# PREVALENCE OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP. IN FRESH MEAT AND MEAT PRODUCTS IN CATALUNYA, SPAIN

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## Introduction

*Escherichia coli* O157:H7 and *Salmonella* spp. are pathogenic bacteria that colonise the intestines of farm animals. They can contaminate meat and meat products, due to cross contamination during slaughtering or meat processing in packing plants. *Salmonella* is the most reported zoonotic pathogen in Europe (EFSA, 2006), as well as in the U.S. (CDC, 2003).

One of the first steps in risk assessment is exposure assessment, which includes an assessment of the actual or anticipated human exposure to the pathogen. Frequency of contamination of foods and the initial contamination of the raw material, including considerations of regional differences, are factors that must be considered for exposure assessment (JEMRA, 1999). Therefore, in order to carry on the risk assessment for these pathogens, there is a need for the acquisition of data on the frequency of contamination of foods at the point of consumption.

The aim of this study is to survey the presence of *E. coli* O157:H7 and *Salmonella* spp. in fresh meat and meat products commonly consumed in Catalunya.

## Materials and Methods

Collection of samples for the detection of *Salmonella* spp.: A total of 1025 samples consisting of 6 refrigerated fresh duck meat samples, 120 refrigerated fresh beef samples, 19 refrigerated fresh goat meat samples, 85 refrigerated fresh rabbit meat samples, 50 refrigerated fresh turkey meat samples, 30 refrigerated fresh quail meat samples, 187 refrigerated chicken samples, 121 refrigerated fresh pork samples, 121 refrigerated fresh lamb meat, 115 fresh pork sausage samples, 30 ground beef samples, 8 ground beef and pork samples, 74 refrigerated beef hamburger samples and 59 refrigerated chicken hamburger samples were collected.

Collection of samples for the detection of *E. coli* O157:H7: A total of 943 samples consisting of 6 refrigerated fresh duck samples, 119 refrigerated fresh beef samples, 18 refrigerated fresh goat meat samples, 54 refrigerated fresh rabbit meat samples, 29 refrigerated fresh turkey meat samples, 30 refrigerated fresh quail meat samples, 168 refrigerated chicken samples, 121 refrigerated fresh pork samples, 113 refrigerated fresh lamb samples, 114 fresh pork sausage samples, 30 ground beef samples, 8 ground beef and pork samples, 73 refrigerated beef hamburger samples and 60 refrigerated chicken hamburger samples were collected. All samples weighed 300g approximately, were representative of the most consumed fresh meat and meat products in the area, and were collected from retail stores or food industry in Catalunya, Spain, from 1998 to 2004. These samples were brought to the laboratory in a cooler, and immediately analyzed for the presence of *E. coli* O157:H7 or *Salmonella* spp. For the detection of *Salmonella*, 25 g of sample were placed in a sterile plastic bag with 225ml of buffered peptone water, and blended for 2 minutes in a stomacher lab blender. After incubation at 37ºC for 18 ± 2h, 0.1ml of the blend was placed in Muller Kauffmann tetrathionate-novobiocin (MKTTn) broth and was incubated at 37 ± 1ºC for 24 ± 3h. Ten ml of the blend was placed in Rappaport Vassiliadis soya (RVS) broth and was incubated at 41.5 ±1 ºC for 24 ± 3h. Aliquots from MKTTn broth and RVS broth were surface plated on xylose lysine deoxycholate (XLD) agar and Rambach agar and were incubated at 37 ±1ºC for 24 ± 3h. One to five typical colonies from each plate were inoculated in Kligler agar slants, and incubated at 37 ± 1ºC for 24 ± 3h. Typical colonies were further confirmed with API 20E strips, or were grown on nutritive agar at 37ºC for 24h for serological confirmation.

For the detection of *E. coli* O157:H7, a 25-g sample was placed in a sterile plastic bag with 225 ml of trypticase novobiocin broth, and blended for 2 minutes in a stomacher lab blender. After incubation at 37ºC for 23 ± 1 h, it went through an immunomagnetic separation using Dynabeads coated with polyclonal antibodies against *E. coli* O157. The obtained cell suspension was plated on tellurite-cefixime-sorbitol McConkey agar and incubated at 37ºC for 24h. Typical colonies were inoculated on Kliger agar slants and further incubated at 37ºC for 24h. Positive presumptive isolates where tested for β-D-glucuronidase activity. Negative isolates were further confirmed by latex agglutination test and positive cultures to latex agglutination test were confirmed using API 20E strips, or were grown on nutritive agar at 37 ºC for 24h for serological confirmation.

## Results and Discussion

Results of analysed samples are shown in Table 1. *E. coli* O157:H7 was isolated from 4 samples out of 943 samples of fresh meat and meat products. This pathogen was present in 3.3 % of refrigerated quail samples, but only in 0.6% of refrigerated chicken samples and 0.8 % of refrigerated pork and lamb samples. *E. coli* O157:H7 was not isolated from ground beef, a mix of ground beef and pork, beef and chicken hamburgers or fresh pork sausage.

*Salmonella* was isolated from 91 samples out of 1025 samples. This pathogen was present 0.8% of refrigerated beef samples, 2% of refrigerated turkey meat samples, 2.5% of refrigerated pork samples, 2.7% of refrigerated beef hamburgers, 3.3% of ground beef samples, 6.1% of fresh pork sausage, 11.8% of refrigerated rabbit samples, 12.5% of mixed ground beef and pork samples, 16% of refrigerated chicken samples, 22% of refrigerated chicken hamburger and 73.3% of refrigerated quail samples (Table 1).

**Table 1:** Prevalence of *E. coli* O157:H7 and *Salmonella* in fresh meat and meat products in Catalunya, Spain.

| Meat sample | *E. coli* O157:H7 | | *Salmonella* | |
|---|---|---|---|---|
| | Positive samples/analyzed samples | % positive samples | Positive samples/analyzed samples | % positive samples |
| Refrigerated duck | 0/6 | 0 | 0/6 | 0 |
| Refrigerated beef | 0/119 | 0 | 1/120 | 0.8 |
| Refrigerated goat | 0/18 | 0 | 0/19 | 0 |
| Refrigerated rabbit | 0/54 | 0 | 10/85 | 11.8 |
| Refrigerated turkey | 0/29 | 0 | 1/50 | 2 |
| Refrigerated quail | 1/30 | 3.3 | 22/30 | 73.3 |
| Refrigerated chicken | 1/168 | 0.6 | 30/187 | 16 |
| Refrigerated pork | 1/121 | 0.8 | 3/121 | 2.5 |
| Refrigerated lamb | 1/113 | 0.8 | 0/121 | 0 |
| Fresh pork sausage | 0/114 | 0 | 7/115 | 6.1 |
| Ground beef | 0/30 | 0 | 1/30 | 3.3 |
| Ground beef/pork | 0/8 | 0 | 1/8 | 12.5 |
| Refrigerated beef hamburger | 0/73 | 0 | 2/74 | 2.7 |
| Refrigerated chicken hamburger | 0/60 | 0 | 13/59 | 22 |

Results show that prevalence of *E. coli* O157:H7 and *Salmonella* in refrigerated quail meat was the highest of the analysed meat samples. Quails are consumed as fresh meat, they are not used to elaborate meat products and undergo a thorough cooking process before consumption. Given the small size of quails, it is very likely that the heat treatment reaches all the carcass points, and therefore the health significance of the high prevalence of these pathogens is reduced. On the other hand, results also show a high prevalence of *Salmonella* in fresh rabbit, fresh chicken and chicken hamburgers, all of which are food products that are cooked before consumption, and therefore, the probability of the actual intake of *Salmonella* is reduced. However, the probability of cross contamination in the processing plant should be taken into account.

## Conclusions

Microbial risk assessment involves exposure assessment (CAC, 2004), which is the degree of pathogen intake likely to occur. It also involves estimating the population at risk. Obtaining data on the prevalence of pathogens in different food products and different geographical areas should be a previous step in risk analysis. In Catalunya, *E. coli* O157:H7 was detected only in quail, chicken, pork and lamb, when analyzing fresh meat and raw meat products. However, the prevalence of *Salmonella* on fresh meat and raw meat products detected over 7 years, ranges from 0.8% to 73.3%. The pathogen was not isolated from fresh duck, goat and lamb.

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