Growth and Virulence of *Salmonella typhimurium* Grown in Different Foods

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Experimental data indicate that the virulence of *Salmonella typhimurium* grown in different foods varies. The possibility exists, therefore, that ingestion of foods containing the same number of salmonellae may not constitute a similar health hazard.

The principal reservoir of *Salmonella* is the intestinal tract of vertebrate animals. It is from this source that the organism may be spread into air, water, soil, animal feed, human food, to invade other hosts (5). Further, a large number of salmonellae are necessary to cause food infection. The number present in a food, as a result of direct contamination, is normally not sufficient to cause salmonellosis. For salmonellosis to occur, therefore, it is essential that multiplication occur in the food (5). Although salmonellae can grow on a variety of foods, several physical and chemical characteristics of the growth menstruum have been shown to affect not only the amount and rate of growth of salmonellae (1, 3) but also their virulence (4).

In studying the effect of natural food menstrua on growth and virulence of *Salmonella typhimurium*, fresh 2- to 3-lb broilers (approximately 746 to 1,120 g) were deboned and subsequently roasted loosely covered with aluminum foil at 325°F for 40 to 50 min per pound. The chicken was then cooled and aseptically ground up. Portions of 30 to 40 g were dispensed into separate sterile plastic bags. Portions (30 to 40 g) of canned barbecue sauce (Cordon Bleu) (pH 5.2) and of canned spaghetti sauce with meat (pH 4.2) (Chef-Boy-Ar-Dee) were also dispensed into sterile plastic bags.

All portions were inoculated with from 4 to 8 cells per g (the number being established from preliminary experiments) from the second consecutive 48-h *S. typhimurium* ES 878 (2) grown in brain heart infusion broth (Difco). The inoculated products were stored at 20°C. No growth of cells occurred in the spaghetti sauce, even when high inoculum levels (7.9 × 10⁸/g) were used. The growth of cells (determined using standard techniques of homogenization-dilution in 0.85% saline followed by surface plating on plate count agar [Difco] and brilliant green agar [Difco]) in the barbecue sauce and chicken meat was similar, reaching the transitionary late log to early stationary phase of growth (10⁸ cells/g) after 48 h. The pH of the foods did not change appreciably during the incubation period.

At the different time intervals, portions of both foods were removed and homogenized in a Waring blender. Dilutions were made for enumeration of cells and for inoculation into the yolk sac of day-old chicks to assess virulence (2). With barbecue sauce, cells of an older physiological age were less virulent than cells of a younger physiological age (Table 1). The virulence of cells grown on ground cooked chicken meat did not vary significantly with

| Food commodity | Age of Cells | LD₅₀ values |
|----------------|--------------|-------------|
|                | Chronological (h) | Physiological |             |
| Barbecue sauce (pH 5.2) | 6 Early log | 5.0 × 10⁶ |
|                  | 24 Mid log | 4.0 × 10⁵ |
|                  | 48 Late log | 4.8 × 10⁴ |
|                  | 72 Stationary | 1.5 × 10⁴ |
| Ground, cooked chicken meat (pH 7.0) | 12 Early log | > 2.4 × 10⁴ |
|                  | 24 Mid log | 3.4 × 10⁴ |
|                  | 48 Late log | 4.3 × 10⁴ |
|                  | 72 Stationary | 4.8 × 10⁴ |

* Results are the average of three separate experiments run in duplicate. No deaths occurred in control chicks inoculated with either saline or uninoculated food.
age. This is in contrast to our previous results (4) in which cells grown at acid pH in culture medium increased in virulence with age, and cells grown at neutral pH decreased in virulence with increase in age. The surviving chicks all exhibited lower weight gains than the controls, and after sacrificing were found to contain viable salmonellae in their tissues. Infection, therefore, had occurred in all instances in chicks inoculated with \textit{S. typhimurium} (4).

From the present results, it would appear that extrapolation of virulence activity data, obtained with cells grown in one laboratory medium at different pH to indicate virulence activity in various natural foods at different pH, would be in error. Although pH may have in some manner influenced the virulence of the salmonellae in these more complex systems, the composition of the food, no doubt, also played an important role. Nevertheless, the results do clearly indicate that the virulence of \textit{S. typhimurium} (and perhaps other salmonellae as well) grown in different foods, inoculated in the same manner and with the same number of cells grown under identical conditions for the same period of time, may be markedly different. This being the case, the possibility may also exist that the ingestion of the one food could result in salmonellosis, whereas ingestion of the other, containing the same number of organisms, would not.

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