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INCIDENCE OF YERSINIA IN RAW MILK AND KAREISH CHEESE WITH SPECIAL REFERENCE TO YERSINIA ENTERCOLITICA

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ABSTRACT

A Total of sixty random samples of raw milk and kareish cheese (30 of each) were collected randomely from various markets in Gharbia governorate for detection of Yersinia organisms.

The obtained results revealed that Yersinia species were isolated from 23.33% and 16.67% of the examined raw milk and kareish cheese samples respectively ,Yersinia enterocolitica also isolated from 2(6.67%) and 3(10%) samples of raw milk and kareish cheese respectively.

Furthermore, the isolated strains of Yersinia enterocolitica were sensitive to streptomycin, sulphamethoxazole, gentamycin, , danox and enrofloxacin, while they were resistant to pencillin, ampicillin and cefatoxine.

Public heath significance and possible sources of contamination of milk and its products with such organisms as well as some recommendations to ensure maximum margin of human safety were discussed.

INTRODUCTION

Yersinia may contaminate milk and milk products during processing, distribution and marketing as well as storage ***(Donnelly, 1990 and Pritchard et al., 1995).***

Raw milk may play an important role for Yersiniosis in human being if it is consumed without sufficient heat treatment and / or used for

manufacture of raw dairy products ***(Jayarao and Henning., 2001).*** *Yersinia species* can grow in a food item at refrigeration temperature (Psychrotrophic microorganisms) resultting in food borne outbreaks ***(Kirov et al., 1993)*** and destroyed by high temperature short time pasteurization condition of 71.8 ^{o}C for 18 seconds easily kill *Yersinia enterocolitica* ***(Tacket et al., 1984 , Schiemann,1989 and Padilha et al., 2001).***

Kareish cheese is a raw milk product which manufactured by farmers at home from naturally fermented milk by lactic acid bactria, this method may expose the product to contamination with certain *Yersinia species* constituting public health hazard ***(Hamama et al., 1992).***

Therefore, the aim of the present study was planned to determine the incidence of Yersinia species in raw milk and kareish cheese and to study the effect of lowering pH (acidity) on viability of *Yersinia enterocolitica* in raw milk kept at room temperature .

MATERIALS AND METHODS

1- a. Sampling :

Sixty random samples of raw milk and Kareish cheese (30 each) were collected from street vendors in Ghariba governorate. The collected samples were transferred to the laboratory in sterile bags without delay for detection of Yersinia microorganisms

b. Preparation of samples:

All milk samples were tested by peroxidase test to exclude heat-treated milk samples.Then, 25 ml of raw milk or 25 gm of kareish cheese were thoroughly mixed with 225 ml Trypticase Soya Broth (TSB), flasks were incubated at 22^{o}C for 24 hours ***(Schiemann, 1983).*** One ml from each of the pre-enrichment broth was added to 9 ml of sterile Pepton-Sorbitol-Bile(PSB) broth supplemented with Novobiocin (10 μ g/ml) and Polymexin (5 I.U./ml) and incubated at 22^{o}C for 48-72 hours ***(Landgraf et al., 1993).***

c. Isolation and identification of yersinia:

A loopful from each PSB broth was streaked on to Cefsulodin-Irgasan-Novobiocin (CIN) agar plate and incubated at 30 ^{o}C for 18-20 hours. Characteristic colonies (dark red center, with transparent border) were picked up and subcultured onto slope nutrient agar for further identification according to ***Seelinger and Jones (1986).***

2- Experimental study in raw milk:

Yersinia enterocolitica isolated culture was maintained on Trypticas Soya Agar (TSA) at 4 ^{o}C. then acolony of Yersinia enterocolitica was picked and grown on Trypticase Soya Broth(TSB)at 30 ^{o}C overnight. One ml of the culture was serially diluted in 0.1% pepton water to attain the desired inoculum level and then added to raw milk to yeild a concent-ration of 2.5 x 10^{7} C.F.U/ml . The inoculated milk was incubated at 30^{o}C and examined after 6, 12, 18, 24, 30 and 36 hours. Ten ml of the milk sample was added to 90ml of 0.1 % sterile peptone and , 100 μl., was inoculated onto Cefsulodin – Irgasan – Novobiocin agar**.**The plates were incubated at 30^{o}C for 48 hours and countable plates were selected and counted.

3- Determination of pH:

The pH of inoculated milk was determined by using pH-meter model (HANNA, HI 8014).

4- Antibiotic susceptibility test:

The isolated strains of *Yersinia enterocolitica* were tested against Streptomycin 10 μg, Enrofloxacin 10 μg, gentamycin GM, Ampicillin 10 μg, Sulphamethoxzole 25 μg, danox 5 μg ,cefataxine 30 μg and pen-cillinG10 μg.The antibiotic discs were placed on the surface of Trypticas soya agar plate and incubated overnight at 37^{o}C,the diameter of inhibition zone of *Yersinia enterocoltica* were measured and interpreted by referring tables recommended by National Committee for Clinical Laboratory Standards (NCCLS) ***(Finegold and Martin, 1982).***

RESULTS

Table (1): Incidence of Yersinia spp.in examined samples of raw milk and kareish cheese. (No. 30).

Yersinia spp	Raw milk		Kareish cheese	
	Positive samples		Positive samples	
	No.	%	No.	%
Y.enterocolitica	2	6.67	3	10.00
Y. Pestis	4	13.33	1	3.33
Y. pseudotuberculosis	1	3.33	1	3.33
Total	**7**	**23.33**	**5**	**16.67**

Table (2): viability of isolated Yersinia enterocolitica in raw milk kept at room temperature.

Time	PH	Count	Multiplication or reduction %
Zero time	6.8	2.5×10^7	-
6 hours	6.7	4.0×10^7	60
12 hours	6.6	3.0×10^7	20
18 hours	6.5	2.6×10^7	4
24 hours	5.5	1.2×10^7	-52
30 hours	5.2	8.0×10^6	- 68
36 house	4.5	5.0×10^6	- 80

Table (3): Antibiotic sensitivity test for the isolated strains of Yersinia enterico-litica

Antibiotic discs in μg.	Sensitive	Inter-Mediate	Resistant
Penicillin G (10)	- ve	-ve	+ ve
Enrofloxacin EN (10)	+ve	- ve	-ve
Danox DNF (5)	+ve	- ve	-ve
Gentamycin GM	+ve	- ve	-ve
Ampicillin Amp (10)	-ve	+ve	-ve
Cefataxine ctx (30)	-ve	+ve	-ve
Sulphamethoxazole (25)	+ve	-ve	-ve
Streptomycin S (10)	+ve	-ve	-ve

Table (4): Incidence of compitative microorganisms like Yersinia spp. grown on CIN agar.

Compitator organisms	Raw milk		Kareish cheese	
	Positive	Samples	Positive	Samples
	No.	%	No.	%
Proteus species	4	13.33	1	3.33
P. mirabilis	1	3.33	-	-
P. rettgeri	1	3.33	-	-
P. vulgaris	2	6.67	1	3.33
Providencia alcalifaciens	2	6.67	-	-
Pseudomonas fluorescens	3	10	1	3.33
Serratia rubidanea	1	3.33	-	-

DISSCUSSION

The results outlined in table (1) **indicate** that the incidence of *Yersinia* species in examied samples of raw milk and kareish cheese was 23.33 % and 16.67% , from which 6.67% and 10% were identified as *Yersinia enterocolitica*, 13.33% and 3.33% as *Yersinia pestis* and 3.33% and 3.33% as *Yersinia pseudotuberculosis*, respectively.

Nearly similar findings were reported by ***Karplyk et al., (1985)*** who detected Yersinia enterocolitica in 0.2% of raw milk samples, while higher percentages were obtained by ***Franzin et al., (1984), Mmoh et al., (1984)*** and ***El-Sherbini et al., (1993).*** Yersinia organisms are widely distributed in nature and a variety of food including milk and milk products. Most food isolates were non pathogenic and known as environ mental strains ***(Adams and Moss, 1995).***

Table (2) show that the effect of natural acidity on the survival of *Yersinia enterocolitica* in raw milk. The number increased from 2.5×10^7 to 4.0×10^7 C.F. U/ml after 6 hours, then decreased gradually over a period of 12 – 36 hours tell reached to 5.0×10^6 C.F.U / ml after 36 hours when the raw milk completely fermented and the pH measured 4.5 . These finding agree with ***karaioannoglou et al., (1985)*** who reported that *Yersinia enterocolitica* can grow and multiply at pH 5.3 – 5.5 over a period of 30 days.

Table (3) reveal that the isolated strain of *Yersinia enterocolitica* was sensitive to streptomycin, sulphamethoxazole, Gentamycin, danox and enrofloxacin, while resistant to pencillin and varied in susceptibility to ampicilline & cefataxine. ***Henin and Kaldas (1995)*** reported that, the isolated strains of Yersinia enterocolitica were highly sensitive to gentamycin, neomycin, nalidixic acid and amikacin. Moreover, ***Abdel-Khalek (1998)*** reported that, the isolated strains of *Yersinia enterocolitica* were sensitive to gentamycin and chloramphenical, resistent to pencillin, while varied in susceplibility to erythromycin & Neomycin .

Inspection of table (4) reveal that high incidence of compitative microorganismes that have the same morphological characters on CIN agar as Yersinia organisms, those bacteria were *Proteus spp.*, *Providencia alcalifaciens*, *Pseudomonas fluorescens* and *Serratia rubidanee* . The obtained results were similar to that obtained by ***Walker, (1989a)*** and ***El-Sherbini (1990).*** Psychrotrophs can produce extracellular thermostable proteolytic and lipolytic enzymes which responsible for off flavor and yield loss in cheese manfacture. ***(Prieto et al., 2002).***

Yersinia enterocolitica is an enteric pathogen of emerging importance. It causes illness *characterized* by gastrointestinal disorder, pseudo - appendicular syndrom, arthritis, erythema nodsum and septicemia which cause death ***(Morse et al., 1984 ; Tacket et al, 1984 and IDF , 1994).*** Accordingly,the present results allow to conclude that consumption of raw milk and milk products cotaminated with *Yersinia enterocolitica* has a potential health hazard. Utilization of pasteurized milk in manufacturing of milk products is safe and free from contamination is of great significance to avoid such serious organisms to gain access in these milk products. Also proper cleaning and sanitation of dairy equipments where *Yersinia* is more susceptible to chlorine than other enteric bacteria with concentration of 0.25 ppm chlorine dioxide for 5 min, that can reduce Yersinia by 4 to 5 log cycles.

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مدي تواجد ميكروب اليرسينيا في اللبن الخام والجبن القريش في محافظة الغربية مع التركيز علي ميكروب اليرسينيا انتيروكولتيكا

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المعمل الفرعي بمرسي مطروح – المعمل الفرعي بطنطا

معهد بحوث صحة الحيوان

تتعرض الا لبان ومنتجاتها للتلوث بمختلف أنواع الميكروبات التى منها ما يسبب الفساد ومنها ما يمثل خطورة صحية على المستهلك.

لذلك قامت هذه الدراسة على جمع عدد ستين عينة (60) من اللبن الخام والجبن القريش (30 من كل نوع) من الاسواق المختلفة بمحافظة الغربية وذلك لتحديد مدى تلوثها بميكروبات اليرسينيا.

وقد دلت الدراسة على تواجد ميكروبات اليرسينيا بنسبة 23.33% ، 16.67 % ، وكانت العترات المعزولة هى يرسينيا انتيروكوليتكا (6.67%،10%) يرسينيا بستس (13.33 % ،3.33%) ويرسينيا سيدوتيوبركلوزس (3.33%،3.33%) من عينات اللبن الخام و الجبن القريش على التوالي.

هذا وقد تم إجراء اختبار الحساسية لمعرفة مدى تأثير المضادات الحيوية على ميكروب اليرسينيا انتيروكوليتكا ، وقد وجد ان هذا الميكروب حساس لكل من الانروفلوكساسين والدانوكس والجنتاميسين والكلورمفينيكول والسلفاميسوكال والستربتوميسين ، وعلى العكس فإن كل من البنسلين والامبسلين والسيفاتكسين كان تأثيرها ضعيفا على الميكروب.

وقد اهتمت الدراسة بمناقشة الأهمية الصحية لميكروبات اليرسينيا و المصادر المحتملة لتلوث الألبان ومنتجاتها بهذه الميكروبات،وتم وضع بعض التوصيات لحماية المستهلك من تلك الميكروبات.