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Growth and survival of *Escherichia coli* O157: H7 in meat, poultry and vegetables mixed with different concentrations of mayonnaise

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Abstract: In the last 20 years *Escherichia coli* O157: H7 has emerged as a new pathogen, causing worldwide disease, death and economic loss. Different studies have revealed important survival characteristics of this pathogen, although there are divergent criteria about its ability to survive in various mayonnaise formulations. We studied the effect of different mayonnaise concentrations (0 %, 18 %, 37 % and 56 %) (weight/weight) over the survival of the bacterium in common foods from a neotropical environment (Costa Rica). High [10^7-10^8 Colony Forming Units (CFU)/ml] and low *E. coli* populations (10^4-10^6 CFU/ml) were inoculated, (three replicates) in meat, chopped cabbage and poultry, and mixed with commercial mayonnaise to obtain the concentrations specified. They were incubated at 12 ºC for 24, 48 and 72 hr. The *E. coli* O157: H7 enumeration was done according to a standard methodology. Populations of *E. coli* O157: H7 showed an increasing trend during the first incubation period (48 hr), in all the preparations, regardless of the fat concentration used. Our data indicate that *E. coli* O157: H7 is capable of surviving and growing in meat, cabbage and poultry mixed with mayonnaise, independently of its concentration.

Key words: *Escherichia coli* O157: H7, mayonnaise, meat, cabbage, poultry, survival.

In the last 20 years *Escherichia coli* O157: H7 has emerged as a new pathogen, causing worldwide disease, death and economic loss. Its severity is reflected in the outcome of recent outbreaks, for instance, over 5 000 Japanese schoolchildren became sick after the consumption of contaminated radish sprouts (Anonymous 1997); the recall of 25 million pounds of ground beef in the US (Mead and Griffin 1998), and seven cases in children in Costa Rica, of whom two died (Herrera 1998).

The infection caused by this bacterium has been reported from over thirty countries in six continents. It is more common in urban than rural areas and it appears seasonally, being more frequent during the warm summer months, both in the northern and southern hemispheres (Mead and Griffin 1998).

Different studies have revealed important survival characteristics of this bacterium. Its resistance to acidic environments has been well documented by several studies, including the works of Zhao *et al.* (1993) who showed that the bacterium survived in apple cider for as long as 31 days at 8 ºC, pH 3.6-4; Miller and Kaspar (1994) showed survival in same substrate pH 3.7-4.1 for 14-21 days at 4 ºC and a strain even survived for 24 hr in an trypticase soy broth pH 2.

The behavior of this bacterium at different storage temperatures and incubation periods shows resistance and multiplication at low temperatures, especially when stored at 12-22 ºC. Doyle (1991) has described the survival of this bacterium in frozen ground beef. Richert *et al.* (2000) report its survival on produce held at 4 ºC and its growth at 15 ºC and previous

Costarrican work demonstrates the survival and multiplication of this bacterium even at 0 ºC (Arias *et al.* 2000)

Sperber (1983) has reported that *E. coli* O157: H7 generally does not survive at a water activity (aw) < 0.95. Contrasting with this fact, an outbreak in 1993 in Oregon was linked to salad dressing made with commercial mayonnaise and in 1994 dry-cured salami was implicated in an outbreak of haemorrhagic colitis caused by this bacterium. Since the 1993 outbreak, questions regarding the ability of this bacterium to survive in various mayonnaise formulations have been raised. Nevertheless, the results have been controversial.

In Costa Rica, as in many other countries, mayonnaise is part of customary diet of the population. Therefore, this study was designed to evaluate the survival of low (10^4-10^6 CFU/g) and high (10^7 – 10^8 CFU/g) populations of *E. coli* O157: H7 inoculated in samples of meat, poultry and vegetables, mixed with different mayonnaise concentrations.

MATERIALS AND METHODS

Inoculum preparation: A strain of *E. coli* O157: H7 isolated from a human being was used. Stock cultures were maintained at –70 ºC on tryptic soy agar slants (Oxoid) and were activated in tryptic soy broth (pH 7.0) at 35 ºC. Culture was transferred by loop inocula twice at 24 hr intervals to 100 ml of tryptic soy broth in 250 ml Erlenmeyer flasks. Appropriate dilutions were performed in peptone water 0,1 % in order to obtain high inoculum level (10^7-10^8 CFU/ml) and low inoculum level (10^4-10^6 CFU/ml) populations.

Foods: Meat, poultry and packed chopped cabbage were obtained from a local distributor in San José, Costa Rica, between November 1999 and March 2000. All foods were kept at 2-5 ºC from the time of purchase to the onset of experiments, not more than 4 hr later. Meat and poultry were chopped aseptically in order to avoid large bacterial loads and standardizing the size and width variables.

Commercial mayonnaise that contained soybean oil, eggs, vinegar, water, egg yolk, salt, sugar, lemon juice [pH 3.8 and water activity (aw) 0.925] was also acquired from a detail local grocery, and was used to prepare salads containing 0 to 56 % wt/wt mayonnaise.

Procedure for preparing and inoculating foods: Approximately 1 kg of meat, chopped cabbage and poultry was divided in four portions (250 g each), placed in sterile polyethylene bags, and mixed with enough mayonnaise in order to obtain 18 %, 37 % and 56 % (wt/wt) mixtures. A control without mayonnaise was included in each experiment.

Each food sample was inoculated with 25 ml of high population of *E. coli* O157: H7 and pummeled in Stomacher for 2 min. Immediately thereafter, an initial *E. coli* O157: H7 count was performed, and samples were incubated at 12 ºC for 24, 48 and 72 hr. Temperature was chosen to simulate common salad bar temperatures.

Same procedure was used for meat, chopped cabbage and poultry inoculated with 25 ml of low level inoculum each.

Three independent trials were performed for each food analyzed. For each trial, a non inoculated control was included.

Enumeration of *E. coli* O157: H7: The *E. coli* O157: H7 enumeration was done according to the methodology described in the Bacteriological Analytical Manual (Anonymous 1995). Immediately after each incubation period, 25 g samples of salad were removed and mixed with 225ml of sterile peptonated water 0.1 % in 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). SMA plates were incubated at 35 ºC for 20 to 22 hr before colonies were counted. The same procedure was used for control samples (FDA 1995).

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

Aw measurements: The aw of each food mixture was measured according to the AOAC official method 978.18 (FDA 1995).

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

RESULTS

Meat: Meat samples with 18 % mayonnaise, inoculated with 10^{6-8} *E. coli* O157: H7/g, showed a significant increase ($p < 0.05$) in bacterial population after 48 hr incubation. This population was reduced by almost one logarithm after 72 hr incubation. A different behavior was observed in samples containing 37 % fat, where *E. coli* O157: H7 population showed a constant and significant increase ($p < 0.05$); after 72 hr of incubation an increase of almost 4 log units was observed. The population of *E. coli* O157: H7 showed a reduction only in the samples having 56 % fat after 72 hr incubation.

In the low inoculum samples, the population of *E. coli* O157: H7 showed an increase of almost 1 log after 72 hr incubation. This increase was independent of the fat concentration. In the samples with 18 % and 37 % fat, the population present at 72 hr of incubation was slightly lower than the one determined at 48 hr incubation.

The averaged pH showed a slight drop during the storage period (6.7 to 5.7). The averaged aw was 0.987.

Poultry: A significant increase ($p < 0.05$) in *E. coli* O157: H7 was determined in high and low inoculum samples. The increase was independent of fat concentration. On the average, an increase of 2 log units was observed after 72 hr incubation. This increase was steady at low inoculum samples. On the contrary, in high inoculum samples, the population evidenced at 72 hr incubation was slightly lower than the one determined at 48 hr.

The averaged pH showed a slight drop during the storage period (6.9 to 5.9). The averaged aw was 0.983.

Vegetables: The high population inoculum of *E. coli* O157: H7 in the vegetable samples with 37 % or 56 % fat was constant after 72 hr incubation. Only the samples having 18 % fat showed and important reduction of 2 log units in the population ($p < 0.05$) after 72 hr.

In the low population inoculum sample with 37 % fat, a one log reduction was observed after 72 hr. In the samples containing 18 % and 56 % fat, the population increased approximately by 1 log unit after 72 hr incubation.

The averaged pH showed a slight drop during the storage period (4.1-3.4), and the averaged aw was 0.995.

DISCUSSION

The ability of *E. coli* O157: H7 to survive in different environmental conditions has been well documented (An-Hung *et al.* 1995, Ruscica and Sobol 1995, Arias *et al.* 2000). Deng *et al.* (1998) emphasized the ability of this bacterium to survive in dry foods, with a wide range of aw and pH values, particularly at refrigeration temperatures. Nevertheless, the capacity of this pathogen to survive in high concentration fat foods, such as mayonnaise, has been controversial.

Weagant *et al.* (1994), Zhao and Doyle (1994) and Hatchox *et al.* (1995) demonstrated that *E. coli* O157: H7 could survive in mayonnaise commercially prepared for up to 35 days at refrigeration temperatures. However, Raghubeer *et al.* (1995) did not detect this bacterium in inoculated mayonnaise incubated at 22 ºC after 96 hr.

Our results show that *E. coli* O157: H7 can survive and even multiply in meat, poultry and vegetables mixed with different concentrations of mayonnaise. Both inocula evaluated demonstrated a multiplicative trend in the different foods evaluated, despite the concentration of mayonnaise used during the first 48 hr of incubation at 12 ºC. Even more, the low inoculum population tested showed multiplication even after 72 hr incubation. The high inoculum population shows a slight decrease after 48 hr

incubation, a behavior that can be explained based on the competition for nutrients and the effect of different emerging metabolites.

Recently, Abdul Raouf *et al.* (1993), have reported that populations of *E. coli* O157: H7 (10^5 CFU/g) inoculated in ground, roasted beef salads containing up to 40 % mayonnaise and held at 5 ºC showed no changes even after 72 hr of incubation. They have suggested that the dilution of antimicrobial effects of mayonnaise, owing to added food, can be one of the factors that explains the microorganism's survival.

The present study was done at 7 ºC above the incubation temperature defined by Abdul Raouf *et al.* Our results show that this slight increase of incubation temperature (from 5 to 12 ºC) significantly stimulates bacterial growth and not only survival. We propose that, together with the dilution of antimicrobial effects of the agents present in mayonnaise, the principal cause of survival and growth of this bacteria at such high fat concentrations is due to the aw increase in the mixture. The aw of mayonnaise is < 0.95, a level where *E. coli* O157: H7 can not survive (Sperber 1983). Nevertheless, in the food-mayonnaise mixture evaluated, the aw increased significantly up to 0.98 or more, optimal for microorganism growth (Rocelle *et al.* 1996).

Acetic acid, present in most mayonnaise formulations at concentrations ranging from 0.31 to 0.32 % has been defined as an agent capable of delaying microbial growth. Swaminathan *et al.* (1981) reported that mayonnaise had a significant inhibitory effect over the growth of *Salmonella typhimurium* in turkey meat sandwiches. Likewise, *Listeria monocytogenes* showed a rapid decrease in number when mixed with commercial, reduced-calories mayonnaise (Glass and Doyle 1991). Such was not the case with *E. coli* O157: H7 in this study. Although the mayonnaise evaluated contained acetic and citric acid, it had no effect over the bacterial survival and growth as described by Buchanan and Edelson (1999), who showed the acidic resistance response to malic, citric, lactic and acetic acid adjusted to pH 3 with HCl.

It is unlikely that the bacterial concentrations tested in this study can naturally occur. However, *E. coli* O157: H7 can survive in different concentrations of mayonnaise, despite of the final pH or storage temperature. Findings from our study on meat, poultry and vegetables mixtures with up to 56 % mayonnaise indicate that the acidity and pH achieved in such formulation can not be relied upon to control the growth of *E. coli* O157: H7.

Diverse authors, including Farber (1991) and Buchanan and Doyle (1997) conclude that the most effective way of reducing the risk associated with *E. coli* O157: H7 and other pathogens is through implementation of the "Hazard Analysis Critical Control Point" (HACCP) system in food industries. At the same time, the prevention of cross contamination and unsanitary handling practices by food manufacturers and consumers are critical in controlling the contamination of food and minimizing the risk of human infection.

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RESUMEN

En los últimos 20 años, *Escherichia coli* O157: H7 ha emergido como un nuevo patógeno, causando enfermedad, muerte y pérdidas económicas mundialmente. Diversos estudios han revelado que este patógeno posee importantes características de sobrevivencia, no obstante, existe criterio divergente respecto a su habilidad para sobrevivir en varias formulaciones basadas en mayonesa. Se procedió a estudiar el efecto de diversas concentraciones de mayonesa (0 %, 18 %, 37 % y 56 %) (p/p) en la sobrevivencia de la bacteria en alimentos comunes de un ambiente neotropical (Costa Rica). Una población alta de *E. coli* (10^7-10^8 UFC/ml) y una baja (10^4-10^6 UFC/ml) fueron inoculadas, en tres ocasiones diferentes, en carne picada, repollo picado y pollo picado y mezcladas con mayonesa comercial, con el fin de obtener las concentraciones estipuladas. Estas muestras fueron incubadas a 12 ºC por 24, 48 y 72 hr. 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 una tendencia a aumentar durante las primeras horas de incubación (48 hr) a pesar de las concentraciones de grasa utilizadas. Los hallazgos de este estudio indican que la *E. coli* O157: H7 es capaz de sobrevivir y crecer en ensaladas de carne, repollo, y pollo. Se debe tener precaución para evitar el falso sentimiento de seguridad derivado del uso de mayonesa en ensaladas.

REFERENCES

Abdul-Raouf, U., L. Beuchat & M. Ammar. 1993. Survival and growth of *Escherichia coli* O157: H7 in ground, roasted beef as affected by pH, acidulant and temperataure. Appl. Environ. Microbiol. 59: 2364-2368.

An-Hung, F., J. Sebranek & E. Murano. 1995. Survival of *Listeria monocytogenes, Yersinia enterocolitica* and *Escherichia coli* O157: H7 and quality changes after irradiation of beef steaks and ground beef. J. Food Sci. 60: 972-977.

Anonymous. 1995. Bacteriological analytical manual. Food and Drug Administraton, Maryland, 400-429 p.

Anonymous. 1997. Verocytotoxin producing *Escherichia coli* (enterohemorrhagic *E. coli*) infections, Japan, 1996-1997. Infect. Agents Surveil. Rep. 18: 153-154.

Arias, M.L., R. Monge, C. Chaves & F. Antillón. 2000. Effect of different storage temperatures on the growth and survival of *Escherichia coli* O157: H7 inoculated in beef, milk, vegetables and chicken giblets in Costa Rica. Rev. Biol. Trop. 49: 517-524.

Buchanan, R. & S. Edelson. 1999. pH –dependent stationary-phase acid resistance response of enterohemorrhagic *Escherichia coli* in the presence of various acidulants. J. Food Prot. 62: 211-218.

Buchanan, R. & M. Doyle. 1997. Foodborne disease significance of *Escherichia coli* O157: H7 and other enterohemorrhagic *Escherichia coli*. Food Tech. 5: 69-76.

Deng, Y., J Ryu & L. Beuchat. 1998. Influence of temperature and pH on survival of *Escherichia coli* O157: H7 in dry foods and growth in reconstituted infant rice cereal. Int. J. Food Microbiol. 45: 173-184.

Doyle, M. 1991. *Escherichia coli* O157: H7 and its significance in foods. Int. J. Food Microbiol. 12: 289-302.

Farber, J. 1991. *Listeria monocytogenes*. J. AOAC 74: 701-701.

Glass, K.A. & M. Doyle. 1991. Faint of *Salmonella* and *Listeria monocytogenes* in commercial reduced calorie mayonnaise. J. Food Prot. 54: 691-695.

Hathcox, A.L. Beuchat & M. Doyle. 1995. Death of enterohemorrhagic *Escherichia coli* O157: H7 in real mayonnaise and reduced calorie mayonnaise dressing as influenced by initial population and storage temperature. Appl. Environ. Microbiol. 61: 4172-4177.

Herrera, M.L. 1998. Incidencia de casos de *Escherichia coli* O157: H7 en Costa Rica. Rev. Hosp. Nac. Niños.

Mead, P & P. Griffin. 1998. *Escherichia coli* O157: H7. Lancet 352: 1207-1212.

Miller, L. & W. Kaspar. 1994. *Escherichia coli* O157: H7 acid tolerance and survival in apple cider. J. Food Prot. 57: 460-464.

Raghubeer, E., K Campbell & S. Meyer. 1995. Fate of *Escherichia coli* O157: H7 and other colifoms in commercial mayonnaise and refrigerated salad dressing. J. Food Prot. 58: 13-18.

Richert, K., J. Albrecht, L. Bullerman & S. Sumner. 2000. Survival and growth of *Escherichia coli* O157: H7 on broccoli, cucumber and green pepper. Dairy Food Environ. Sanit. 20: 24-28.

Ruscica, M. & R. Sobol 1998. *Escherichia coli* enterohemorrágica. Alim. Latinoamer. 208: 35-40.

Sperber, W. 1983. Influence of water activity on foodborne bacteria. A review. J. Food Prot. 46: 142-150.

Swaminathan, B., J. Howe & B. Essming. 1981. Mayonnaise sandwiches and *Salmonella.* J. Food Prot. 44: 115-117.

Weagant, S., J. Bryant & D. Bark. 1994. Survival of *Escherichia coli* O157: H7 in mayonnaise-based sauces at room and refrigerated temperature. J. Food Prot. 57: 629-631.

Zhao, T. & M. Doyle. 1994. Fate of enterohemorrhagi *Escherichia coli* O157: H7 in commercial mayonnaise. J. Food Prot. 57: 780-783.

Zhao, T., M. Doyle & R. Besser. 1993. Fate of enterohemorrhagic *Escherichia coli* O157: H7 in apple cider with and without preservatives. Appl. Environ. Microbiol. 59: 2526-2530.