Occurrence of *Salmonella* spp and *Escherichia coli* O157:H7 in raw meat marketed in São Paulo city, Brazil, and evaluation of its cold tolerance in ground beef

Ocorrência de *Salmonella* spp e *Escherichia coli* O157:H7 em carnes cruas comercializadas na cidade de São Paulo, Brasil e tolerância a baixas temperaturas em carne moída

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Miyoko JAKABI[1*]; Dilma Scala GELLI[1]; Christiane Asturiano RISTORI[1]; Ana Maria Ramalho DE PAULA[1]; Harumi SAKUMA[1]; Giselle Ibette S Lopez LOPES[1]; Sueli A. FERNANDES[2]; Renata B. LUCHESI[3]

* Corresponding author: Instituto Adolfo Lutz, Av. Dr.Arnaldo nº 355, Cerqueira César, 01246-902, São Paulo, S.P. Brasil. e-mail: mijakabi@ial.sp.gov.br

[1] Food Microbiology Laboratory
[2] Enteropathogens Laboratory
[3] Culture Medium Laboratory

ABSTRACT

Among foodborne diseases, salmonellosis has been considered one of the major public health problems in many countries worldwide. *Escherichia coli* O157:H7 has been another important foodborne pathogen due to its increasing incidence as a human disease agent and its association with various kinds of foods, especially those from animal origin. Incidence of *Salmonella* spp. and *E. coli* O157:H7 in the raw meat (bovine, swine and chicken) marketed in São Paulo - Brazil was determined in 253 samples. Twenty-three samples (9.1%) were positive for *Salmonella* spp. Among these, 11 different serovars were identified, and *S.* Enteritidis was found to be the most prevalent. All the tested samples were negative for *E. coli* O157:H7. *Salmonella* Enteritidis (the predominant serovar) and *E. coli* O157:H7 were also tested for their cold-temperature sensitiveness in ground beef kept under refrigeration (4ºC) for 120 days and under freezing (-18ºC) for up to 90 days. *E. coli* O157:H7 inoculated into ground beef was more sensible to refrigeration and freezing temperatures than *S.* Enteritidis, but both maintained viability under freezing condition up to 90 days.

Key Words. *Salmonella* spp.; *Escherichia coli* O157:H7; raw meat; resistance; freezing; refrigeration; cold tolerance.

RESUMO

Mundialmente, as salmoneloses são consideradas um grande problema de saúde pública. *Escherichia coli* O157:H7 é outro importante patógeno devido ao aumento da incidência de doenças em humanos e sua associação com vários tipos de alimentos, principalmente os de origem animal. Foi determinada a incidência de *Salmonella* spp. e *E. coli* O157:H7 em 253 amostras de carnes cruas (bovina, suína e de frango) comercializadas em São Paulo, Brasil. Vinte e três amostras (9,1%) foram positivas para *Salmonella* spp. Entre estas 11 diferentes sorovares foram identificados e *S.* Enteritidis foi o mais prevalente. Todas as amostras foram negativas para *E. coli* O157:H7. A sobrevivência de *S.* Enteritidis (o sorovar predominante) e *E. coli* O157:H7 foi avaliada em carne bovina moída mantida sob refrigeração (4ºC) por 120 horas e congelamento (-18ºC) por até 90 dias de armazenamento. *E. coli* O157:H7 foi mais sensível que a *S.* Enteritidis nas temperaturas de refrigeração e congelamento, mas os dois patógenos se mantiveram viáveis até 90 dias de estocagem sob congelamento.

Palavras-Chave. *Salmonella* spp.; *Escherichia coli* O157:H7; carne crua; resistência; congelamento, refrigeração.

INTRODUCTION

Salmonellosis is considered a major public health problem in many countries worldwide. Many foods, particularly those of animal origin, are important vehicles for *Salmonella* transmission. Animal carriers have an important epidemiological role in salmonellosis, as the microorganism is continuously discharged into the environment through animal feces. Conditions associated with beef production, transportation to slaughterhouses, and meat processing may favor dissemination of pathogenic microorganisms. Inside processing plants and food preparation areas, several operations may also favor cross-contamination[4]. At the food preparation area, cross-contamination could occur if storage time and temperature conditions are abusive[33].

Nowadays, *Salmonella* is one of the microorganisms most frequently involved in foodborne disease outbreaks[2,24]. Tavechio et al[31] reported that in S. Paulo, Brazil, between 1991 and 1995, *S.* Enteritidis was predominant from non human source. This serovar appeared in 1994 associated with foodborne disease outbreaks[17]. In 2000, in S. Paulo were notified 27(13,7%) outbreaks of foodborne diseases caused by *Salmonella* with 602(12,2%) cases[6].

E. coli O157:H7 is another important foodborne pathogen because of its increasing incidence as a human disease agent and because of its association with various kinds of food, mainly those of animal origin, in many countries. Since 1982, when *E. coli* O157:H7 was first identified as a cause of foodborne illnesses in the United States, many outbreaks have been reported[3,5,12].

This bacterium is an important pathogen in the USA, Europe, and Japan and in some South Hemisphere countries such as Argentina, Australia, Chile, and South Africa. In Argentina, *E. coli* O157:H7 has been associated with hemolytic uremic syndrome (HUS) patients and children presenting bloody diarrhea[19]. In S. Paulo, in 1990, one strain of *E. coli* O157:H7 was isolated from an AIDS patient presenting diarrheal disease. In 2001, two cases of *E. coli* O157:H7 illness were reported, both in Campinas, São Paulo State, in June and July[15]. However, the isolation of the pathogen as an agent responsible for outbreaks of foodborne diseases has not yet been reported[7].

It is now recognized that refrigeration is not sufficient to avoid the growth of a number of foodborne pathogens, including enterohemorragic *E. coli*.

The purpose of this study was to verify the incidence of *Salmonella* spp. and *E. coli* O157:H7 in the raw meat marketed in S. Paulo city, Brazil, and to test the most frequent serovars of *Salmonella* and *E. coli* O157:H7 for their cold resistance in ground beef under refrigeration and freezing.

MATERIAL AND METHODS

Samples

There was a total of 253 samples of raw meat (bovine, swine and chicken) purchased at local supermarkets in São Paulo, Brazil. Samples were placed into plastic bags, and transported under refrigeration to the Food Microbiology Laboratory of Instituto Adolfo Lutz, in São Paulo, Brazil.

Isolation and Identification of *Salmonella.*

Conventional methods were used, as recommended by ISO[16], although there was some modification in the incubation temperature of selenite cystine broth.

Pieces of beef, pork, and chicken meat were analyzed through homogenization technique and chicken carcasses through rinsing.

Pre-enrichment was performed by incubating buffered peptone water (BPW) overnight at 35ºC. Selective enrichment was performed using selenite cystine (SC) and modified Rappaport-Vassiliadis (RV) broth incubated at 42ºC/24-48h[14]. Each enrichment broth was streaked onto selective agar plates: *Salmonella-Shigella* agar (SS), brilliant green agar (BGA) and bismuth sulfite agar (BSA), and incubated for 24h at 35ºC (SS and BGA) and for 48h at 35ºC (BSA). Typical colonies from each plate were biochemically tested using Instituto Adolfo Lutz presumptive medium (IAL), developed for Enterobacteriaceae characterization[25], incubated for 24h at 35ºC, and serologically tested using polyvalent somatic and flagellar antisera produced by the Bacteriology Laboratory of Instituto Adolfo Lutz. The positive strains were sent to that Laboratory for complete serotyping[26].

Isolation and Identification of *E. coli* O157:H7

Conventional methods were used, as recommended by the American Public Health Association (APHA)[13], including the step of resuscitation[21,33].

Meat samples were homogenized with BPW and incubated at 35ºC for 6h. Each sample was plated onto MacConkey-sorbitol agar and incubated at 35ºC for 24h. At least three colonies were subjected to biochemical and serological tests.

Evaluation of refrigeration and freezing effects on *Salmonella* Enteritidis and *E. coli* O157:H7 inoculated in ground beef.

Samples

Samples of ground beef were purchased at local supermarkets in São Paulo, Brazil.

Strains

Salmonella Enteritidis was isolated in the Food Microbiology Laboratory of Instituto Adolfo Lutz, São Paulo, Brazil, from food involved in foodborne disease outbreaks.

E. coli O157:H7 IAL 1848 was obtained from the Culture Collection Laboratory at the same Institute.

Preparation of cultures/Sample contamination

S. Enteritidis and *E. coli* O157:H7 strain were cultivated in Brain Heart Infusion (BHI) at 35ºC for 18-24 hours, and after incubation, serial dilutions until 10^{-4} were made in BPW, and

4,5 mL of this dilution was inoculated into 450g of ground beef. Each pathogen was cultivated and inoculated into ground beef separately.

Following homogenization by hand massaging of the plastic bags, samples were separated into 10 portions of 25g and placed into sterile plastic bags. One portion was analyzed immediately and the others held for 24, 48, 72 and 120 hours (at 4ºC) and 1, 7, 30, 60 and 90 days (at -18ºC) before analytical quantification.

Analysis of inoculated ground beef samples

Each 25g portion of the ground beef sample was homogenized with 225 mL of a BPW in a stomacher 400 Laboratory Blender (Seward Medical Ltd., London, England) and subsequent decimal dilutions were made using the same diluent. Enumeration of *S.* Enteritidis and *E. coli* O157:H7 was performed using Most Probable Number (MPN) procedure according to APHA[23]. Isolation and identification were performed according to the same procedures as described in 2 and 3, above.

Three separate trials were performed for each strain of *S.* Enteritidis and *E. coli* O 157:H7.

RESULTS AND DISCUSSION

From 253 samples of raw meat, 23 (9.1%) were positive for *Salmonella* spp. Out of these 23 samples, 19.6% were swine sausage, 8.7% were swine meat, 7.3% were bovine meat, and 4.6% were chicken (Table 1). A total of eleven serovars of *Salmonella* spp. were isolated (Table 2). *S.* Enteritidis was isolated from 8 samples, followed by the *S.* Typhimurium and *S.* I 4,5,12:i:- both isolated from three samples. According to Tavechio et al[31], during the period from 1991 to 1995, *S.* Enteritidis was largely isolated from human sources.

Several researchers investigated the prevalence of *Salmonella* spp. in raw meat and isolated various *Salmonella* serovars, with findings appointing *S.* Enteritidis as the most prevalent[9,11,20,31]. In São Paulo city, Paula et al[22] detected 22.5% *Salmonella* spp. in 200 samples of raw meat, and *S.* Enteritidis was the most frequent serovar (28.8%).

All the samples tested in this study were negative for *E. coli* O157:H7. Silveira et al[30] analyzed 886 samples of hamburguers from different slaughterhouses in the South and Southeast regions of Brazil, and Silva et al[29] analyzed 340 samples of meat products and the industrial environment of meat manufacturers in the same regions. They did not find the pathogen. In Brazil the prevalence of *E. coli* O157:H7 in healthy dairy cattle has also been very low[8].

Figures 1 and 2 show the results of refrigeration on *E. coli* O157:H7 and *Salmonella* Enteritidis, respectively, inoculated in ground beef, and Figures 3 and 4 illustrate the freezing effects. Our findings showed that the population of *E. coli* O157:H7 was sensitive to refrigeration and freezing temperatures. In both cases, the initial population was reduced in 5,4 and 5,6 log10 MPN/g respectively, and data from literature indicates that *E. coli* O157:H7 populations are also affected by freezing[28]. Those researchers concluded that the death of *E. coli* O157:H7 in ground beef after freezing and thawing ranged from 0.62 to 2.52 log10 CFU/g. On the other hand, Saad and Franco[27], observed that the counting of *E. coli* O157:H7 inoculated in ground beef kept under refrigeration was relatively constant throughout the 96 hours the experiment lasted, and Doyle and Schoeni[10] showed that *E. coli* O157:H7 in ground beef can survive up to nine months at –20ºC. The presence of high levels of total aerobes (>7 $\log_{10}$ CFU/g) in ground beef stored at 2ºC could result in unfavorable changes in the environment and it could consequently affect the survival of *E.coli* O157:H7[1].

A population of *Salmonella* Enteritidis subjected to refrigeration was reduced in 0,3 log10 MPN/g after 120 hours of experiment. When subjected to freezing, the initial population was reduced in 0,6 log10 MPN/g. Figure 2 illustrates the growth of *S.* Enteritidis in the samples up to 48 hours after the beginning of the experiment. According to Varnam and Evans[33], the growth of *Salmonella* strains at temperatures below 7ºC depends on strain type and serovar, and populations are reduced when subjected to freezing temperatures. Teagasc[32] observed the

Table 1. Incidence of *Salmonella* spp. in raw meats at retail level in São Paulo city, Brazil

Samples	Analyzed Samples	Positive Samples
Beef	96	7
Chicken	65	3
Swine	23	2
Raw pork sausage	56	11
Raw chicken sausage	13	0
Total	253	23

Table 2. Serovars of *Salmonella* isolated from raw meats at retail in São Paulo city, Brazil

Serovars	Samples (number)
S. Enteritidis	8
S. Typhimurium	3
S. I 4.5,12:i:-	3
S. London	2
S. Infantis	1
S. Agona	1
S. Derby	1
S. Panama	1
S. Senftenberg	1
S. I 13.23:z:-	1
S. Emek	1
Total	23

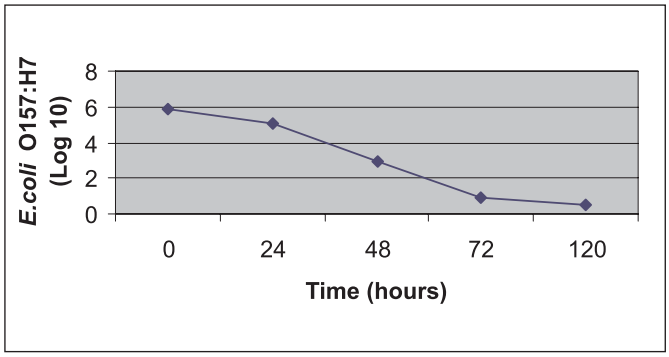

Figure 1. Survival of *E. coli* O157:H7 inoculated into ground beef stored at 4ºC for 120 hours.

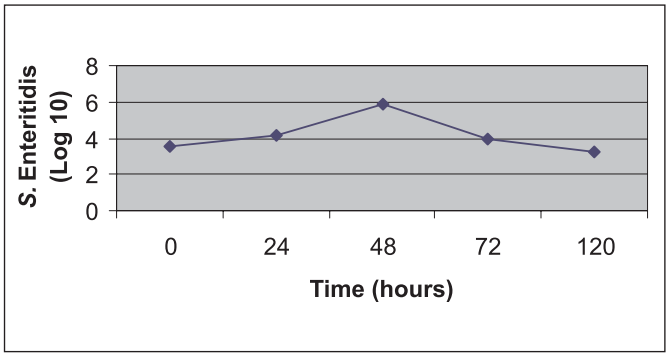

Figure 2. Survival of *Salmonella* Enteritidis inoculated into ground beef stored at 4ºC for 120 hours.

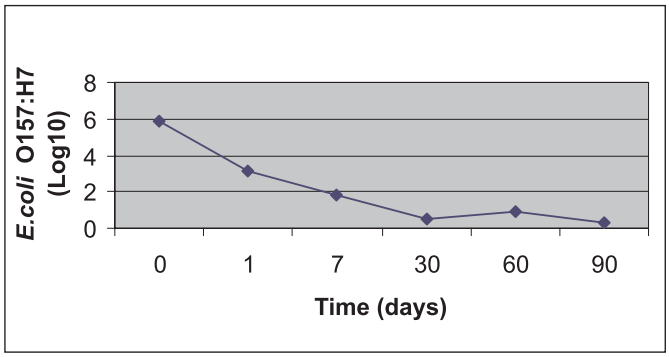

Figure 3. Survival of *E. coli* O157:H7 inoculated into ground beef stored at -18ºC for 90 days.

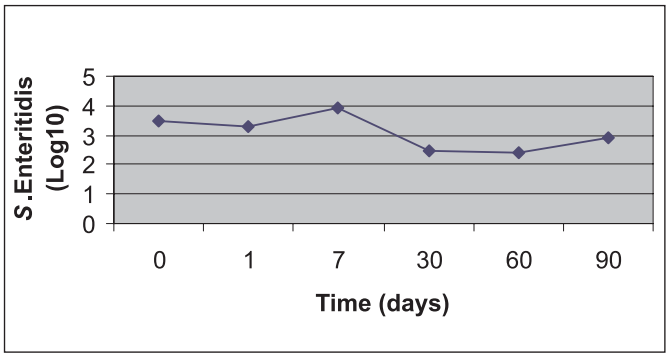

Figure 4. Survival of *S.* Enteritidis inoculated into ground beef stored at -18ºC for 90 days.

influence of different types of meat (minced beef, pork and lamb) and of varied fat levels in frozen minced beef (adding 5, 10, 20, 30, and 50% of fat to the meat) on the survival of *Salmonella* Kentucky and *Staphylococcus aureus* subjected to freezing (-35ºC) and stored for 10 weeks. Both pathogens showed increased survival rates when the level of fat was up to 30% for *S.* Kentucky and 20% for *S. aureus*. A fat level of 50%, however, caused a reduction in the survival rates, which were then similar to the rates observed in lean meat. The pathogen survival rates also varied according to the meat type, being highest on pork and lowest on beef. These results show that, at first, fat had a cryoprotective effect, but increased levels of it caused a reduction in the pathogen survival rate.

Freezing sensitivity of microorganisms belonging to the same genera and species can vary depending on strain type, type of freezing and freezing temperature[18], cell age (i.e. exponential or stationary phase), bacterial type, Gram negative or positive, nature of suspending medium (i.e. fish, meat or vegetables), storage length, and thawing conditions[32]. Among genera, however, this sensitivity can differ, as was shown in the present study, with *Salmonella* presenting higher resistance than *E. coli* O157:H7.

The survival of *E. coli* O157:H7 and *S.* Enteritidis in ground meat stored at refrigeration and freezing temperatures indicates that this food should be considered hazardous if it is contaminated with these pathogens.

CONCLUSIONS

All analyzed samples were negative for *E. coli* O157:H7, which is in agreement with current literature. In Brazil, there has been no report on the occurrence of foodborne outbreaks due to shiga-like toxin producing *E. coli*.

S. Enteritidis was the predominant serovar isolated from raw meat.

One strain of *S.* Typhimurium and one of *S.* Emek were lysine decarboxylase negative (LDC); this occurrence is not frequent, but it is an important consideration for its identification, since it can lead to it being misdiagnosed as *Citrobacter* sp.

Results of the current study suggest that the population of *S.* Enteritidis in experimentally inoculated ground beef were not influenced by refrigeration and freezing temperatures.

E. coli O157:H7 was sensitive to refrigeration and freezing temperatures, but survived up to 90 days under freezing.

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