Dept. of Food Hygiene,
Animal Health Research Institute, Dokki.

EFFECT OF IRRADIATION ON PREVALENCE OF E.COLI O157: H7 IN RAW MILK AND SOME MILK PRODUCTS

(With 6 Tables)

By

R.H. ABDEL DAYEM and SAHAR R. MOHAMED*

* Bacteriology Dept., Animal Health Research Institute, Dokki.

(Received at 24/12/2005)

تأثير التشعيع على تواجد الميكروب القولونى H7: O157 فى الألبان وبعض منتجاتها

ربيع حماد عبدالدايم ، سحر رشدى محمد

تـم جمـع (١٣٠) عينة عشوائية من المحلات والسوبر ماركت من عينات الألبان وبعض منـتجاتها. ثلاثـون عينة من الزبادى وخمسون عينة من كل من اللبن والجبن. تم نقل هذه العيـنات إلـى المعمل مبـاشرة حيث تم فحصها للميكروب القولونىH7:O157. تم عزل المـيكروب القولونى H7:O157 من عينة واحدة (٢%) من كل من اللبن والجبن. ولم يتم عـزل المـيكروب مـن أى عيـنات الزبادى. تم اختبار ضراوة الميكروب على حيوانات الـتجارب ووضح تأثيره فى إحداث الانيميا النزيفية والوفاه وكذلك إفرازه للسموم المعوية. وفى الجزء التجريبى من البحث تم حقن عينات من الألبان ومنتجاتها (الزبادى والجبن) التى ثبـت خلوها من الميكروب موضوع الدراسة بعترة من الميكروب القولونى H7:O157 إلى جرعات مختلفة من اشعة جاما. وبعد ذلك تم حفظ العينات فى الثلاجة عند درجة حـــرارة ٥°م لمدة ثلاثة أيام وعند فحص العينات وجد أن الميكروب القولونى H7:O157 فقد قدرته على إحداث الوفاه أو إفراز السموم المعوية.

SUMMARY

A total of one hundred and thirty random milk and some milk products samples were collected from different markets, and were examined for the presence of *Escherichia coli* O157:H7. The organism could be isolated from one sample (2%) of each of milk and cheese samples, but it failed to detect in yoghurt samples. An important cause of haemolytic

anaemia syndrome and lethal effect on mice was detected, while enterotoxin of strain gave maximum secretion in rabbit ligated ileal loop. Raw milk and milk products samples (cheese and yoghourt) free from *E. coli* O157:H7 were inoculated by strain of *E. coli* O157:H7 obtained from a Bacteriology Veterinary Lab. The effects of irradiation on the inoculated milk and milk products stored at 8°C and 5°C were determined. As survivor level of *E. coli* O157:H7 following gamma irradiation appeared completely discarded in refrigerator on 1, 2 & 3 days of incubation at 5°C. The cytotoxin production was completely stopped by irradiation at 0.25-0.53 kgy.

Key words: *Irradiation, E. coli 0157:H7, milk, milk products*

INTRODUCTION

The increasing number of foodborne bacteria and the worldwide outbreaks of bacteria, particularly *E. coli,* have increased interest in irradiated food, which is a promising technique for the elimination of this bacterium (Dussa *et al.,* 2004). The unpasteurized milk was first recognized as a vehicle of transmission of *E. coli* O157:H7 by Chapman *et al.* (1993).

The bovine intestinal tract is a known reservoir of *E. coli* O157:H7; hence, feces may contaminate milk by the organism (Montenegro *et al.,* 1990) Furthermore, enteropathogenic *E. coli* O157: H7 has been incriminated in many cases of foodborne disease outbreaks, travelers diarrhea and colibacillosis in adults. Verocytotoxin producing *E. coli* O157:H7 was subsequently found to be common cause of diarrhea haemorrhagic colitis, haemolytic uramic syndrome (Yamamoto *et al.,* 2003 and Vernozy. *et al,* 2005).

Rediation is the process of emission of energy by exited atoms. loizing radiation is capable of converting atoms and molecules to ions via the removal of electrons, also radiation can be energetic charged particles such as electrons or high energy photons such as X-Rays or Gamma Rays. (Thayer 1995). Also, at has been stated that irradiation is an excellent method for the reduction and/ or elimination of pathogenic food borne microorganisms (Monk *et al.,* 1995).

Therefore, this work was carried out to isolate *E. coli* O157:H7 as well as to study the effect of the irradiation on this bacterium on this bacterium.

MATERIALS and METHODS

Survey of *E. coli* O157:H7 in milk and some milk products:

One hundred and thirty samples of milk and milk products were collected from different markets; including 30 samples of yoghourt, and 100 samples of raw milk and cheese, (50 each) and the samples were examined for the presence of *E.coli* O157:H7 according to the method recommended by Adesiyun *et al.* (1997).

Assay for cytotoxin:

Assay for cytotoxin was performed by modification of the method of Klipstein *et al.* (1983). Fresh trypsinized cells were diluted 1: 5 in eagle minimal essential medium plus 2% foetal calf serum. One hundred µl samples were placed in 96 well microtiter plates to which 100 µl bacterial free supernatant were added to each well. The plates were incubated for 18 hrs at 36°C in 5% CO_2 and these were fixed with methanol, then stained with Giemsa stain. The results are reported as the last dilution that showed > 50% rounded cells.

Rabbit ligated ileal loop:

The technique recommended by Klipstein *et al.* (1983) was applied. Toxins preparation in 250 µl of buffer were placed for 16 hrs into single 10 cm ligated ileal loops of fasting rabbit weighting about one kg.

The results reported for each data point were the mean ± the standard error of the mean for the volume/ length ratio V/ L into rabbit of each strain.

Mouse lethality:

The applied method stated by Ratman *et al.* (1980) was done as follows:

Mouse lethality assayed by intraperitoneal injection of toxin diluted in normal saline into 15- 20 g BAL B/ C mice death between 2^{nd} and 7^{th} days.

Screening raw milk for *E. coli* O157: H7 to be used in irradiation experiment:

The method reported by Massa *et al.* (1999) was applied. One hundred raw milk samples were collected from dairy farms. The samples were taken and maintained at 4°C until analysis within 5 hrs of sampling by direct plating method. 25 ml of each sample were separately homogenized in 0.1% peptone water. Serial diluations were made. One hundred microliters of appropriate dilutions were spread on sorbitol MacConkey agar plates. The inoculated plates were incubated at 37°C.

After 18- 24 hrs, the plates were examined for non sorbitol fermenting presumptive colonies which were confirmed by latex agglutination using *E. coli* latex kit (Oxoid, DR 320). The milk products samples were manufactured from pasteurized milk.

Irradiation protocol:

The technique of irradiation recommended by Lucht *et al.* (1997) was done. For gamma irradiation, sterile scrow cap test tubes (16 X 125 mm) containing 0.5 ml culture were immersed in 2- L beaker containing crushed ice. An aluminum disc assembly with holes at the circumference was used to position of the test tubes for electron beam irradiation, the samples (0.5 ml, 1 X 10^8 C.F.U./ ml) in screw- cap. Test tubes were horizontally placed in a plastic tray on a layer of crushed ice.

Damage in *E. coli*:

Damage in *E. coli* was immediately examined following the gamma irradiation (0.44 and 0.76 kgy) survivors were serially diluted and surface spread (0.1 ml) onto plates containing yeast extract agar (BYEA).

This medium described by Lucht *et al.* (1997):

Ingredients	G/ L
KH_2PO_4.	1.0
$(NH_4)_2$ HPO_4	4.0
$MgSO_4$	7.0
Sodium citrate	0.5
Dextrose	10
Yeast extract	20.0
Agar	15
PH	6.8

The series was initially incubated at 18°C for 0, 1, 2, 4, 5, 24, 28 and 48 hrs. The ratio of CFU was obtained.

Survival of *E. coli* O157: H7 strain in raw irradiated milk and milk products (Massa *et al.*, 1999):

Whole milk was collected from a local dairy farm, stored at 4°C and used within 2 hrs of collection. The used strain of *E.coli* O157: H7 in this study was obtained from Bacteriological Veterinary Laboratory. Tryptone soya broth was used for the preparation of the working cultures by growing the strains over night at 37°C with shaking. The strain was diluted for working cultures in saline (0.85%) and then 1ml of the culture was inoculated into 100 ml of raw milk and some milk products

(yoghurt and cheese) at final concentration between about 10^3 and 10^6 (CFU/ ml^{-1}). Milk was held at 8°C and 5°C and then aerobic plate count was determined.

Escherichia coli O157:H7 as counted by serially diluting (1: 10) milk and milk products in 0.1% peptone water and then surface plating. The plates were incubated at 37°C for 18- 24 hrs and selected colonies were confirmed to be *E.coli* O157:H7.

RESULTS

A total of 100 milk samples were assayed for *E. coli* O157:H7 and cytotoxins. The organism and cystotoxins were not found in any samples. The behaviour of *E.coli* in milk and milk products held at 8°C as in Table 2 essentially showed no changes in viable population by direct plating method. The colony forming units ranged from 10^4-10^6 CFU/ml^{-1}. The initial pH of the milk was 6.7, which is the normal value of freshly drawn milk and had declined to 4.8.

Challenged rabbit with enterotoxin in ileal loop, cytotoxicity was detectable more often in the proximal and distal small intestine, it is also found to mice cytotoxin as lethal.

Irradiation at 0.15, 0.53, 0.77 and 0.85 KGY caused a damage in minimal time. Furthermore, the damage was increased with increase in time as in Table 5. In Table 6, irradiated products, refrigerated at 8°C and 5°C, showed no growth of *E.coli* O157:H7 for 3 days.

Table 1: Serovars of pathogenic *E.coli* in raw milk and milk products.

Serovar / Source	O26		O55		O157:H7	
	No.	%	No.	%	No.	%
Milk	2	4	1	2	1	2
Yoghourt	0	0	0	0	0	0
Cheese	2	4	2	4	1	2

Table 2: Survival of *Escherichia coli* in raw milk and milk products after inoculation by 1 ml of *E.coli* O157: H7 at 8°C.

Strain	Hours	*E. coli* O157: H7 CFU/ ml^{-1}	pH
O157: H7	0	5×10^4	6.7
	24	4.8×10^6	6.7
	72	2.4×10^6	6.5
	144	2.3×10^6	5.9
	216	2.1×10^6	5.5
	264	2.1×10^6	4.8

Table 3: Biological proportion of toxin extract from *E.coli* O157: H7 in mouse.

Day of inspection	Mouse leathality				
	40 µg	50 µ	60 µg	70 µg	80 µg
1	0/ 5	0/ 5	1/ 5	2/ 5	3/ 5
2	0/ 5	1/ 5	1/ 5	2/ 5	4/ 5
3	0/ 5	1/ 5	3/ 5	5/ 5	5/ 5

Table 4: Pathogenic properties of *E.coli* O157: H7.

Virulent strain	Pathogenic	Properties
O157: H7	Degree of cytotoxicity	+++
	Cytotoxicity in titre	128
	Secretion in ileal loop V/ L	210 ± 7

V/ L Volume length ratio of Challenged Rabbits legated Leal loop by enterotoxin

Table 5: Efficacy of gamma radiation doses on O157:H7 in milk and milk product.

Irradiated dose (KGY)	Milk	Yoghourt	Cheese
0.25	2.3	2.20	2.3
0.53	- ve	- ve	- ve
0.77	- ve	- ve	- ve
0.85	- ve	- ve	- ve

Table 6: Shelf life of refrigerated irradiated raw milk and some milk products.

	Day of inspection		
	1st	2nd	3rd
Milk	- ve	- ve	- ve
Cheese	- ve	- ve	- ve
Yoghourt	- ve	- ve	- ve

DISCUSSION

From the obtained data, it could be concluded that the cheese is the most contaminated product with pathogenic *E. coli*. This product may be subjected to contamination during its preparation. The results of the present study are in agreement with those reported by Ansay and Kaspar. (1997). However, Adesiyun *et al.* (1997) found that 17 (18.5%) of 94 strains of *E. coli* isolated from bulk milk products.

A total of 100 milk samples were assayed for *E. coli* O157: H7 and cytotoxins. The organism and cytotoxins were not found in any samples. The behaviour of *E. coli* in milk and milk products held at 8°C as in Table 2 essentially showed no changes in viable population by direct plating method. The colony forming unit ranged from 10^4 - 10^6 CFU/ ml^1. The initial pH of the milk was 6.7, which is the normal value of freshly drawn milk and had declined to 4.8. Challenged rabbit with enterotoxin in ileal loop, cytotoxicity was detectable more often in the proximal and distal small intestine, it is also found lethal to mice. Following irradiation at 0.15, 0.53, 0.77 and 0.85 at 0.7°C, it caused damage in minimal time and further damage with increase in time occurred as in Table 5. In Table 6, refrigerated and irradiated products at 8°C and 5°C showed no growth of *E.coli* O157:H7 for 3 days.

Verototoxin producing *Escherichia coli* O157:H7 is now recognized as an important enteric pathogen for humans and has been implicated as the causative agent of several food associated outbreaks of disease (Doyle, 1991).

Raw milk was first recognized as a vehicle of transmission of *E.coli* O157:H7 (Anonanys, 1994). *E.coli* O157:H7 organisms are reinforcing the observation that they are not very common, the results of the present study are in agreement with those reported by Hancock *et al.*, (1994).

The behaviour of *E.coli* in milk and some milk products held at 8°C as shown in Table 2, reveal that the initial colony forming units ranged from 10^3- 10^6CFU ml^{-1}. The results obtained in the study confirmed the ability of *E.coli* O157:H7 to grow or maintain themselves at both low temperature and pH.

In this respect, Palumbo *et al.* (1995) found that haemorrhagic strain of *E.coli* O157: H7 grew in brain heart infusion broth at 8°C and produced cytotoxin at 10°C. On the other hand, Sabra (2000) examined 50 local human and animal isolates of Enteropathogenic *E.coli* for

production of verotoxin, the highest verotoxin producers were *E.coli* of bovine origin.

The present study has revealed that cytotoxins have lethal effect to mice and LD_{50} varied from 60 to 75 µg protein in different cytotoxins produced from the isolated *E.coli*. This agrees with Ratman *et al.* (1980) who recorded that LD_{50} of cytotoxin in mice varied from 660 to 90 µg protein.

The obtained results in Table 4 showed that the toxins of *E. coli* O157: H7 isolated from milk and milk products had distinct cytopathic effect with titre 12. These findings are supported by results of Massa *et al.* (1999) who stated that the cytoxin producing *E. coli* is recognized as an important enteric pathogen for human and has been implicated as a causative agent of several food associated outbreaks.

The obtained results in Table 4 showed that *E. coli* enterotoxins isolated from milk, yoghurt and cheese showed a maximum secretion in ligated ileal loop. This result is similar to that recorded by Waker *et al.* (1988) who found that all *E. coli* proved to be toxin positive and caused a fluid accumulation in ligated ileal loop.

The rate and total amount of repair in injured microorganisms are dependent on various environmental factors, including freezing, drying, acid or salt treatment has been shown to repair within 30 min, in contrast, heat injured cell have been reported to take from 3 to 4 hrs (Ray, 1989).

However, the observation that optimal repair of *E. coli* following irradiation occurred when initially maintained at suboptimal temperature over via of data presented by Ray (1989). Moreover, Monk *et al.* (1995) suggested that the ability of irradiated *E.coli* to survive treatment as dependent on to counter- current metabolic processes one process, involved in synthesis was thought to promate repair and operated at temperatures up to 18°C. Also Hammad *et al.* (1998) found that irradiation dose at 4 KGY greatly reduced the initial microbial counts.

The other process was promoted damage and or permanent injury leading to death and operated at temperatures higher than 1°C.

Although the exact nature of this destructive process is thought to promote thermolability of various cellular enzyme mediated physiological reaction and/ or structural components. Also Chapman *et al.* (1993) confirmed that the pathogen is unable to multiply or produce cytotoxins if the milk or milk product kept refrigerated at the correct temperature (5°C).

In conclusion, potentially lethal damage to *E.coli* O157:H7 appeared by gamma irradiation regarding cytotoxicity was susceptible to irradiation.

The findings suggests that, irradiation is an effective means to kill the bacteria also irradiation differentially inactivates some activities of cytotoxin.

REFERENCES

Adesiyun, A.A.; Wett, L.A.; Eomain, H. and Kaminjolo, J.S. (1997): "Prevalence and characteristics of stains of *Escherchia coli* isolated from milk and feces of cows on dairy farms." J. Food Protection, 60: 1174-1181.

Anonanys (1994): "Outbreak of *E.coli* O157:H7 associated with contaminated milk." International Food Safety, 3: 74.

Ansay, S.E. and Kaspar, C. (1997): "Survey of rataib cheeses dairy processing, environments and raw milk for *Escherichia coli* O157:H7." Letters in Applied Microbiology 25, 131- 134.

Champan, P.A.; Wright, D.J. and Higgins, R. (1993): "Untreated milk as a source of verotoxigenic *E. coli* O157:H7." Veterinary Record, 133: 171- 172.

Doyle, M.P. (1991): "*Escherichia coli* O157:H7 and its significance in foods." International Journal of Food Microbiology 13, 207- 216.

Dussa, J.C.; Denfeld, R.W.; Simon, J.C. and Matrin, S.F. (2004): "UV irradiated densritic cell fail to tolerize murine effector T cells." J. Invest. Dermatol., 122 (4): 942- 52.

Hammad, A.A.; Abdel–Aai and Rify, M.N. (1998): Radiation, decontamination of some important species from it is bacterial load. Egypt, APP. Sci. Dec. 273.

Hancock, D.D.; Besser, T.E.; Kinsel, M.L. and Tarr, P.I. (1994): "The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle." Epidemiology and Infection 113, 199- 207.

Klipstein, F.A.; Engert, R.F. and Hughten, R.A. (1983): "Protection in rabbit immunized with vaccine of *Escherichia coli* heat stable toxin- cross linked to the heat labile toxin." Inf. Imm. 40 (3): 888- 8893.

Lucht, L.; Blank, G. and Bors, J. (1997): "Recovery of *Escherichia coli* from potentially lethal radiation damage." J. Food Safety, 7: 261- 271.

Massa, S.; Goffredo, E.; Altieri, C. and Natoia, K. (1999): "Fate of *Escherichia coli* O157: H7 in unpasteurized milk." Letters in Applied Microbiology 28, 89- 92.

Monk, D.J.; Beuchat, L.R. and Doyle, M.P. (1995): "Irradiation inactivation of food born microorganisms. "J. Food Protection, 58: 197-208.

Montenegro, M.A.; Bulte, M. and Trumpf, T. (1990): "Deflection and characterization of faecal verotoxin producing *Escherichia coli* from healthy cattle." J. Clinical Microbiology 28, 1417- 1421.

Palumbo, S.A.; Cald, J.E. and Scoltz, F.J. (1995): "Minimum and maximum temperatures for growth and verotoxin production by haemorrhagic strain of *Escherichia coli.*" Journal of Food Protection 58, 352- 356.

Ratman, S.; March, S. and Ahmed (1980): "Characterization of *Escherichia coli* serotype O157:H7." J. Clin- Microbiol., 26 (1): 2006- 2012.

Ray, B. (1989): "Enumeration of injured indicator bacteria from foods. In injured Index and Pathogenic bacteria." J. Food Protection, 49, 651- 655.

Sabra, S.M. (2000): "Studies on virulence factors of *E.coli.*" Ph. D. Thesis, Fac. Vet. Med., Cairo Univ.

Thayer, D.W. (1995): Use of irradiation to kill enteric pathogens. J. Food. Safety 15:181

Vernozy, M.P.; Montent, M. Berardin, C. and Beutin, L. (2005): Isolation and characterization of shiga toxin – producing Escherichia coli strains from raw milk. Letters in Applied microbiology 41, 236-241 .

Waker, C.W.; Upson, R. and Warren, R.E. (1988): "Haemorrhagic colitis detection of vero toxin producing *Escherichia coli* O157:H7 in a clinical microbiology laboratory." J. clin. Pathol., 41: 0- 84.

Yamamoto, T.; Kojio, S.; Taneike, I. and Iakura, N. (2003): "Irradiation of shiga toxin producing *Escherichia coli* induces StX phage." FEMA Microbiol Letter, 16: 222 (1): 115- 21.