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THE FATE OF *ESCHERICHIA COLI* 01.57:H7 IN RAW MILK

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The survival of E. coli 0157:H7 was examined in artificially contaminated raw milk kept at 7^{0}C,18^{0}C and 20^{0}C for 21 days. E. coli 0157:H7 survived more than 21 days in samples kept at 7^{o}C, 18^{0}C and 20^{0}C for 21 days. E. coli 0157: H7 survived more than 21 days in samples kept at 7^{0}C and 18^{0}C and for 14 days at 20^{0}C. In the first 3 days in the milk kept at 7^{0}C the number of E. coli 0157:H7 increased from 5.4 log CFU/ml to 7.0 log CFU/ml. and did not change up to day 14, but slightly decreased to 5.9 log CFU/ml at the end of the study. In parallel to the change of E. coli 0157:H7 counts in raw milk, total bacterial counts also changed. In the first 7 days total bacterial counts increased from 7.3 log CFU/ml to 8.9 log CFU/ml, then decreased to 7.8 log CFU/m at 14 days and after that the numbers decreased to 6.9 log CFU/ml at the end of the study. During the experiment pH decreased from 6.56 to 5.58.

In the milk kept at 18^{0}C, in the first 3 days, the numbers of E. coli 0157:H7 increased from 5.7 log CFU/ml to 7.3 log CFU/ml and then decreased to 5.7 log CFU/ml at 14 days followed by a slow decrease to 4.8 log CFU/ml on the 21 st day. In the first 24 h, total bacterial counts increased from 7.2 log CFU/ml to 8.4 log CFU/ml, remained at the same value and then slightly decreased to 7.0 log CFU/ml, at 21 days. During the experiment pH decreased from 6.56 to 3.94.

During storage of the milk at 20^{0}C an increase of the number of E. coli 0157:H7 from 5.5 log CFU/ml to 7.3 log CFU/ml was determined in the first 3 days. This value remained up to 4 days. After that the number of E. coli 0157:H7 rapidly decreased to 5.9 log CFU/ml at the 7 th day remaining at that value up to the 14 th day. Total bacterial counts in the first 24 h increased from 7.3 log CFU/ml to 8.5 CFU/ml, and then decreased to 8.1 log CFU/ml after 14 days. The pH decreased from 6.56 to 3.97 at the end of the investigation.

Key words: E. coli 0157:H7, pH, raw milk, fate, total bacterial counts

INTRODUCTION

E. coli O157:H7 has emerged as a significant human pathogen, especially in young children (Riley et al. 1983). It is an enteric pathogen known to cause bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Okrend et al. 1992). In 1982, two outbreaks of acute bloody diarrhea led to the discovery of *E. coli* 0157:H7 as a food pathogen (Neill, 1997). Milk has been identified as an important vehicle of infections (Martin et al. 1986). Fecal contamination of milk is one likely route of transmitting *E. coli* 0157:H7 to humans. Dairy cattle are a probable reservoir of the organism (Borczyk et al. 1987., Chapman, 1993). In addition to foodborne transmission *E. coli* 0157:H7 may be spread by person to person contact (Griffin and Tauxe 1991). Two children from separate families in Wisconsin developed hemorrhagic colitis and HUS after drinking raw milk from dairy farms. *E. coli* 0157:H7 was isolated from the stools of both patients and feces of healthy heifers on both farms. In the outbreak associated with the consumption of pasteurized milk, which occurred in Scotland in 1994, more than 100 people were affected (Simmons, 1997). This serotype is unable to ferment sorbitol within 24 h, and also does not possess the enzyme β-glucuronidase. *E. coli* 0157:H7 is MUG assay negative, indicating that β-grucuronidase activity is not phenotypically expressed by these organisms. Morever, it does not grow well at 44^{0}C, which distinguishes it from most other *E. coli* isolates (Doyle and Schonei, 1984). Refrigeration was observed to enhance the survival of *E. coli* in an acid environment, but it could not grow at low pH, even though it survived in acid foods for several weeks (Garcia-Graells et al. 1993). When enterohemorrhagic *E. coli* was added to raw milk the number of *E.coli* remained constant in milk held ar 8^{0}C and 12^{0}C. The background flora rapidly increased in number, reaching 10^6 *E. coli/ml* in four to six days (Palumbo et al. 1996).

Raw milk can be contaminated with *E. coli* 0157:H7 as a result of fecal contamination. The purpose of this study was to determine the fate of *E. coli* 0157:H7 in artificially contaminated raw milk depending on storage temperature.

MATERIALS AND METHODS

Preparation of *E. coli* inoculum. *E. coli* 0157:H7 was grown at 37^{0}C in brain heart infusion broth. The test culture was subjected to a minimum of three successive 20-24h transfers.

Inoculation and analysis of milk. Raw milk (4% fat, pH 6.56) was inoculated with a dilution (made in sterile 0,9% saline) to yield a starting count of ca. 10^5 *E. coli* 0157:H7/ml. The inoculated milk was stored at 7^{0}C, 18^{0}C and 20^{0}C for a period of 21 days.

Before inoculating milk with *E. coli* 0157:H7 background microbioal populations and pH were determined by plating serially diluted milk in sterile 0,9% saline on to total bacterial count agar plates (Torlak-Yugoslavia). The agar plates were incubated at 30-32^{0}C for 72 h. In addition the initial pH was determined using a pH meter (Kranj-Iskra, MA 5735).

Samples of artificially contaminated raw milk were taken at 0, 24h, 48h, 72 h, 7 days, 14 days and 21 days for microbial analysis and pH measurement.

Enumeration *E. coli* 0157:H7. Duplicate samples of the milk (20 ml) were dispersed in 180 ml sterile 0,9% saline. Decimal dilutions of milk were plated on to Fluorocult *E. coli* 0157:H7 agar plates (Merck-Germany). The plates were incubated in air at 37^{0}C for 24 h and typical greenish sorbitol negative colonies were counted and confirmed by biochemical tests.

Enumeration of total bacterial counts. The bacterial counts in raw milk were determined in decimal dilutions of milk in sterile 0,9% saline which were plated on to total bacterial count agar plates (Torlak-Yugoslavia) and incubated at 30-32^{0}C for 72 h.

RESULTS

The results concerning the fate of *E. coli* 0157:H7 in raw milk stored at 7^{0}C for 21 days are shown in Table 1.

Table 1. Change in *E. coli 0157:H7* numbers, total bacterial counts and pH in raw milk during storage at 7^{0}C.

Time (days)	*E. coli 0157 : H7** (log CFU/ml)	pH*	Total bacterial counts* (log CFU/ml)
0	5.418	6.56	7.252
1	5.829	6.36	7.559
2	6.757	6.29	7.910
3	7.002	6.24	7.930
7	7.055	6.10	8.881
14	7.154	5.95	7.765
21	5.952	5.58	6.932

*Mean values of three determminations

In raw milk kept at 7^{0}C *E. coli* 0157:H7 survived for 21 days. In the first 3 days the number of *E. coli* 0157:H7 increased from 5.4 log CFU/ml to 7.0 log CFU/ml, and then remained at the nearly same level up to 14 days. After that the number of *E. coli* 0157:H7 decreased to 5.9 log CFU/ml at the end of the study. In the first 7 days total bacterial counts increased from 7.2 log CFU/ml to 8.9 log CFU/ml. A decrease of total bacterial counts to 6.9 log CFU/ml was noted at the end of the study. During storage of the milk pH decreased from 6.56 to 5.58 at the end of the study.

The fate of *E. coli* 0157:H7 in raw milk during storage at 18^{0}C is shown in Table 2.

Table 2. Change in *E. coli 0157:H7* numbers, total bacterial counts and pH in raw milk during storage at 18°C

Time (days)	*E. coli 0157 : H7** (log CFU/ml)	pH*	Total bacterial counts* (log CFU/ml)
0	5.671	6.56	7.227
1	6.430	6.21	8.447
2	6.960	6.53	8.094
3	7.319	4.70	8.198
7	7.314	4.47	8.423
14	5.723	4.12	8.368
21	4.755	3.94	7.011

*Mean values of three determinations

In the first 3 days the number of *E. coli 0157:H7* increased from 5.7 log CFU/ml to 7.3 log CFU/ml. A decrease to 5.7 log CFU/ml was determined at 14 days. After that the number of *E. coli 0157:H7* decreased further to 4.8 log CFU/ml at 21 days. In the first 24 h, total bacteria counts increased from 7.2 log CFU/ml to 8.4 log CFU/ml, and then remained nearly at the same levels up to 14 days. Total bacterial counts continued to decrease to 7.0 log CFU/ml at 21 days. During storage of the milk the pH decreased from 6.56 to 3.94 at the end of the study.

The fate of *E. coli 0157: H7* in raw milk during storage at 20°C is shown in Table 3.

Table 3. Change in *E. coli 0157:H7* numbers, total bacterial counts and pH in raw milk during storage at 20°C

Time (days)	*E. coli 0157 : H7** (log CFU/ml)	pH*	Total bacterial counts* (log CFU/ml)
0	5.471	6.56	7.252
1	6.886	5.72	8.513
2	7.320	4.62	8.341
3	7.234	4.56	8.440
7	5.949	4.46	8.796
14	5.898	3.97	8.104
21			

*Mean values of three determinations

In the first 2 days the number of *E. coli 0157:H7* increased from 5.5 log CFU/ml to 7.3 log CFU/ml. After 3 days storage numbers decreased from 7.2 log CFU/ml to 5.9 log CFU/ml at 7 days. The number of *E. coli 0157:H7* then remained at the same value to the end of the study. In the first 24h total bacterial counts increased from 7.3 log CFU/ml to 8.5 log CFU/ml. After 14

days total bacterial counts decreased to 8.1 log CFU/ml. During storage of the milk the pH decreased from 6.56 to 3.97.

DISCUSSION

The results showed that *E. coli 0157:H7* survived in the artificially contaminated raw milk at 7^{0}C and 18^{0}C for more than 21 days and at 20^{0}C for 14 days. During storage of milk at 7^{0}C the decrease in *E. coli 0157:H7* numbers was slightly lower in the first 3 days, than in the milk kept at 18^{0}C and 20^{0}C. After 7 days the increase in *E. coli 0157:H7* in raw milk stored at 7^{0}C was higher than in raw milk kept at 18^{0}C and 20^{0}C. In parallel to the change of *E. coli 0157:H7* counts, there was a change in total bacterial counts.

The increase in total bacterial counts at 18^{0}C and 20^{0}C was higher than in raw milk kept at 7^{0}C. Thus, number of *E. coli 0157:H7* was a function of the number of background microflora. During storage of the milk at 7^{0}C the pH decreased from 6.56 to 5.58. With increased pH, as a result of bacterial multiplication the number of *E. coli 0157:H7* increased. During storage of the milk at 18^{0}C, pH changed from 6.56 to 3.97. In the milk kept at 20^{0}C, the pH also decreased from 6.56 to 3.94. *E coli 0157:H7* survived at pH 3.94. The rate of growth of *E. coli 0157:H7* in milk depends on the ecological conditions. Palumbo et al. (1997) found that, in pasteurized milk from the supermarket, the response of the strains was a function of level of background flora.

Our results indicated that *E. coli 0157:H7* can grow in milk at 7^{0}C, which is not an uncommon teperature for holding refrigerated milk at retail or in consumers' homes. Wang et al. (1997) found that *E. coli 0157:H7* can grow in pasteurized milk at 8^{0}C, but did not grow at 5^{0}C. In our investigation the response of the strains was a function of the level of background flora.

Although *E. coli 0l57:H7* cannot grow at low pH, it survived several days or weeks in acidic environments (Garcia-Graells et al 1998). However, storage temperature is also an important factor that influences the safety and quality of milk. Wang at al. (1997) recommended holding milk at ô 5^{0}C for prevention of growth of this pathogen.

A c k n o w l e d g m e n t

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PREŽIVLJAVANJE *ESCHERICHIA COLI 0157:H7* U SIROVOM MLEKU

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SADRŽAJ

Ispitivano je preživljavanje i razmnožavanje *E. coli 0157:H7* u eksperimentalno kontaminiranom sirovom mleku čuvanom 21 dan pri 7^{0}C, 18^{0}C i 20^{0}C. *E. coli 0157:H7* je preživljavala duže od 21 dan, pri temperaturi od 7^{0}C, 18^{0}C, a 14 dana pri 20^{0}C. U mleku čuvanom pri 7^{0}C, u prva tri dana, broj *E. coli 0157:H7* se povećao sa 5.4 log CFU/ml na 7.0 log CFU/ml i na skoro istom nivou se održao do 14. dana, a 21. dana se smanjio na 5.9 log CFU/ml. Paralelno sa promenom broja *E. coli 0157:H7* menjao se i ukupan broj bakterija. U prvih sedam dana ukupan broj bakterija povećao se sa 7.3 log CFU/ml na 8.9 log CFU/ml, posle 14 dana se smanjio na 7.8 log CFU/ml, a na kraju ispitivanja je iznosio 6.9 log CFU/ml. Tokom ispitivanja pH vrednost se snižavala sa 6.56 na 5.58.

Tokom čuvanja mleka pri temperaturi do 18^{0}C u prva tri dana broj *E. coli 0157:H7* se povećavao sa 5.7 log CFU/ml na 7.3 log CFU/ml, 14. dana je

iznosio 5.7 log CFU/ml, a 21. se smanjio na 4.8 log CFU/ml. Ukupan broj bakterija se u prvih 24 h povećavao sa 7.2 log CFU/ml na 8.4 log CFU/ml i na približno istoj vrednosti se održavao do kraja ispitivanja kada je iznosio 7.0 log CFU/ml. Tokom ispitivanja pH vrednost se snižavala sa 6.56 na 3.94.

U prva tri dana čuvanja mleka pri 20^{0}C broj *E. coli 0157:H7* se povećavao sa 5.4 log CFU/ml na 7.3 log CFU/ml, na toj vrednosti se održavao uz neznatne varijacije do 4. dana, a zatim se smanjio na 5.9 log CFU/ml sedmog dana, i do 14. dana ostao gotovo nepromenjen. Ukupan broj bakterija u prvih 24 sata se povećao sa 7.3 log CFU/ml na 8.5 log CFU/ml, a zatim se smanjio na 8.1 log CFU/ml, posle 14 dana. Tokom ispitivanja pH vrednost se smanjivala sa 6.56 na 3.97.