INCIDENCE OF SALMONELLAE AND ESCHERICHIA COLI IN KAREISH CHEESE WITH SPECIAL REFERENCE TO HEAT STABLE ENTEROTOXIN PRODUCING ESCHERICHIA COLI USING POLYMERASE CHAIN REACTION

BY

***El-Bagoury, A.M. and **Mosaad, A. A.**

- *Dept. of Food Hygiene and Control, ** Dept. of Microbiology,*
- *Fac.Vet. Med., Sadat City, Minoufiya Univ.*

SUMMARY

Thirty five samples of kareish cheese (Unripened skim milk soft cheese) were collected from private markets in Sadat City, Minoufiya Governorate, Egypt, to be examined for the presence of salmonellae and Escherichia coli. Salmonella Typhimurium and Escherichia coli could be isolated from one sample (2.85%) and 18 (51.42%) of the examined kareish cheese samples, respectively.The isolated E.coli were belonged to 8 serovars, including O_{44} :H_{34}, O_{55}:H_6, O_{78} :H_{11}, O_{27} :H_{20}, O_{128} :H_{12}, O_{124} :H_{32}, O_{127} :H_{29} and O_{159} : H_{20}. Meanwhile, 2 E.coli isolates were untypable with the available antisera. Five strains out of the eleven enterotoxigenic E. coli strains isolated from the examined kareish cheese samples proved to produce heat-stable toxin (ST), by using polymerase chain reaction (PCR) technique. E.coli O_{157} : H_7 could not be detected in all examined kareish cheese samples.

INTRODUCTION

Kareish cheese is considered as the most popular variety of unripened soft cheese in Egypt . It is made mainly at farmer's houses from naturally soured raw skimmed milk . Although such type of cheese is palatable and of high nutritive value , yet it is considered as a possible vehicle for transmission of many pathogenic microorganisms as salmonellae and E.coli.

Salmonellae are considered among the most important enteric foodborne pathogens where their presence at any level in the food constitutes a severe health hazard. As proper pasteurization kills such organisms, most outbreaks of human illness have been associated with the consumption of raw or inadequately heat treated milk and / or the dairy products made from raw milk as kareish cheese *(Evans and Maguire, 1996 and Ellis et al. 1998).*

Several serovars of salmonellae including *Salmonella Typhimurium* were recorded as the cause of many food poisoning outbreaks due to consumption of cheese made from raw milk *(Ahmed et al., 2000; De Valk et al.,2000 and De Buyser et al.2001).*

Escherichia coli is an important organism in the microbiology of food; besides being involved in foodborne gastroenteritis, It is considered a good indicator of possible faecal contamination and hence contamination with other pathogenic enteric bacteria.

Escherichia coli is considered as a part of the normal microflora of the intestinal tract of human and warm-blooded animals and may get into cheese through direct or indirect faecal contaminations (*Synge, 2000*).
Several serovars of *Escherichia coli* have been implicated in foodborn disease outbreak due to consumption of raw milk and / or cheese made from raw milk *(Coia et al., 2001 and De Buyser et al.2001).*

E. coli $O_{157}:H_7$ (enterohaemorrhagic *E.coli*)is probably the most important in terms of foodborne diseases and it causes a very serious threat, particularly as related to raw milk or cheeses made from raw milk, which have been identified as vehicles of food related outbreaks *(Doyle and Padhye,1989 and Coia et al., 2001*). It constitutes a public health hazard particularly for young childern and elderly persons as it may cause diarrhoea, haemorrhagic colitis and life threating post-diarrhoeal disorders of haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (*Morgan et al., 1993 and Vernozy-Rozand, 1999).*

Enterotoxogenic *E. coli* are being associated with diarrhoea (infantile diarrhoea and teraveller's diarrhoea), where they can produce heat- labile and / or heat –stable enterotoxin after colonization of the cells of the proximal small intestine (*Candrian et al.,1991*).

Therefore the objective of the present study was to determine the incidence of salmonellae and *E. coli* in Kareish cheese , identification of heat- stable enterotoxin producing *Escherichia coli* using polymerase chain reaction and to discuss the public health significance of the isolated organisms.

MATERIAL AND METHODS

Thirty five random samples of Kareish cheese were collected from private markets in Sadat City, Minoufiya Governorate, Egypt. Collected samples were prepared according to *APHA, 1992*, before being examined bacteriologically for the following organisms .

1-Salmonella organisms:

Isolation of salmonellae from examined Kareish cheese samples was done according to the technique recommended by *APHA,1992*, using peptone water 0.1% as pre-enrichement medium, tetrathionate broth and selenite cystine broth as

selective pre-enrichment media and Salmonella- Shigella (S-S) agar and xylose lysine deoxycholate (XLD) agar as plating media.The isolated salmonellae were identified biochemically according to *Kreig and Holt (1984)* and serologically according to Kauffman- White Scheme *(Kauffman, 1974)*.

2- Escherichia coli:

2.1- E. coli other than E.coli O_{157}:H_7:

Isolation of *E.coli* other than *E.coli* O_{157}:H_7 was done according to the technique adopted by APHA, 1992, where inoculated EC broth tubes were incubated at 44.5°C for 48 hours. Loopfuls from gassing EC tubes were streaked onto Eosin Methylene Blue (EMB) agar plates incubated at 35 °C for 24 hours. Isolated purified colonies of suspected *E. coli* was identified morphologically and biochemically according to *Kreige and Holt (1984)*. Serotyping of the isolated *E. coli* strains were done by using the slide agglutination technique as recommended by APHA, 1992 using the available poyvalent and monovalent antisera.

2.2 -E.coli O_{157}:H_7

Isolation of E.coli O_{157}:H_7 was done according the technique recommended by *APHA, 1992* where the inoculated EC broth tubes were incubated at 37°C for 24 hour. Loopfuls from positive EC broth tubes were streaked onto Sorbitol MacConkey agar plates (Oxoid, CM813).

The suspected colonies were purified and identified biochemically according to *Kreige and Holt (1984)*.

2.3 -Detection of Heat Stable Enterotoxin (ST1)producing E.coli strains by using Polymerase Chain Reaction (Genotyping method)

2.3.1-Preparation of bacterial template DNA:

DNA template was prepared from some of the isolated E.coli cells according to *Kon Jung (1999)*.

2.3.2- Oligonucleotide primers :

Primers were dissolved in nuclease -free water to obtain the required concentration *(Sambrook et al., 1989 and Baumforth et al.,1999)*.

2.3.3- Programing the thermal cycler:

The thermal cycler was programmed according to *Baumforth et al., (1999)*.

2.3.4- Polymerase Chain Reaction Protocol and detection of PCR products:

The reaction was conducted according to the technique recommended by *Sambrook et al., (1989)*.

RESULTS AND DISCUSSION

The obtained results of this investigation were summarized in tables (1,2 &3) .Results recoded in table (1) revealed that Salmonella organisms could be isolated from only one (2.85%) of examined kareish cheese samples.

Serological typing proved that the isolated salmonella was *S. typhimurium*, with antigenic structure of 1,4,5,12 phase 1:i, phase 2:1,2 (Table, 2). Nearly similar incidence of salmonella species including *S.typhimurium* from kareish cheese was reported by *Moursy and Nasr (1964)* and *Ahmed et al.(1988)*. On the contrary, *Tawfeek et al.(1989)* and *El-Kady (1995)* reported that Salmonella organisms were failed to be detected in all examined kareish cheese samples.

Salmonellae continue to be a major concern for the dairy industry because these bacteria have caused recent outbreaks of illness and have been isolated from various products in the market place . Most milk borne salmonellosis has been associated with raw milk, inadequetly pasteurized milk and / or milk contaminated after pasteurization as well as cheese made from raw milk *(Bryan, 1983; D'Aoust,1989; El-Gazzar and Marth, 1992 and Ahmed et al.,2000).*

Salmonella typhimurium has been recorded as a cause of recent food poisoning outbreaks due to consumption of raw milk soft cheese (*De Valk et al.,2000).*

It is evident from the results recorded in table (1) that *E. coli* could be isolated from 18 (51.82%) of examined kareish cheese samples. Nearly similar incidence of *E.coli* in retail soft cheese samples was reported by Ansay and Kaspar (1997), while comparatively lower incidence was reported by *El-Bassiony (1975)* and *Ahmed et al.; (1987)* and relatively higher incidence was reported by *Ahmed et al.(1988)*.

The results recorded in table (2) point out that the isolated *E.coli* were belonged to 8 serovars; $O_{27}:H_{20}$, $O_{44}:H_{34}$, $O_{55}:H_6$, $O_{78}:H_{11}$, $O_{124}:H_{32}$, $O_{127}:H_{29}$, $O_{128}:H_{12}$, $O_{159}:H_{20}$, while 2 strains were untyped. Nearly similar *E.coli* serovars were isolated from kareish cheese samples by *El-Kholy (1989) and Abdel-Haleem (1990).*

Escherichia coli constitutes a puplic health importance as it is capable of eliciting gastrointestinal illness among young childern and it has been emphasized by many authors, as one of the food poisoning causative agents (Kornacki and Marth (1982).

Enteropathogenic *E.coli* serovars isolated in this work ; $O_{44}:H_{34}$, $O_{55}:H_6$, $O_{127}:H_{29}$ and $O_{128}:H_{12}$ have been incriminated in many outbreaks of infant diarrhoea (*Robins-Browne, 1987 and Doyle and Padye, 1989*). While *E.coli* serotype $O_{124}:H_{32}$ (Enteroinvasive) was recorded to be associated with shigella- like illness among young children (*Levine,1989*).

Enterotoxigenic *E.coli* serotypes recovered from the examined kareish cheese samples O_{27}:H_{20}, O_{78} :H_{11} and O_{159} :H_{20} were considered a major cause of traveller's diarrhoea and infantile diarrhoea (*Levine, 1989*).

The data recorded in table (3) indicates that 3 out of 4 E.coli O_{27}:H_{20} , 2 out of 4 *E.coli* O_{78} :H_{11} and none of 3 O_{159} :H_{20} serovars proved to produce heat stable toxin(ST1) using Polymerase chain reaction.The obtained results agree to some extent with that reported by *MacDonald et al. (1985) and Candrion et al.,(1991).*

Fig.(1): The polymerase chain reaction (PCR) was used to amplify DNA sequences from mal B operon of *E.coli* . All tested *E.coli* strains yielded the specific DNA fragment . The enterotoxigenic E.coli strains (ETEC) were identified with additional primer pairs specific for the gene coding for the heat –stable toxin type 1(ST1).

Results in table (1) point out that *E.coli* O_{157} :H_7 could not be detected in all examined Kareish cheese samples The obtained results run parallel to that recorded by Ansay and Kaspar (1997).

In conclusion, the data given by this study assured that kareish cheese does not satisfy the consumer demand of safety as it represents a good vehicle for transmission of many food-borne pathogens and their related disease to their consumers. Polymerase chain reaction (**PCR**) should be used as a rapid technique and specific for the diagnosis , detection and differentiation of the food-relevant pathogens .

REFERENCES

Abdel-Haleem, M.E. (1990): Studies on sanitary condition of kareish and Mich cheese . M.V.Sc. Thesis , Dept. of Food hygiene and control, Fac.Vet.Med., Zagazig Univ.

Ahmed, A. H.; Abdel-Rahman, HG. A. and Moustafa, M. K. (1988): Incidence of Enterobacteriaceae in some selected food stuffs. Assuit Vet. Med. J. 20 (20): 105-108

Ahmed, A. H. A.; Moustafa, M.K. and Abdel-Hakiem, E.A. (1987): Sanitary condition of kareish cheese manufactured in Assuit City. Assuit Vet. Med. J. 19 (37) : 76- 80

Ahmed, R.; Soule, G.; DemcZuk,WH.; Clark,C.;Khakhria,R. ;Ratnam,S.; Marshal,S.; Woodward,DL.; Johnson,WM. and Rodgers,FG. (2000): Epidemiologic typing of *Salmonella enterica serotype entertidis* in a Canada- wide outbreak of gastroenteritis due to contaminated cheese. J.Clin. Microbiol., 38 (6): 2403 – 2413.

Ansay, S.E. and Kaspar, C.W. (1997): Survey of retail cheese, dairy processing environments and raw milk for *E. coli* O_{157}:H_7 . Lett Appl. Microbiol., 25 (2): 131-140

American Public Health Association ((APHA)) (1992): Compendium of methods for the microbiolgical examination of foods . 2nd Ed. American Public Health Association, Washington, D.C., USA.

Baumforth, K. R. N.; Nelson, P. N.; O' Neil, J. D. and Murry, P.G. (1999): The polymerase chain reaction. J.Clin.Pathol. Mol.Pathol., 25: 1-10

Bryan, F.L. (1983): Epidimiology of milk-borne disease. J.Food Prot., 46: 637- 643

Candrian, U.; Furrer, B.; Hofelein,C.; Meyer,R.;; Jermini,M. and Luthy,J. (1991): Detection of *Escherichia coli* and Identification of enterotoxigenic strains by Primer – directed enzymatic amplification of specific sequences. Int. J. Food Microbiol. 12 (4): 339-350

Coia, JE.; Johnston, Y.; Steers, NJ. And Hanson, MF. (2001): A survey of the prevalence of *Escherichia coli* O_{157} in raw meats, raw cow's milk and raw- milk cheese in South – East Scotland. Int.J. Food Microbiol. 21; 66 (1/2): 63-75

D'Aoust, J.Y. (1989): Salmonella. Page 332 in Food borne Bacterial pathogens. M.P. Doyle. Ed. Marcel Dekker, Inc., New York.

DeBuyser, ML.; Dufour,B.; Maire, M. and Lafarge, V. (2001): Implication of milk and milk products in food-borne diseases in France. Int.J. Food Microbiol., 20; 67 (1/2): 1-17.

De Valk, H.; Delarocque- Astagneau,E.; Colomb, G.; Ple,S.; Godard, E.; Vaillant, V.; Haeghebaert, S.; Bauvet,PH.; Grimont,P. and Deseclose, G. (2000): A comunity- wide outbreak of *Salmonella enterica serotype Typhimurium* associated with eating raw milk soft cheese in France. Epidemiol. Infect., 124(1):1-7.

Doyle, M.P. and Padhye,V.V. (1989): *Escherichia coli* .In: M.P. Doyle(Ed.), Food borne bacterial pathogens. Marcel Dekker, Inc., New York, PP. 235- 281.

El-Bassiony, T.A. (1975): Studies on kareish cheese in upper Egypt. M.V.Sc..Thesis, Food Hygiene and control, Fac. Vet. Med., Assuit Univ.

El-Gazzar, F.E. and Marth, E.H. (1992): Salmonellae and salmonellosis and dairy foods : A review. J. Dairy Sci. ,75: 2327- 2343.

El-Kady, A.M. (1995): Studies on the sanitary condition of kareish cheese in Ismailia. M.V.Sc. Thesis, Food hygiene and control Dept., Fac.Vet.Med., Suez Canal Univ.

El-Kholy, A.M. (1989): Enterobacteriaceae in Egyptian soft cheese and their public health significance . Assuit Vet. Med. J. 21 : 42 .

Ellis,A.; Preston,M.;Borczky,A.; Miller,B.; Stone,P.; Hotton, B.; Chagla, A. and Hockin.J.(1998): A community outbreak of Salmonella berta associated with a soft cheese product. Epidemiol. Infect., 120, 1 : 29-35.

Evans,H.S. and Maguire,H.(1996): Outbreaks of infectious disease in schools and nurseries in England and Wales 1992 to 1994. CDR- weekly, 6 (7): R 103-R108.

Kauffman, F.(1974): Kauffman- White Scheme . WHO- BC 172, L., Rev.I. Acta. Path. Microbiol., Scand. 61 : 381.

Kon Jung, H. (1999): Identification of serotype by use of serologic assay and detection of enterotoxin gene of *E. coli* by mean of PCR assay for isolates from Pigs , chickens and cows. Am. J. Vet. Res., 60 (4): 468- 472.

Kornacki, J. L. and Marth, E. H.(1982): Foodborne illness caused by *Escherichia coli*. A review .J. Food Prot., 45 : 1051-

Kreig, N.R. and Holt, J.G.(1984): Bergey's Manual of Systemic Bacteriology. Vol.1, Williams and Wilkins, Baltimor, USA.

Levine, M.M. (1987): *Escherichia coli* that cause diarrhoea : Enterotoxigenic; Enteropathogenic; Enteroinvasive; Enterohaemorrhagic and Enteroadherant. J. Infect. Dis. 155 : 377- 399.

MacDonald, K.L.; Eidson, M.; Strohmeyer, C. ; Levy, M. E. ; Wachsmuth, K.; Hargrett, N.T. and Cohen, M. L.(1985): A multistate outbreak of gastrointestinal illness caused by enteropathogenic *Escherichia coli* in imported semisoft cheese. J. Infect. Dis.151 (4): 716- 730

Morgan,D.; Newman, C.B.; Hutchinson, D.N.; Walker, H.M.; Rowe, B. and Majid, F. (1993): Verotoxin- Producing *Escherichia coli* O157:H7 infections associated with the consumption of Yoghurt. Epidmiol. Infect., 111, 181-187.

Moursy, A. W. and Nasr, S.M. (1964): Studies on the sanitary condition of fresh kareish cheese with special reference to the incidence of food poisoning organisms . J.Arab.Vet.Med. Asso., 24-99.

Robins-Browne, R. M. (1987): Traditional enteropathogenic *Escherichia coli* of infantile diarrhoea . Infect. Dis. 9 : 28- 53.

Sambrook. J.; Fritsh, E.F. and Maniatis, T. (1989): Mollicular cloning . A laboratory manual , 2nd Ed., Cold Spring Harbar Laboratory Cold Spring, New York.

Synge,B.A. (2000): Verotoxin- Producing *E.coli* : A Veterinary view. J. Applied Microbiol. Symposium Suppl. 88, 315- 375.

Tawfeek, N.F.; Sharaf.O.M. and Hewedy, M.M. (1989): Incidence of several pathogens and staphylococcal enterotoxins in kareish cheese. Offental Gesundheitswes, 51 (1) : 27-36

Vernozy-Rozand, C. (1999): Verotoxin- Producing *Escherichia coli* (VTEC) and *Escherichia coli* O_{157}:H_7 in medicine and food industry. Ann.Biol.Clin. (Paris) 57 (5) : 507-515

Table (1): Incidence of isolated salmonellae and Escherichia coli strains from examined kareish cheese samples:

Isolated organisms	Positive samples	
	No	%
Salmonellae	1	2.85
Escherichia coli	**18**	**51.42**
E. coli O_{157}:H_7	**0**	**0 .00**

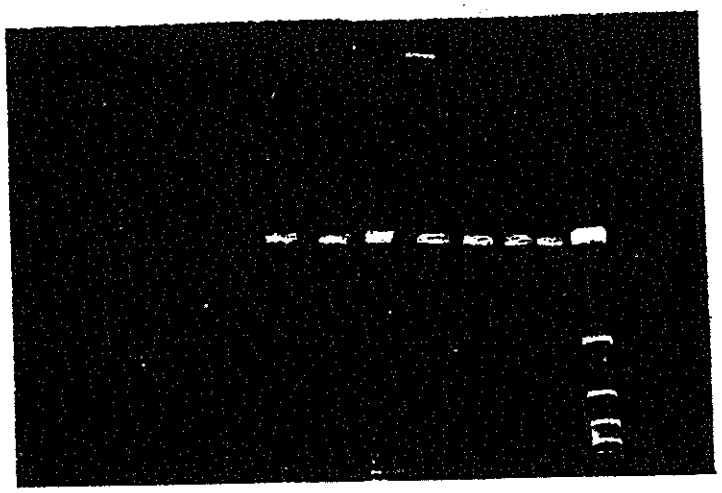
- The number of the examined kareish cheese samples = 35

Tables (2): Frequency distribution of salmonella and E.coli serovars isolated from examined kareish cheese samples:

Serovars	No. of isolates	%
Salmonella		
S. typhimurium:		
1,4,5,12		
Phase1:I /Phase 2:1,2	1	100
Escherichia coli:		
O_{27} : H_{20}	4	14.29
O_{44} : H_{34}	4	14.29
O_{55} : H_6	3	10.71
O_{78} : H_{11}	4	14.29
O_{124} : H_{32}	3	10.71
O_{127} : H_{29}	2	7.14
O_{128} : H_{12}	3	10.71
O_{159} : H_{20}	3	10.71
Untypable strains	2	7.14
Total	28	100

Table (3): Frequency distribution of heat stable toxin (ST1)-Producing enterotoxigenic E. coli serovars isolated from examined kareish cheese samples .

Enterotoxigenic *E.coli* serovars	No, of tested strains	Heat stable toxin(ST1)-producing srains	
		No	%
O_{27} : H_{20}	4	3	27.27
O_{78} : H_{11}	4	**2**	18.18
O_{159} : H_{20}	3	0	0.00
Total	11	5	45.45



7 6 5 4 3 2 1 M

Fig.1 : Electrophoresis of PCR products of enterotoxigenic *E. coli*
M = 100 DNA base ladder marker.
Lanes from 1-7 = PCR positive isolates

الملخص العربى

مدى تواجد السالمونيللا والأشيريشيا كولاى فى الجبن القريش مع الأهتمام الخاص بعترات الأشيريشيا كولاى المفرزة للإنتيروتوكسين المقاومة لتأثير الحرارة بإستخدام تقنية تفاعل البلمرة

عبد الرحمن محمود الباجورى و عبد العزيز عبد الخالق مسعد

تم فحص عدد ٣٥ عينة من الجبن القريش المجمعة من الأسواق المنتشرة بمدينة السادات – محافظة المنوفية وذلك لتحديد مدى تواجد ميكروبات السالمونيلا والأشيريشيا كولاى ٠

وقد أسفرت نتائج البحث عن تواجد ميكروب السالمونيلا تيفيميوريم فى عينة واحدة والأشيريشيا كولاى فى عدد ١٨ عينة ، بنسب ٢.٨٥ % و ٤٢.٥١% على التوالى وبالتصنيف السيرولوجى لعترات الأشيريشيا كولاى تبين أنها تنتمى إلى ثمانية انواع مختلفة وهى $O_{44}:H_{34}$, $O_{55}:H_6$, $O_{78}:H_{11}$, $O_{27}:H_{20}$, $O_{128}:H_{12}$, $O_{124}:H_{32}$, $O_{127}:H_{29}$ and $O_{159}:H_{20}$. بالأضافة إلى عدد إثنين عترة لم يتم تصنيفها بالأنتيسيرا المستخدمة وبإستخدام تفاعل البلمرة (PCR) وجد ان خمسة عترات من بين الأحدى عشرة عترة المفرزة للأنتيرتوكسين والمصنفة سيرولوجيا كانت من النوع المقاوم للحرارة " كما أسفرت التحاليل الميكروبيولوجية عن خلو العينات المختبرة من وجود الأشيريشيا كولاى (o157:H7)

هذا وقد أثبتت الدراسة من ان الجبن القريش هى منتج غير آمن من الناحية الميكروبيولوجية حيث انها تعتبر من اهم المصادر التى تنقل العديد من الأمراض ومسبباتها للانسان.

ومن هذه الدراسة تبين انه يجب إستخدام التقنيات الحديثة مثل تفاعل البلمرة كطريقة سريعة ونوعية لتحديد وتشخيص وتضيف معظم الميكروبات الممرضةوالتى تنتقل عن طريق الأغذية ٠