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STUDIES ON YERSINIA ENTEROCOLITICA IN RAW MILK AND ICE-CREAM

(With 3 Table)

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دراسات عن ميكروب البارسينيا أنتيروكوليتيكا في اللبن الخام والآيس كريم

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أجريت هذه الدراسة على ١٢٥ عينه من اللبن الخام وعدد ٥٠ عينه من الآيس كريم جمعت من مدينة أسيوط لمعرفة تواجد ميكروب البارسينيا أنتيروكوليتيكا فيها. تم عزل الميكروب من ١١ عينه من اللبن الخام ومن ٤ عينات من الآيس كريم وتم تصنيفها بيوكيميائياً بنوع ٤ وقد تم مناقشة النتائج وأهمية وخطورة تواجد هذا الميكروب على الصحه العامه وما يجب إتخاذه لمنع إنتشار هذا الميكروب.

SUMMARY

A total of 175 random samples (125 raw milk and 50 Frozen ice-cream) from different localities in Assiut city were examined for the occurrence of *Y.enterocolitica*. The organism was isolated from 8.8 and 8% of raw milk and ice-cream samples respectively. All isolates belonged to biotype 4. The importance of *Y.enterocolitica* as a public health hazard was disscussed.

INTRODUCTION

Yersinia enterocolitica is now widely recognized as a cause of disease in man and occosinally other species *(LANGFORD, 1972; WINBLAD, 1973 b and BOTTONE, 1977)*. Acute gastroentritis or enterocolitis is the most frequent clinical form of this infection, followed by an acute syndrome of the right iliac fossa (pseudoappendicitis, mesenteric lymphadenitis or terminal ileitis). Other clinical conditions which were described are: septicemia, polyarthritis, erythema nodosum, abscesses. The orgnism is usually isolated from feaces and less frequently from appendix, mesenteric lymph nodes, abscesses, blood, urine and from asymptomatic carriers *(SCHIEMANN and TOMA, 1978)*.

Epidemiological study of human Yersinia infections has implicated water, animals, food and other environmental sources as reservoirs of the organisms *(MORRIS and FEELEY, 1976)*.

To the best of our knowldge there is a lack of literature about isolation of *Y.enterocolitica* in Egypt, *MOUSTAFA, 1990* reported that the incidince of *Y.enterocolitica* in raw milk was 10% from 100 samples examined, while it has been isolated by many workers from raw milk *(HUGHES, 1979; DELMAS & VIDON, 1982; CHRISTENSEN, 1982; MOUSTAFA et al., 1983; FRANZIN & FANTIONO, 1984; BOER et al., 1986 and MERCADO & IBANEZ, 1986)*.

Although freezing of *Y.enterocolitica* is known to cause cell inactivation *(GREEZ and EL-ZAWAHRY, 1984)*, the organism has been isolated from ice-cream by *WAUTERS, 1970; MOLLARET et al., 1972; DELMAS et al., 1985; BOER et al., 1986 and MOUSTAFA, 1990)*.

The aim of our study was to determine the incidence of *Y.enterocolitica* in raw milk and ice-cream, and the role of the organism as a public health hazard.

MATERIAL and METHODS

Collection and preparation of samples:

125 raw milk samples (50 ml each) in sterile MacCarteny bottles and 50 random samples of frozen ice-cream were collected from different farms, retailers and super markets in Assiut city. The samples were carried in Freezer box to the laboratory with a minimum of delay.

Milk samples were tested for heat treatment according to *(RICHARADSON, 1985)* before centrifuged while ice-cream samples were brought to room temperature by setting the containers in warm water bath, then thorouhly mixed *(RICHARDSON, 1985)*.

Isolation and Identification of *Y.enterocolitica*:

One ml of each prepared samples from, the sediment of milk and thoroughly mixed ice-cream was transfered to 10 ml phosphate buffer saline (PBS, M/15, pH 7.6) and incubated at 4°C for 14 days for enrichment. Enrichment samples were inculated into Cefsulodin-Irgasan-Novobiocin (CIN) agar (Oxoid) *SCHIEMANN (1979 b, 1979 c)*.

The creteria used for presumptive identification of *Y.enterocolitica* was a colony having a deep red center "bullseye" surrounded by a transparent border. The edge of the colony was either entire or irregular. All presumptive colonies were transfered to nutrient agar slant and stored after incubation at 32°C for 24 hrs. in refregerators at 4°C for later biochemical reactions as shown in Table 1 *(SCHIEMANN and DEVENISH, 1982)*, which recommended that the reaction on Kligler Iron Agar, together with tests for urea hydrolysis and sucrose and salicin fermentation is sufficient for differentiating *Y.enterocolitica* from other organisms that are capable of growing on CIN medium and also for distinguishing pathogenic forms.

The strains were biotyped according to *WAUTERS (1970)*. Table 2.

Table 1: Typical Reactions of Pathogenic *Yersinia enterocolitica*.

Test	Incubation temperature	Test result
Kliger Iron Agar	35°C	Alkaline/acid butt no gas or H_2S
Urea hydrolysis	35°C	positive
Sucrose fermentation	22°C	positive
Salicin fermentation	35°C	Negative

Table 2: Wauters biotyping of *Yersinia enterocolitica*

	w1	2	3	4	5
Lecithinase	+	-	-	-	-
Indole	+	+	-	-	-
Trehalose	+	+	+	+	-

RESULTS

The obtained results are summarized in Table 3.

Table 3: Isolation rate of *Yersinia enterocolitica* from examined raw milk and ice-cream samples.

Type of samples	No. of examined samples	No. of positive samples	Biotyping	%
Raw milk	125	11	4	8.8
Ice-cream	50	4	4	8

DISCUSSION

Y.enterocolitica is an organism which has been gaining widdespread recognition in recent years as a source of human infection *(WINBLAD, 1973 b).*

The results of this study showed that the incidence of Y.enterocolitica among examined raw milk samples was found to be 8.8%. This rate of isolation was nearly similar to those results reported by other investigators, such as *CHRISTENSEN (1982)* in Denmark and *MOUSTAFA (1990)* in Egypt.

On the other hand high isolation rates of *Y.enterocolitica* were obtained by *SCHIEMANN and TOMA (1978)* in Southern Ontairo U.S.A. and *DELMAS & VIDON (1982)* in Alsace, they were 32 and 54.5% respectively.

Also, *Y.enterocolitica* were recovered from 4(8%) samples of ice-cream, the results are somewhat similar to those obtained by *MOUSTAFA (1990)* who found it 8.9% in 67 ice-cream samples.

A greater prevalence of *Y.enterocolitica* in ice-cream 22% was found by *DELMAS* et al. *(1985)* in the north eastern region of France. While lower incidence 5% was reported by *BOER* et al. *(1986)* in the Netherlands.

The difference in these results may be attrributed to various temperatures used in storing the products, as freezing

to-18 and-75°C resulted in 7 and 42% cell inactivation respectively *(GRECZ and EL-ZAWAHRY, 1984).*

From the forementioned studies it was noticed that the isolation rates of Y.enterocolitica were high in the temperate countries. This may be explained as due to the importance of cold temperature on growth, survival, pathogenicity and toxin production of that organism.

As regards the biotypes, all strains of *Y.enterocolitica* isolated from raw milk and ice-cream in this study belonged to biotype 4, the same biotype was isolated from more than 105 cases of gastro enteritis in Saint-Justin Hospital Montreal, from 1967-1972 *(LAFLEUR, 1973).*

From these findings the consumption of raw milk and other dairy products from raw milk as ice-cream are practices that further allow for the transmission of human yersiniosis.

As the organism is associated with food borne infection *(H.W.C., 1976 and BLACK* et al., *1978)* it is surprising that high incidence of *Y.enerocolitica* in raw milk and ice-cream may be due to either the origin of organism in the animal or the environment. Even a slight contamination of milk with Yersinia could ultemately result in high cell denisties since milk is a good growth medium and *Y.enterocolitica* is able to multiply at refrigeration temperature *(LEE, 1977 b).*

The contamination of raw milk and ice-cream with *Y.enterocolitica* sugests that attention to hygienic milk-handling practices may be an important preventive measure. Likewise, avoidance of direct contact with excreta from domestic animals that may potentially harber the orgnism *(INOUE and KUROSE, 1975).* Proper heat treatment of raw milk would eliminate the risk of infection by this organism.

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