffalo milk containing 5, 7.5 and 10% sodium Chloride. Raw milk was inoculated with saline suspension of *Br. melitensis* harvested culture to give an initial inocula of 6.8×10^6 CFU/ml milk. A sample was taken after inoculation to determine the *Br. melitensis* count. The inoculated raw milk was divided into three equal portion. Every portion was salted by addition of sod. chloride to obtain the desired concentration. The proceddure described by FAHMI and SHARARA (1950) was used to manufacture Damietta cheese. Raw buffalo milk used for this experiment was obtained from the Dairy Technology Dept., Animal Production Research Institute, Dokki, Giza. After manufacture, (each type of infected cheese), the cheese and the whey were divided into two portions; the first one was stored at room temperature (22°C), while the second portion was kept in a refrigerator (4°C). Every type of salted cheese and its whey was tested periodically for the presence of viable brucella organisms, as well as for sodium chloride and acidity percentages.

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Bacteriological examination of inoculated cheese:

Isolation of *Br. melitensis* was carried out by culturing the cheese and whey separately on brucella agar using the technique adopted by ALTON *et al.* (1975).

Sodium chloride percent:

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

Acidity percent:

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

RESULTS

Are presented in tables 1 and 2.

DISCUSSION

Cheese made from raw milk containing brucella organisms should be subjected to adequate heat treatment during manufacture. Depending on the initial infecting dose and characteristics of the dairy products, brucella organisms may remain viable for long period.

In some countries the sale of certain dairy products made from raw milk is prohibited until after a reasonable storage time to allow many pathogenic bacteria to die. Concerning the viability of the local field strain of *Br. melitensis* in Damietta cheese and whey stored at room temperature, the results represented in Table(1) show that the inoculated organism was present up to 15, 15 and 10 days in cheese containing 5, 7.5 and 10% added sod. chloride, respectively. While, the organism survived for 3 days in the whey of all types of salted cheeses. On the other hand, the results in Table (2) indicate that *Br. melitensis* remained viable for 25, 25 and 22 days in different types of salted cheeses stored in refrigerator (4°C), respectively. While, it survived for 8 days in the whey of different types of salted cheeses.

The majority of the researches on the survivability of *brucellae* in cheese has been carried out in Europe with other cheese varieties. ALIVISATOS (1947) inoculated *Br. melitensis* into raw milk and pasteurized milk used for the manufacture of soft Feta cheese. According to the same author the organism survived in raw milk cheese for 4-16 days and for 10-26 days in pasteurized milk cheese. GARGANI (1952) showed that

Br. melitensis inoculated into Pecorino cheese did not survive for more than 90 days. Many outbreaks of brucellosis in USA and France were traced to cheese made from unpasteurized goat milk (STILES, 1945 and BOYER et al., 1957).

The effect of storage temperature on the longevity of the microorganisms is striking. Storage temperature obviously affect the survival rate, as *Br. melitensis* strain survived longer at refrigerator temperature (4°C). If the temperature is high, it favours the growth of lactic acid producing bacteria, thus increasing the acidity and causing the death of the organism. On the contrary, when it is low the aciduric bacteria grow more slowly, thus allowing the organism to survive longer. It is also noted that the organism survived in cheese (solid phase) for longer periods than in whey (liquid phase), as the organism tends to survive in liquid media for shorter periods than it does in solid media (ALTON et al., 1975).

The acidity or pH is an important factor that influences the growth rate of microorganisms. The results of acidity percentages of cheese recorded in the same Tables show that as the storage period advanced the acidity percentage increased and this consequently affects the survival of the organism in cheese. This observation agrees with that of KUZDAS and MORSE (1954) and SALEM et al. (1974).

Salt concentration is one of the main factors that control the growth of microorganisms. Sodium chloride is used in Egypt in preparation of some cheeses (Damietta and Kareish cheeses) with different concentrations. It is evident from the obtained results recorded in the same Tables that as the storage period advanced, the sodium chloride percentage of cheese increased, this may be attained to the dryness of cheese during storage. Sodium chloride with low concentrations has little inhibitory effect on the growth rate of pathogenic microorganisms in sterile milk, but it has no effect when raw or pasteurized milk was used. This may be due to the inhibitory effect of sod. chloride on other normal flora that are not salt tolerant (IBRAHIM et al., 1981). However, HIGNETT et al. (1967) found that Ringer's solution had a markedly adverse effect on the viability of *Br. abortus* when used as a preservative or as a diluent.

The higher concentration of sod. chloride has little germicidal action on pathogenic organisms, hence the action of sod. chloride depends to a large extent on the medium in which it is dissolved, where it is more active when dissolved in distilled water than when it is dissolved in a medium containing protein as milk. This was attributed to the fact that many cations of sod. chloride combine with proteins to

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form an insoluble albuminate, hence the content of free sodium ions in the medium is diminished (WILSON and MILLES, 1975).

On conclusion, the obtained results proved that the survival period of *Br. melitensis* strain under experiment has a significant public health importance. Acidity percent and the storage temperature as well as the time of storage has a direct effect on the viability of the organism. On the other hand, the concentration of sod. chloride has a little effect on the longevity of the organism. Furthermore, the method used to make cheese do not kill brucellae. These findings encourage us to recommend that soft cheese should be manufactured from pasteurized milk, as the pasteurization temperatures were found to inactivate brucellae. In case of Damietta cheese manufactured from raw milk, it should not be consumed before at least 15 days after preservation in room temperature or 25 days if kept in refrigerator (4°C).

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Table (1): Viability of the local field *Br. melitensis* biovar 3 in Damietta cheese stored at room temperature (22 °C).

Storage Period (day)	Salt concentration											
	5% Na Cl				7.5% Na Cl				10% Na Cl			
	Surv. Ch	Salt W	Acid %	%	Surv. Ch	Salt W	Acid %	%	Surv. Ch	Salt W	Acid %	%
1	+	+	3.5	0.12	+	+	5.84	0.22	+	+	7.15	0.27
2	+	+			+	+			+	+		
3	+	+			+	+			+	+		
5	+	-			+	-			+	-		
8	+	-	3.54	0.32	+	-	6.01	0.41	+	-	7.45	0.48
10	+	-			+	-			-	-		
13	+	-			+	-			-	-		
15	+	-	3.81	0.51	+	-	6.24	0.68	-	-	8.56	0.87
18	-	-			-	-			-	-		
20	-	-			-	-			-	-		

Ch : Cheese W : Whey Surv. : Survivability

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Table (2) : Viability of the local field *Br. melitensis* biovar 3 in Damietta cheese stored in refrigerator (4 °C) .

Storage Period (day)	Salt concentration											
	5%Na Cl				7.5% Na Cl				10% Na Cl			
	Surv. Ch	Salt W	Acid %	Acid %	Surv. Ch	Salt W	Acid %	Acid %	Surv. Ch	Salt W	Acid %	Acid %
1	+	+	3.5	0.12	+	+	5.84	0.22	+	+	7.01	0.27
2	+	+			+	+			+	+		
5	+	+			+	+			+	+		
8	+	+	3.54	0.20	+	+	6.01	0.29	+	+	7.42	0.32
10	+	-			+	+			+	-		
13	+	-			+	+			+	-		
15	+	-	3.81	0.31	+	+	6.23	0.38	+	-	8.56	0.43
18	+	-			+	+			+	-		
20	+	-			+	+			+	-		
22	+	-	4.12	0.33	+	+	6.83	0.47	+	-	8.66	0.57
23	+	-			+	+			-	-		
24	+	-			+	+			-	-		
25	+	-			+	+			-	-		
26	-	-	4.12	0.51	-	-	6.84	0.72	-	-	8.66	0.86

Ch : Cheese W : Whey Surv. : Survivability