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**BEHAVIOR OF VIRULENT YERSINIA ENTEROCOLITICA  
IN DAMIETTA CHEESE**  
(With Two Tables)

By  
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سلوك ميكروب الـ Yersinia enterocolitica الممرض  
في الجبن الدمياطى

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أجريت هذه الدراسة لمعرفة نمو وبقاء ميكروب الـ Y. enterocolitica أثناء عملية تصنيع وتخزين الجبن الدمياطى لما لهذا الميكروب من خطورة كبيرة على الصحة العامة . وقد تم تصنيع الجبن الدمياطى من لبن مبستر معملها مضاف إليه ٥% ملح وآخر مضاف إليه ١٠% ملح وقد تم إضافة ميكروب الـ Yersinia (٠×١) (أمل) وذلك قبل إضافة الملح والمنفعة . وقد تم عد الميكروب المتواجد وكذلك قياس الرقم الهيدروجينى ونسبة الرطوبة والملح دوريا أثناء عملية التصنيع وكذلك أثناء فترة التخزين . وقد وجد أن ميكروب الـ Yersinia تزايد قليلا فى العدد أثناء عملية التصنيع وقد كانت الزيادة ملحوظة فى الجبن المصنع من لبن مضاف إليه ٥% ملح . ثم بدأ عدد الميكروب يتناقص سريعا خلال فترة التخزين حيث لم يمكن إكتشافه بعد إسبوعين فى كل من النوعين من الجبن كذلك لوحظ تناقص الرقم الهيدروجينى للجبن وكذلك نسبة الرطوبة بينما تزايدت نسبة الملح فى الجبن أثناء التخزين . وقد تم مناقشة الخطورة الصحية لهذا الميكروب وكذلك الطرق الصحية الواجب إتباعها .

**SUMMARY**

A virulent strain of Yersinia enterocolitica was inoculated into pasteurized milks. The inoculated milks salted with 5 or 10% sodium chloride, were used for making Damietta cheese. The cheeses were stored in their whey at 30±1°C, and were examined periodically for Yersinia counts, pH value, moisture and salt contents. A slight increase in number, of Y. enterocolitica occurred during preparation of cheeses. However, the growth rate of the organism in cheese made from milk with 5% added salt, was higher than that recorded in cheese made from milk with 10% added salt. Numbers of Y. enterocolitica sharply decreased in both types of cheeses during storage period, and could not be detected by the end of the second week.

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**INTRODUCTION**

Yersinia enterocolitica is a zoonotic microorganism capable of causing foodborne enteritis in humans (ZEN-YOJI, 1973; OLSOVSKA, et al. 1975; HEALTH and WELFARE Canada 1976, and BLACK, et al. 1978). Furthermore, the organism can produce a variety of clinical symptoms, particularly mesenteric lymphadenitis, terminal ileitis, artheritis, septicemia, erythema nodosum, and liver and spleen abscesses (SONNENWIRTH & WEAVER, 1970 and WINBLAD, 1973).

Y. enterocolitica could be isolated from milk and milk products, including raw and pasteurized milk, cream, ice-cream, and cheese (ALDOVA, et al. 1973; LEE, 1977; SCHIEMANN, 1978; SCHIEMANN and TOMA, 1978; HUGHES, 1979; MOUSTAFA, et al. 1983 a, and FRANZIN, et al. 1984). However, few surveys on presence and survival of Y. enterocolitica in cheese have been made (SCHIEMANN, 1978 and MOUSTAFA, et al. 1983 b). Several reports have indicated that Y. enterocolitica could produce toxins in food (BOYCE, et al. 1979; FRANCIS, et al. 1980 and KAPPERUD and LANGELAND, 1981). These enterotoxins may be able to resist the temperatures used in food processing and storage (BOYCE, et al. 1979).

Although, there are no documented outbreaks of foodborne illness caused by Y. enterocolitica and associated with cheese, this study was planned to evaluate the safety of Damietta cheese artificially contaminated with virulent Y. enterocolitica strain.

**MATERIAL and METHODS****Cultures:**

Y. enterocolitica strain (2635 serotype 0:8) that caused the chocolate milk outbreak (BLACK, et al. 1978), was used in this study. The strain was obtained from the Food Research Institute, University of Wisconsin, Madison, USA. Culture of Y. enterocolitica was grown in Trypticase Soy broth (Difco) at  $23 \pm 1^\circ\text{C}$  for 18 hours before inoculation into milk.

**Preparation of Damietta cheese:**

Raw milk obtained from the dairy farm of the faculty of Agriculture, Assiut Univ., was locally pasteurized at  $63^\circ\text{C}$  for 30 min. Pasteurized milk was inoculated with Y. enterocolitica to give the desired number of bacterium per ml. ( $1 \times 10^6$ ). The inoculated pasteurized milk was divided into two equal portions, and were salted by addition of sodium chloride to give concentrations of 5 and 10%. The procedure described by FAHMY and SHARARA (1950) was used to manufacture the cheese. Two control blocks of Damietta cheese were prepared from pasteurized milk containing the same percentages of salt without addition of Yersenia culture. Contaminated cheeses as well as controls were kept at  $30 \pm 1^\circ\text{C}$ , and tested periodically.

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### Sampling:

Samples for detection of Yersinia counts and determination of pH value were taken from milk before and after addition of the inoculum and immediately after curdling. Samples were taken from the finished cheeses and controls after preparation and weekly thereafter for pH determination, Yersinia counts, moisture and salt contents.

### Preparation of cheese for examination:

Cheese samples were prepared for examination according to Standard methods (MARTH, 1978).

### Enumeration of Y.enterocolitica:

Surface plating onto cefsulodin-Irgasan Novobiocin (CIN) agar (SCHIEMANN, 1979) was done for each sample and its dilutions. The plates were incubated at 27°C for 48 hours, and typical colonies presumed to be Y.enterocolitica were counted. Confirmatory tests were done on each isolate suspected to be Y.enterocolitica as described by FEELEY, et al. (1976).

### pH determination:

The pH values of milk and cheese were determined by using a pH meter (an orion, Model 701) equipped with standard combination electrode.

### Moisture and salt content:

Moisture was determined according to Standard Methods (MARTH, 1978). The salt content was determined by the method described by AHERTON and NEULANDER (1977).

## RESULTS

The obtained results were recorded in Tables (1 & 2).

## DISCUSSION

As recorded in Table (1), an increase in salt (water phase) and a decrease in moisture contents of cheeses during storage period. These findings were comparable to the results obtained by AMER, et al. (1979) and AHMED, et al. (1983).

Data in Table (2), reveal that a slight increase in numbers of Y.enterocolitica occurred during manufacturing of cheese made from milk with 5% added salt. The organism achieved its maximum population ( $1 \times 10^7$  cells/g) by the end of cheese making Y.enterocolitica began to lose its viability rapidly and could not be detected ( $< 100$  cells/g) by the end of the second week of storage. The pH value of cheese after preparation was 6.2 and slowly decreased to reach a low value of 4.6 by the end of storage.

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Concerning the results obtained from cheese made from milk with 10% added salt (Table 2), no change could be observed in the number of Y. enterocolitica during manufacture of cheese. The number of organisms decreased rapidly during the storage period of cheese, and could not be detected ( $< 100$  cells/g) by the end of the second week.

According to the data obtained by HANNA, et al. (1977 b) and STERN, et al. (1980) Y. enterocolitica could grow and survive at pH value ranging from 4.6-9, it is obvious from the obtained results (Table 2) that pH values of both types of cheese were favorable for growth and survival of Y. enterocolitica during manufacturing and storage of the product. However, the growth rate of the organism was lower than that obtained by MOUSTAFA, et al. (1983 b) who reported that Y. enterocolitica increased rapidly 1000-fold to about  $1 \times 10^6$ /g of curd during the manufacturing process of Colby-like cheese. This probably resulted from the high percentage of salt content of Damietta cheese, which had a negative effect on growth and multiplication of Y. enterocolitica during cheese making. Furthermore, the gradual increase in salt content of cheese during storage was accompanied with extensive reduction in numbers of Y. enterocolitica (Table 1 & 2), which could not be recovered from cheese by the end of the second week. Different results were recorded by SCHIEMANN (1978), who reported that Y. enterocolitica disappeared from cheese curd samples stored at 4°C after 8 weeks. Also in another study, Y. enterocolitica serotype 0:8 could persist in Colby-like cheese at numbers in excess of 200/g after 8 weeks of storage at 3-1°C (MOUSTAFA, et al. 1983). These differences could be attributed to the type of cheese used, amount of added salt and the variation in storage temperatures.

The results obtained by MORRIS and FEELEY (1976) have suggested that about  $3.9 \times 10^9$  Y. enterocolitica must be consumed to cause illness in human. If an average person consumes 200 g of cheese at one time, about  $2 \times 10^7$  Y. enterocolitica/g are required to make one ill. Damietta cheese is oftenly ripened in its salted whey for about 8 weeks before sale. This period of ripening presumed to be enough for destruction of potential disease agents which may be present. Therefore, the numbers of Y. enterocolitica retained in the experimental Damietta cheese after two weeks of storage were lower than the level needed to cause illness.

On conclusion, contamination of Damietta cheese by Y. enterocolitica emphasizes again the importance of stringent sanitation which must be followed during cheese making. Presence of even small numbers of virulent Y. enterocolitica presents a public health hazard and should not be ignored.

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Table (1)  
Changes in chemical composition of Damietta cheese during storage

Storage period (weeks)	Cheese prepared from milk with 5% added salt			Cheese prepared from milk with 10% added salt		
	T.S. %	Moisture %	Salt <sup>a</sup> %	T.S. %	Moisture %	Salt <sup>a</sup> %
0	33	67	5.5	34.7	65.3	8.7
1	35	65	5.9	37	63	9.5
2	42	57	6.33	41	59	10

Table (2)  
Counts of *Y. enterocolitica* strain 0:8 and changes in pH during manufacture and storage of Damietta cheese.

Storage period (weeks)	Cheese prepared from milk with 5% added salt		Cheese prepared from milk with 10% added salt	
	Yersinia/g	pH	Yersinia/g	pH
Inoculum/ml.	$1 \times 10^6$	6.6	$1 \times 10^6$	6.6
Curd	$2 \times 10^6$	6.3	$1.3 \times 10^6$	6.5
0 time	$1 \times 10^7$	6.2	$1 \times 10^6$	6.3
First week	$1 \times 10^6$	5.3	$5 \times 10^2$	5.7
Second week	/ *100	4.6	/ *100	5.3

\* : No colonies could be detected on the plate.

## REFERENCES

- Ahmed, A. A-H.; M.K. Moustafa, and E.H. Marth (1983): Growth and survival of *Staphylococcus aureus* in Egyptian Domiati cheese. J. Food Prot. 46: 412-415.
- Amer, S.N.; A.H. Fahmi, and M.A. El-Batawy (1979): A comparison between some properties of Domiati cheese made with adult bovine and calves rennet as affected by different salt concentrations. Egyptian J. Dairy Sci. 7: 55-62.
- Aldova, E.; J. Cerna, and M. Janeckova (1973): *Yersinia enterocolitica* and its demonstration in food. Ceskoslovenska Hygiene 8: 395-404 (Cited after Swaminathan et al. 1982).

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- Atherton, H.V. and J.A. Newlander (1977): Chemistry and testing of dairy products, 4th ed. AVI Publishing company, Inc. Westport, Connecticut.
- Black, R.E.; R.J. Jackson; I. Tasi; M. Medvesky; M. Shyegani; J.C. Feeley; K.I.E. Macleod, and A.M. Wakelee (1978): Epidemic Yersinia enterocolitica infection due to contaminated chocolate milk. *N. Engl. J. Med.* 298: 76-79.
- Boyce, J.M.; D.J. Evans; D.G. Evans and Dupont, H.Z. (1979): Production of heatstable, methanol-soluble enterotoxin by Yersinia enterocolitica. *Infect. Immun.* 25: 532-537.
- Fahmy, A.H. and H.A. Sharara (1950): Studies on Egyptian Domiat cheese. *J. Dairy Res.* 17: 312-227.
- Feeley, J.C.; W.H. Lee; G.K. Morris (1976): Yersinia enterocolitica. pp. 351-357. In M.L. Speck (ed). Compendium for methods for the microbiological examination of foods. American Public Health Association, Washington D.C.
- Francis, D.W.; P.L. Spaulding, and J. Lonet (1980): Enterotoxin production and Thermal resistance of Yersinia enterocolitica in milk. *Appl. Environ. Microbiol.* 40: 174-176.
- Franzin, I.; P. Fantino, and V. Vidotto (1984): Isolation of Yersinia enterocolitica like organisms from raw milk in Italy. *Current Microbiol.* 6: 357-360 (*Dairy Sci. Abstr.* 47, 3: 1985).
- Hanna, M.O.; J.C. Stewart; Z.L. Carpenter and C.V. Vanderzant (1977 a): Effect of Packaging methods on development of Yersinia enterocolitica on beef steaks. *J. Food Safety.* 1: 29-37.
- Hanna, M.O.; J.C. Stewart; Z.L. Carpenter and C.V. Vanderzant (1977 b): Effect of heating, freezing, and pH on Yersinia enterocolitica like organisms from meat. *J. Food Prot.* 40: 689-692.
- Health and Welfare, Canada (1976): Yersinia enterocolitica gastroenteritis outbreak-Montreal. *Can Dis. Weekly Rep.* 2: 73-74.
- Hughes, D. (1979): Isolation of Yersinia enterocolitica from milk and a dairy farm in Australia. *J. Appl. Bacteriol.* 46: 125-130.
- Kapperud, G. and G. Langeland (1981): Enterotoxin production at refrigeration temperature by Yersinia enterocolitica like bacteria. *Curr. Microbiol.* 5: 119-122.
- Lee, W.H. (1977): An assessment of Yersinia enterocolitica and its presence in Foods. *J. Food Prot.* 40: 486-489.
- Marth, E.H. (ed). (1978): Standard methods for the examination of dairy products, 14th ed. American Public Health Assoc., Washington, DC.
- Morris, C.K. and J.C. Feeley (1976): Yersinia enterocolitica a review of its role in food hygiene. *Bull. W.H.O.* 54: 79-85.
- Moustafa, M.K.; A.A-H. Ahmed and E.H. Marth (1983 a): Occurrence of Yersinia enterocolitica in raw and pasteurized milk. *J. Food Prot.* 46: 276-278.
- Moustafa, M.K.; A.A-H. Ahmed and E.H. Marth (1983 b): Behavior of virulent. Yersinia enterocolitica during manufacture and storage of Colby-like cheese. *J. Food Prot.* 46: 318-320.
- Olsovsky, Olsakova, Z.V. Chobot, S. and Siviridov, V. (1975): Mass occurrence of Yersinia enterocolitica in two establishments of collective care of children. *J. Hygiene, Epidemiology, Microbiology and Immunology* 19: 22-29.

## YERSINIA IN CHEESE

- Schiemann, D.A. (1978): Association of Yersinia enterocolitica with the manufacture of cheese and occurrence in pasteurized milk. *Appl. Environ. Microbiol.* 36: 274-277.
- Schiemann, D.A. (1979): Synthesis of a selective agar medium for Yersinia enterocolitica. *Can. J. Microbiol.* 25: 1298-1304.
- Schiemann, D.A. and S. Toma (1978): Isolation of Yersinia enterocolitica from raw milk. *Appl. Environ. Microbiol.* 35: 54-58.
- Sonnenwirth, A.C. and Weaver, R.E. (1970): Yersinia enterocolitica. *New Engl. J. Med.* 283, 1468. (Cited after Hanna, et al. 1977 a).
- Stern, N.J.; M.D. Pierson and A.W. Kotula (1980): Effects of pH and sodium chloride on Yersinia enterocolitica growth at room and refrigeration temperature. *J. Food Sci.* 45: 64-67.
- Swaminathan, B.; M.C. Harmon and I.J. Mehlman (1982): A review. Yersinia enterocolitica. *J. Appl. Bacteriol.* 52: 151-183.
- Winblad, S. (1973): The clinical panorama of human Y. enterocolitica. In *Contribution to Microbiology and Immunology*, Vol. 2, Yersinia, Pasteurella and Francisella. karger, Basel.
- Zen-Yoji, H.; Maruyama, T.; Sakai, S.; Kimura, S.; Mizuno, T. and Momose, T. (1973): An outbreak of enteritis due to Yersinia enterocolitica occurred at a Junior school. *Jpn. J. Microbiol.* 17: 220-222.