

# **Campylobacter Transfer from Naturally Contaminated Chicken Thighs to Cutting Boards Is Inversely Related to Initial Load**

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## ABSTRACT

Foods prepared in the kitchen can become cross-contaminated with *Campylobacter* by contacting raw products, particularly skinned poultry. We measured the percent transfer rate from naturally contaminated poultry legs purchased in supermarkets. Transfer of *Campylobacter* from skin ( $n = 43$ ) and from meat ( $n = 12$ ) to high-density polyethylene cutting board surfaces was quantitatively assessed after contact times of 1 and 10 min. The percent transfer rate was defined as the ratio between the number of *Campylobacter* cells counted on the cutting board surface and the initial numbers of *Campylobacter* naturally present on the skin (i.e., the sum of *Campylobacter* cells on the skin and board). Qualitative transfer occurred in 60.5% (95% confidence interval, 45.5 to 75.4) of the naturally contaminated legs studied and reached 80.6% (95% confidence interval, 63.0 to 98.2) in the subpopulation of legs that were in contact with the surface for 10 min. The percent transfer rate varied from  $5 \times 10^{-2}\%$  to 35.7% and was observed as being significantly different (Kruskall-Wallis test,  $P < 0.025$ ) and inversely related to the initial counts on poultry skin. This study provides quantitative data describing the evolution of the proportion of *Campylobacter* organisms transferred from naturally contaminated poultry under kitchen conditions. We emphasize the linear relationship between the initial load of *Campylobacter* on the skin and the value of the percent transfer rate. This work confirms the need for modeling transfer as a function of initial load of *Campylobacter* on leg skin, the weight of poultry pieces, and the duration of contact between the skin and surface.

*Campylobacter* is a gram-negative microaerophilic zoonotic pathogen that is recognized as a major cause of bacterially mediated diarrheal disease worldwide (24) even when low quantities of the bacterium (20) have been ingested. Most clinical cases spontaneously resolve, but they can evolve to more severe nervous symptoms, such as Guillain-Barré syndrome, which is an acute, inflammatory, demyelinating polyneuropathy (28). *Campylobacter* is recognized as playing a major role in the occurrence of food-borne diseases. Risk factor studies (5, 27) or outbreak investigations (8) of infections with *Campylobacter jejuni* have identified consumption and handling of raw chicken as an important risk factor for campylobacteriosis. Similar conclusions have been provided by attribution studies comparing clinical and food isolates of *Campylobacter* (6, 12) by using different typing strategies (6, 16). At the farm level, the flock prevalence of *Campylobacter* is suspected to be elevated in most European countries (2, 7), justifying Commission Decision 2007/516/EC, which mandates that data on the prevalence of *Campylobacter* in poultry ceca during slaughter should be obtained (4). Given the physiological needs of *Campylobacter*, growth does not occur during food processing (10), and the presence of *Campylobacter* in end products typically results from cross-contamination (1). When considering *Campylobacter* risk for consumers, a quantitative approach is crucial in determining

realistic exposure to humans. This quantitative approach was proposed in risk assessment studies of human cases of campylobacterioses associated with broiler meat in The Netherlands, where a farm-to-fork Quantitative Microbiological Risk Assessment (QMRA) model for broiler production was available (19, 20). One of the objectives was to define *Campylobacter* criteria in the food chain, testing whether the values were realistic options for risk management. Currently, there are too few studies that provide quantitative data on *Campylobacter* cross-contamination during meat handling (15) to be able to run the QMRA, and some of the most recent exposure analyses have used indirect assessments of *Campylobacter* transfer (20) or artificially contaminated samples (25). Nowadays, quantification of *Campylobacter* transfer from poultry to cutting boards is recognized as an underinvestigated but essential part of all risk assessments for this pathogen (20). This study focuses on analyses simulating a typical situation in the consumer's kitchen. Naturally contaminated poultry pieces were used with or without skin and placed in contact with a cutting board for 1 or 10 min. The objective of this work was to obtain transfer rate data sets and to assess the evolution of the percent transfer rates depending on initial concentration. Parameters tested included contact time and poultry leg surfaces (meat or skin) in contact with a typical high-density polyethylene board.

## MATERIALS AND METHODS

**Product.** Broiler legs were purchased from neighborhood supermarkets near the poultry producers. All poultry originated from

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flocks reared under free-range conditions in order to increase the probability of obtaining samples positive for *Campylobacter*. A total of 43 legs generated 55 analyses: 43 analyses for skin and 12 analyses for meat.

**Experimental procedure.** Domestic high-density polyethylene cutting boards were used in this study. The boards were cut in square pieces (5 by 5 cm) and aseptically applied to the surface of the skin or meat with a 200-g deadweight for 1 or 10 min. The effective contact surface was estimated to be 9 cm<sup>2</sup>. Sterile scalpels and grips were used to cut the 25-cm<sup>2</sup> sample (skin or meat) surfaces under the board. The skin, meat, and board were placed in separate sterile stomacher bags for further quantitative bacteriological examination.

**Bacteriological analyses.** *Campylobacter* was quantified by adding 50 ml of Preston broth (AES, Bruz, France) to the bags containing the boards, whereas the skin or meat fragments were diluted 1:10 (wt/wt) in Preston broth. All samples were homogenized for 30 s in a Pulsifier (AES), after which 500 µl of the suspension and serial dilutions of the suspension were plated in triplicate on Karmali agar (AES) giving a detectable limit of 20 CFU/g for the skin and meat and 90 CFU for the entire board (about 10 cells per cm<sup>2</sup> of contact). After initial plating, the bags containing Preston broth were enriched using the ISO 10272-1 (April 2006) method for *Campylobacter* detection and further plated on Karmali agar and Butzler no. 2 (Virion agar) (9).

Suspected colonies of *Campylobacter* on selective media (Karmali agar and Virion agar) were examined under a phase-contrast microscope and identified as *Campylobacter* when characteristic spiral morphology and corkscrew motility were observed.

**Data analysis.** Transfer was defined as the detection of *Campylobacter* on a board after contact with the naturally contaminated skin or meat surface. The quantitative percent transfer rate was calculated as follows: percent transfer rate =  $100 \times a/(a + b)$ , where  $a$  is the number of *Campylobacter* cells on the board (*Campylobacter* CFU/ml  $\times$  50 [volume in milliliters of the initial suspension from the boards]), and  $b$  is the initial number of *Campylobacter* cells naturally present on the skin (or meat) (*Campylobacter* CFU/cm<sup>2</sup>  $\times$  9 [estimated surface of skin or meat actually in contact with the board]).

For samples positive for *Campylobacter* only after enrichment, the retained values for transfer were defined as immediately under the threshold (19 CFU/g or 90 CFU per board).

Nonparametric statistical comparisons of the proportions (Fisher's exact test) or values (Kruskall-Wallis) of the percent transfer rates were made using Systat software.

## RESULTS

**Qualitative transfer.** Overall, 25 of 31 (80.6%; 95% confidence interval, 63.0 to 98.2%) leg skins in contact with the cutting board for 10 min yielded transferable *Campylobacter*. The proportion of observed transfers was significantly lower (2 of 24 positive; Fisher's test,  $P < 0.05$ ) when skin or meat surfaces were applied to the board for only 1 min. Having confirmed that contamination levels of the skin samples analyzed were not significantly different from their initial values (Fisher's test,  $P = 0.652$ ), we confirmed that transfer proportions depend on the duration of contact with the board (Fisher's test,  $P < 0.001$ ).

**Quantitative transfer.** Although *Campylobacter* was detected on every sample of our collection, the initial skin

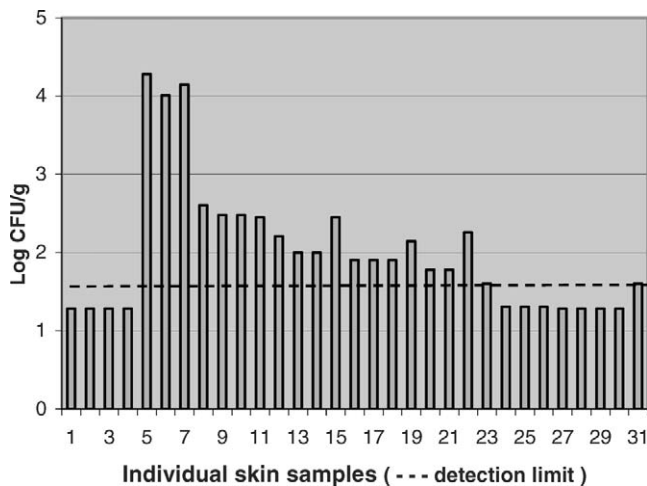


FIGURE 1. Distribution of *Campylobacter* contamination on skin samples.

surface contamination measured below the threshold value of 1.3 log CFU/g in 8 of the 31 cases. Contamination levels reached a maximum of 4.3 log CFU/g, with only 3 skin samples presenting values of  $>4$  log CFU/g while the majority (24 of 31) of the sampled skins presented contamination levels between 1.3 and 2.6 log CFU/g, with an average of 2 log CFU/g (Fig. 1).

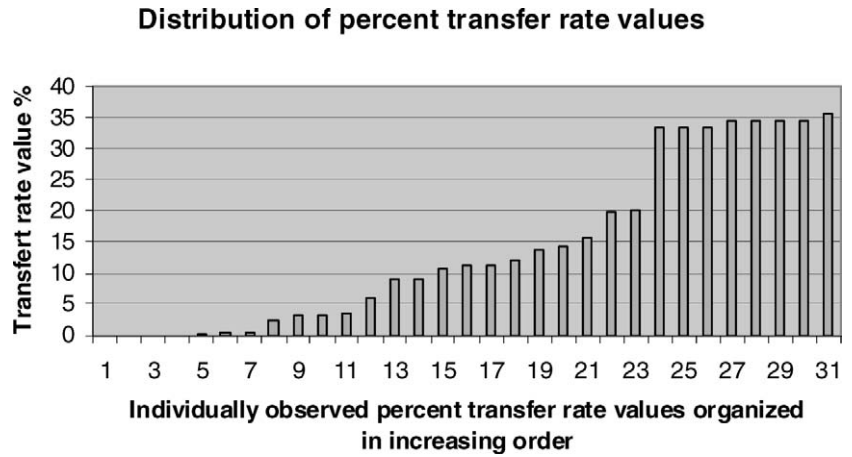
In 4 of the 31 cases, no transfer to the board was detected after a 10-min contact time despite the confirmed presence of *Campylobacter* on the skins. In these four cases, the skins presented low initial contamination levels ( $<20$  CFU/g of skin, or  $<180$  *Campylobacter* CFU available for transfer). Transfer was detected in 27 cases, and in 8 cases the amount of *Campylobacter* on the board was determined to be  $>2.7$  log CFU per board.

The percent transfer rate was defined as the ratio of the number of *Campylobacter* CFU counted on the cutting board surface multiplied by 100, divided by the initial quantity of *Campylobacter* naturally present on the skin in contact with the board. Percent transfer rates, varying from  $5 \times 10^{-2}\%$  to 35.7%, can be visually distributed into three groups of samples:  $<10\%$  of the *Campylobacter* transferred; between 10 and 20% of the *Campylobacter* transferred; and a last group for which  $>20\%$  of the *Campylobacter* organisms available on the skin were transferred to the high-density polyethylene board (Fig. 2).

Table 1 represents initial skin contamination based on transfer rate. The proportion of *Campylobacter* transferred to the board is inversely related to the initial bacterial concentration on the skin. While the number of *Campylobacter* on the board increases with initial concentration on the skin, the proportion of transferred bacteria is significantly higher for lower initial skin concentrations (Kruskall-Wallis,  $P = 0.025$  and  $<0.05$ ).

Figure 3 describes the percent transfer rates of *Campylobacter* as a function of initial concentrations of the bacteria on the skin. Graphical analyses suggest that the initial count of *Campylobacter* on the skin is inversely related to the proportion of *Campylobacter* transferred to the cutting

FIGURE 2. Distribution of the *Campylobacter* percent transfer rate after a 10-min contact.



board by a linear function, as confirmed by the Pearson product-moment correlation coefficient ( $r = -0.96$ ).

**DISCUSSION**

Previous studies (13, 19, 23) have shown how risk-based *Campylobacter* microbiological criterion determination or mitigation options can be derived from the application of a farm-to-fork QMRA model. Due to the huge impact of the information stemming from these studies, these assessments need to be particularly accurate. From a public health and industrial point of view, this is especially true when defining microbial criteria that will be used to prevent consumer exposure. As a result, such models should be fitted with accurate data. As emphasized by several authors (13, 19), the models show that, in addition to the prevalence of the contamination, numbers of *Campylobacter* have to be explicitly incorporated into the model to precisely assess the effect of an intervention. Consequently, studies providing quantitative data such as this one are of particular interest.

The transfer of *Campylobacter* from contaminated carcasses to different surfaces in a domestic kitchen during food preparation has been previously described in the work of Cogan et al. (3). QMRA models have emphasized that such cross-contaminations can significantly contribute to the risk of *Campylobacter* infection (18). In order to evaluate the human risk, these studies considered the cutting board as a frequently contaminated place in the kitchen and as the central parameter in QMRA models, respectively. The probability of human exposure (20) was shown to be dramatically conditioned by the modification of the probability of washing the board during food preparation.

While other studies have provided variable counts of *Campylobacter* contamination levels of carcasses (22),

these were not available in the work of Cogan et al. (3). Although results of the current baseline study being conducted in Europe (Commission Decision 2007/516/EC (4)) have yet to be made public, it is expected that the contamination of carcasses at levels higher than 1 log CFU/cm<sup>2</sup> will be found to occur frequently. In our study, contamination levels reached a maximum of 3 log CFU per board. This strongly indicates that our experimental procedure is relevant to the assessment of percent transfer rates in kitchen conditions and that its results are coherent with natural contamination values of breast fillets, which have been found to be between 100 and 1,000 CFU/g (14). In a study by Lubber et al. (15), transfer results under kitchen conditions were reported with *Campylobacter* counts that did not exceed 20 CFU per board, but the transfer protocol had not been standardized. In the study by Lubber et al. (15), the disadvantages of previous transfer studies that were performed with chicken skin samples inoculated with *Campylobacter* (11) were discussed. There is evidence that using a high number of bacteria for inoculation leads to biased transfer ratios, as suggested in the case of *Enterobacter aerogenes* (17). In our study, we describe how percent transfer rates vary significantly depending on the initial natural contamination levels of poultry legs. Contact time, the crucial impact of which has been recently reviewed (21), is another parameter that must be taken into account. Our

TABLE 1. Initial contamination of the skin surface associated with percent transfer rate

Transfer rate (%)	Mean initial skin contamination (log CFU/g ± SD)
>20	1.34 ± 0.11 (n = 9)
10–20	1.97 ± 0.25 (n = 10)
<10	2.86 ± 0.90 (n = 11)

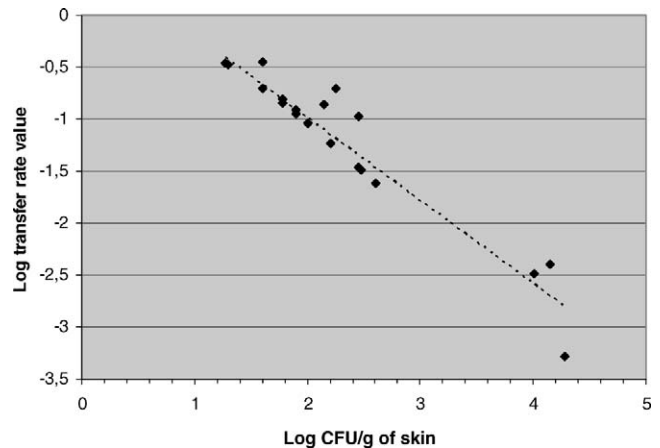


FIGURE 3. Graphical relationship between log transfer rate and the initial *Campylobacter* count on skin (log CFU per gram).



work clearly indicates that transfer ratios are higher for 10-min contact times than for 1-min contact times. Moreover, it has been described in the literature that percent transfer rates vary with the weight applied to the board during cutting operations (26) or with the weight of the carcass as it lies on the board (15). Our results were not biased by this last parameter, since the pressure applied to the skin was standardized with the use of a 200-g deadweight.

The present study quantifies percent transfer rates of *Campylobacter* from commonly consumed chicken parts to the cutting board under kitchen conditions. The use of data obtained from the study of naturally contaminated broiler legs has enabled a more precise evaluation of the influence of cross-contamination routes in the kitchen in terms of human illnesses (18). To our knowledge, the linear relationship inversely associating the initial load of *Campylobacter* and the value of the percent transfer rate had not been previously described. One study, which focused on the transfer of *Enterobacter* sp. from artificially contaminated products (17), corroborates the coherence of our results, which were obtained from *Campylobacter* naturally available under kitchen conditions. This is of particular interest because we can speculate that in the near future the results of these studies could lead to *Campylobacter* load reduction on carcasses. Our results, which demonstrate that the transfer of bacteria is inversely proportional to the initial count of *Campylobacter* on poultry skin, should be taken into account for an accurate evaluation of human exposure probabilities. In particular, the results of our study complete the transfer estimate proposed by Kusumaningrum et al. (11) and provide accurate data for QMRA models (18) presently available. According to the present study, percent transfer rates appear to be a complex function of the initial contamination of the skin, the duration of contact, and the applied weight. These three parameters will be further studied in order to model their individual effects and interactions. Such a polynomial function would be of particular interest for the optimization of QMRA studies.

In a farm-to-fork QMRA approach, authors have promoted mitigation measures at the farm level to control *Campylobacter* in the production chain (23). They estimate that a 2-log CFU/g reduction of *Campylobacter* in fecal excretion could lead to a 30-fold reduction of *Campylobacter*-induced human foodborne diseases. The definition of such a quantitative objective at the farm level appears to be realistic regarding this human pathogen. The use of more precise QMRA models, as helped by our work, will allow to better define microbiological criteria and to specify the performance objective of mitigation options in farms.

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