The cross-contamination of *Salmonella enteritidis* on sterile and non-sterile meat

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**Abstract**

The ability of two strains of Salmonella enteritidis to cross-contaminate from inoculated egg droplets on surfaces of beef (sterile or non-sterile) was investigated. When the samples were placed on these surfaces where egg droplets were still wet, cross-contamination occurred within 1s onto every piece of food. It took at least 1 min for all the food pieces to be contaminated when egg droplets had been allowed to dry. Both strains were capable of rapid growth on beef (sterile or non-sterile) at 20°C, but growth rates appeared to be slowed by pre-exposure to either 4 or -18°C.

**Keywords:** *Salmonella enteritidis*, cross-contamination, egg, sterile, non-sterile, beef

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**1. Introduction**

Salmonella enteritidis phage type (PT) 4 continues to be of major importance in human foodborne salmonellosis, particularly in Europe (Schmidt 1995). Contaminated chicken meat and eggs have been identified as the most frequently implicated vehicles (Schmidt 1995). As with other salmonellas (Roberts 1986) cross-contamination in the kitchen may be an important contributory factor in Salm. enteritidis outbreaks. Previous work at this laboratory (Humphrey et al. 1994) demonstrated that, when contaminated egg contents were homogenized, Salm. enteritidis could be recovered from work surfaces over 40 cm away from the mixing bowl. The same study also showed prolonged survival of the bacteria in these droplets. Such practices could, therefore, facilitate cross-contamination of salmonellas onto other previously safe foodstuffs.

Contaminated surfaces are unlikely to be a direct hazard and for an outbreak to occur salmonellas must be able to move onto foodstuffs placed on such a surface and, perhaps, be given the opportunity to multiply.

This was investigated in the study reported in this paper using two strains of Salm. enteritidis PT4 with markedly different survival profiles on surfaces (Humphrey et al. 1995).

**2. Materials and methods**

*Salmonella isolates.* Two Salm. enteritidis PT4 isolates were used. Isolate E, which has been shown to have enhanced tolerance to heat, acid, hydrogen peroxide and to survive well on surfaces, came from a human case (Humphrey et al. 1995). Isolate I, which is significantly more sensitive to the above conditions (Humphrey et al. 1995), was obtained from a chicken carcass.

**Culture conditions.** Cultures were maintained on Blood Agar plates at 4°C with subculture onto fresh plates every 2 d. These were incubated at 37°C overnight and then stored at 4°C until required. These stock cultures were used in all of the following experiments.

**Broth cultures.** A single colony from the stock culture was inoculated into 9 ml of Lemco broth (Oxoid) and incubated at 37°C. Cultures were used 16–18 h after initial incubation and were in stationary phase. Approximately 5*10^8 cells ml\(^{-1}\) were present at this stage.

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Preparation of roast beef. Raw beef was purchased from a retail outlet and roasted in foil the same day. Standard cooking procedure was adhered to (25 min per 2.2 kg of meat plus another 25 min in an oven at 180°C). The outer layer was discarded and the those inner portion sliced and cut into 2 cm² pieces with three or five squares being placed in sterile petri dishes. Squares were inoculated with a nalidixic acid-resistant mutant of PT4 isolate E from a fresh culture broth, or a culture broth which had been held at either -18 or 4°C for 24 h. Contamination levels were either $10^2$, $10^3$ or $10^4$ cells per beef square. Contaminated squares were placed at 20°C with samples being removed at 0, 2, 4, 6 and 24 h for enumeration of the salmonellas by plating on xylose lysine desoxycholate (XLD) agar (Oxoid) containing 100 µg ml⁻¹ nalidixic acid. Plates were incubated at 37°C for 24 h.

Total counts were also performed by plating on CLED medium, which was incubated at 30°C for 24 h.

Cross-contamination experiments. Formica squares (2 cm²) were contaminated with the PT4 isolates suspended in homogenized whole egg as described previously (Humphrey et al. 1994). Contamination levels were either $10^5$ or $10^4$ cells per square. Squares were either used immediately or following storage at 20°C for 24 h. Sterile beef pieces were placed onto the contaminated egg droplets and removed after 1, 5, 10 and 30 s or 1, 3, 5, 10 and 30 min. The food pieces were examined for the presence of salmonellas by enrichment culture in BPW followed by plating on XLD which was incubated at 37°C for 18–24 h. Salmonella-like colonies were identified using standard serological techniques.

3. Results and discussion

Growth on beef. Since many foods are not sterile, it was considered important to determine whether *Salm. enteritidis* PT4 was able to compete with other, naturally occurring bacterial contaminants.

A nalidixic acid-resistant mutant of strain E was used so that the *Salm. enteritidis* cultures could be isolated from the other microorganisms on the beef. The mutant grew to high numbers on beef slices stored at 20°C. Growth appeared to be unaffected by the size of the inoculum and was essentially the same whether $10^2$ or $10^4$ cells were used. These observations are in agreement with those of Gill and DeLacey (1990) who reported growth of *Salm. typhimurium* on high pH beef contaminated with other bacteria. Marked variations were found in the levels of indigenous contamination between different cooked beef slices and between different areas on individual slice. The growth rate of PT4 isolate E was unaffected by differences in the levels of other microorganisms. When growth was measured over 24 h, growth on beef was unaffected by pre-exposure to either 4 or -18°C for 24 h. During the first 6 h, however, growth rates were measurably slower than that of control cultures with contamination levels remaining largely unchanged.

Cross-contamination. All samples of beef placed on either fresh or dry salmonella-positive egg droplets for longer than 1 min became salmonella-positive. This was unaffected whether PT4 isolate E or I was used or whether the initial contamination level in the egg droplets was $10^4$ or $10^5$ cells. However, samples placed on dry egg droplets containing isolate E were more likely to become salmonella-positive and this almost certainly is the result of this isolate’s ability to survive well in dried food materials adhering to surfaces (Humphrey et al. 1995).

4. Conclusions

Roberts (1972), Van Schothorst *et al.* (1976), De Wit *et al.* (1978) and De Boer and Hahne (1990) demonstrated that salmonellas from frozen broiler chicken carcasses can contaminate food preparation areas. Similar results were seen when contaminated egg was the vehicle (Humphrey *et al.* 1994).
The work presented in this paper demonstrates the ease with which organisms can transfer to and grow on foodstuffs held at ambient temperature. These observations give support to the work of Roberts (1986) who detailed the importance of cross-contamination and preparation too far in advance in outbreaks of salmonellosis. Not surprisingly, this present study also demonstrated that PT4 isolates better able to survive are more likely to cross-contaminate. It is of interest that isolate E came from a human case and has recently been shown to be significantly more virulent than isolate I in a mouse model (Humphrey et al. 1996). Pre-exposure to either 4 or -18°C, to mimic food exposure, did not affect the ability of either PT4 isolate to survive on food but did slow growth during the first few hours.

It could be argued that the use of stationary phase cultures, which are known to survive better under a range of damaging environments (Kolter et al. 1993; Grau 1994), might have exaggerated the possibilities of cross-contamination. Growth phase has no effect on the survival characteristics of either PT4 isolates E or I, although heat and acid tolerance, for example, were very different between log and stationary phase cultures (Humphrey et al. 1995).

References


