Research Note

Quantification of the Contamination of Chicken and Chicken Products in The Netherlands with *Salmonella* and *Campylobacter*

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ABSTRACT

The research described in this contribution provides quantitative data on contamination levels with *Salmonella* and *Campylobacter* in chicken and chicken products in The Netherlands at retail level using the most probable number method and direct counting. Most samples contained $<10$ *Salmonella* per carcass, both in fresh (89%) and frozen (68%) products, contamination levels with *Campylobacter* varied from $<10$ (18%) to more than 5,500 (18%) per fresh carcass. Most frozen samples (57%) contained $<10$ *Campylobacter* per carcass.

In The Netherlands, various studies have been performed to estimate the incidence of gastroenteritis and the relative importance of different pathogens. In the study from 1987 to 1991 in collaboration with general practitioners (GP) in two cities, an incidence was found of 15 GP consultations for gastroenteritis per 1,000 person years (8). In a national study done in cooperation with GPs from The Netherlands Institute of Primary Health Care (NIVEL) in 1992 and 1993 the incidence was estimated at nine GP consultations for gastroenteritis per 1,000 person years (6). During the first 2 years of a study on gastroenteritis in sentinel practices in The Netherlands (NIVEL) in 1996 to 1999 an incidence was calculated of 5.8 GP consultations for gastroenteritis per 1,000 person years (4).

*Salmonella* spp. were found in 5, 4, and 3.7% of stool samples in the study from 1987 to 1996 (8), the national study in 1992 and 1993 (6), and the first 2 years of the study in 1996 to 1999 (4), respectively; for *Campylobacter* spp. the corresponding percentages were 14, 15, and 10%.

*Salmonella* and *Campylobacter* are zoonotic pathogens, with many animal species serving as reservoirs. Since 1997 a monitoring system was implemented to obtain reliable data on the prevalence and trends of zoonotic agents in farm animals in The Netherlands. During the first year of monitoring of fecal droppings at flock/herd level of poultry, calves, and dairy cows, *Salmonella* spp. were isolated from 22.0% of the broiler flocks, from 15.3% of the layer flocks, and from 1.6% of the veal calves herds. *Salmonella* was not detected in dairy cattle herds. *Campylobacter* spp. were only monitored in broiler flocks, and 29.8% of the broiler flocks were found positive for *Campylobacter* spp. (7).

Since the beginning of the 1990s preventive measures have been introduced to reduce the number of *Salmonella* infections in production animals (1, 2, 5). More recently a model was developed that describes the transmission of *Salmonella* through the poultry meat production chain, including slaughter, for exposure analysis (11). From this study it was concluded that processes in the retail and consumer phase have to be a subject of further study and that quantitative data are lacking for performing exposure analysis.

Exposure assessment, as part of risk assessment (12), is essential to gaining insight in the risk of infection with a pathogen after consumption of a certain amount of contaminated product. In this paper we describe our attempt to quantify numbers of *Salmonella* and *Campylobacter* on chicken and chicken products at retail level in The Netherlands. The quantification was performed in two ways: first, by rinsing chickens (fresh and frozen) and using the rinsing fluid for a most probable number (MPN) method; second, after rinsing, samples of 10 cm$^2$ skin were used for direct counting.

MATERIALS AND METHODS

**Chickens and chicken products.** Fresh and frozen chickens were obtained from local stores. Fresh chickens were stored at 4°C and tested within a few days. Fresh chicken tested ($n = 45$) consisted of eight samples of chicken parts and 38 complete carcasses.

Frozen chickens ($n = 44$), whole carcasses only, were stored at −20°C until the start of the test. The storage time at −20°C differed from a few days to a few weeks. For thawing, frozen chickens were placed in a metal box and stored overnight at room
temperature. Fresh and frozen (after thawing) chickens (or chicken products) were cut into two equal pieces.

**Salmonella, MPN method.** One part was rinsed with 250 ml of buffered peptone water (BPW). After washing for 2 min, BPW was divided in 3 × 50 ml, 3 × 5 ml, and 3 × 0.5 ml (the 3 × 0.5-ml portions were added to 5 ml fresh BPW) and incubated at 37°C for 18 ± 2 h. Subsequently, from the BPW cultures, 0.1 ml was added to 10 ml Rappaport Vassiliades broth followed by incubation for 24 ± 2 h and 48 ± 2 h at 42°C. After 24 and 48 h, Rappaport Vassiliades cultures were streaked on brilliant green agar. Brilliant green agar was incubated for 24 ± 2 h at 37°C. Suspected colonies were tested on triple sugar iron medium with ureum agar and on lysine decarboxylase broth. After confirmation, the number of *Salmonella* present in the rinsing fluid was calculated using the MPN table of de Man (3).

**Salmonella, direct count.** As for *Salmonella*, after rinsing, a 10-cm² sample of skin was prepared, added to 10 ml Preston medium, and ground for 1 min by using ultra-turrax. After making decimal dilutions in peptone physiological salt, 0.1 ml of these dilutions was spread on CCDA plates. The CCDA plates were incubated microaerophilically at 42°C. The CCDA plates were screened for colonies suspected as *Campylobacter* spp. These colonies were tested via phase-contrast microscopy for the typical form (cork screw) of *Campylobacter*. The number of organisms present in the rinsing fluid was calculated by using the MPN table of de Man (3).

**Campylobacter, MPN method.** To the second part of the carcass or product, 250 ml of Preston medium was added, followed by washing for 2 min. The Preston medium was divided into 3 × 10 ml, 3 × 1 ml, and 3 × 0.1 ml. The 1- and 0.1-ml portions were brought into 10 ml fresh Preston medium. The inoculated media were incubated under microaerophilic conditions, using BBL CampyPak (Becton Dickinson, Paramus, N.J.) at 42°C for 18 ± 2 h, followed by streaking on charcoal cefoperazone deoxycholate agar (CCDA) plates. The CCDA plates were incubated under microaerophilic conditions for 48 ± 4 h at 42°C.

The CCDA plates were screened for colonies suspected as *Campylobacter* spp. These colonies were tested via phase-contrast microscopy for the typical form (cork screw) of *Campylobacter*. The number of organisms present in the rinsing fluid was calculated using the MPN table of de Man (3).

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**Statistical analysis.** MPN data according to de Man (3) give the number of bacteria present in the largest volume of a series of samples volumes. In order to calculate the number of bacteria present on a carcass, the number from the table is multiplied by [(total volume)/largest sample volume], giving the number of bacteria per half carcass, times 2, giving the number of bacteria per chicken or chicken product. The 95% confidence limits were multiplied by the same factor.

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**RESULTS AND DISCUSSION**

Using the MPN method we found that most of either fresh or frozen chicken carcasses or products (89, respectively 68%) contained <10 *Salmonella* per carcass (Table 1), assuming a random distribution of bacteria over each half of the carcass. Only a small percentage (2% fresh and 2% frozen) contained more than 1,100 *Salmonella* per
chicken. For one sample, no MPN could be estimated due to an unlikely range of positive results. After rinsing we were not able to detect Salmonella via direct count on the skin.

For fresh carcasses or products we found that approximately 38% was contaminated at a level of <100 Campylobacter per carcass. Thirty-four percent of the examined chicken contained more than 1,000 Campylobacter. Frozen carcasses were less contaminated with Campylobacter spp., 71% of the samples contained <100, and 9% contained >1,000 of this pathogen. For a relative large percentage of samples (16 and 14% of fresh, respectively, frozen), no MPN could be estimated, due to an unlikely set of results. This was probably due to the presence of a large number of competitive bacteria, whose growth was not suppressed sufficiently by selective agents in Preston medium and thus might have outcompeted Campylobacter in the largest sample volume. Confidence limits (95%) of MPNs are shown in Figure 1. By direct counting, we were able to detect 100 to 500 Campylobacter in six samples (Table 1).

Kotula and Pandya (9) performed a quantitative study on the presence of Salmonella spp. and Campylobacter spp. on the skin of broiler chickens directly after killing in the processing plant. The mean counts ranged for Salmonella from 5.4 to 6.9 $\log_{10}$ g$^{-1}$ skin and for Campylobacter from 5.7 to 7.9 $\log_{10}$ g$^{-1}$ skin. This is significantly higher than our results. It must be mentioned that they took samples before scalding. Uyttendaele et al. (13) conducted during four subsequent years a semiquantitative study on the contamination level of poultry and poultry products with Salmonella, at the beginning of the retail phase. Their results seem to be comparable with our findings although their method could only discriminate between samples containing none, low (1 CFU/100 cm$^2$ or 25 cm$^2$ or g), and high (>1 CFU/cm$^2$ or g) Salmonella spp. With our method it is possible to quantify infected samples more accurately, which is thus more suitable for exposure assessment.

The difference in level of contamination between fresh and frozen products with respect to Campylobacter can be explained by the protective microenvironment of the skin (10). Inclusion of Campylobacter during the defeathering into the feather follicles could provide protection against drying, oxygen, or other forms of stress.

Direct counting results indicate that there were at most a few attached organisms present; in only six cases Campylobacter was counted in the range of 100 to 500 (Table 1). However, the absence of countable colonies on the isolation plates could have been caused by overgrowth by...
competitive organisms. Therefore, in the case of low infection levels, isolation methods have to be improved in order to generate more reliable quantitative data.

Compared to Salmonella, chicken is more contaminated with Campylobacter, a finding that correlates with the level of incidence of gastroenteritis caused by either of these two pathogens. Regarding these contamination levels as shown in Table 1 and the fact that these kinds of products normally are consumed after being prepared well-done, cross-contamination directly from raw poultry or indirectly via contaminated surfaces or niches in the household kitchen of ready-to-eat products is the most likely mode of infection. Further studies to quantify the level of infection with Salmonella and Campylobacter of raw poultry in the consumer phase in performing exposure assessment should take into account the effect of the kitchen environment on the behavior of Salmonella and Campylobacter in food prepared by the consumer.

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REFERENCES