Effect of storage temperatures on growth and survival of *Escherichia coli* O157: H7 inoculated in foods from a neotropical environment

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Abstract: *Escherichia coli* O157: H7 has emerged as a new pathogen and is found worldwide. We studied the effect of several storage temperatures on the survival of this bacterium in common foods from a neotropical environment (Costa Rica) because at least seven clinical cases have been reported from the country, and no epidemiological link or probable food association has been described. High (10^6-10^8 CFU/ml) and low (10^4-10^6 CFU/ml) populations of *E. coli* were inoculated (three replications) in ground meat, chopped cabbage, chicken giblets and pasteurized milk and incubated at 0, 6 and 12ºC for 24, 48 and 72 h. Vegetables and milk were also stored at 22ºC for the same periods. The *E. coli* O157: H7 enumeration was done according to the methodology described in the Bacteriological Analytical Manual. Populations of *E. coli* O157: H7 showed either an increasing or decreasing trend, according to temperature, time or food base. Our data indicate that *E. coli* O157: H7 is capable of surviving and growing in meat, cabbage, milk and chicken giblets; food items commonly consumed by Costa Ricans.

Key words: *Escherichia coli* O157: H7, meat, milk, cabbage, chicken giblets, survival.

The epidemiology of food-born diseases has changed widely in the last decades. New pathogens have emerged and spread worldwide; *Escherichia coli* O157: H7 is an example. This bacterium was first recognized as a food born pathogen in 1982, when it was associated with two food related outbreaks of haemorrhagic colitis in the United States (Riley *et al*. 1983). It currently appears to occur worldwide and has three different manifestations: haemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Doyle and Palhye 1989, Doyle 1991, Anonumous 1995).

The intestinal tract of ruminants is its prime reservoir. Therefore, food of animal origin has been linked as a vehicle.

Nevertheless, this bacterium has also been associated with illness caused by dairy products, vegetables, salads, apple juice and even water (Wilshaw *et al*. 1994). Person to person spread also occurs.

Recovery frequency from food is low (Sekla et al. 1990), as is the contamination levels of food associated with outbreaks (10-6200 CFU/g) (Todd *et al*. 1988). At the same time, its infecting dose has been described as low, in some cases, two cells / 25 g of food have been known to cause illness (Sekla *et al*. 1990) and a high acidic tolerance has also been described (Wilkinson 1997). Strict commercial regulations have been developed worldwide, including a zero tolerance level for food imported by the US (Anonymous 1997).

Human infection with *E. coli* O157 has been reported from over 30 countries in six continents. Annual incidence rates of 8 per 100 000 population or greater have been described from the USA, Canada and Argentina, in regions characterized for having four seasons. Developing countries, especially those from the tropical belt of the planet, have climatic conditions that favor the proliferation of contaminating bacteria, a fact reflected in the high prevalence of diarrheic diseases, thus, the incidence of *E. coli* O157: H7 should be higher in the tropics than in temperate regions. Maybe because of the recent emergence of this agent, there are almost no reports of its isolation in tropical countries. In Costa Rica at least seven clinical cases in children, two of them fatal, have been reported, but no epidemiological link or food association has been described (Herrera 1998). There are no reports from the country evaluating *E. coli* O157: H7 in risky food: it is vital to evaluate bacterial behavior at different storage conditions, to minimize transmission by food.

The purpose of this study was to determine the effect of different storage temperatures on the survival of *E. coli* O157: H7 in milk, beef, vegetables and chicken giblets.

MATERIALS AND METHODS

Foods: Meat, pasteurized milk, packaged chopped cabbage and chicken giblets were purchased from a retail grocer in San José, Costa Rica. All foods were kept at 2 to 5ºC between the time of purchase and initiation of experiments, which never exceeded 4 h. Meat was chopped aseptically to avoid the great bacterial charge present in ground meat and standardizing the size and extent of variables.

Inoculum preparation: A human isolated strain of *E. coli* O157: H7 was used. Stock cultures were maintained at –70ºC on tryptic soy agar slants (Oxoid) and activated in tryptic soy broth (pH 7.0) at 35ºC. Culture was transferred by loop inocula twice at 24 h intervals to 100 ml of tryptic soy broth in 250 ml Erlenmeyer flasks. The last culture was considered as the large-

population suspension (106 to 108 CFU/ml) of *E. coli* O157: H7 for inoculating test foods. A low-population suspension (10^4-10^6 CFU/ml) was prepared by adding 1 ml of the large-population suspension to 99 ml of tryptic soy broth.

Procedure for inoculating foods: Approximately 1 kg of ground meat, chopped cabbage and chicken giblets and 1 L of pasteurized milk was separately placed in a sterile polyethylene bag and inoculated with 50 ml of large-population suspension of enterohaemorrhagic *E. coli*. Each food was homogenized in a Stomacher for 2 min. Immediately, three 200 g (or ml) samples of each food were taken and incubated at 0, 6 and 12ºC for 24, 48 and 72 h. Vegetables and milk were also stored at 22ºC for the same periods. A 25g sample of each food was taken before incubation in order to perform an initial count of *E. coli* O157: H7.

The same procedure was used with another 1 000 g of ground meat, chopped cabbage and chicken giblets and another 1 000 ml of pasteurized milk inoculated with 50 ml of small-population suspension.

Three independent trials were performed for each food. For each trial, a non-inoculated sample was used as control.

Procedures for enumeration of *E. coli* O157: H7: Immediately after each incubation period, 25 g samples were taken and combined with 225 ml of sterile peptonated water 0.1% in a sterile polyethylene bag and pummeled with a Stomacher for 2 min. Wash fluid was serially (1: 10) diluted and surface plated (0,1 ml) on duplicate sorbitol McConkey agar (SMA) (Oxoid). The SMA plates were incubated at 35ºC for 20 to 22 h before colonies of *E. coli* O157: H7 were counted. The same procedure was used with the control samples (Anonymous 1995).

pH measurements: The pH of the primary diluent in which food samples were pummeled was measured at each microbiological analysis.

Statistical analysis: A Duncan multiple range test was used. Each value represents the mean of six values (duplicate values for each sample analyzed from three independent trials).

RESULTS

Meat: Populations of *E. coli* O157: H7 on ground meat stored at 0, 6 and 12ºC increased significantly (p< 0.05) during the first 48 hours, whereas the populations decreased around 1 logarithm within 72 hours at the same storage conditions (Fig. 1). The averaged pH showed a slight drop during the storage period (6.1 to 5.4)
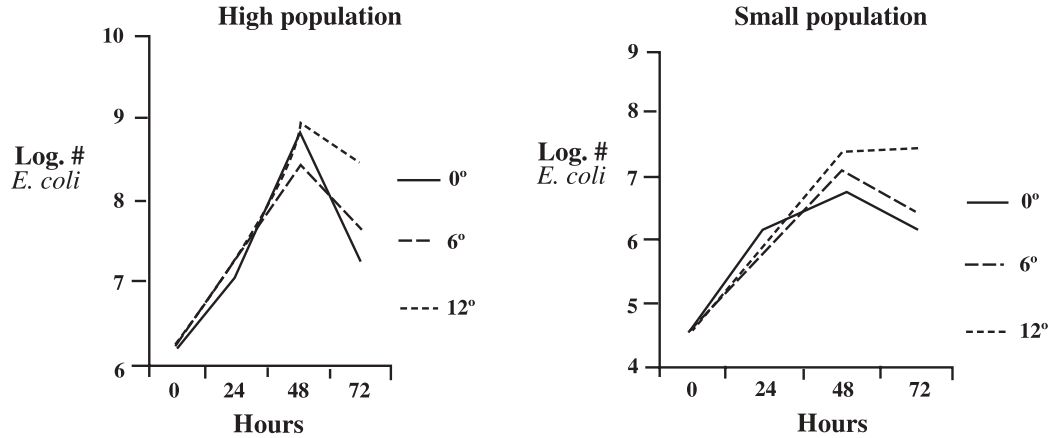

Fig. 1. Logarithm of the number of *E. coli* O157: H7 present in meat according to storage temperature and incubation period.

Milk: Unlike the studies of ground meat, populations of *E. coli* O157: H7 declined significantly (p<0.05) within 24 hours when milk initially containing 10^8 CFU/ml was kept at 0 and 6ºC. However, increases were detected within 48 hours of storage at 6 and 12ºC (Fig. 2). Within 72 hours of incubation at 0 and 6ºC, a significant decrease of *E. coli* O157: H7 was detected in milk samples containing a low-population inoculum. On the contrary, populations of *E. coli* O157: H7 on milk stored at 12 and 22ºC increased significantly (p<0.05) after 48 h.
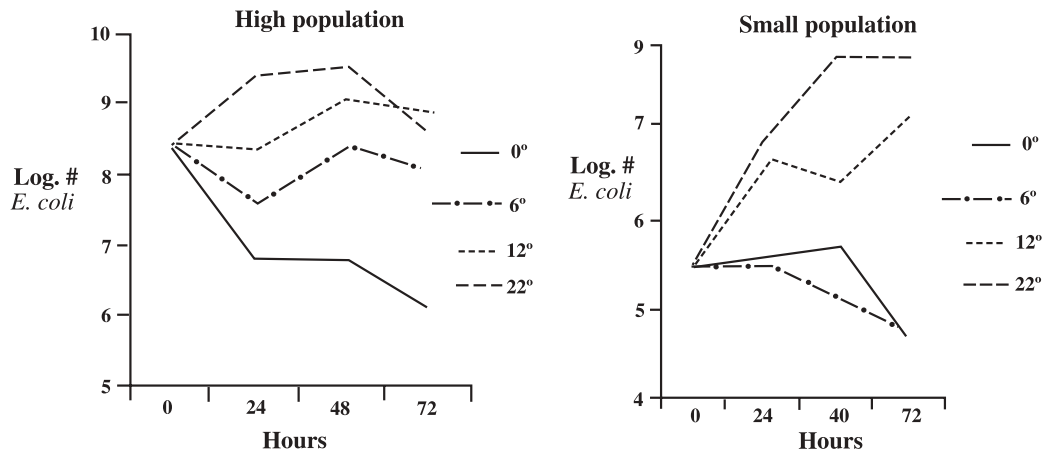

Fig. 2. Logarithm of the number of *E. coli* O157: H7 present in milk according to storage temperature and incubation period.

Cabbage: Populations of *E. coli* O157: H7 decreased significantly on chopped cabbage stored at 22ºC after 24 hours, decreasing 3 log within 72 hours. An increase after 48 h of storage at 6ºC was followed by significant (p<0.05) decrease at 72 h in the chopped cabbage initially containing a large inoculum (108 CFU/g). On the other hand, populations on cabbage stored at 12ºC increased significantly within 72 h. A significant increase (p<0.05) of *E coli* population was detected on chopped cabbage initially containing a low inoculum (105 CFU/g) after 72 h of storage at 0ºC. On the contrary, a significant decrease was observed in cabbage stored at 6 and 12ºC for 72 h (Fig. 3). The shift in pH at 0, 6 and 12ºC ranged between 7.5 to 6.0; the most marked rate of decrease in pH occurred in vegetables stored at 22ºC (7.5 to 5.3).
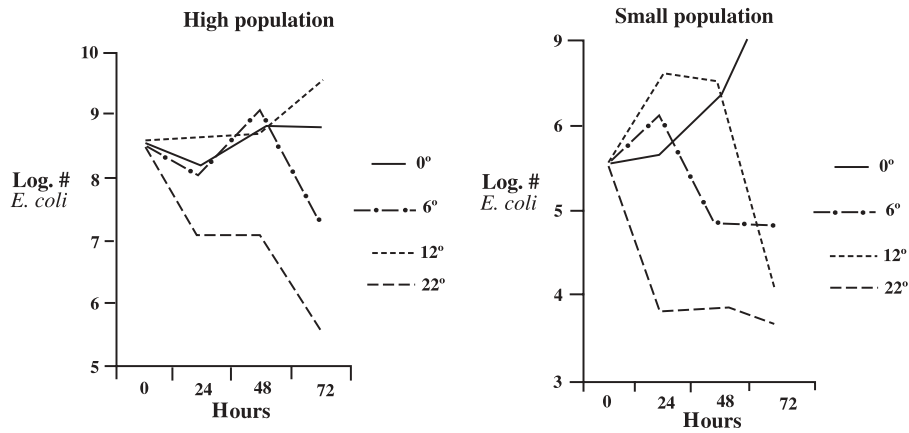
Fig. 3. Logarithm of the number of *E. coli* O157: H7 present in cabbage according to storage temperature and incubation period.

Chicken giblets: Populations of *E. coli* O157: H7 decreased significantly in chicken giblets stored at 0, 6 and 12ºC for 24 h, in samples initially containing low and large inoculum. Within 48 h incubation of storage at 12ºC a significant increase of *E. coli* O157: H7 was detected in chicken giblets initially inoculated with a large population. A similar behavior was observed in chicken giblets containing low inoculum within 48 h of storage at 0ºC. Populations in low and large inoculum samples stored at 0ºC declined significantly after 48h (Fig. 4). The pH shifted between 6.5-5.9.
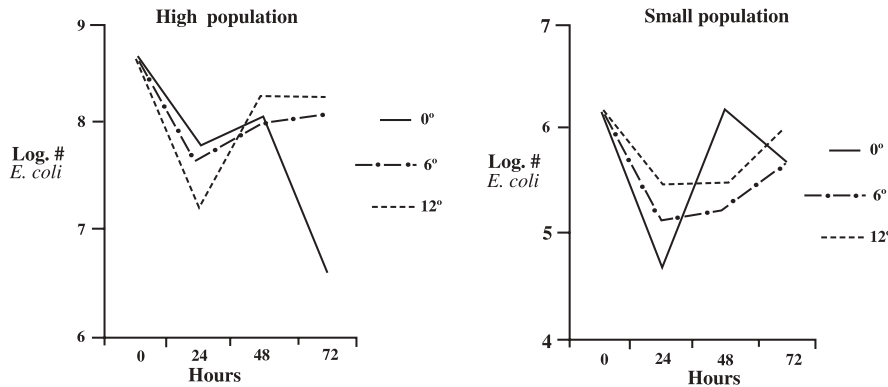
Fig. 4. Logarithm of the number of *E. coli* O157: H7 present in chicken giblets according to storage temperature and incubation period.

DISCUSSION

Foods of animal origin have been described as primary sources of enterohaemorrhagic *Escherichia coli* (EHEC) infections (Abdul-Raouf *et al*. 1993) and meat has been described as the principal vehicle of transmission of this bacterium to human beings (Szabo *et al*. 1990, Roberts *et al*. 1995). *E. coli* O157: H7 has been isolated from dairy cattle, calves, chickens, swine and even from sheep, and from their retail meat (Doyle and Schoeni 1987, Mermelstein 1993, An-Hung *et al*. 1995). Several authors have described its survival in ground beef during frozen storage (Doyle and Schoeni 1987). Our results coincide with these observations, independently of the population inoculated to meat, or the storage temperature, the numbers of *E. coli* increased significantly ($p<0.05$) during the first 48 hours of incubation.

Our results also show survival and multiplicative capability in chicken giblets stored at 0, 6 and 12ºC. Griffin and Tauxe (1991) did not recover this bacterium from raw chicken, but our results show that contaminated or cross contaminated chicken giblets may harbor important numbers.

Consumption of unpasteurized milk has also been associated with *E. coli* O157: H7 outbreaks (Borczyk *et al*. 1987, Bielaszewska *et al*. 1997). Behavior of this bacterium at several incubation temperatures and times shows important resistance to low temperatures, and multiplication especially when stored at 12 and 22ºC; the shift in pH also failed to inhibit growth. These results paralel those of Massa et al. (1999) who report increase in the viable population of EHEC inoculated into milk and stored at 8ºC.

On the other hand, our results, like those of Ruscisa and Sobol (1995) show that *E coli* O157: H7 can grow on refrigerated packed vegetables, as has been reported for *Listeria monocytogenes* (Berrang *et al.* 1989, Beuchat and Bracket 1990, Monge and Arias 1997), Salmonella (Rosas *et al*. 1984) and Aeromonas hydrophila (Berrant *et al*. 1989). During the first 24 hours, *E. coli* incubated at 0, 6 and 12ºC showed an increase in number, nevertheless, at 22ºC it presented a significant decrease in numbers probably due to competition and the important pH change (7,5-5,3). The decrease in pH can be attributed to the fermentative capability of this microorganism and to competition, including lactic bacteria. Similar results have been obtained by Faith *et al*. (1997) who found reductions in number of bacteria of 2-4 log 10 CFU/g when stored in pepperoni at 21ºC for 14 days.

Vegetables and fruits can become contaminated through cattle manure used in soil or through the use of contaminated water for irrigation or washing (Cieslat *et al*. 1993). At the same time, usual maintenance of packed vegetables is at refrigeration temperatures (8-10ºC), that have no effect on this bacterium. Richert *et al*. (2 000) report similar results, since they observed survival of EHEC on produce held at 4ºC and growth at 15ºC.

A recent outbreak of enterohaemorrhagic infection in kindergarten children from Japan is evidence of bacterial survival and multiplication on vegetables. The contaminated food was identified as a potato salad (Makino *et al*. 1997).

Increased attention to hygiene would be useful, but better risk reduction can be achieved through controlling points of potential contamination in the field and during the processing and handling of the products.

Findings made in this study indicate that *E. coli* O157: H7 is capable of surviving and growing in common food: meat, cabbage, milk and chicken giblets under the conditions that are normal in tropical countries such as Costa Rica.

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RESUMEN

Escherichia coli O157: H7 ha emergido como un nuevo patógeno y se encuentra mundialmente. En Costa Rica, por lo menos ha informado de siete casos clínicos, y no se ha encontrado un lazo epidemiológico o asociación con alimentos. El propósito de este estudio fue determinar el efecto de diferentes temperaturas de almacenamiento sobre la sobrevivencia de esta bacteria en alimentos de uso común. Una población alta de *E. coli* (10^6-10^8 UFC/ml) y una baja (10^4-10^6 UFC/ml) fueron inoculadas (tres réplicas) en carne picada, repollo picado, vísceras de pollo y leche pasteurizada e incubadas a 0, 6 y 1ºC por 24, 48 y 72 h. Los vegetales y la leche también fueron incubados a 22ºC por los mismos períodos. La enumeración de *E. coli* O157: H7 se realizó de acuerdo a la metodología descrita en el Bacteriological Analytical Manual. Las poblaciones de *E. coli* mostraron tendencias a aumentar o disminuir, dependiendo de la temperatura, tiempo y base alimenticia. Nuestros datos indican que la *E. coli* O157: H7 es capaz de sobrevivir y crecer en carne, repollo, leche y vísceras de pollo, alimentos de uso común en Costa Rica.

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