

ORIGINAL ARTICLE

Cross-contamination in the kitchen: estimation of transfer rates for cutting boards, hands and knivesE.D. van Asselt^{1,2}, A.E.I. de Jong^{1,2}, R. de Jonge¹ and M.J. Nauta¹

1 Laboratory for Zoonoses and Environmental Microbiology, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

2 Marketing and Consumer Behaviour Group, Wageningen University, Wageningen, the Netherlands

Keywords*Campylobacter jejuni*, consumer practices, *Lactobacillus casei*, quantification.**Correspondence**

Esther van Asselt, RIKILT – Institute of Food Safety, PO Box 230, 6700 AE Wageningen, the Netherlands.

E-mail: esther.vanasselt@wur.nl

Present addressE.D. van Asselt, RIKILT – Institute of Food Safety, Wageningen, the Netherlands.
A.E.I. de Jong, Food and Consumer Product Safety Authority (VWA), Amsterdam, the Netherlands.

2007/1680: received 19 October 2007, revised 14 March 2008 and accepted 1 April 2008

doi:10.1111/j.1365-2672.2008.03875.x

Abstract**Aims:** To quantify cross-contamination in the home from chicken to ready-to-eat salad.**Methods and Results:** Based on laboratory scenarios performed by de Jong *et al.* (2008), transfer rates were estimated for *Campylobacter jejuni* and *Lactobacillus casei* as a tracer organism. This study showed that transfer characteristics for both micro-organisms were comparable when washing regimes and transfer via items (cutting board, hands and knives) were compared. Furthermore, the study showed that the use of separate transfer rates for transfer from chicken to items and from items to salad will lead to an overestimation of campylobacteriosis risk. Applying good hygienic practices resulted in final levels of bacteria in the salad below the detection limit. Our study showed that it is important to include these data points in model fitting.**Conclusions:** Results obtained in observational studies with *Lact. casei* can be translated to *Camp. jejuni* using the transfer rates obtained in this study. Cross-contamination by hands, cutting boards and knives was equally important.**Significance and Impact of the Study:** Cross-contamination should be incorporated in microbiological risk assessments. The present study contributes to this by quantifying transfer of *Camp. jejuni* and *Lact. casei* from raw chicken via various contact surfaces into the ready-to-eat product.**Introduction**

Up to 87% of reported foodborne outbreaks is associated with food prepared or consumed in the home. However, there are large differences between countries, and most countries report between 10% and 50% of outbreaks associated with private homes (Redmond and Griffith 2003). Despite these differences, it is clear that cross-contamination in the home is an important factor that should be included in microbiological risk assessments (MRAs). In the exposure assessment part of an MRA, the steps from farm-to-factory are usually relatively well documented and growth or inactivation of pathogens can be estimated for each step by applying predictive models. However, once the product has left the factory and enters the consumer phase, conditions are less well defined,

although it is recognized that the main factors determining food safety at home are adequate cooking, proper storage, personal hygiene and preventing cross-contamination (Medeiros *et al.* 2001). It is, however, largely unknown how these factors influence the final level of bacteria at the point of consumption.

In order to quantify the effect of consumer behaviour on food safety, Mylius *et al.* (2007) developed a model on cross-contamination during food preparation, which has been applied in the Carma model, a risk assessment on *Campylobacter* (Nauta *et al.* 2007). This study clearly showed that parameter values necessary to quantify cross-contamination are scarce. Although some data are available on contamination routes, such as contamination via cutting boards and hands (Chen *et al.* 2000; Montville *et al.* 2001; Moore *et al.* 2003; Kusumaningrum *et al.*

2004; Luber *et al.* 2006), these data focus on only one part of a contamination route. Therefore, de Jong *et al.* (2008) studied actual transfer of bacteria from raw ingredients to a prepared meal in a realistic setting to gain insight in the overall transfer of bacteria. The effect of various contamination routes (cutting boards, hands and knives) was studied using a variety of laboratory scenarios with and without washing of the items used for preparing a ready-to-eat salad.

In the study of de Jong *et al.* (2008), *Campylobacter jejuni* was chosen as a model pathogen, as it belongs to the top five of pathogens causing most infections worldwide (Zwietering and van Asselt 2005). In the Netherlands, there are an estimated 65 000 campylobacteriosis cases per year (Kemmeren *et al.* 2006). Although only 20–40% of these cases are associated with chicken meat, consumption of chicken is still the predominating factor for campylobacteriosis (EFSA 2006; Humphrey *et al.* 2007). Illnesses via chicken meat can occur either by undercooking or by cross-contamination. de Jong *et al.* (2008) therefore used a chicken curry salad because this recipe offered possibilities for cross-contamination and undercooking.

In this paper, we aimed to quantify cross-contamination routes at home, based on laboratory experiments of de Jong *et al.* (2008). As the results presented in this paper are part of a transdisciplinary project in which natural and social scientists cooperated to study the effect of consumer behaviour in the home on food safety (Fischer *et al.* 2005, 2007), a tracer organism needed to be found for *Camp. jejuni* for ethical reasons. de Jong *et al.* (2008) found that *Lactobacillus casei* showed comparable cross-contamination behaviour in laboratory tests and could therefore be used as tracer organism for *Camp. jejuni*. Therefore, in the current study, transfer rates for both organisms were compared so that results from a consumer study with *Lact. casei* can be translated to *Camp. jejuni*. The obtained transfer rates can then be applied in MRAs to determine the quantitative effect of consumer behaviour on human health risks (Nauta *et al.* 2008).

Materials and methods

Cross-contamination model

Transfer rates were estimated based on laboratory results from de Jong *et al.* (2008). In their study, they used a chicken curry recipe that offered possibilities for cross-contamination. The recipe consisted of the following: first cut a chicken breast fillet in half (by which the chicken can contaminate various items), then boil it in water for 10 min. Cut the chicken to smaller pieces, cut the fruit and add spices and cream. Details of the recipe can be found in de Jong *et al.* (2008). It was assumed that cross-

contamination in this recipe is only possible through hands, knives and cutting board. Various cross-contamination scenarios were tested in the laboratory. Scenarios in which one item was studied (washed with or without soap or not washed) and scenarios in which all items were either not washed (worst case scenario) or in which all items were decontaminated between cutting raw chicken and the salad [best case (BC) scenario]. Each scenario was repeated at least four times. The various contamination routes are depicted in Fig. 1.

For simplicity reasons, it was assumed that contamination from hands, cutting board and knife to the boiled chicken is comparable to contamination from these items to the fruit particles. Therefore, in the model, cooked chicken and fruits were combined in the term 'salad'.

The number of bacteria found in the prepared salad depended both on the number of bacteria transferred through cross-contamination and the number of bacteria surviving the cooking step. A first order inactivation was assumed for the boiling process. So overall:

$$N_S = \sum_{i=h,b,k} [t_{ci}(1-t_{iw})t_{is}N_0] + (1-t_{ch}-t_{cb}-t_{ck})N_0 \exp(-k\tau) \quad (1)$$

(cross-contamination) (cooking)

where N_S : number of bacteria in the salad [colony forming units (CFU) per salad]; N_0 : number of bacteria on the raw chicken breast fillet (CFU per fillet); t_{ci} : transfer rate from raw chicken to item i (which can be either hands (h), cutting board (b) or knife (k)); t_{iw} : transfer rate from item i to the sink because of washing; t_{is} : transfer rate from item i to salad; k : inactivation rate (1 min^{-1}); τ : boiling time (min).

The inactivation rate k is based on heating experiments with *Camp. jejuni* and *Lact. casei* on chicken breast fillet, which resulted in $D_{100^\circ\text{C}}$ -values of 1.90 and 1.93 min, respectively (de Jong *et al.*, submitted). Chicken inoculation in these heating experiments was performed as described by Bergsma *et al.* (2007) who also found comparably high heat resistance levels of *Camp. jejuni* at frying temperatures between 105–167°C ($D = 1.95 \text{ min}$). de Jong *et al.* (in prep) boiled the chicken in water in their heating experiment instead of frying in margarine as was carried out by Bergsma *et al.* (2007), as the boiling method was better reproducible. The D -values found resulted in inactivation rates of ($k = \ln(10) \text{ per } D$) 1.21 and 1.19 min^{-1} for *Camp. jejuni* and *Lact. casei*, respectively.

Transfer rates were based on the number of surviving bacteria in the final salad, as determined by de Jong *et al.* (2008) both for *Camp. jejuni* and *Lact. casei*. Only initial and end point cell numbers were measured (N_S and N_0) and none at points in-between. Based on these

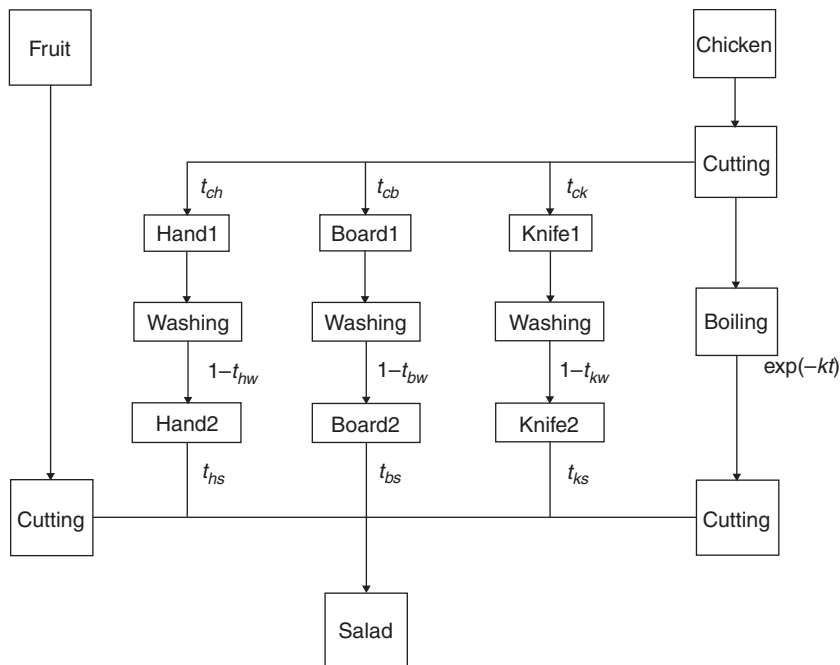


Figure 1 Cross-contamination routes with transfer rates used in the model. t_{ci} : transfer rate from raw chicken to item i , which can be either hands (h), cutting board (b) or knife (k); t_{iw} : transfer rate from item to sink because of washing; t_{is} : transfer rate from item to salad.

measurements, only overall transfer rates could be estimated, i.e. the multiplication of $t_{ci}t_{is}$ and not the separate parameter values. As described by de Jong *et al.* (2008), this was carried out to mimic consumer practices so that no interventions during cooking were needed. Furthermore, by measuring final bacterial cell levels in the salad an accurate contamination level of the salads is determined, in contrast with estimated levels based on multiplication of contamination levels at intermediate points. Transfer rates could be estimated by comparing various cross-contamination scenarios. For example, transfer via the cutting board ($t_{cb}t_{bs}$) could be estimated by comparing the BC scenario (in which the chicken was not touched by hands and decontaminated knives and cutting boards were used to cut the boiled chicken) with a scenario where the same cutting board was used for raw and boiled chicken. The effect of washing could be determined by comparing preparation scenarios with and without washing of an item. An example is given in the Appendix.

Reduction levels (N_s/N_0) of the various cross-contamination scenarios were estimated based on the average of multiple log-transformed data points of one scenario and by fitting a normal distribution through the log-transformed data points. In some cases (e.g. in the BC with hand washing), some of the data points were below the detection limit (so-called censored data). This was incorporated by using a statistical technique for this type of data (Lorimer and Kiermeier 2007). The mean (μ) and standard deviation (σ) of this normal distribution was estimated using the maximum likelihood estimation

(MLE). The estimates for μ and σ were obtained by combining the probability density function for count data and the cumulative density function for censored data.

Transfer rates from eqn (1) were estimated using Monte Carlo simulations in @Risk software (Palisade, Newfield, NY, USA) with 10 000 iterations. The above-mentioned approach with incorporation of levels below the detection limit was used to determine the various transfer rates. Transfer rates from literature were fitted using Bestfit (@Risk software, Palisade).

Statistical analysis

The effect of washing the various items was tested for its significance with ANOVA on the log-transformed data in SPSS (SPSS, Chicago, IL, USA). A significance level of 0.05 was used.

Results

The detection limit

In various laboratory scenarios, either one or all contamination routes combined (hands, cutting board and knives) were studied in order to determine its effect on the final level of bacteria in the salad. The lowest number of bacteria that could be enumerated in the final salad was approximately 25 CFU per salad, which is thus the detection limit. Depending on the initial level of bacteria on the chicken fillet (below 9 log CFU per fillet), the highest

Table 1 Log difference between initial bacterial cell levels on the raw chicken and final levels in the salad in log N_0/N_5 for various scenarios. Experimental details can be found in de Jong *et al.* (2008)

Scenario	<i>Campylobacter jejuni</i>				<i>Lactobacillus casei</i>			
	Mean*	SD*	N† (total, <dl)‡	Normal (μ , σ)§	Mean*	SD*	N† (total, <dl)‡	Normal (μ , σ)§
BC with cooking	>7.32	0.03	8, 8	–¶	>7.40	0.02	8, 8	–¶
BC without cooking	2.29	0.38	3, 0	2.29, 0.31	0.49	0.86	3, 0	0.49, 0.70
WC with cooking	3.07	0.76	4, 0	3.07, 0.66	1.84	0.92	3, 0	1.84, 0.75
WC without cooking	0.79	0.10	4, 0	0.79, 0.09	0.40	0.14	4, 0	0.40, 0.13
BC without hand washing	2.97	0.67	6, 0**	2.88, 0.68	1.77	0.47	4, 0	1.77, 0.41
BC with hand washing (cold)	6.27	1.15	4, 1	6.46, 1.26	3.62	0.94	4, 0	3.62, 0.81
BC with hand washing (cold water and soap)	6.93	0.62	4, 1	7.04, 0.66	5.62	1.39	4, 0	5.62, 1.20
BC without knife washing	3.06	0.63	4, 0	3.06, 0.56	2.48	0.88	4, 0	2.48, 0.76
BC with knife washing (cold)	6.76	0.67	6, 5	9.16, 2.26	4.74	0.53	7, 0	4.74, 0.49
BC with knife washing (hot)	>7.17	0.01	3, 3	–¶	5.83	0.27	3, 0	5.83, 0.22
BC with knife washing (hot water and soap)	>7.07	0.01	3, 3	–¶	6.89	1.69	3, 2	9.15, 3.48
BC without board washing	3.15	0.55	5, 0	3.15, 0.49	1.44	0.39	4, 0	1.44, 0.33
BC with board washing (cold)	3.51	0.43	4, 0	3.51, 0.43	2.20	0.49	3, 0	2.20, 0.42
BC with board washing (hot)	7.22	0.05	3, 2	7.26, 0.05	5.47	0.49	3, 0	5.47, 0.40
BC with board washing (hot water and soap)	6.06	1.37	3, 1	6.40, 1.54	3.59	0.60	3, 0	3.59, 0.49

BC, best case, where chicken is not touched by hand and new cutting boards and knives are used to cut the boiled chicken and fruit.

WC, worst case, where hands, cutting board and knife were not washed after cutting the raw chicken.

Cold, items were washed with running cold water.

Hot, items were washed with running hot water (10 s for board and 2 s per side for knife).

Soap, items were washed with soap (board and knife were rinsed for 2 s with hot water then thoroughly brushed away from the tap and rinsed again, hands were washed with cold water and soap).

*Calculated average and SD based on the log reductions (log N_0/N_5) in the salad (values below the detection limit were set at the detection limit).

†Number of samples taken.

‡Number of data points below the detection limit (dl) out of the total number of samples taken.

§Fitted normal distribution, including values below the detection limit.

**One sample contained more bacteria than could be counted. This was incorporated as 1-normdist (x , μ , σ , 1) (see Materials and Methods section).

¶All data were below the detection limit. It was not possible to fit a normal distribution.

detectable reduction level was approximately 7 log CFU. Assuming log reductions in the salad were normally distributed, a normal distribution was fitted on the obtained data from de Jong *et al.* (2008). Both the calculated mean and standard deviation of the data and those obtained by fitting normal distribution for each scenario are given in Table 1 for both *Camp. jejuni* and *Lact. casei*. The mean and standard deviation in Table 1 were calculated assuming that values below the detection limit are at the detection limit. The mean and standard deviation used in the normal distributions were estimated using the MLE as described in the Materials and Methods section so that values below the detection limit could be included. It can be seen that in case there are values below the detection limit, the first method resulted in a lower average of log reductions (Table 1, Fig. 2), which is to be expected. When there were no values below the detection limit, the

estimated standard deviation of the normal distribution was slightly different from the calculated standard deviation on the data because of the use of the maximum likelihood method. In further analysis, we used the fitted normal distribution.

Cross-contamination

Transfer rates were estimated based on the results of the laboratory scenarios performed by de Jong *et al.* (2008). The BC scenario (no cross-contamination possible) resulted in all values below the detection limit. It was, therefore, assumed that the BC scenario was negligible compared with the other scenarios (see Appendix). Mean values for the transfer rates of each cross-contamination route can be found in Table 2. For knife washing with hot water or soap, all values for *Camp. jejuni* were below

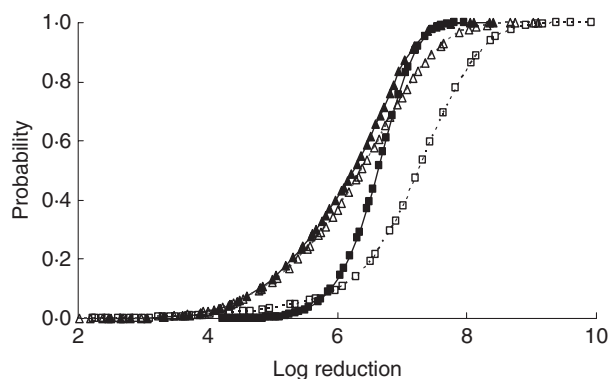


Figure 2 Distribution of the log difference between initial bacterial levels on the raw chicken and final levels in the salad (log reductions in the salad) for *Campylobacter jejuni* in case the knife (squares) or hands (triangles) were washed with cold water. Solid lines and closed symbols represent a normal distribution using the mean and standard deviation as given in Table 1 (assuming that values below detection limit are at the detection limit) and dotted lines and open symbols represent a normal distribution as determined in Table 1 (including values below the detection limit using the MLE).

the detection limit (see Table 1). Because these scenarios were not significantly different ($P > 0.05$) from the BC scenario (where cutlery was changed instead of washed), the transfer rates for knives washed with hot water [$t_{kw(hot)}$] and soap [$t_{kw(soap)}$] were set to 1 (100% removal). When one or more mistakes were made in the kitchen (no hand washing, no knife washing and/or no board washing), this resulted in low log differences between initial and final levels in the salad (approximately 3 log CFU for *Camp. jejuni* and approximately 2 log CFU for *Lact. casei*). When items were washed instead of replaced, this resulted (in most cases) in only a partial removal of bacteria giving reduction levels in between 3 and 7 log CFU for *Camp. jejuni* and 2 and 7 log CFU for *Lact. casei*. Table 2 shows that transfer rates ($t_{ci}t_{is}$) for

Lact. casei are a factor of 10 higher than for *Camp. jejuni*, meaning that cross-contamination with *Lact. casei* resulted in higher numbers in the final salad. Washing of the various items showed that *Camp. jejuni* was removed more easily from knives and hands than *Lact. casei*.

Comparison with literature

As mentioned earlier, limited data are available to quantify cross-contamination. For *Camp. jejuni* only data on board transfer were found (Moore et al. 2003; Kusumaningrum et al. 2004; Luber et al. 2006) and limited data on knife transfer (Luber et al. 2006). For hand transfer and hand washing, only data on *Enterobacter aerogenes* are present, which is used as surrogate organism for *Salmonella* (Chen et al. 2000; Montville et al. 2001). Mylius et al. (2007) and Nauta et al. (2007) used all available literature data to quantify cross-contamination with *Camp. jejuni* and estimated human risk for campylobacteriosis. In order to determine whether the choice of pathogen and the distribution used influences the outcome of a risk assessment, our data were compared with fitted distributions on literature data for board and hand transfer. In our study, normal distributions on log-transformed data were used to determine transfer rates. This distribution is used more often in literature (Chen et al. 2000; Montville et al. 2001). Another possible distribution is the beta distribution, as the probability of a micro-organism to transfer from one item to the next is between 0 and 1. Therefore, transfer rates from literature were fitted with either a normal distribution through log-transformed data or a beta distribution on nontransformed data and compared with our transfer rates. In this way, the difference between data sets and the effect of model choice could be assessed.

Data on contamination routes are usually determined separately: either from chicken to items or from items to

Table 2 Estimated mean transfer rates for *Campylobacter jejuni* and *Lactobacillus casei* based on the normal distribution of the data from Table 1

	Hand		Board		Knife		Overall	
	<i>Camp. jejuni</i>	<i>Lact. casei</i>	<i>Camp. jejuni</i>	<i>Lact. casei</i>	<i>Camp. jejuni</i>	<i>Lact. casei</i>	<i>Camp. jejuni</i>	<i>Lact. casei</i>
$(t_{ch} + t_{cb} + t_{ck})$							0.92	0.22
$t_{ci}t_{is}$	4.5×10^{-3}	2.7×10^{-2}	1.3×10^{-3}	4.9×10^{-2}	2.0×10^{-3}	1.6×10^{-2}	2.7×10^{-3}	6.5×10^{-2}
$[1 - t_{iw(cold)}]$	1.9×10^{-2}	8.0×10^{-2}	5.2×10^{-1}	3.0×10^{-1}	9.1×10^{-3}	3.9×10^{-2}		
$[1 - t_{iw(hot)}]$	—*	—*	1.5×10^{-4}	1.9×10^{-4}	0†	2.4×10^{-3}		
$[1 - t_{iw(soap)}]$	7.0×10^{-4}	6.7×10^{-3}	4.6×10^{-2}	1.8×10^{-2}	0†	4.2×10^{-2}		

$t_{ci}t_{is}$, Total transfer from chicken to item (hands, cutting board or knife) and from item to salad.

$1 - t_{iw}$, Fraction remaining on the item after washing the item.

*Not determined.

†No significant difference with best case, assumed no bacteria remaining on the item.

Table 3 Comparison of transfer rates

Transfer rate	Micro-organism	Distribution fitted through data	Mean	Data fitted from
$t_{hw(soap)}$	<i>Campylobacter jejuni</i>	$1 - \frac{10^{Normal(-7.04,0.66)}}{10^{Normal(-2.88,0.68)}}$	0.9994	This study*
	<i>Enterobacter aerogenes</i>	$10^{Normal(0.0044,0.0096)}$	0.9899	(Chen et al. 2000)†
	<i>Ent. aerogenes</i>	Beta(25.68,0.26)	0.9901	(Chen et al. 2000)†
$t_{ch}t_{hs}$	<i>Ent. aerogenes</i>	$1 - Beta(0.24,6.67)$	0.9653	(Mylius et al. 2007; Nauta et al. 2007)‡
	<i>Camp. jejuni</i>	$10^{Normal(-2.88,0.68)}$	4.46E-3	This study
	<i>Ent. aerogenes</i>	$10^{Normal(-1.40,0.56)} \times 10^{Normal(-1.75,1.07)}$	3.97E-2	(Chen et al. 2000; Montville et al. 2001)
	<i>Ent. aerogenes</i>	Beta(0.61,5.71) \times beta(0.33,2.44)	1.13E-2	(Chen et al. 2000; Montville et al. 2001)
$t_{cb}t_{bs}$	<i>Ent. aerogenes</i>	Beta(1.78,41.1) \times beta(0.6,2.3)	8.63E-3	(Mylius et al. 2007; Nauta et al. 2007)‡
	<i>Camp. jejuni</i>	$10^{Normal(-3.15,0.49)}$	1.34E-3	This study
	<i>Camp. jejuni</i>	$10^{Normal(-2.11,0.62)} \times 10^{Normal(-0.51,0.32)}$	8.26E-3	(Moore et al. 2003; Kusumaningrum et al. 2004; Lubert et al. 2006)
	<i>Camp. jejuni</i>	Beta(0.72,40.27) \times beta(1.42,2.20)	6.98E-3	(Moore et al. 2003; Kusumaningrum et al. 2004; Lubert et al. 2006)
	<i>Camp. jejuni</i>	$10^{Normal(-1.90,0.61)} \times 10^{Normal(-0.47,0.32)}$	1.54E-2	(Mylius et al. 2007; Nauta et al. 2007)‡
	<i>Ent. aerogenes</i>	$10^{Normal(-0.89,0.37)} \times 10^{Normal(-1.21,0.60)}$	2.98E-2	(Chen et al. 2000)
	<i>Ent. aerogenes</i>	Beta(1.73,8.59) \times beta(0.74,5.09)	2.13E-2	(Chen et al. 2000)

*As described in the appendix.

†Only data from conventional washing used.

‡ $t_{hw(soap)}$, Data from Chen et al. (2000) for conventional and nonhand operated washing; $t_{ch}t_{hs}$ data from (Montville et al. 2001); $t_{cb}t_{bs}$ data from (Kusumaningrum et al. 2004).

salad (Chen et al. 2000; Montville et al. 2001; Kusumaningrum et al. 2004). In order to compare the outcome of our overall transfer rates ($t_{ci}t_{is}$) with literature, the overall transfer from chicken to salad was calculated by multiplying the transfer rate from chicken to hands or boards (t_{ci}) with the transfer rate from boards or hands to salad (t_{is}). The results of the fitted distributions are given in Table 3. These fitted distributions were subsequently used to estimate the log difference between the initial level of 10^9 CFU per fillet on raw chicken and the level of bacteria in the final salad. The following scenarios were used for the calculation: BC with hand washing using soap [effect of $t_{ch}(1 - t_{hw})t_{hs}$], no board washing (effect of $t_{cb}t_{bs}$) and no hand washing (effect of $t_{ch}t_{hs}$). The mean log reductions of the various data sets are given in Fig. 3, which gave a representative view of the distributions found for each data set and scenario. In order to illustrate this, the distributions of one scenario (no board washing) are given in Fig. 4.

Figure 3 shows that the model choice (normal or beta distribution) only had a small effect (approximately 0.5 log difference) on BC with hand washing. The influence of the type of micro-organism used (*Ent. aerogenes* or *Camp. jejuni*) could only be assessed for board transfer. This showed that for these micro-organisms, modelling transfer via cutting board is not greatly influenced (less than 0.5 log difference) by the type of micro-organism used. When comparing our data with literature, the results were comparable for hand transfer but more log reductions were found for board transfer and hand wash-

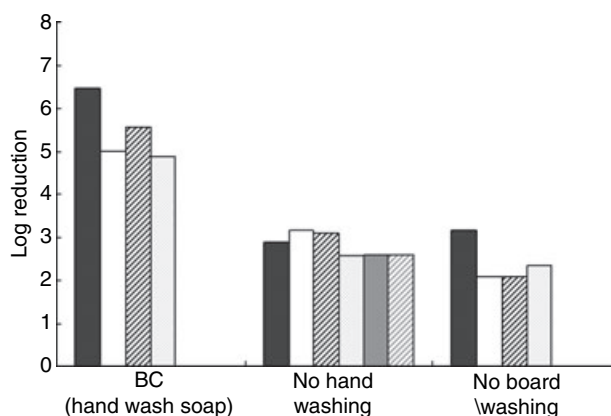


Figure 3 Mean log difference between initial bacterial levels on the raw chicken and final levels in the salad (log reduction) for various scenarios (BC, best case) based on data from this study and literature data fitted with a lognormal or a beta distribution as described in Table 3. Columns in order of appearance: lognormal distributions as used in this study (black bars), lognormal distributions based on data from Chen et al. (2000) for *Enterobacter aerogenes* (white bars), beta distribution on the same data (black dashed bars), combined distributions as used in Nauta et al. (2007) (dotted bars), lognormal distribution on data from Kusumaningrum et al. (2004), Lubert et al. (2006) and Moore et al. (2003) for *Campylobacter jejuni* (grey bars) on cutting boards and a beta distribution on the same data (grey dashed bars).

ing than expected from literature. In order to assess the effect of these differences on the estimation of human health risks, the obtained transfer rates from our study (as given in Table 3) were used as input in the Carma

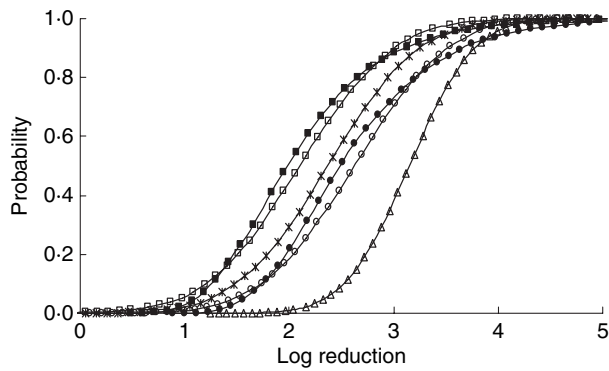


Figure 4 Cumulative distributions of the log reductions in the salad (difference between initial levels on the raw chicken and final levels in the salad) in case the cutting board was not washed (no board wash as given in Fig. 3). Reductions were estimated for lognormal and beta distributions based on data from Chen *et al.* (2000) for *Enterobacter aerogenes* (open and closed squares respectively), combined distributions as used in Nauta *et al.* (2007) (stars), lognormal and beta distribution on data from Kusumaningrum *et al.* (2004), Luber *et al.* (2006) and Moore *et al.* (2003) for *Campylobacter jejuni* (open and closed circles respectively) and lognormal distributions as used in this study for *Camp. jejuni* (triangles).

model from Nauta *et al.* (2007). The estimated number of *Campylobacter* cases per year became half the number of cases based on the literature transfer rates from Chen *et al.* (2000) and Kusumaningrum *et al.* (2004) as used in Mylius *et al.* (2007) and the Carma model from Nauta *et al.* (2007).

Discussion

Detection limit

Figure 2 shows that including values below the detection limit is especially important when almost all values are below the detection limit (for knife washing five out of six samples were below the detection limit). The large difference between the solid and dotted line (data at or below the detection limit, respectively) in this case was mainly because of one data point, which was more than 1 log higher than the detection limit. Therefore, the estimation of μ and σ was uncertain in this case. In case only one out of four samples was below the detection limit, this did not greatly influence the distribution found (as is seen for hand washing with soap). The results from Table 1 and Fig. 2 show that inclusion of data points below the detection limit in the fit of the data had an influence on the estimated log reductions and, therefore, gave a more realistic approach than by assuming that data below the detection limit are at the detection limit, which is usually performed in microbiology. Lorimer and Kiermeier

(Lorimer and Kiermeier 2007) also concluded that using the MLE for values below the detection limit results in a better estimation of the true microbial levels present.

Cross-contamination

The estimated number of bacteria transferred from chicken to hands, cutting board and knife ($t_{ci} = t_{ch} + t_{cb} + t_{ck}$) was higher for *Camp. jejuni* than for *Lact. casei* (Table 2), indicating that *Camp. jejuni* was more easily transferred from raw chicken to items. Therefore, transfer from chicken to salad ($t_{ci}t_{is}$) was expected to be higher for *Camp. jejuni* than for *Lact. casei*. However, Table 2 shows that $t_{ci}t_{is}$ is a factor 10 lower for *Camp. jejuni*. This means that transfer from items to the salad is lower for *Camp. jejuni* than for *Lact. casei*. A possible explanation may be that once *Camp. jejuni* is attached to a surface, it can hardly be removed and, therefore, transfer from an item to the salad is lower than for *Lact. casei*. If *Camp. jejuni* is indeed more firmly attached, it would be expected that removal of this bacterium from a surface by washing would be more difficult than for *Lact. casei*. However, our results showed that *Camp. jejuni* is removed more easily from knives and hands during washing (less bacteria remain on the various items) than *Lact. casei* (Table 2). A more plausible explanation for the lower transfer rates is that *Camp. jejuni* is injured on the various surfaces because it is known to be vulnerable to dryness (de Boer and Hahne 1990; Mattick *et al.* 2003). Furthermore, fruits are cut with the items, which have a low pH causing a further reduction in the already injured *Camp. jejuni*, but less in *Lact. casei* as these bacteria are more acid tolerant.

The overall transfer rates ($t_{ci}t_{is}$) for hands, cutting boards and knives proved to be in the same order of magnitude as the overall transfer rate for the worst case scenario, both for *Camp. jejuni* and *Lact. casei*. This indicated that one mistake (e.g. not washing your hands) was as important as doing everything wrong (worst case scenario). The fact that transfer via various contact surfaces is comparable was also found by Martínez-Tomé *et al.* (2000) and Luber *et al.* (2006). In a previous study, Mylius *et al.* (2007) analysed the impact of cross-contamination from chicken meat to salad during food preparation for *Campylobacter* risk assessment. They found that cross-contamination was most likely to occur via the hands of the cook, but that nevertheless the behavioural variation between consumers in board washing made the latter route more important. Their approach was different as they used a combination of transfer rates for separate routes as obtained from various literature sources (Chen *et al.* 2000; Montville *et al.* 2001; Kusumaningrum *et al.* 2004) and for various micro-organisms (*Ent. aerogenes*

and *Camp. jejuni*; see Table 3). This makes comparisons between transfer rates for board and hand transfer difficult. In addition, the frequencies of human behaviour (like hand and board washing), derived from literature data, were included to derive a risk estimate. In our study, we did not include the impact of behavioural frequencies, which complicates comparisons between the two studies. In the study described in this paper, the same experimental set-up was used for various cross-contamination routes using the same micro-organism resulting in similar transfer rates for hand and board transfer.

Comparison transfer rates with literature

Figure 3 shows that, for the scenario without board washing and BC with hand washing, higher log reductions were found in the salad than expected based on literature data. Numerous factors can explain these results. First of all, the experimental set-up was different in the sense that in this study overall transfer rates were determined instead of separate measurements of transfer from chicken to item and a separate study for transfer from item to salad. Usually, items like cutting boards and knives are swabbed or rinsed to determine the number of attached cells (Chen *et al.* 2000; Luber *et al.* 2006). This number is then used to calculate the transfer rate from chicken to cutting board or knife and from cutting board or knife to salad. By swabbing or rinsing a surface, it is likely that different amounts of bacteria are removed than by cutting a chicken on a cutting board. Another explanation may be that, in this study, not only chicken was cut on the board, but also fruits, which may have caused inactivation of *Camp. jejuni*. Although this can be seen as a draw back in the experimental set-up, it does give a better reflection on real-life practices in the domestic kitchen (de Jong *et al.* 2008).

Both the model choice (beta or lognormal) and the type of micro-organism used (*Ent. aerogenes* and *Camp. jejuni*) did not have a large effect on the log reductions found in the salad (less than 0.5 log difference). In microbiological experiments, differences smaller than 0.5 log CFU are regarded as acceptable because of experimental error. Furthermore, although a difference of 0.5 log CFU in a meal will influence the final number of *Campylobacter* cases estimated, it will not change the order of magnitude. As model calculations are not accurate representations of reality, this is seen as acceptable.

Implications for public health risk

As log difference between initial and final levels in the salad were higher in this study than based on literature (Fig. 3), estimated transfer rates and subsequently the number of estimated campylobacteriosis cases is lower

than found in literature where separate transfer rates are used. Therefore, it seems that the use of separate transfer rates (t_{ci} and t_{is}) in a risk assessment model, instead of overall transfer rates ($t_{ci}t_{is}$), results in an overestimation of the public health risk of *Campylobacter*. The advantage of the experimental set-up as used by de Jong *et al.* (2008) is that real-life practices are mimicked more closely than when separate transfer rates are used. The obtained overall transfer rates are thus more realistic than adding separate transfer rates and will give a more accurate estimation of the effect of cross-contamination on the microbiological status of a meal prepared in the domestic kitchen.

In order to include the determined transfer rates in a MRA, one needs to know how often items are washed or not washed. For this purpose, Fischer *et al.* (2007) performed an observational study using *Lact. casei* as tracer organism in order to determine what errors are most common in domestic cooking. Although log reductions for *Camp. jejuni* and *Lact. casei* were different, the trends were comparable implicating that the use of *Lact. casei* is justified as tracer organism for *Camp. jejuni*.

In conclusion, the obtained transfer rates for *Camp. jejuni* can be used to quantify cross-contamination routes in the home.

Acknowledgements

The authors would like to thank Harsi Kusumaningrum for providing us the raw data of her transfer experiments. Arie Havelaar and Marcel Zwietering are kindly thanked for critically reading this manuscript. The authors would like to acknowledge the support from ZonMW (grant 014-12-033) and the Dutch Ministry of Public Health, which allowed us to conduct this study.

References

- Bergsma, N.J., Fischer, A.R.H., Van Asselt, E.D., Zwietering, M.H. and De Jong, A.E.I. (2007) Consumer food preparation and its implication for survival of *Campylobacter jejuni* on chicken. *Brit Food J* **109**, 548–561.
- de Boer, E. and Hahne, M. (1990) Cross-contamination with *Campylobacter jejuni* and *Salmonella* spp. from raw chicken products during food preparation. *J Food Prot* **53**, 1067–1068.
- Chen, Y., Jackson, K.M., Chea, F.B. and Schaffner, D.W. (2000) Quantification and variability analysis of bacterial cross contamination rates in common foodservice tasks. *J Food Prot* **64**, 72–80.
- EFSA (2006) *2005 Zoonoses in the European Union*. Parma, Italy: EFSA.
- Fischer, A.R.H., de Jong, A.E.I., de Jonge, R., Frewer, L.J. and Nauta, M.J. (2005) Improving food safety in the domestic

- environment: the need for a transdisciplinary approach. *Risk Anal* **25**, 503–517.
- Fischer, A.R.H., De Jong, A.E.I., Van Asselt, E.D., De Jonge, R., Frewer, L.J. and Nauta, M.J. (2007) Food safety in the domestic environment: an interdisciplinary investigation of microbial hazards during food preparation. *Risk Anal* **27**, 1065–1082.
- Humphrey, T., O'Brien, S. and Madsen, M. (2007) *Campylobacter* as zoonotic pathogens: a food production perspective. *Int J Food Microbiol* **117**, 237–257.
- de Jong, A.E.I., van Asselt, E.D., Zwietering, M.H., de Jonge, R. and Nauta, M.J. (submitted). Extreme heat resistance of food borne pathogens *Campylobacter jejuni*, *Escherichia coli* and *Salmonella typhimurium* during cooking of chicken breast fillet. *J. Food. Prot.*
- de Jong, A.E.I., Verhoeff-Bakkenes, L., Nauta, M. and de Jonge, R. (2008) Cross-contamination in the kitchen: effect of hygiene measures. *J Appl Microbiol* **105**, 615–624.
- Kemmeren, J.M., Mangen, M.-J.J., van Duynhoven, Y.T.H.P. and Havelaar, A.H. (2006) *Priority Setting in Food Borne Pathogens No. 330080001/2006*. Bilthoven: National Institute for Public Health and the Environment (RIVM).
- Kusumaningrum, H.D., van Asselt, E.D., Beumer, R.R. and Zwietering, M.H. (2004) A quantitative analysis of cross-contamination of *Salmonella* and *Campylobacter* spp. via domestic kitchen surfaces. *J Food Prot* **67**, 1892–1903.
- Lorimer, M.F. and Kiermeier, A. (2007) Analysing microbiological data: Tobit or not Tobit? *Int J Food Microbiol* **116**, 313–318.
- Luber, P., Brynestad, S., Topsch, D., Scherer, K. and Bartelt, E. (2006) Quantification of *Campylobacter* species cross-contamination during handling of contaminated fresh chicken parts in kitchens. *Appl Environ Microbiol* **72**, 66–70.
- Martínez-Tomé, M., Vera, A.M. and Murcia, M.A. (2000) Improving the control of food production in catering establishments with particular reference to the safety of salads. *Food Control* **11**, 437–445.
- Mattick, K., Durham, K., Domingue, G., Jorgensen, F., Sen, M., Schaffner, D.W. and Humphrey, T. (2003) The survival of foodborne pathogens during domestic washing-up and subsequent transfer onto washing-up sponges, kitchen surfaces and food. *Int J Food Microbiol* **85**, 213–226.
- Medeiros, L.C., Kendall, P., Hillers, V., Chen, G. and Dimascola, S. (2001) Identification and classification of consumer food-handling behaviors for food safety education. *J Am Diet Assoc* **101**, 1326–1339.
- Montville, R., Chen, Y. and Schaffner, D.W. (2001) Glove barriers to bacterial cross-contamination between hands to food. *J Food Prot* **64**, 845–849.
- Moore, C.M., Sheldon, B.W. and Jaykus, L.A. (2003) Transfer of *Salmonella* and *Campylobacter* from stainless steel to romaine lettuce. *J Food Prot* **66**, 2231–2236.
- Mylius, S.D., Nauta, M.J. and Havelaar, A.H. (2007) Cross-contamination during food preparation: a mechanistic model applied to chicken-borne *Campylobacter*. *Risk Anal* **27**, 803–813.
- Nauta, M.J., Jacobs-Reitsma, W.F. and Havelaar, A.H. (2007) A risk assessment model for *Campylobacter* in broiler meat. *Risk Anal* **27**, 845–861.
- Nauta, M.J., Fischer, A.R.H., van Asselt, E.D., de Jong, A.E.I., Frewer, L.J. and de Jonge, R. (2008) Food safety in the domestic environment: the effect of consumer risk information on human disease risks. *Risk Anal* **28**, 179–192.
- Redmond, E.C. and Griffith, C.J. (2003) Consumer food handling in the home: a review of food safety studies. *J Food Prot* **66**, 130–161.
- Zwietering, M.H. and van Asselt, E.D. (2005) The range of microbial risks in food processing. In *Handbook of Hygiene Control in the Food Industry* ed. Lelieveld, H.L.M., Mostert, M.A. and Holah, J. pp. 31–45 Cambridge: Woodhead Publishing Limited.

Appendix

Transfer via cutting boards, hands and knives to the final salad

Transfer rates (t) can be determined by comparing various scenarios. For example, transfer via the cutting board can be determined by comparing the best case (BC) scenario (in which the chicken is not touched by hand and new knives and cutting boards are used to cut the boiled chicken) with a scenario where the same cutting board is used for raw and boiled chicken.

Scenario 1. Best case:

$$\left(\frac{N_S}{N_0}\right)_1 = (1 - t_{ch} - t_{cb} - t_{ck}) \exp(-k\tau)$$

Scenario 2. BC with same (unwashed) cutting board ($t_{bw} = 0$):

$$\left(\frac{N_S}{N_0}\right)_2 = t_{cb}t_{bs} + (1 - t_{ch} - t_{cb} - t_{ck}) \exp(-k\tau)$$

As N_S and N_0 are known for both scenarios, $t_{cb}t_{bs}$ can be estimated by subtracting the two:

$$t_{cb}t_{bs} = \left(\frac{N_S}{N_0}\right)_2 - \left(\frac{N_S}{N_0}\right)_1$$

As in the BC all data were below the detection limit, $\left(\frac{N_S}{N_0}\right)_1$ is assumed to be negligible.

Quantifying the washing effect

The effect of washing was determined by comparing scenarios with and without washing. For example, to determine transfer from the cutting board after washing with cold water, the following scenario was used:

Scenario 3. BC with board washing (with cold water only):

$$\left(\frac{N_S}{N_0}\right)_3 = t_{cb}(1 - t_{bw(\text{cold})})t_{bs} + (1 - t_{ch} - t_{cb} - t_{ck}) \exp(-k\tau)$$

Transfer from the cutting board to the sink because of washing with cold water is then:

$$t_{bw(\text{cold})} = 1 - \frac{\left(\frac{N_S}{N_0}\right)_3 - \left(\frac{N_S}{N_0}\right)_1}{\left(\frac{N_S}{N_0}\right)_2 - \left(\frac{N_S}{N_0}\right)_1}$$

Transfer from chicken to items

Transfer from chicken to items (t_{ci}) is determined based on the BC scenario without cooking. In this scenario, the chicken is put in cold bouillon for 10 min and afterwards cut on a

clean cutting board with a clean knife. In this case, transfer from raw chicken to items takes place two times: before and after putting the chicken in cold bouillon. Therefore, t_{ci} can be determined as:

$$\left(\frac{N_S}{N_0}\right)_4 = (1 - t_{ch} - t_{cb} - t_{ck}) \exp(-k\tau)(1 - t_{ch} - t_{cb} - t_{ck})$$

transfer before bouillon transfer after bouillon

As cooking time $\tau = 0$, t_{ci} can be determined as:

$$t_{ch} + t_{cb} + t_{ck} = 1 - \sqrt{\left(\frac{N_S}{N_0}\right)_4}$$