How safe is the cheeses sold in Beni-suef city

Saadia H. El.Shinawy, Mona H. A. Tolba.

Department of Food hygiene, Faculty of Veterinary Medicine, Beni-Suef , University, Beni-Suef, Egypt.

The incidence of *E. coli* O157, *Salmonella typhimurium, Listeria monocytogenes,* and *Yersinia enterocolitica* were studied in some locally produced cheeses. Thirty samples of each kind of the cheese were tested for the presence of some pathogens, which are frequently associated with food born disease. *E. coli* O157*:* IMS; *Listeria monocytogenes* were enumerated on modified Oxford agar; Salmonella was enumerated using standard procedures from Dynal manual using anti-Salmonella antibodies. *Y. enterocolitica* was enumerated on Yersinia selective agar base. Romano and processed cheese were found to be free from all tested pathogens but Kareish and Feta cheese were found to harbor *E. coli* and *Y. entertocolitica*. The results indicated the presence of some food borne pathogens in our food supply. Certain types of locally produced cheese still pose a significant health threat for the consumers. The finding of this study warrant the need for educational programs for dairy producers about the risks associated with consumption of certain cheeses manufactured from raw or insufficiently heated milk.

Dairy products have been recognized as being susceptible to post processing contamination. The predominant contaminants of processed dairy products have their origins from the raw milk supply. If one examines a list of bacterial pathogens commonly associated with raw milk, *E.coli, Salmonella typhimurium, Listeria monocytogenes, Campylobacter jejuni,* and *Yersinia enterocolitica* are just a few of many contaminants (Donnelly, 1990).

The ability of *E.coli* to survive in fermented dairy products made from raw milk (McIngvale *et al*., 2000 and Maher *et al*., 2001) is of major concern because the consumption of such products have led to human infection (Morgan *et al*., 1993 and Durch *et al*., 2000)

The first description of food borne listeriosis in human was associated with consumption of raw milk (Beckers *et al*., 1987) and listeriosis outbreaks from milk and cheeses have generated an interest in defining the survival of *Listeria monocytogens* in cheeses (Abdallah *et al*., 1993). *Yersinia enterocolitica* is related to *Yersinia pestis*, the organism responsible for bubonic plague. However, its virulence potential is not as great as that of *Y. pestis*. Food borne yersiniosis causes acute to severe gastrointestinal discomfort with pseudoappendecitis that is frequently observed in young children.

This study was designed to explore the microbiological characteristics of some locally produced cheeses that sold in Beni-Suef city.

Material and methods

Thirty samples of Kareish, processed, Feta and Romano cheese were collected randomly from different area in Beni-Suef governorate. Samples under cooling condition were sent to the lab for bacteriological examination. 25 gm of each sample was weighted and added to 225 ml of sterile peptone water in a stomached bag. The sample was homogenized in a lab-Tek 400 stomacher (Tekmar, Cincinnati, OH). Further serial dilution of the initial homogenate were prepared in dilution fluid and 0.1 ml volumes of appropriate dilutions spread in duplicate onto selected media for enumeration of pathogen to determine the total microbial load. Aerobic plate counts were made on plate count agar (Difco, Detroit, Michigan).

E. coli O157: IMS was performed on one ml of enriched cultures and 20 µl of magnetic beads coated with an antibodies prepared to the lipopolysaccharided of *E. coli* O157 (Dyna-beads anti-*E. coli* O 157, Dynal INC., lake success, NY) were transferred in a 1.5 ml micro centrifuge tube. The beads were suspended, mixed, incubated at room temperature and rotated at 30 rpm for 30 min on an Orbitron Rotator II (Fisher Scientific, Mississauga, ON). Samples were placed in a magnetic particle concentrator (MPC 10, Dynal) for three minutes and washed twice in lambda buffer (2.5 gm Mg SO_4. 7 H_2O, 0.006 g gelatin, 6 ml 0.1 M tris buffer, pH. 7.2, in 11 distilled water) (Chapman *et al*., 2001) After the final wash a final volume

of 100 µl was obtained. After IMS the final sample was plated on Sorbitol MacConkey agar. Plates were incubated for 24 h at 37°C.

Listeria monocytogens was isolated after enrichment on listeria enrichment broth (Difco) on modified Oxford agar (Oxoid, Unipath Ltd, Basingstoke, England) according to (McClain and Lee 1989). MOX plates were incubated at 35°C for 24 h after which 5 presumptive colonies were streaked onto BHI for purification and incubated at 35°C for 24 h. Presumptive Listeria isolates were confirmed and identified based on the results of Gram stain, catalase reaction, typical umbrella motility, and fermentation of mannitol, rahmnose and xylose. A modified CAMP test for enhanced haemolysis in the presence of *Staphylococcus aureus* alone was performed (McKellar, 1994). The species of all isolates were confirmed by use of API-Listeria (BioMerieux) identification kit.

Salmonella was isolated by using standard procedure from Dynal Manual using anti-Salmonella antibodies. After IMS, the final samples were plated onto xylose lysine desoxycholate agar (Merck). Plates were incubated at 35 ºC for 24 h. Colonies presumptive for Salmonella were inoculated onto triple sugar agar (Difco) and urease agar (Difco). Organism was then identified by API-20E identification kit (Biomerieux) (Andrews *et al*., 1992).

Y. enterocolitica was isolated on Yersinia selective agar base (Difco, Detroit, Michigan) containing the CIN (ceftsulodin-irgasan-novobiocin) supplement (Difco) after 48 h incubations at 25°C. Colonies with characteristics of Yersinia were examined with API-20E identification kit (BioMerieux) (Weagant *et al*., 1992). The results in Table 1revealed that high aerobic plate count was obtained for all types of examined cheeses. Highest number obtained for Kariesh cheese as the mean of $\log_{10}$ (9.97 CFU/gm) and the lowest for Romano cheese mean $\log_{10}$ (7.1CFU/gm). This may be due to the unhygienic condition under which Kariesh cheese was handled and the insufficient heat treatment. Kariesh cheese was also found to harbor *E. coli* O157:H7, Listeria spp. and *Y. enterocolitica* where 2 (6.7%), 5 (16.7%), 1 (3.3%) were detected respectively. Feta cheese was found to contain 2 (6.7%) Listeria spp. and 3 (10%) *Y. enterocolitica*.

Processed cheese and Romano cheese were found to be negative for all the tested pathogens. The ability of *E. coli* to grow and survive during manufacture in fresh (Arocha *et al*., 1992), hard (Reitsma and Henning, 1996) and Camembert (Ramsaran *et al*., 1998) cheeses investigated, in fresh cheese, *E. coli* has grown from an initial level of about 10^5 cfu/ml to final number about 10^7 cfu/g during the manufacture. However, during the heating phase, total inactivation occurred. *L. monocytogenes* was not detected in any of the examined samples. The only detected Listeria spp. in Kariesh cheese was *Listeria innocua* and the result was higher than those obtained by (Fathi and Nagah, 1992). *Listeria innocua* is the species that frequently isolated from raw milk, (Lovett *et al*., 1985; Farber *et al*., 1988 and El-Leboudy and Fayed 1992). Abd El-Gawad (1998) reported the presence of *Listeria monocytogenes* in 1% of examined raw milk samples but could not isolate the pathogenic strain in any kind of the tested cheeses. The possibility that the growth of *L. monocytogenes* was suppressed by the associated microflora (McLauchin *et al*., 1990), pointed out that the

Results and discussion

Table (1): Surveillance of food borne pathogens in selected cheeses.

Type of samples	No. of sample examined	APC* (Mean $\log_{10}$)	*E. coli* O157: H7	Listeria Spp.	Salmonella Spp.	*Y. enterocolitica*
Kariesh cheese	30	9.97	2 (6.7%)	5 (16.7%)	0	1 (3.3%)
Processed cheese	30	8.6	0	0	0	0
Feta cheese	30	7.9	0	2 (6.7%)	0	3 (10%)
Romano cheese	30	7.1	0	0	0	0

detection of Listeria other than *L. monocytogenes* likely to indicate an increased risk of contamination by *L. monocytogenes*, because the physiology and habitat of different species are very similar.

Yersinia incidence in our samples is lower than that is reported before (Hamama *et al*., 1992) they tested a total of 227 samples of milk and dairy products, Yersinia species were recovered from 11 out of 30 raw milk samples (36.6%), one out of 20 pasteurized milk samples (5%), 15 out of 63 traditional fermented milk samples (23.8%), 7 out of 94 cheeses and 1 out of 20 cream samples (5%). Jayarao and Henning (2001) examined the prevalence of food borne pathogen in milk and found that *Campylobacter jejuni*, shiga-toxin producing *E. coli*, *L. monocytogenes*, Salmonella spp. and *Y. enterocolitica* were detected in 9.2, 3.8, 4.6, 6.1, and 6.1 % of examined milk samples respectively.

The mechanism of raw milk contamination is unclear but in vitro studies have suggested that teat contamination could lead to intra-mammary infection. Faecal contamination, rumen contents, saliva and the farm environment are other routes of transmission. Contamination of the milk will result in contamination of the equipment used for milking, filtering, cooling, farm personnel and storage as well as subsequently produced dairy products.

Prevention and control of food borne disease constitute a problem of worldwide concern. Identification of sources of microbial contamination in raw products, processing environment and ingredients, the entrance of pathogens to processed food products leads to better control. These measures in combination with strict sanitation programs and improved testing procedure will ultimately enable the control of the incidence of food borne diseases.

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