

THE FATE OF ESCHERICHIA COLI 0157:H7 IN PASTEURIZED MILK

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The survival of E. coli 0157:H7 was examined in artificially contaminated pasteurized milk stored at 7°C and 20°C for 21 days. E. coli 0157:H7 survived for more than 21 days in samples of pasteurized milk. The number of E. coli 0.157:H7 in the milk was directly correlated with the total bacterial counts. In the first 24h at 7°C the number of E. coli 0157:H7 slowly decreased from 43×10^3 /ml to 14×10^3 /ml, and then increased from 94×10^3 /ml on the second day to $3\ 160 \times 10^3$ /ml on day 14. After that the number of E. coli 0157:H7 slightly decreased to $2\ 250 \times 10^3$ /ml, at the end of the study. In the first three days total bacterial counts slowly increased, while pH also did not change during the same time. After 7 days total bacterial counts increased from 520×10^3 /ml to $31\ 000 \times 10^3$ /ml, while pH declined from 5.98 to 4.96 after 21 days

Within the first four days of storage of pasteurized milk at 20°C, the number of E. coli 0157:H7 increased from 47×10^3 /ml, to $1\ 260 \times 10^3$ /ml and then rapidly fell to day 7. After that the milk was spoiled and the number of E. coli 0157:H7 continued to decrease to 26×10^3 /ml on day 14. Then numbers rapidly rose to 660×10^3 /ml after 21 days. In parallel to the change of E. coli 0157:H7 counts in pasteurized milk, total bacterial counts in the first 24h increased, and after that slowly decreased from 920×10^3 /ml to 520×10^3 /ml. The results also showed increased total bacterial counts from 1040×10^3 /ml on the 7th day to $192\ 000 \times 10^3$ /ml after 21 days. During storage of the milk the pH decreased from 6.70 to 3.94 after 14 days, and then rose to 4.80.

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

INTRODUCTION

E. coli 157:H7 has emerged as a significant human pathogen, especially in young children (Riley et al. 1983; Palumbo et al. 1997). It is an enteric pathogen known to cause bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome and thrombocytopenia purpura (Okrend et al. 1992). Since 1982, this pathogen

and other verotoxigenic *E. coli* have been involved in foodborne outbreaks associated with consumption of undercooked ground beef and unpasteurized milk (Bleumink, 1997). Milk has been identified as an important vehicle of infections (Martin, et al. 1986). Thermally processed fluid milk has also been implicated as a source of human illness where inadequate pasteurization and pretreatment contamination factors participate in many incidents (Sharp, 1987). In the outbreak associated with the consumption of pasteurized milk, which occurred in Scotland in 1994, more than 100 people were affected (Simmons, 1997). Epidemiological investigations have revealed that dairy cattle, especially young animals, are a principal reservoir of *E. coli* 0157:H7 (Borczyk et al. 1987; Montenegro et al. 1990; Wells et al. 1991; Chapman, et al. 1993; Hancock et al. 1994; Zhao et al., 1995.). Results of a longitudinal study of *E. coli* 0157:H7 in a dairy herd, thought to be the source of a small outbreak of human infections, showed that about 1% of more than 6000 animals and about 1% of more than 5000 herds tested were positive for this pathogen (ACMSF, 1995). In addition to foodborne transission, *E. coli* 0157:H7 may be spread by person to person contact (Graffin et al. 1991). This serotype is unable to ferment sorbitol within 24 h, and also does not possess the enzyme β -glucuronidase. Moreover, it does not grow well at it all, at 44°C, which distinguishes it from most other *E. coli* isolates. *E. coli* can grow at pH levels ranging from 4 to 9 (Garlant et al. 1994). Others have observed that refrigeration enhances the survival of *E. coli* in acid environments. It cannot grow at low pH, but can survive for several days or even weeks in acid foods (Garlant, et al. 1994; Garcia-Graells et al., 1998). When enterohemorrhagic *E. coli* was added to raw milk the number of *E. coli* remained constant in milk held at 8°C and 12°C. The background flora rapidly increased in number, attaining 10⁶ *E. coli*/ml in four to six days (Palumbo et al. 1997).

Raw milk can be contaminated with *E. coli* 0157:H7 and inappropriate pasteurization or postpasteurization contamination with the pathogen may be present in milk. Because of that, the purpose of this study was to determine the fate of *E. coli* 0157:H7 in artificially contaminated pasteurized milk, depending on storage temperature.

MATERIALS AND METHODS

Preparation of *E. coli* inoculum. *E. coli* 0157:H7 was grown at 37°C in brain heart infusion broth. The test culture was subjected to a minimum of three successive 20-h transfers before the experiment.

Inoculation and analysis of milk. Pasteurized milk (2.8% fat, pH 6.70) was inoculated with a dilution (made in sterile 0.9% saline) to yield a starting count of ca. 10³ *E. coli* 0157:H7/ml. The inoculated milk was stored at 7°C and 20°C for a period of 21 days.

Before inoculating milk with *E. coli* 0157:H7, background microbial populations and pH were determined by plating serially diluted milk in sterile 0.9% saline on to total bacterial count agar plates (Torlak-Yugoslavia) and incubating at 30-32°C for 72^h. In addition, the initial pH was determined using a pH meter (Kranj-Iskra, MA5735).

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

Enumeration *E. coli* 0157:H7. Duplicate samples of milk (20 ml) were dispersed into 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°C for 24 h and typical greenish sorbitol negative colonies were counted and confirmed by biochemical tests. Isolates were also tested for β -glucuronidase activity using 4-methylumbelliferyl- β -D-glucuronide.

Enumeration of total bacterial counts. The bacterial counts in pasteurized 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°C for 72h.

RESULTS

The results concerning the fate of *E. coli* 0157:H7 in pasteurized milk stored at 7°C for 21 days are shown in Table 1.

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

Time (days)	<i>E.coli</i> 0157:H7 (10 ³ /ml)	pH	Total bacterial Counts (10 ³ /ml)
0	43	6.70	68
1	14	6.70	100
2	94	6.70	116
3	120	6.70	400
7	600	5.98	520
14	3160	5.65	9600
21	2250	4.96	31000

*Mean values of three determinations

In pasteurized milk held at 7°C *E. coli* 0157:H7 survived for 21 days. In the first 24 h the number of *E. coli* 0157:H7 slowly decreased from 43x10³/ml to 14x10³/ml and then increased from 94x10³/ml on the second day to 3 160x10³/ml on day 14. After that the number of *E. coli* 0157:H7 slightly decreased to 2 250x10³/ml at the end of the study. In the first three days total bacterial counts slowly increased and pH also did not change during the same time. After 7 days total bacterial counts increased from 520x10³/ml to 31 000 x 10³/ml while pH fell from 5.98 to 4.96.

The fate of *E. coli* 0157:H7 in pasteurized milk during storage at 20°C is shown in Table 2.

Table 2. Change in *E. coli* 0157:H7 numbers, total bacterial counts and pH in milk during storage at 20°C

Time (days)	<i>E. coli</i> 0157:H7 (10 ³ /ml)	pH	Total bacterial counts (10 ³ /ml)
0	47	6.70	68
1	320	4.73	920
2	560	4.63	790
3	1260	4.52	520
7	100	4.50	1040
14	26	3.94	10400
21	660	4.80	192000

*Mean values of three determinations

In the first four days of storage of pasteurized milk at 20°C, the number of *E. coli* 0157:H7 increased from 47x10³/ml to 1 260x10³/ml, and then rapidly decreased to 7 days. After that the milk was spoiled and the number of *E. coli* 0157:H7 continued to decrease to the level of 26x10³/ml at 14 days and then rapidly rose to 660x10³/ml at 21 days. In parallel to the change of *E. coli* 0157:H7 counts in pasteurized milk, total bacterial counts in the first 24h increased, and after that slowly decreased from 920x10³/ml to 520x10³/ml. The results also showed that total bacterial counts increased from 1 040x10³/ml at 7 days to 192 000x10³/ml after 21 days. During storage of the milk the pH decreased from 6.70 to 3.94 after 14 days and then rose to 4.80.

DISCUSSION

The results showed that *E. coli* 0157:H7 survived in the artificially contaminated pasteurized milk at 7°C and 20°C. The increase of *E. coli* 0157:H7 counts during storage at 7°C was related to total bacterial numbers. During storage at 20°C in the first three days *E. coli* 0157:H7 counts were higher than in pasteurized milk kept at 7°C. There was an increase in *E. coli* 0157:H7 at 7°C after a further four days to levels higher than in the milk kept at 20°C. The increase in total bacterial counts in the milk kept at 20°C was higher than in pasteurized milk kept at 7°C. Although the pH was low after 14 days in the milk kept at 20°C. *E. coli* 0157:H7 survived and multiplied in the first three days when the pH was 4.52. After that numbers decreased to 14 days when the pH was lowest (3.94). With increased pH as a result of bacterial multiplication the number of *E. coli* 0157:H7 increased. Some others have noticed that refrigeration may lead to unchanged survival of *E. coli* in acidic environments (Garcia-Graells et al. 1998). This may be due to reduced permeability of the cell membrane to protons and or reduced metabolic activity.

Thus, 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 the level of background flora.

Our results indicated that *E. coli* 0157:H7 can grow in milk at 7°C, which is not an uncommon temperature 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°C, but did not grow at 5°C. Palumbo et al. (1997) determined that most *E. coli* 0157:H7 isolates grow in brain heart infusion broth at 10°C and some strains grow at 8°C.

In addition to that, we can conclude that the most important control measures to ensure milk safety are proper pasteurization and the avoidance of postpasteurization contamination. However, storage temperature is also an important factor that influences the safety and quality of milk. Wang et al. (1997) recommended holding milk at $\leq 5^{\circ}\text{C}$ for prevention of growth of this pathogen.

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PREŽIVLJAVANJE *ESCHERICHIA COLI* 0157:H7 U PASTERIZOVANOM MLEKU

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SADRŽAJ

Praćeno je preživljavanje i razmnožavanje *E. coli* 0157:H7 u veštački kontaminiranom mleku čuvanom 21 dan pri 7°C i 20°C. *E. coli* 0157:H7 je preživljavala u eksperimentalno kontaminiranom pasterizovanom mleku duže od 21 dan. Promena broja *E. coli* 0157:H7 u mleku, tokom čuvanja je zavisila, od temperature i dužine čuvanja mleka, broja mikroorganizama i promene pH vrednosti u mleku. U mleku čuvanom pri 7°C, broj *E. coli* 0157:H7 se neznatno smanjivao sa 43×10^3 /ml na 14×10^3 /ml posle 24 h, a zatim se povećavao i 14 dana iznosio 3.160×10^3 /ml. Posle 14. dana broj *E. coli* 0157:H7 se smanjivao i na kraju ispitivanja iznosio 2.250×10^3 /ml. Ukupan broj bakterija se povećavao sa 100×10^3 /ml na 31.000×10^3 /ml posle 21 dan. pH vrednost se nije menjala prva tri dana, a zatim se smanjivala i 21 dana iznosila 4.96. U prva četiri dana čuvanja mleka pri 20°C broj *E. coli* 0157:H7 se povećavao sa 47×10^3 /ml na 1.260×10^3 /ml, sedmog dana se smanjio na 100×10^3 /ml, 14. dana na 26×10^3 /ml, a 21. dana se povećavao na 660×10^3 /ml. Sedmog dana mleko je bilo promenjeno. Ukupan broj bakterija u mleku čuvanom pri 20°C povećavao se, a pH vrednost se snižavala sa 6,70 na 3,94 posle 14 dana, a zatim se povećavala na 4,80 posle 21 dan.