-Preliminary Report-

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Joint FAO/WHO Activities on Risk Assessment of Microbiological Hazards in Foods:

-Preliminary Report-

Hazard identification, hazard characterization and exposure assessment of *Campylobacter* spp. in broiler chickens

prepared by

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1. Introduction

The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) assembled an expert drafting committee in early in 2001 to begin developing a risk assessment framework for *Campylobacter* spp. in broiler chickens. The framework is composed of hazard identification, hazard characterization and exposure assessment with the intention of completing the risk characterization portion in the year 2002. This initial framework will be presented to the Codex Committee on Food Hygiene for comment in the Fall of 2001. The work conducted by the drafting committee builds upon the work of three major risk assessments for *Campylobacter* in chicken conducted by Canada (Fazil *et al.,* 2000), Denmark (Christensen *et al.,* 2001) and the United Kingdom (Hartnett *et al.,* a; b; c). The review and analysis of current scientific information and the computer model can be used by member countries to conduct a country-specific risk assessment or to identify and collect the needed information and research for inclusion in a risk assessment.

The purpose of the 'Draft Hazard Identification, Hazard Characterization and Exposure Assessment of *Campylobacter* spp. in broiler chickens' was to conduct a thorough examination of the current and available scientific information to identify and characterize the risk posed by *Campylobacter* spp. in broiler chickens. The drafting group was asked by the Codex Alimentarius Committee to consider the same risk management questions as asked for Salmonella in broilers/chickens: The questions requested the drafting committee to provide estimates for: 1) The risk of exposure and illness given exposure from *Campylobacter* in broilers/chickens and 2) The change in exposure and illness likely to occur for different interventions in primary production, in processing and in food handling. In these initial stages of development our framework does not evaluate the risk or adverse outcomes, such as illness for a human population. However, risk and the likelihood of adverse outcomes will be addressed in the risk characterization for *Campylobacter* spp. in broiler chickens work that will be done in the year 2002.

In our analysis, human exposure to *Campylobacter* from consumption of chicken is estimated through the careful evaluation of the variety of pathways ranging from practices on the farm, during processing and production, through consumer handling that may contribute to contamination of chicken with the organism. Furthermore, we assess and compare the effects of mitigation measures at various stages of production and handling on the prevalence and levels of *Campylobacter* on broiler chickens throughout the farm-to-fork continuum to evaluate their value in reducing the prevalence and/or concentration of organisms on broiler chickens.

1.1 Approach: Exposure assessment

Our approach for the exposure assessment portion of the model was to develop a model that details the prevalence and concentration of *Campylobacter* throughout the production line from farm to fork based on the models already prepared by Canada, UK, and Denmark. The exposure assessment addresses:

- Farm and production practices: This component evaluates the pathways on the farm during rearing and transportation by which chickens may acquire *Campylobacter*, and spread of the organism to uninfected birds. The impact of different intervention strategies that may reduce the prevalence of positive flocks or the amount of organism is evaluated.

- Slaughterhouse and processing: The processing component scrutinizes the various impact of various processes on prevalence, concentration and cross-

contamination of other birds with the organism. Of special interest are 5 major practices – scalding, defeathering, evisceration, washing, and chilling- which provide opportunities for cross-contamination with *Campylobacter* to other broiler carcasses in close proximity. The effectiveness of different intervention strategies that may reduce the prevalence and, or concentration on slaughtered carcasses is also examined.

- Post-processing and Consumer handling practices: This component of the model considers the impact of cooking and cross-contamination and their potential contributions to human exposure to *Campylobacter*.

As concrete demonstrations of how the computer model can be used for country-specific broiler systems, it will be run with input data from a developed country (referred to as Country A) and, if available and possible, also with input data from a developing country.

1.2 Approach: Hazard characterization

Hazard characterization describes the dose-response relationship, which when combined with the estimated dose derives a probability of the percentage of individuals that may become ill. The FAO/WHO guidelines on hazard characterization were followed when identifying the elements that should be represented in the hazard characterization section. This section describes:

- The characteristics of the pathogen, host, and food matrix. The characteristics of the pathogen that will be discussed include the influences of infectivity, virulence and pathogenicity of the organism on the ability of the organism to elicit infection and illness. Host characteristics that could influence acquisition of an infection include susceptibility, sex, environmental factors, genetics, underlying disease and concurrent medication. The influence of the food matrix is also addressed.

- The public health outcomes addressed will include the likelihood for infection, gastrointestinal illness, septicemia, and also non-gastrointestinal sequelae such as Guillain-Barre syndrome and reactive arthritis.

- Susceptible populations: Although susceptible populations are likely an important factor in determining who acquires illness that progresses to more severe consequences, there are few data on susceptible sub-populations that can be used in this portion of the model. However, susceptible subpopulations may be addressed in the risk characterization portion of the *Campylobacter* spp. in broiler chickens risk assessment.

- The approach used in setting up a dose-response model was to set up a model based on the data from Black *et al.* (1988) (human feeding trial). The data will be fitted to a number of relationships including the Beta-poisson model to determine which one best fits the available data.

In the risk characterization the completed model will use the dose-response models to link the hazard characterization and exposure assessment components to derive an estimate for risks of *Campylobacter* illness to human populations.

1.3 Campylobacter species included

In many countries *Campylobacter jejuni* is isolated as the agent responsible for more than 90% of cases of campylobacteriosis. *Campylobacter coli* is often the second most often isolated

species. For the most part when this report is discussing *Campylobacter* spp., especially human clinical isolates, we are referring to *Campylobacter jejuni*.

1.4 Products included

The products considered in this report include fresh intact broilers or whole chickens, and chicken parts. Frozen chicken is also considered, because freezing of chicken directly after processing as a mitigation to reduce *Campylobacter* is used in many facilities. The drafting committee found no evidence of different prevalences or concentrations of *Campylobacter* on intact broiler chickens versus broiler parts.

2. Hazard identification

The hazard identification portion of the model identifies and characterizes the risks of *Campylobacter* infection arising from the consumption of chicken in the human population.

2.1 Human incidence in developed countries

In the 1970s, with the development of suitable selective media, it was established that thermophilic *Campylobacter* was a common cause of bacterial gastroenteritis in humans (Skirrow, 1977). *Campylobacter* is now the leading cause of zoonotic enteric infections in most developed and developing countries (WHO, 2000). The trend for registered human cases per 100.000 inhabitants caused by thermophilic *Campylobacter* for a number of countries is shown in Figure 2.1. The cases are usually caused by *Campylobacter jejuni* or to a lesser extent by *Campylobacter coli* (Nielsen *et al.,* 1997; Wooldridge & Ketley, 1997; Anon., 1999; Nadeau *et al.,* 2001). Most human *Campylobacter* infections are classified as sporadic single cases or as part of small family related outbreaks. Identified outbreaks are not common.

As it appears from Figure 2.1, the reported incidence of *Campylobacter* infections has markedly increased in many developed countries within the last 20-year period. In some countries there has been a steady increase during the entire 20 years, whereas in other countries the increase has occurred primarily in the 1990s. In countries such as Denmark, Sweden, Finland, Norway, the Netherlands, and the UK the incidence rate has exceeded that of *Salmonella* (Anon., 2001a). In a few countries the incidence rate has started to decline, e.g. in Belgium, the Netherlands, England and Wales, New Zealand, Iceland, and the USA. In Belgium the decline, which started in June 1999, might be attributable to a precipitous drop in chicken consumption triggered by the dioxin crisis, since chicken and other meat products were withdrawn from the shops at that time (Anon., 2001a). In Iceland the significant decrease in the number of human cases from 1999 to 2000 may be explained by the fact that several interventions and mitigation strategies have been introduced in the broiler production during this period.

The incidence rates of *Campylobacter* infections vary widely (Figure 2.1) for example from 12.9 cases per 100.000 inhabitants in Spain in 1999 to 232.5 in New Zealand. Some of the variation may partly be explained by differences in surveillance systems, diagnostic methods and means of reporting, so caution should be used when drawing inferences from the data.

It seems likely that there is under-reporting of *Campylobacter* infections and the true number of cases is actually higher than the registered number of cases. This is because the incidence rates only reflect the laboratory confirmed cases of *Campylobacter* infections, cases where the patients have consulted a general practicing doctor/hospital, and where *Campylobacter* has been detected in a stool sample from the patient, i.e. only a fraction of the true number of infections. The true rate of infection is considered to be higher than the number of reported cases (from 7.6 up to 100 times as high) (Skirrow, 1991; Kapperud, 1994; Wheeler *et al.,* 1999, Mead *et al.,* 2000).

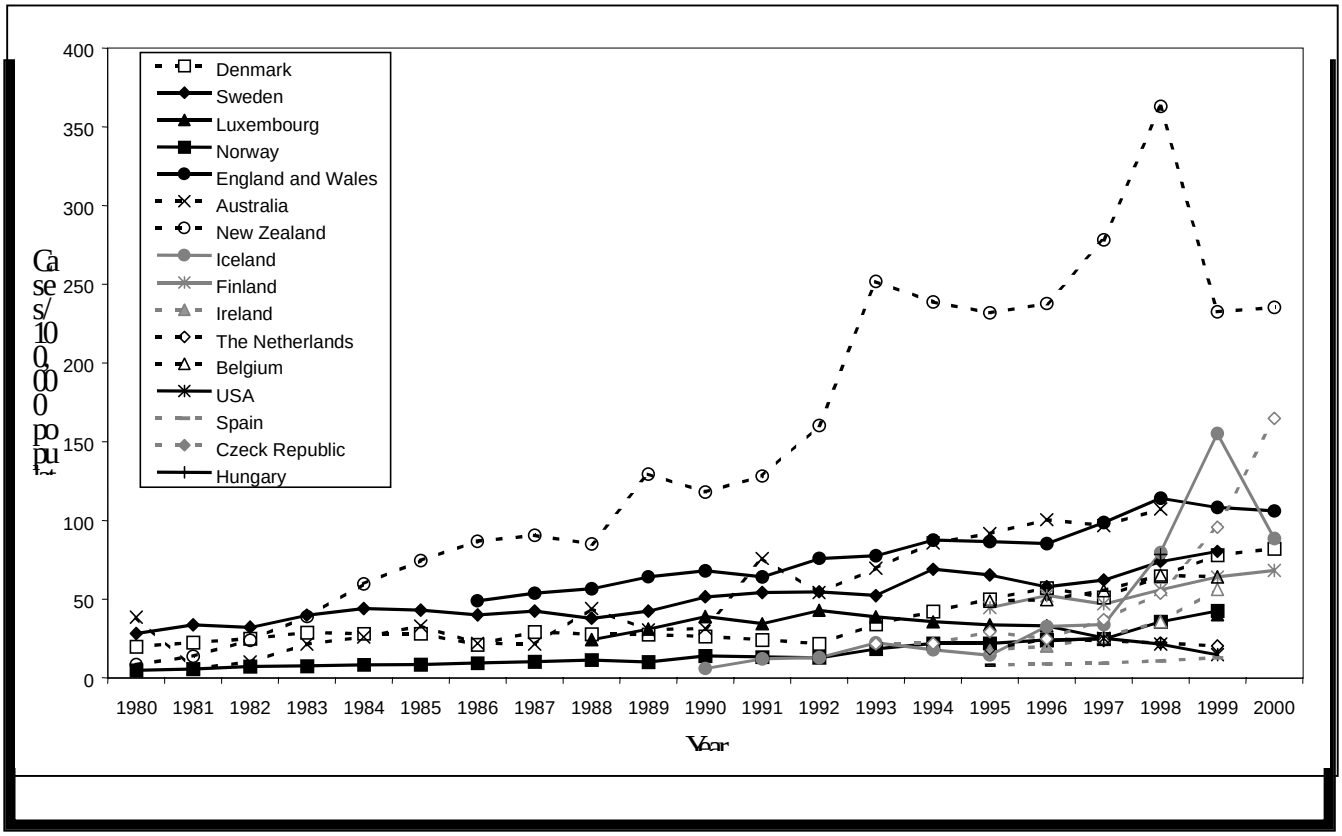

Figure 2.1. The number of registered* human cases per 100.000 population caused by *Campylobacter jejuni/coli*. The data presented are the data available to the drafting group in the summer 2001. (CDC-FoodNet; Anon 1999; Friedman *et al.* 2000a; Anon., 2001a; Anon., 2001b; Anon. 2001c; Anon; 2001d; Anon., 2001e; Benes, 2001; Kruse, 2001; F. Georgsson, *Pers.Comm*.).

*Laboratory confirmed cases of campylobacteriosis are notifiable in Austria, Denmark, Finland, Sweden, and Norway. In Ireland campylobacteriosis is notifiable when detected in the context of food poisoning. In other countries reports are based on laboratory isolates. Besides this the requirement to notify foodborne infections (on a clinical basis, without the causative agent) exists in Italy, England and Wales and Northern Ireland (Anon., 2001a).

(On request of the Expert Consultation, the figure will be updated if more data become available).

2.2 Human incidence in developing countries

The burden of human *Campylobacter* infections is not known in the developing countries, as there is no national surveillance in these countries. However, it is likely that the rate of campylobacteriosis is high especially among children below 2 years of age (Blaser *et al.,* 1983) causing substantial morbidity and eventually morbidity. For example campylobacteriosis contributes significantly to malnutrition in infants, as campylobacteriosis is particularly acute during the weaning period (WHO, 2000). (On request of the Expert Consultation this section will be expanded).

2.3 Characteristics of the organism

Bacteria belonging to the genus *Campylobacter* are non-sporeforming, oxidase-positive, Gram-negative rods. Cells are pleomorphic. Log-phase cells have a characteristic slender, curved or spiral shape and have flagella, usually single, at one or both poles (monotrichate or amphitricate) and are highly motile, spinning around their long axes and frequently reversing direction. As cultures age, spiral or curved forms may be replaced by coccoid forms (Barrow and Feltham, 1993).

In general, *Campylobacter* species do not grow in conventional aerobic or anaerobic culture systems. *Campylobacter* does not ferment or oxidize sugars and are oxygen-sensitive microaerophilic bacteria, with optimal growth in an atmosphere containing 5-10% oxygen. Since *Campylobacter* is sensitive to hydrogen peroxide and superoxide anions produced in media, lyzed blood and FBP (0.025% each of ferrous sulphate, sodium metabisulphite, sodium pyruvate) are added to enrichment broths and selective agars to neutralize these toxic products of oxygen and to increase the aerotolerance of the organisms (ICMSF, 1996).

C. jejuni and *C. coli* are distinguished from most other *Campylobacter* species by their high optimum growth temperature (42°C). *C. jejuni* has two subspecies; subsp. *jejuni* – the familiar cause of enterocolitis in man and subsp. *doylei* – a more fastidious and slower growing organism which does not grow at 43°C. *C. upsaliensis* also appears to be enteropathogenic for man. This species is related to the 'thermophilic' *Campylobacter,* even though not all strains grow at 43°C. *C. upsaliensis* is seldom detected by conventional methods used for *C. jejuni* and *C. coli*. Primary isolation of this organism usually requires the use of selective filtration, non-selective media and incubation at 37°C. Additionally, *C. upsaliensis* requires H_2 or formate for microaerophilic growth (Holt *et al.,* 1994). *C. lari* is 'thermophilic' like *C. jejuni* and *C. coli* but is considered to be of low virulence and is only occasionally encountered in man (Barrow and Feltham, 1993). (On request of the Expert Consultation more species and the influence by media will be included in this section).

2.3.1 Growth

In general, *Campylobacter* spp. grow at 37°C, but not below 32°C (Table 2.1), i.e. it is reasonable to assume that *Campylobacter* spp. do not multiply during slaughtering, post processing, transport and storage. However, the organisms may survive these steps, especially when the temperature is low. In various food items survival has been recorded after several weeks of storage at 4°C and in frozen poultry after several months (Table 2.2). Though *Campylobacter* may persist for prolonged periods in chilled and frozen products, a reduction in the concentration (Table 2.2) and a decline in the viability are observed during storage.

2.3.2 Death or inactivation

Campylobacter is particularly sensitive to drying and reduced pH. For example *Campylobacter* is inhibited at pH values below 5.1. In addition, *Campylobacter* is sensitive to salt concentrations above 1.5% (ICMSF, 1996). *C. jejuni* and *C. coli* are rather sensitive to heat and do not survive

cooking or pasteurisation temperatures (D-values are 0.21-2.25 minutes at 55-60°C) (ICMSF, 1996) (see also Table 2.3). (On request of the Expert Consultation, more test will be added describing the reduction by freezing).

Table 2. 1 Growth characteristics of thermophilic *Campylobacter* species (ICMSF, 1996)

	Minimum	Optimum	Maximum
Temperature (°C)	32	42-43	45
PH	4.9	6.5-7.5	ca. 9
NaCl (%)	-	0.5	1.5
Water activity (a_w)	>0.987	0.997	-
Atmosphere	-	5% O_2 + 10% CO_2	-

Table 2.2 Effect of chilling and freezing on the number of *Campylobacter* in meat products

Substrate	Storage temp. (°C)	Initial decrease (log_{10} cfu/day)	Total decrease (log_{10} cfu/day)	Strains examined	Reference
Chicken carcass	-20	-0.1-1.4/21	-0.5-2.3/84	5 C.j./C.c.	Hänninen, 1981
Chicken drip	-20	-0.1-1.1/21	-0.6-2.5/84	5 C.j./C.c.	Hänninen, 1981
Chicken carcass	-20	-0.5/36	-1.4/64	NF	Oosterom *et al.,* 1983a
Chicken liver	-20	-1/'few'	-1.6/84	NF	Oosterom *et al.,* 1983a
Chicken drumsticks	-20	-1.4/7	-2.7/182	1 C.j.	Yogasundram & Shane, 1986
Chicken breast skin	-20	-2.4/3*	ca. –3.7/56	1 C.j.	Lee *et al.* 1998
Ground beef liver	-20	-0.9-1.4/3	-2.3-2.6/84	5 C.j./C.c.	Hänninen, 1981
Ground beef	-15	-3/3	-3/14	5 C.j.	Stern & Kotula, 1982
Raw chicken breast	2	-	-5-6/24	2 C.j.	Curtis *et al.,* 1995
Raw minced beef	2	-	-5-6/27	2 C.j.	Curtis *et al.,* 1995
Cooked minced beef	2	-	-5-6/49	2 C.j.	Curtis *et al.,* 1995
Patê	2	-	-5-6/15	2 C.j.	Curtis *et al.,* 1995
Ground beef liver	4	-0.0-0.4/6	-	5 C.j./C.c.	Hänninen, 1981
Cooked chicken	4	-0.3-0.7/7*	-	3 C.j.	Blankenship & Kraven, 1982
Chicken carcass	4	-0.6-1/4-7	-	NF	Oosterom *et al.,* 1983a
Chicken drumsticks	4	-0.7/7	-	1 C.j.	Yogasundram & Shane, 1986
Chicken breast skin	4	+1.4/7*	-	1 C.j.	Lee *et al.* 1998
Raw chicken breast	10	-	-5-6/13	2 C.j.	Curtis *et al.,* 1995
Cooked minced beef	10	-	-5-6/23	2 C.j.	Curtis *et al.,* 1995
Patê	10	-	-5-6/6	2 C.j.	Curtis *et al.,* 1995

C.j. = *Campylobacter* jejuni; C.c. = *Campylobacter* coli; *, numbers estimated from a figure presented in the reference; NF, natural *Campylobacter* contamination

Table 2.3. Effect of heat treatment on the number of *Campylobacter* in scald water and chicken products

Substrate	Temp. (°C)	D (min)	pH	Strains exa-mined	Repli-cates	Method of heat[a]	Reference
Scald water	52	0.4±0.02	4.0	1	3	Dir	Humphrey & Lanning, 1987
Scald water	52	8.72±0.12	6.0	1	3	Dir	Humphrey & Lanning, 1987
Scald water	52	11.50±0.2	7.0	1	3	Dir	Humphrey & Lanning, 1987
Scald water	52	6.40±0.28	8.0	1	3	Dir	Humphrey & Lanning, 1987
Scald water	52	2.00±0.41	9.0	1	3	Dir	Humphrey & Lanning, 1987
Scald water	52	1.00±0.22	10.0	1	3	Dir	Humphrey & Lanning, 1987
Cooked chicken	53	4.85-4.49	-	6[b]	3	RT	Blankenship & Kraven, 1982
Cooked chicken	55	2.12-2.25	-	6[b]	3	RT	Blankenship & Kraven, 1982
Cooked chicken	57	0.79-0.98	-	6[b]	3	RT	Blankenship & Kraven, 1982

a, Dir, cells added to heating media at test temperature; RT, cells heated from ambient temperature
b, one strain was tested separately, the other five strains were tested together

2.4 Reservoirs

The principal reservoir of pathogenic *Campylobacter* spp. is the alimentary tract of wild and domesticated mammalians and birds. Several countries have monitoring programs to determine the prevalence of *Campylobacter* in food producing animals and birds. The results of these programmes are reported to the WHO and published by the Comminity Reference Laboratory on the Epidemiology of Zoonoses (BgVV, Berlin). From the BgVV reports it is evident that *Campylobacter* is commonly found in broilers, cattle, pigs, sheep, wild animals and birds, and in dogs (Anon. 2001a)). Other investigations have shown that healthy puppies and kittens (Hald & Madsen, 1997), rhodents (Cabrita *et al.,* 1992; Berndtson, 1996), beetles (Jacobs-Reitsma *et al.,* 1995), and houseflies (Rosef & Kapperud, 1983; Berndtson, 1996) may also carry *Campylobacter*.
(On request of the Expert Consultation seasonality will be included).

C. jejuni and *C. coli* seem to have a favoured reservoir. *C. jejuni* is predominantly associated with poultry (Tauxe, 1992; Anon., 1998a; 1999; 2001a; Nadeau *et al.,* 2001), but has also been isolated from cattle, sheep, goats, dogs and cats (Nielsen *et al.,* 1997; Anon., 1999). *C. coli* is predominantly found in pigs (Rosef *et al.,* 1983; Nielsen *et al.,* 1997), but has also been isolated from poultry, cattle, and sheep (Anon., 1999). In a Norwegian survey, 100 percent of the pigs examined were colonized with *C. coli* (Rosef *et al.,* 1983). In a Danish investigation of faeces from 600 pigs, 94.7% of the animals were colonized by *C. coli* and 0.3% with *C. jejuni* (Sørensen & Christensen, 1996).

Water is also an important part of the ecology of *Campylobacter*. *Campylobacter* has been isolated from surface water, rivers, and lakes at prevalences up to about 50% (Bolton *et al.,* 1987; Carter *et al.,* 1987; Brennhovd *et al.,* 1992; Arvanitidou *et al.,* 1995). Additionally, 45% of sand samples from bathing beaches contained *Campylobacter* (Bolton *et al.,* 1999a). This means that *Campylobacter* may be present in untreated drinking water and bathing water. *Campylobacter* is introduced into the water by sewage and faeces from wild animals and birds. The isolation frequency of *Campylobacter* from water is highest in cold winter months (Carter *et al.,* 1987; Brennhovd *et al.,* 1992). This is explained by a higher survival rate at low temperatures. It has been shown that in water *C. jejuni* survived for one to over four weeks at 4°C, whereas at 25°C the bacterium persisted for only 4 days (Blaser *et al.,* 1980). Another study

has shown that *C. jejuni* remained recoverable for up to four months when suspended in aged, filter-sterilized stream water held at 4°C. At 25°C and 37°C the bacteria became non-culturable within 28 and 10 days, respectively (Rollins and Colwell, 1986). Variations in exposure to daylight may also contribute to the high isolation frequency in winter and low isolation frequency in summer. In seawater, *Campylobacter* has been found to survive for 24 h in darkness and for 30-60 min in daylight (Jones *et al.,* 1990).

In water and other environments with sub-optimal growth conditions, *Campylobacter* may convert into a 'viable but non-culturable state'. The importance of this 'state' in transmission of *Campylobacter* to animals and man is not agreed upon. The question is if the viable non-culturable organisms are still virulent or if they can reverse into a culturable, virulent state after passage through a host. In some studies 'viable but non-culturable' *Campylobacter* organisms have shown to regain culturability after passage through for example chicks (Stern *et al.,* 1994), mice (Jones *et al.,* 1991a), rats (Saha *et al.,* 1991), and embryonated eggs (Cappelier *et al.,* 1999). In other studies it has not been possible to demonstrate that 'viable but non-culturable' *Campylobacter* can regain culturability (Beumer *et al.,* 1992; Medema *et al.,* 1992; Boucher *et al.,* 1994; Fearnley *et al.,* 1996; Korsak & Popowski, 1997). The possible influence of 'viable but non-culturable' *Campylobacter* on human health is not dealt with in the present risk assessment, as their role in the food chain is still unknown.

2.5 Prevalence in retail products

As *Campylobacter* is a common inhabitant of the gastrointestinal tract of warm-blooded animals, faeces content will inevitably contaminate the meat during slaughter and evisceration. As regards cattle and pigs, the concentration of *Campylobacter* has shown to decline during the slaughter processes, probably due to the dehydration of the meat surface that takes place during cooling with forced ventilation procedures (Oosterom *et al.,* 1983a). In 1995 a Danish investigation of 600 pig carcasses showed that the chilling procedure reduced the prevalence and numbers of *Campylobacter* on the carcass surfaces from 43-85% to 11-18% (Sørensen & Christensen, 1996).

Contrary to the reductions observed in the processing of cattle and pigs, broiler chicken processing does not tend to reduce the *Campylobacter* concentration significantly. Scalding, plugging, cooling, freezing and subsequent storage do not eliminate the organism only reduce the concentration (Oosterom *et al.,* 1983b; Izat *et al.,* 1988). Investigations of broiler processing plants have shown that *C. jejuni* is present at all stages of production, when a *Campylobacter*-positive flock has passed the equipment.

Several countries within the EU monitor and report the incidence of *Campylobacter* in food at retail to WHO/ the Community Reference Laboratory on the Epidemiology of Zoonoses (BgVV, Berlin). The BgVV reports (Anon., 1998a; 1999; 2001a) show that especially poultry meat is contaminated with *Campylobacter* (prevalences up to 75% in 1999). A high prevalence (77%) has also been found in lamb liver in a study in Northern Ireland (Anon, 2001a). At low frequencies, *Campylobacter* has been found in beef, pork, other meat products, raw milk and milk products, and in fish and fish products. Other food items, from which *C. jejuni* has been detected, are mushrooms (Doyle & Schoeni, 1986), fresh vegetables such as spinach, lettuce, radish, green unions, parsley and potatoes (Park & Sanders, 1992) and modified atmosphere packaged foods such as unsmoked bacon and salad vegetables (Philips, 1998).

Seasonality seems to influence the *Campylobacter* prevalences in retail chicken products in some countries. Higher recovery rates (P=87%-97%) have been observed during the warmer months of the year in chicken from supermarkets in North Carolina compared to December and January (P=7% and 33%, respectively) (Willis & Murray, 1997). Seasonality in *Campylobacter* prevalences has also been observed in Denmark (Rosenquist & Nielsen, 1999).

2.6 Risk factors in developed countries

Campylobacter may be transmitted from the reservoirs to humans by direct contact with contaminated animals or animal carcasses or indirectly through the ingestion of contaminated food or water.

2.6.1 Food related risk factors

The possible risk factors related to sporadic cases of human campylobacteriosis have been investigated in several case-control studies (Norkrans & Svedheim, 1982; Hopkins *et al.,* 1984; Oosterom *et al.,* 1984; Harris *et al.,* 1986; Deming *et al.,* 1987; Brieseman, 1990; Southern *et al.,* 1990; Lighton *et al.,* 1991; Kapperud *et al.,* 1992; Saeed *et al.,* 1993; Schorr *et al.,* 1994; Adak *et al.,* 1995; Neal & Slack, 1997; Friedman *et al.,* 2000b; Effler *et al.,* 2001; Neimann, 2001). Most studies have identified handling raw poultry and eating poultry products as important risk factors accounting for a variable percentage of cases. Other food related risk factors that have repeatedly been identified include consumption of other meat types, undercooked or barbecued meat, raw seafood, drinking untreated surface water or unpasteurized milk or dairy products. Also eating meat cooked outside the home (at restaurants) has been identified as a risk factor in the USA, New Zealand and Hawai (Brieseman, 1990; Friedman *et al.,* 2000b; Effler *et al.,* 2001).

Other food items that have been related to sporadic cases of human campylobacteriosis are contaminated shellfish (Griffin *et al.,* 1983; Harris *et al.,* 1986) and contaminated cucumbers (Kirk *et al.,* 1997).

Cross-contamination of *Campylobacter* from raw chicken to prepared food has also been identified as a risk factor. Harris *et al.* (1986) observed an association between infection and not washing the kitchen cutting board with soap.

The major risk factors that have usually been associated with outbreaks of campylobacteriosis are consumption of unpasteurized milk, foods – in particular poultry, untreated surface water and contaminated public and private water supplies (Finch & Blake, 1985; Peabody *et al.,* 1997; Engberg *et al.,* 1998; Neimann, 2001).

Outbreaks and sporadic cases seem to have different epidemiological characteristics. For example, the sporadic cases seem to peak in summer, whereas the outbreaks (based on 57 outbreaks in the United States) seem to culminate in May and October (Tauxe, 1992).

2.6.2 Other risk factors

Other risk factors, which have been identified, are travelling, contacts with pets and farm animals, and recreational activities in nature.

Exposure during travel abroad seems to be a common risk factor of human campylobacteriosis in the Northern European countries. In Denmark and UK travelling abroad has been estimated to account for 10-25% of the reported cases (Cowden, 1992; Neal & Slack, 1995; Mølbak *et al.,* 1999). In Sweden and Norway the estimated percentage is 40-60% (Kapperud & Aasen, 1992; Berndtson, 1996). Campylobacteriosis has mainly been associated with travel to the Mediterranean countries and Asia (Kapperud, 1994; Mølbak *et al.,* 1999; Neimann, 2001).

Several investigations have pointed out contact with pets, particularly young pets like kittens and puppies, as a behaviour increasing the risk of acquiring infection by *Campylobacter* spp. (Blaser *et al.,* 1978; Hopkins *et al.,* 1984; Deming *et al.,* 1987; Brieseman, 1990; Kapperud, 1994; Adak *et al.,* 1995; Neimann, 2001). Hald & Madsen (1997) found that 29% of the healthy puppies examined carried *Campylobacter* spp. with a species distribution of 76% *C. jejuni*, 5% *C. coli*,

and 19% *C. upsaliensis*. Only 5% of 42 healthy kittens examined excreted *Campylobacter upsaliensis.*

The information about the risk associated with professional handling of production animals at farm level is contradictory. One study carried out in New Zealand suggested that rural residence associated with live animals did increase the risk of human campylobacteriosis (Brieseman, 1985). Saeed *et al.* (1993) found no increased risk for *Campylobacter* enteritis associated with contact with various animals. However, exposure to diarrhoeic animals was associated with a four-fold increase in the risk of human campylobacteriosis. In addition, Brieseman (1990), Skirrow (1987) and Kist & Rossner (1985) described a higher incidence of campylobacteriosis in the rural population than in the population living in urban areas. In contrast, Adak *et al.* (1995) demonstrated that occupational contact with livestock or their faeces was associated with a decrease in the risk of becoming infected by *Campylobacter* spp.. Another investigation has revealed a higher incidence among the urban population than in the population living in rural areas (Kapperud & Aasen, 1992). Danish results (Neimann, 2001) do not indicate an increased risk of acquiring campylobacteriosis for people handling production animals at farm level.

As a consequence of the presence of *Campylobacter* spp. in the environment and in particular, untreated water, recreational activities taking place in the nature like camping, trekking and bathing could pose a risk of acquiring an infection by *Campylobacter* spp. In a case-control study carried out by Adak *et al.* (1995) it was found that ingestion of untreated water while participating in recreational activities was associated with an increased risk of acquiring campylobacteriosis as also suggested in earlier studies by Hopkins *et al.* (1984) and Skirrow (1987). In Norway, 42 of 96 water samples from streams and lakes were found positive with *Campylobacter* spp.. The distribution of species was *C. jejuni* 71.7%, *C. coli* 21.7%, *C. lari* 3.3% and non-typable 3.3%, indicating that the *Campylobacter* originated from sewage and run off from fields (Brennhovd, 1991).

Studies on the occurrence of *Campylobacter* spp. in seawater and sand from bathing beaches indicate that bathing could also pose a risk. Along the coast of Tel Aviv *C. jejuni* was isolated in levels ranging from 2-13 cfu per 100 ml seawater and 13-20 cfu per g sand (Ghinsberg *et al.,* 1994). In the UK, *Campylobacter* spp. was isolated in 46 out of 92 samples of sand from beaches with non-EEC Bathing Water Directive standard, and in 36 of 90 samples of sand from beaches having EEC standard. Further, *C. jejuni* and *C. coli* was isolated more frequently in sand from beaches that did not meet the EEC standard (Bolton *et al.,* 1999a).

2.6.3 Person to person transmission

In the developed countries person to person transmission is considered to be infrequent (Altekruse *et al.,* 1999). This is because infected humans constitute a minor reservoir for *C. jejuni*, since asymptomatic excretion of *Campylobacter* is uncommon. A few examples of person to person transmission have however been observed. For example in an outbreak in Kansas, USA, among people attending a school luncheon where the only source of transmission of *Campylobacter* seemed to be via the food handler, who happened to have a *Campylobacter* infection (Olsen *et al.,* 2001).

In the developing countries human carriage could play a larger role in the transmission of infection (Blaser *et al.,* 1983).

2.6.4 Relative importance of the risk factors

So far it has not been possible to quantify the number of *Campylobacter* cases related to each of the different risk factors described. (On request of the Expert Consultation a paragraph will be added describing the situation in countries, e.g. Belgium, Iceland, where the number of registered human cases of campylobacteriosis have decreased probably due to interventions

in the poultry production. Also information from case control studies on the estimated role of broiler chickens in human campylobacteriosis will be included).

However, the relative importance of the potential sources of *C. jejuni* for human cases of campylobacteriosis has been investigated in some studies by applying different subtyping methods to isolates of *C. jejuni* obtained from patients and the possible sources described. Similarities in the distribution of serotypes of *C. jejuni* isolated from humans, water, and chickens are reported by Hudson *et al.* (1999). Fricker & Park (1989) demonstrated similarities in the serotypes between isolates of *C. jejuni* originating from humans, offal, beef, sewage and poultry. Further on, Bänffer (1985) found a positive correlation in the frequencies of bio- and serotypes of *C. jejuni* isolated from humans and chickens, whereas isolates from humans and pigs showed no correlation. Frost *et al.* (1999) showed that the distribution of *C. jejuni* serotypes isolated from chicken and lamb was similar to that seen in concurrent human infections. Wareing *et al.* (1999) have described a strain of *C. jejuni* (Penner serotype HS4, 'complex': Preston phage-group 55), which has frequently been associated with human gastroenteritis in the UK. This strain seems to have a global distribution and has been shown to be the causative agent in several milk-borne outbreaks of human campylobacteriosis. Using a PFGE subtyping method Hänninen *et al.* (1999) demonstrated identical genotypes of *C. jejuni* isolated from cases of human infections and retail chicken meat in Finland. In Denmark, similarities between *C. jejuni* serotypes have been demonstrated among isolates from humans, broilers, and poultry products and - to a lesser extent – cattle, with serotype O:2 being the most dominant type (Anon., 1998b; Nielsen *et al.,* 1997; Nielsen & Nielsen, 1999). As regards *C. coli* similarities between serotypes isolated from humans, broilers, pigs and retail poultry products have been described. However, the frequency by which *C. coli* is isolated from humans and from retail poultry products in Denmark is low compared to *C. jejuni.* In a Canadian study macro-restriction profiling has revealed that approx. 20% of human *Campylobacter* isolates were genetically related to genotypes found in poultry (Nadeau *et al*., . 2001).

2.7 Risk factors in developing countries

In developing countries waterborne transmission and direct contact with animals are thought to be the major routes of human infection (Georges-Courbot *et al.,* 1990; WHO, 2000). (On request of the Expert Consultation this section will be expanded).

3. Exposure assessment

The exposure assessment considers the occurrence and number of *Campylobacter* that may be present in chicken and consumption data to determine dose.

3.1 Campylobacter on farm

3.1.1 Introduction

Broiler poultry production is highly specialised and follows a defined structure (ACMSF, 1996). When the birds are 1 day old they are moved to a broiler-growing farm, where they remain until they reach slaughter weight at ages between 30 and 60 days. At this point depopulation occurs; that is birds are removed from the house, and transported to the slaughter facility for processing to produce the sale product.

A typical grow out house contains litter on the floor and troughs which are filled with feed and water in lines. Several birds will feed from a single feed/water trough and the feed and water may even be circulated on belts throughout the house. While on the growing farm, despite the strict controls often in place by a given company, the intensive nature of production means that the birds are exposed to a variety of sources of *Campylobacter*. These may include contaminated farm staff, insects and even persistent contamination of the house itself as a result of a previously positive flock.

3.1.1.1 Campylobacter colonization and infection of poultry

The sources of *Campylobacter* infection of poultry flocks are still debatable. Vertical transmission via contaminated eggs has been documented but remains an area of controversy. Isolation from eggs has been demonstrated as a rare event. In particular, Shanker *et al.,* (1986) obtained only two positive eggs from a sample of 187 eggs from a *Campylobacter* positive breeder flock. The occurrence of only two positive samples is attributed to faecal contamination of the egg shell. Moreover, campylobacters have poor survival rates in egg albumen (Jones *et al.,* 1991b). Therefore it seems that vertical transmission is an unlikely source of infection (Annan-Prah and Jnac, 1988; Van De Giessen *et al.,* 1992).

Transmission from flock to flock, referred to as ‘carry-over’, also seems an unlikely occurrence due to the poor survival of campylobacters in the environment under ambient conditions (Kapperud *et al*., 1993; Jacobs-Reitsma *et al.,* 1995). Further sources such as feed (Humphrey *et al.*, 1993; Mead and Hinton, 1989) and litter (Pokamunski *et al*., 1986; Clark and Bueschkens, 1988) are unlikely as campylobacters are fragile organisms with an intolerance to desiccation. As such they are unlikely to survive well in feed or litter. Most evidence, serotyping and case-control studies (Evans, 1992);(Jacobs-Reitsma *et al*., 1995) suggest that the primary source of introduction into the flock is the external environment. As campylobacters are ubiquitous this hypothesis is intuitive.

Once the flock has been exposed to colonisation, the water and feed play an important role in the dissemination of colonisation throughout the flock. When colonisation is first detected in the birds the feed soon becomes culture positive (Genigeorgis *et al*., 1986). However the organism is rarely found in the water of flocks, which contain colonised birds. The absence of campylobacters in water samples at early stages of colonisation has been attributed to viable but non-culturable forms (VNC). Such forms of *Campylobacter* may be capable of resuscitation in vivo (Mead and Hinton, 1989). Furthermore, the importance of water in the transmission of the organism through a flock has been demonstrated experimentally. Chlorination of the water supply was shown to slow the within flock transmission of the organism (Pearson *et al.,* 1993).

Farm-workers play an interesting role in the epidemiology of flock colonisation. Case-control studies have demonstrated farm staff as a risk factor (Lindblom *et al.,* 1986; Evans, 1992) and external contamination of a flock by catchers has been demonstrated.

The risk of *Campylobacter* colonisation is strongly associated with age (Evans, 1996) with the probability of infection increasing with age. Survival analysis has indicated that a number of management factors acted as predictors of the age at which flocks became colonised but a follow up study reported that intervention methods were only successful in delaying the survival time (Evans, 1996). An interesting feature in the epidemiology of flock infection is the presence of a lag period, which occurs during the first 14 days in the house. During this period no birds can become colonised. This is consistently seen in commercial flocks (Lindblom *et al.,* 1986; Mead and Hinton, 1989) but absent in laboratory experiments (Shanker *et al.,* 1990). The reasons for this difference are unknown.

Seasonality of the colonisation of broiler chickens, i.e. a higher contamination rate during warmer periods, has been reported in certain countries, e.g. in Denmark, Norway, Great Britian and the Netherlands (Kapperud *et al.,* 1993; Jacobs-Reitsma *et al.,* 1994a; Newell *et al.,* 1998; Christensen *et al.,* 2001), but in other countries such as the USA and Canada (Quebec) no evidence of seasonal variation has been found (Gregory *et al.,* 1997; Nadeau *et al.,* 2001).

PFGE-typing has revealed a high genetic diversity among poultry isolates. Usually a flock is colonized by a unique genotype, but flocks raised at different grow-out periods often have different genotypes (Nadeau *et al.,* 2001).

To consider the extent of exposure of humans to contaminated chicken products and methods of control, estimation of the probability of a random chicken destined for human consumption being *Campylobacter* positive at the point of slaughter is required to enable the 'farm to fork' pathway to evolve. This section considers the rearing part or on-farm portion of the first module of the 'farm to fork' framework previously described. The model presented here estimates the probability of a random bird from within the British national flock being *Campylobacter* positive at the time of slaughter, together with an estimation of the uncertainty in this probability.

During the expert consultation it was pointed out that there is no discussion of risk factors and epidemiological studies which have been undertaken. This shall be included at a later date.

3.1.2 Model Description: Rearing or on the farm component

3.1.2.1 Overview

The aim of the rearing module is to estimate the probability that a random bird from the Great Britian poultry flock will be *Campylobacter* positive at the point of slaughter. This probability is defined as P_{pb} and can be estimated as shown in equation (1)

$$P_{pb} = P_{fp} * P_{wfp} \qquad (1)$$

where P_{fp} is the flock prevalence, that is the proportion of the national flock that is positive, and P_{wfp} is the within-flock prevalence of a positive flock at the time of slaughter. A positive flock is defined as a flock that contains one or more birds colonised with campylobacter. Estimation of P_{fp} and P_{wfp} was undertaken as follows.

3.1.2.2 Estimating flock prevalence, P_{fp}

The frequent colonisation of poultry flocks with *Campylobacter* is well documented (Byrd *et al.,* 1998; Gregory *et al.,* 1997). However little data exists on the prevalence of positive flocks within Great Britain or, indeed, world-wide. Currently there are no national surveillance schemes in Great Britian. Although some poultry production companies carry out routine monitoring, the asymptomatic nature of the colonisation means that this a low priority. Consequently this highlights an area of limited data.

Sample data obtained from two fully-integrated poultry companies, an epidemiological study (Evans, 1996) and a published source (Humphrey *et al.,* 1993) were used to obtain an estimate of P_{fp}, as described in Appendix 1.

3.1.2.3 Estimating within-flock prevalence, P_{wfp}

Within-flock prevalence (WFP) is a measure based on the number of birds expected to be colonised with *Campylobacter* within a positive flock. The WFP is directly related to the rate of transmission and is therefore a time dependent phenomenon for a positive flock. It has been reported that the within-flock transmission of *Campylobacter* is rapid and that once *Campylobacter* has been detected the WFP reaches 100% within seven days (Shanker *et al.,* 1990; Jacobs-Reitsma *et al.,* 1995), even in houses where bird movement is restricted (Shreeve *et al*, 2000). However the precise dynamics of *Campylobacter* transmission in poultry flocks is poorly understood.

Mathematical models have been used previously to investigate the pattern of disease epidemics (Bailey, 1975; Fukuda *et al.,* 1984) in both human and animal populations. Here, a mathematical approach has been adopted to describe the transmission of *Campylobacter* within a flock.

As discussed previously, poultry production is highly specialised and follows a defined structure (ACMSF, 1996). Briefly, when the birds are 1 day old they are taken to a broiler-growing farm, where they remain until they reach slaughter weight at ages between 30 and 60 days to become 'table birds'. At this point depopulation occurs; that is birds are removed from the house, and transported to the slaughter facility for processing to produce the sale product.

Upon arrival at the growing farms the birds are placed in a house where they form spatial clusters. This clustering effect is likely to be due to social factors. The display of social behaviour is common to fowl and has been well documented (McBride and Foenander, 1962; Collias *et al.,* 1966; McBride *et al.,* 1969; Wood-Gush *et al.,* 1978; Tribe, 1980; Pamment *et al.,* 1983) and experimental work suggests a similar social behaviour is displayed by birds in the commercial rearing environment (Preston and Murphy, 1989). The area explored by a given bird diminishes with age (Preston and Murphy, 1989) thus enhancing the clustering effect. This reduction can be attributed to the increase in size of birds in a fixed environment.

The transmission of *Campylobacter* in a flock is believed to begin with a single bird becoming colonised. The mechanism by which a single bird becomes colonised and the time at which this occurs is unknown. As discussed previously case-control studies have demonstrated several reservoirs to which a flock may be exposed. These include wild birds, rodents, and cross-contamination from the environment via farm workers (Annan-Prah and Jnac, 1988; Engvall *et al.,* 1986).

Following colonisation of the first bird within the flock, it is likely that transmission will initially be confined to the cluster in which this bird resides. During this process campylobacters are excreted in the faeces of positive birds. As broilers are copraphagic this leads to ingestion of the organisms by other birds in the flock and hence bird to bird transmission. As well as bird to bird transmission, excretion of the organism results in the contamination of the feed and water.

In a short time period (4 days) a threshold will be reached where the contamination level of feed and water is sufficient to cause extensive colonisation in birds as a result of the ingestion of these products. This allows for dissemination of campylobacters throughout the whole flock until all birds are colonised.

Given this description of transmission, it is appropriate to model the time dependent process of flock colonisation in two stages. The first stage is the initial transmission within the cluster containing the first bird that is colonised, and the second stage is the transmission throughout the remainder of the flock.

Within this model it is assumed that the first bird becomes colonised at a time $t = t_{ex}$. This time is defined as the age at first successful exposure of a bird in the flock, that is exposure which leads to colonisation with *Campylobacter*. This time is set to zero, that is $t_{ex} = t_0$. Stage 1 is described by a modified chain binomial model until a threshold time is reached. Experimental studies have shown that, following colonisation of the first bird, campylobacters can be detected in the feed, water and litter after 3 days (Shanker *et al.,* 1990). It is therefore assumed that the levels of contamination become sufficient to allow widespread dissemination of the organism throughout the flock 4 days following colonisation of the first bird. Thus a model for simple epidemic spread can be used to represent the second stage of the colonisation process. Thereafter transmission continues until either all birds become colonised or depopulation occurs at time t_A. The models describing each of the stages are presented by Hartnett *et al.* (a) and are detailed in Appendix 1.

From these models the number of colonised birds within a flock, that is *I(t)*, at time t is estimated. Hence the within-flock prevalence at time *t* since the time of exposure can be calculated directly as follows

$$P_{wfp}(t) = \frac{I(t)}{n}$$

3.1.2.3.1 Possible model modifications

The assumptions on which the model that represents the process of flock colonisation, that is estimation of P_{wfp}, are based are important in the interpretation of the generated results. Within the current model, it is assumed that a flock is initially comprised of birds in clusters. Successful colonisation occurs from a single bird in one cluster. The organism is then disseminated, initially by direct contact with the colonised bird and then via contaminated feed and water. The validity of the assumption that a single bird becomes colonised will depend on the source of infection. For example if *Campylobacter* is introduced into the house as a result of farm staff with, for example, contaminated foot wear, it is likely that there will be a point source of contamination in the house. As a result a single bird near to this point will become colonised first due to the level of exposure or individual bird characteristics such as immune status. In contrast if a contaminated water supply is the source of flock infection the situation is somewhat different.

Campylobacter are frequently isolated from water sources and contaminated water has been associated with human outbreaks of campylobacteriosis (Vogt *et al.,* 1982). If a flock is exposed to contaminated water multiple colonised birds will initiate the colonisation process. Homogeneous mixing could be expected as the water is circulated through the house. This could be described by use of the differential equation for epidemic spread, that is equation (4), in appendix 1, and disregarding the chain binomial model.

In addition it is debatable whether vertical transmission of *Campylobacter* can occur (Cox *et al.,* 1999; Jacobs-Reitsma, 1997). Certainly, if vertical transmission does occur it is likely to be an infrequent event with only up to 10 out of 1000 birds being colonised via this route. Such an occurrence would result in multiple colonised birds and multiple initial clusters containing colonised birds. This can be modelled by use of multiple chain binomial models (Ng and Orav, 1990) as follow.

3.1.2.3.2 Source of infection is contaminated feed and/or water

When the source of *Campylobacter* that a flock is exposed to is contaminated feed and/or water it can be assumed that the whole flock will be exposed. In this situation there will be random appearance of colonised birds beginning from the time that the feed and/or water enters the house. This is the situation presented above in stage two of the model and therefore the colonisation process can be described by use of the model described in Appendix 1.

3.1.2.3.3 Source of infection is via vertical transmission

It is currently debatable whether or not vertical transmission occurs. However if this mode of transmission is possible it has implications regarding the model described in this section. Initially there will be a number of birds that are colonised and as such begin the infection process. Each of these birds will then initiate colonisation of it's social cluster and consequently colonisation of the whole flock.

Both the above sources of infection can be described by modifications to the current mathematical models. These adjustments are described in Appendix 1.

3.1.3 Parameter Estimation

The parameters and their estimated distributions are listed in Table 3.1. There is extensive published work on campylobacter, however the number of studies that investigate the dynamics of within flock transmission of this organism is limited. As a result, values for A, R, and n_c are based upon expert opinion. Experts, including a veterinary epidemiologist, an avian ecologist and a broiler farm manager, selected for their experience with broiler flocks, were asked to provide estimates for minimum, most likely and maximum values for A, R, and n_c. These estimates have been used to define triangular distributions and opinions are combined within a discrete distribution as described in Vose (2000). More specifically, by using Discrete($\{E_1,E_2,\ldots,E_n\},\{w_{E1},w_{E2},\ldots,w_{En}\}$) where E_1, $E_2,\ldots,E_n$ are n individual experts opinions, defined by the associated triangular distributions, and w_{E1}, $w_{E2},\ldots,w_{En}$ are the associated weights of each opinion. Each expert is given equal weighting.

Table 3.1 Probability distributions and associated parameter values used in the model to estimate the probability distribution for a random bird selected from the UK chicken flock being *Campylobacter* positive at the point of slaughter

Parameter	Symbol	Probability Representation
Experimental Data		
Transmission rate per day	b	Uniform(0.1,0.3)
Expert Opinion		
Number of contacts a bird makes with other birds in one day	A	RiskDiscrete($\{\alpha,\beta,\gamma\},\{P_\alpha,P_\beta,P_\gamma\}$) Where: α~Triang(12,100,500)* β~Triang(30,50,120)* γ~Triang(20,45,100)*

Number of times a bird comes into contact with a given bird in one day	R	RiskDiscrete({λ,σ},{P_λ,P_σ}) Where: λ~Triang(3,5,6)* δ~Triang(2,6,8)*
Size of Cluster	n_c	RiskDiscrete({μ,ω},{P_μ,P_ω}) Where: μ~Triang(N/12,N/10,N/8)* ω~Triang(100,300,1000)*
Industrial Data		
Flock size	n	Triang(7800,30750,41596)
Age at depopulation in days	t_A	Triang(28,42,64)
Age at first exposure to *Campylobacter* in days	t_{ex}	Uniform(14,t_A)

* These parameters are Triangular distributions based on expert estimates

The biological transmission rate for *Campylobacter*, *b*, is based upon experimental studies (Stuart *et al.,* 1997; Shanker *et al.,* 1990). These studies involved the placing of a colonised bird in a group of un-colonised birds. Samples were then taken daily to measure the change in the number of colonised birds over time. From these studies two values for the transmission rate were estimated and used to define a uniform distribution; that is, all values between the two values of *b* are assumed equally likely to be the estimated value for a given flock. Ideally, more information is required, for example the most likely value of *b* within the range of these two values. If this information were available the use of a triangular distribution would allow values within the range to be weighted, providing a more realistic estimate for this parameter. The value of b_B is proportional to b as previously described. The proportionality factor is equal to $\frac{1}{10n}$. Due to the absence of data, experts in the area of the colonisation of chickens with *Campylobacter* agreed with this factor by inspection of the resulting epidemic curve.

The age at first successful exposure, t_{ex}, is an unknown parameter in the model. Several studies have shown that campylobacters are rarely isolated from commercial flocks under three weeks of age. One explanation of this is that the colonisation process probably begins with a single bird and it is possible that it takes time before positive birds are detectable in large commercial flocks. It is assumed that the time until the number of birds colonised is large enough to allow detection, after exposure to campylobacters, is one week. Therefore, the time of exposure, t_{ex}, is assumed to be a uniform random variable between fourteen days and the age at depopulation.

Finally, distributions for flock size (*n*), and time of depopulation (t_A) are derived directly from data involving several industrial sources that together are representative of approximately 50% of the national flock.

Currently the model does not explicitly consider rearing in developing countries. However, after discussions at the expert consultation, this model is capable of describing the infection of a flock reared in such a country. This shall be made more explicit at a later date. Further, the model will be extended to allow for a range of possible rearing strategies in developed and developing countries.

3.2 Contamination of chickens on the farm and during transport

3.2.1 Introduction

It is well recognised that the presence of pathogenic organisms in the gut of food-producing animals provides the potential to contaminate food products and hence result in exposure of the human population. As such it is necessary to quantify the level of *Campylobacter* likely to be

present in the gut of a colonised bird at the point of entry into the processing facility, where the slaughter process begins. This will enable a full estimation of the risk posed to the population as a result of such colonisation. However, there is a further reservoir of organisms which may enter the supply chain, these are organisms which contaminate the exterior of the birds. Such organisms may also result in the contamination of food products, hence the levels of such contamination are also required to enable estimates of exposure and ultimately risk from *Campylobacter* for the human population from chicken meat/products.

When a bird becomes exposed to, and ingests a level of *Campylobacter*, if the level is sufficiently high the organisms will establish, and reproduce within the gut of the bird. This process will continue until equilibrium is reached and the level of colonisation will be maintained leading to a stable bacterial population. Once colonised, broiler birds remain colonised. This is referred to a maximal colonisation. The level of organisms sufficient to initiate this process, is currently unknown. The source of *Campylobacter* on a farm is often undetermined during investigations into flock colonisation. Subsequently the levels of exposure in the farm setting are unknown. Further the viability of the organisms may be dependent upon the reservoir from which they originate. For example, organisms that originate from a contaminated puddle may be more efficient at colonising the gut than organisms from contaminated soil, or vice versa.

Once a bird is colonised with *Campylobacter* it will excrete large numbers of campylobacters in it's faeces. Contact with the faeces of such bird is one mechanism by which the organisms spread throughout a flock, as discussed in section 3.1. However, there is a second consequence of this excretion of organisms, namely the contamination of the exterior of the birds. There are two important factors in the contamination of the exterior. Contamination that occurs while the birds are on the farm and contamination that occurs during the transportation to the slaughter facility.

In this section a model is described which estimates the number of *Campylobacter* in a colonised bird. Further, an estimate of the level of contamination that occurs on the farm, and the extent of cross-contamination during transport are made, the result being the level of external contamination for a random bird at the point of slaughter.

During the expert consultation it was suggested that consideration be given to extending the model described in this section to include actual modes of entry of *Campylobacter* into the flock. This will be addressed at a later date and the model described above expanded accordingly.

3.2.2 Model development: Extent of colonisation and external contamination.

3.2.2.1 Contamination on the farm

Given a bird is colonised, it seems biologically consistent that such a bird will also be contaminated on its exterior. However, in a flock, which contains colonised birds but has a within-flock prevalence of less than 1, there is the opportunity for the birds which are not colonised to become contaminated on their exteriors. This can occur as a result of contact with either a colonised, and hence contaminated bird, or contaminated faeces.

The probability that a non-colonised bird will become contaminated on it's exterior can be expected to be related to the within-flock prevalence of the flock. Consider the within-flock transmission dynamics discussed in section 3.1.

In brief, once a flock is exposed and a single bird is successfully colonised, transmission ensues amongst the bird with which the first colonised bird makes contact with on a daily basis that is within the bird's social cluster. Contact continues until a threshold time is reached where the level of contamination in the feed, and water supply is sufficient to result in the colonisation of

an exposed bird. From here onwards colonised birds appear randomly throughout the entire flock. This process continues until either all the birds become colonised or depopulation occurs and the birds are removed for slaughter.

Under the circumstances described above it can be seen that there are two stages of transmission to consider. The initial stage of within-flock transmission, that is transmission amongst the social cluster with which the first colonised bird interacts. Under such circumstances the probability that a random bird in the flock becomes contaminated on it's exterior is assumed to be the probability that the bird is within the cluster containing the first positive bird. Hence the bird has the opportunity to come into contact with colonised and contaminated birds. Once transmission enters the second stage, birds appear in a random fashion throughout the flock. It is now highly likely that a random bird will come into contact with either a contaminated bird or contaminated faeces. Therefore it is assumed that the probability that a bird is contaminated during this stage of transmission is equal to 1.

3.2.2.2 Contamination during transportation to slaughter facility

Once the birds in a given house have reached the desired slaughter weight the birds are caught, loaded onto a vehicle and transported to the slaughter facility. Commonly the birds are loaded into baskets. The baskets are grouped together in modules, each module containing three rows of four baskets. The modules are placed in the vehicle in two rows, stacked one on top if the other, containing between nine and eleven modules, depending upon the size of the vehicle. Each module has a solid metal floor, but the baskets are designed such that the floor of the bask*et al*lows any excrement to pass away from the birds. One vehicle holds 5000 to 6000 birds, depending on the size of the vehicle and weight of the birds. Therefore, multiple vehicles are used for any given flock.

During transportation to the slaughter facility the stress of the process results in changes of the consistency of the faeces to a more liquid nature. This causes the contamination of the exterior of a large proportion of the birds in the transport vehicle despite the metal sheeting separating the modules. It is likely that in any given section of the vehicle there will be contamination of the birds with faeces resulting from the excrement of birds in rows above and also from the modules adjacent. However, in the current context this contamination is only of interest if there are birds present, which are excreting campylobacters. Therefore the probability that a bird becomes contaminated during transport is a function of the number of rows that contain colonised birds and the location of these birds within the vehicle in relation to non-colonised birds.

When estimating the level of contamination on the exterior of a bird in arrival at the slaughter facility there are two distinct situations to consider. These are the transportation of a *Campylobacter* positive flock and the resulting cross contamination that may occur within that flock, and the transportation of negative flocks. Within a *Campylobacter* negative flock by definition there are no colonised birds, hence no birds are shedding the organism. Each of these situations will now be discussed in turn.

3.2.2.2.1 The transportation of positive flocks

Within a positive flock the level of cross-contamination that occurs during transport is governed by the location of the birds, which are shedding the organisms within the vehicle. When a flock is caught and loaded onto the vehicle the lights in the house are dimmed such that bird movement is minimised. Therefore, the order in which the birds are loaded onto the vehicles is related to their location in the house. The birds nearest to the front of the house will be loaded on the first vehicle and the birds at the back of the house onto the last vehicle.

3.2.2.3 Model description: On the farm and during transport

A model has been presented by Hartnett *et al.* (b) and is as follows.

Initially each flock is assigned a *Campylobacter* status defined as θ_f where $\theta_f \in \{0,1\}$such that θ_f =1 means that the flock was positive for *Campylobacter* and θ_f = 0 means that the flock was negative for *Campylobacter*. The condition θ_f =1 occurs with probability P_{pf}, the probability that a random flock is *Campylobacter* positive, therefore θ_f = 0 occurs with probability 1-P_{pf}.

The flock is represented by an $a \times b$ lattice structure where a represents the horizontal distance within the house and b represents the vertical distance. The total number of birds within the flock is $N = a \times b$. At a particular time t each bird in the flock is in a colonisation state defined as $c_x \in \{0,1\}$ where $x = (a,b)$, such that $c_x = 1$ means that the bird at location x is colonised with campylobacter, and $c_x = 0$ means that the bird at location x is not colonised with campylobacter. For $t < t_{ex}$, $c_x = 0$ for all $x = (a,b)$. Once a bird is colonised, $c_x = 1$ it cannot change status, once a bird is colonised with *Campylobacter* it remains colonised. Note that if θ_f = 0 then $c_x = 0$ for all $x = (a,b)$ over all t.

At time $t = t_{ex}$, t is set to $t = 0$ and a random bird is located by selection of a random $a \times b$ location on the lattice. This bird is designated as the first bird to become colonised within the flock, that is $c_x = 1$. The cluster to which this bird belongs is then allocated on the lattice and each bird is assigned a cluster status, defined as $cl_x \in \{0,1\}$, where $cl_x = 1$ if the bird is in the cluster, and $cl_x = 0$ if the bird is not in the cluster. For $t = 1,..,4$ a bird within the flock at any x location changes status with probability $p_c(t; t = 1,..,4)$ given by

$$p_c(t; t = 1,...,4) = \begin{cases} cl_x \times p(t) & c_x = 0 \\ 0 & c_x = 1 \end{cases}$$

where $p(t)$ is the probability that a susceptible bird in the cluster becomes colonised according to the chain binomial model, as described in section 3.1.

Once $t > 4$, the number of birds that will change colonisation status is governed by the differential equation described in Appendix 1 (equation 3). Birds, which become colonised, are picked at random locations throughout the flock, sampling without replacement. Therefore, given a bird at location x the probability it will change status is given by

$$p_c(t; t > 4) = \begin{cases} I_n(t)/N & c_x = 0 \\ 0 & c_x = 1 \end{cases}$$

Here $I_n(t)$ is the number of newly colonised birds at time t, $I_n(t) = I_b(t) - I_b(t-1)$ where $I_b(t)$ is calculated by use of equation 3 as described in Appendix 1. This process continues until $t = t_A$, the time for depopulation. Given a bird is colonised, the number of organisms in the gut of the bird is defined as Λ_s.

Consider the contamination of the exterior of a bird in position x at the point of depopulation. As described previously, the contamination status of a bird is dependent upon both the cluster status of the bird and the stage of within-flock transmission. The contamination status of a bird in position x is defined as $CD_x \in \{0,1\}$ such that $CD_x = 0$ means that a bird in position x is not contaminated at depopulation, and $CD_x = 1$ means that this bird is contaminated at depopulation. This status is governed by the following condition

$$CD_x = \begin{cases} 0 & t <= 4;\ cl_x = 0 \\ 1 & t <= 4;\ cl_x = 1 \\ 1 & t > 4 \end{cases}$$

The result of the model described above is a coordinate for each bird in the flock and an associated colonisation status and contamination status at the point of depopulation. Given a bird is contaminated at depopulation, the level of contamination on the exterior is defined as Ω_d.

An illustration of the implementation of this model is shown in Figure 3.1. This demonstrates the use of the model in Excel with the model programmed using Visual Basic for Applications. The flock used to illustrate the model is assigned parameter estimates as shown in Table 3.2.

Table 3.2: Parameter values for the flock used to illustrate the spatial spread of *Campylobacter* within a flock in Figure 3.1.

Parameter description	Symbol	Parameter value
Flock size	n	8010
Cluster size	n_c	467
Number of contacts a bird makes in one day		109
Number of times a bird comes into contact with any given bird in one day		55
Probability of making contact with another bird	P_c	0.143
Biological transmission rate	b	0.2

It can be seen, that as predicted by the model, when the time since the flock colonisation process began is less than 5 days the colonised birds are confined to a cluster. In this particular case the whole cluster becomes colonised by $t = 3$. This is because as the flock is unusually small, the cluster size is also small and as such less time is required for the birds in that cluster to become fully colonised.

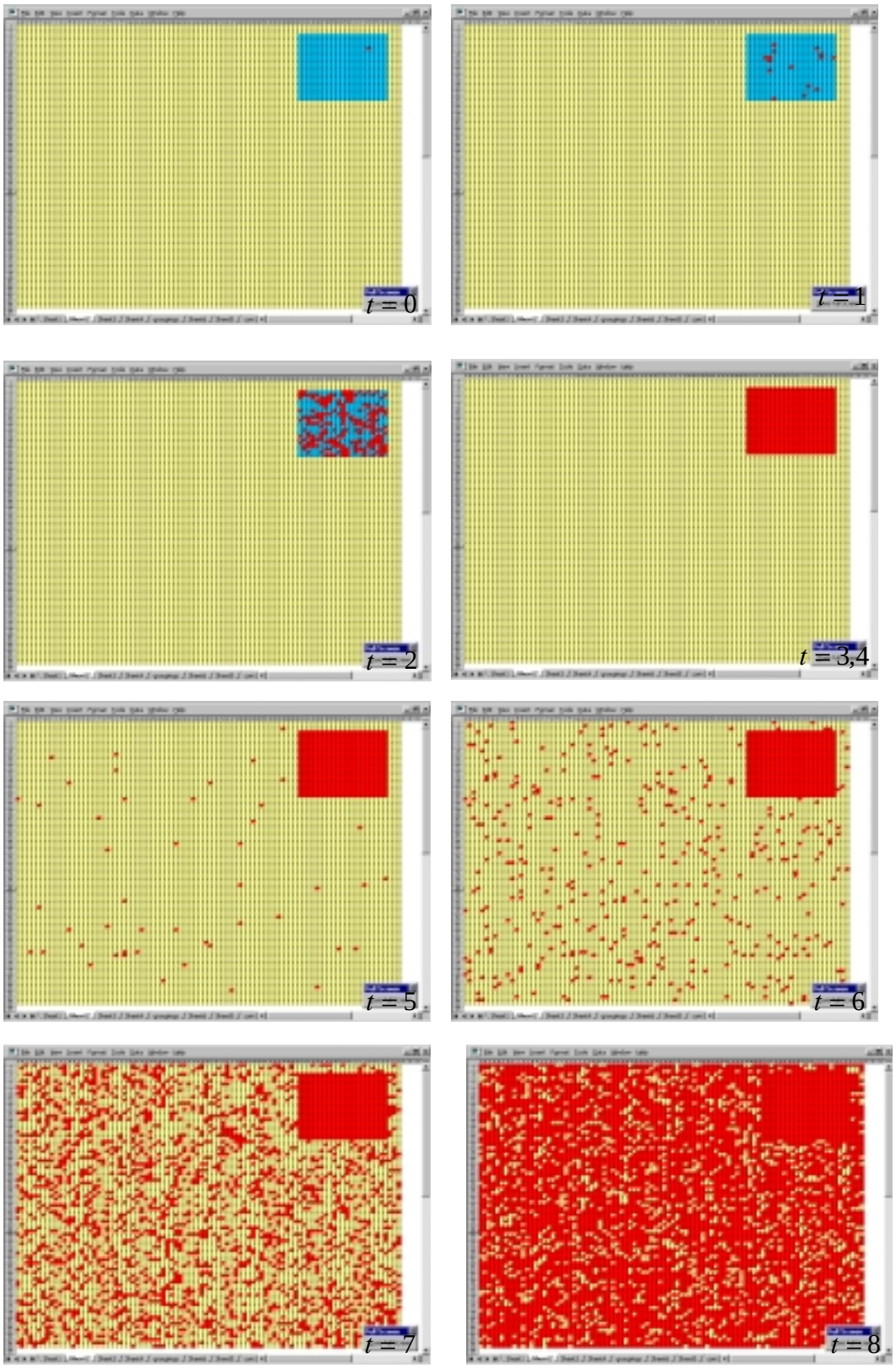

Figure 4.1. Illustration of the spatial model, which predicts the location of positive birds within a flock over time. Negative birds are represented by yellow squares, birds which are assigned to the cluster are identified by blue squares and positive birds are represented by red squares.

The next stage of the model is to place the birds into the transport vehicles. It is assumed that for all flocks, a basket contains 100 birds. The birds are placed in the order that they are in the house onto the transport vehicles, in groups of 100. One end of the house is allocated as the front of the house therefore the further away from the front of the house a bird is, the higher the number vehicle the bird will be transported in. Each vehicle has a maximum capacity if 60 groups of birds, assuming that all vehicles carry 10 modules. The number of vehicles required for any flock is therefore $N/6000$. Once all the birds are placed into the transport vehicles the probability that a random bird from the flock is contaminated during transport, defined as P_{ct} can be calculated, as discussed below.

There are no data available which provide estimates of the probability that a bird will become contaminated during transport in relation to the location of the bird within the vehicle. Therefore, it is assumed that there are two modes of contamination. First, that which occurs as a result of a bird being in a position below colonised birds. Second, contamination as a result of the adjacent sections of the vehicle containing contaminated birds and hence has the potential for horizontal spread of the organisms.

Consider a random bird in module i and row j. The probability that transmission occurs vertically, that is from the birds in module i, rows 1 to j is defined as $C_T(d)_{i,j}$. This probability is dependent upon the distance, that is number of rows, between the selected bird and the nearest colonised birds above. It is assumed that the probability that a bird becomes contaminated is given by the reciprocal of this distance, more specifically

$$C_T(d)_{i,j} = \frac{1}{i - \max\{C_{z,j}.z\}} \qquad z = 1,..,i-1$$

Next, consider cross-contamination from the adjacent birds that is birds in row j, modules 1 to 10. It is assumed that the probability that a given bird will become contaminated by this route is given by the product of the probability that birds in any one of the modules 1 to 10 is contaminated and the reciprocal of the distance between this module and the selected module. More specifically

$$C_T(V)_{i,j} = \left[\sum_{z=1}^{j-1} C_T(d)_{i,z}.\frac{1}{j-z+1}\right] + \left[\sum_{z=j+1}^{10} C_T(d)_{i,z}.\frac{1}{z-j+1}\right]$$

Therefore, the probability that a random bird located in module i, row j will become contaminated during transport, that is $P(CT)_{i,j}$ is given by

$$P(CT)_{i,j} = C_T(V)_{i,j} + C_T(d)_{i,j} - C_T(V)_{i,j}.C_T(d)_{i,j}$$

Hence, on arrival at the slaughter facility, each bird has an associated status for the occurrence of contamination during transport, defined as $CT_{i,j} \in \{0,1\}$ such that $CT_{i,j} = 1$ means that the bird located, located within a vehicle in position (i, j) became contaminated externally during transport, and $CT_{i,j} = 0$ means that this bird did not become contaminated during transport. The condition $CT_{i,j} = 1$ occurs with probability $P(CT)_{i,j}$, therefore $CT_{i,j} = 0$ with probability $1 - P(CT)_{i,j}$.

Given a bird which has become contaminated during transport the level of contamination on the birds exterior is defined as Ω_t.

3.2.2.4 Levels of contamination at slaughter for positive flocks

The level of external contamination that is present upon a bird on arrival at the slaughter facility is significantly different (P<0.05) than that which is present before the flock is transported (Stern *et al.,* 1995), thus suggesting that transportation allows cross contamination within the flock. However, as previously described in section 3.1, the probability that a random bird will become contaminated during transport is a function of the location of the colonised birds in the flock within the transport vehicles, in relation to the location of the selected bird. As such the level of contamination on the exterior of a bird is governed by the probability that the bird became contaminated during transport. When the birds are placed in the transport vehicle each bird has a contamination status at the point of depopulation, (CD), and a status for the occurrence of contamination during transport, (CT). The level of contamination at the point of slaughter, that is Ω_s is governed by these two factors, as described by equation (3.1).

$$\eta_{ext} = \begin{cases} 0 & CD = 0; CT = 0 \\ \Omega_d & CD = 1; CT = 0 \\ \Omega_t & CT = 1 \end{cases} \qquad (3.1)$$

Here, Ω_d is the level of contamination on the exterior of a bird at depopulation, Ω_t is the level of contamination on a bird after transport, and η_{ext} is the level of contamination on a random bird from positive flocks at the point of slaughter.

3.2.2.4.1 The transportation of negative flocks

Consider a negative flock, that is a flock, which has not been exposed to *Campylobacter* at a level sufficient to result in the colonisation of any birds in the flock. Given that birds within a negative flock by definition contain no colonised birds, there are therefore no birds shedding the organisms during transport. As such the above model description does not apply.

Given the absence of colonised birds it may be assumed that within such a flock there is no opportunity for bird to become contaminated on their exteriors. This is not the case. Experimental data suggests that there are at least two occasions when birds in negative flocks may become contaminated. First, it has been hypothesised that when the birds are caught the catchers hands may be contaminated with organisms as a result of previously catching a positive, and hence contaminated, flock. Second, it has been reported in the literature that the baskets within which the birds are transported may be contaminated with campylobacters. The baskets are routinely cleaned once the birds are removed at the slaughter facility however such cleaning may be inadequate to remove all the organisms present.

3.2.2.5 Levels of contamination at slaughter for negative flocks

There are no data available that enable the estimation of either the probability that a negative flock will become contaminated or the extent of such contamination. However, given the two opportunities for contamination described above, it can be seen that the probability that a flock will become contaminated is dependent upon either the catchers or the crates coming into contact with a positive flock at some point previous to contact with the negative flock. Therefore an assumption is made that the probability that a negative flock becomes contaminated is equal

to the national flock prevalence, that is $P_{nc} = P_{pf}$. Here the flock prevalence is as described in section 3.1.

The extent to which a bird from a negative flock will become contaminated is related to the level of contamination in the positive flocks. Consider contamination by catchers hands. Experimental work looking at the cross-contamination of organisms from surfaces to hands and hands to surfaces suggests transfer rate of 10% (Zhao *et al.,* 1998). That is given one contact with a contaminated surface approximately 10% of the organisms will be transferred. For a bird to become contaminated via catcher's hands two things must occur, first the catchers hands come into contact with a contaminated bird and hence become contaminated. Second the contaminated hands transfer the organisms to a previously uncontaminated bird. Therefore an assumption is made that the level of contamination that a random bird in a negative flock receives is 1% of the contamination on the exterior of a random positive bird.

The same assumption is made with regards to contamination via crates as the birds must contaminate the crates, and then the contaminated crates must come into contact with the exterior of a bird from a negative flock. As such there are two points of contact and therefore it is assumed that the transfer rate is 1% of the level of exterior contamination of a positive flock.

Therefore, the contamination level on the exterior of a bird selected at random from the national flock at the point of slaughter, defined as η_{ext}, is given by equation (3.2) (Hartnett *et al*, b).

$$\eta_{ext} = \begin{cases} \Omega_s & \theta_f = 1 \\ 0.01\Omega_s & \theta_f = 0 \end{cases} \qquad (3.2)$$

3.2.3 Parameter estimation and simulation

The level of colonisation within the caeca of several birds within random flocks is reported by Stern *et al*; (1995). This data set recorded levels of colonisation before and after the birds had been transported. This data suggests that there is no significant difference in colonisation levels before and after the birds have been transported. There are several other sources in the literature which give an indication of colonisation levels in positive birds (Stern, 1988; Aho and Hirn, 1988; Jacobs-Reitsma *et al.,* 1994; Berndston *et al.,* 1996; Atabay and Corry, 1997). However such reports are commonly just the mean value, or maximum colonisation observed. This data does not enable the definition of a variability distribution to described colonisation levels in random birds. Given this, only the data set from Stern *et al.* (1995) is utilised to define the variability distribution describing the colonisation level at slaughter, that is Λ_s. This data set is shown in Table 3.3. It can be seen that this data consists of a small number of samples. As such there is uncertainty associated with the form and extent of the variability distribution. Further, the data points reported are means of several samples. There are numerous combinations of colonisation levels that could have lead to the reported mean for any given farm. Therefore, this data is used to define a non-parametric, second-order distribution as described in section 3.1. The mean of this second-order distribution will be the reported data set. As such all possible ways by which the reported data could have occurred is accounted for by the two-dimensional nature of the distribution. It can be seen by comparison with reports of colonisation levels in the literature birds that this data set is consistent with other findings.

Table 3.3 The number of campylobacters colonising the caeca of broilers at slaughter from Stern *et al.* (1995).

Farm number	Mean Log cfu/gram ceacal contents post transport per farm
1	7.08
2	5.74
3	5.11
4	7.00
5	5.40
6	6.38
7	7.28
8	6.28
9	4.11

There is little information in the published literature with regards to the level of contamination on the exterior of birds either before or after transport. An investigation by Stern and colleagues (Stern *et al.* 1995) recorded measurements of external contamination with campylobacters both before and after transport. This data set is shown in Table 3.4. The data consist of mean counts taken from 10 farms, which were under experimental control (numbered 1 to 10) and duplicate samples taken form 5 farms, randomly chosen, not under experimental control (numbered 11 to 15 denoted by *). Given that the cross-contamination that occurs during transport is not controlled on the farms classed as under experimental it is appropriate to pool the two data sets. It can be seen from Table 3.4 that upon 5 occasions that no campylobacters were retrieved from the birds prior to transport, yet a high level of contamination was recorded post-transport further strengthening the importance of cross-contamination during transportation.

As with colonisation levels this is a small sample size hence there is uncertainty with regards to the extent of the variability in the population. Therefore second-order non-parametric distributions for the number of organisms contaminating the exterior of a bird before and after transport were derived from the data shown in Table 3.3. These distributions are then correlated. Using Spearman's rank order the correlation coefficient, ρ, is given by

$$\rho = 1 - \left(\frac{6\sum (\Delta R)^2}{n(n^2 - 1)} \right)$$

where ΔR is the difference in rank of the data in a data pair and n is the number of data pairs (Vose, 2000). Using the data shown in Table 3.4, ρ = 0.895. This indicates that the number of organisms before and after transport that contaminate the exterior of a bird as positively correlated, as expected.

Table 3.4 The number of campylobacters (mean log cfu per carcass) contaminating the exterior of broilers prior and post transport taken from Stern *et al.* (1995).

Farm Number	Mean Log cfu/carcass prior to transport	Mean Log cfu/carcass post-transport
1	ND	7.53
2	ND	ND
3	ND	7.05
4	6.16	7.48
5	6.09	8.18
6	6.38	8.66
7	5.97	7.34
8	5.81	7.34
9	6.23	7.75
10	ND	6.82
11*	2.4	5.8
11*	4.3	6
12*	2.65	5.53
12*	ND	4.93
13*	6.23	9.62
13*	6.15	ND
14*	2.37	6.61
14*	ND	6.36
15*	ND	ND
15*	2.88	6.67

Note that the data points reported in Table 3.3 and Table 3.4 are means of several samples for a given broiler farm. There are numerous combinations of colonisation levels that could have lead to the reported mean for any given farm. Therefore it is appropriate to use non-parametric second-order distributions to describe the variability present. The mean of these second-order distributions will correspond to the reported data sets. As such all possible ways by which the reported data could have occurred is accounted for by the two-dimensional nature of the distributions.

During the expert consultation the importance of the length of time of transport was raised. It is intuitive that if birds are subjected to transport times of, for example, 30 minutes, the extent of external contamination occurring during transport may be much lower than if the birds were subjected to longer transport times. The transport time is likely to be highly variable, not only between producers but also countries. Further, there is currently no data available to quantify this effect. However, the model described above will be adjusted in such a manner that should the data become available at a future date the model can accommodate this, possibly important, factor.

3.3 The slaughter and processing of chicken

3.3.1 Introduction

The processing of chicken meat consists of a highly controlled sequence of events, beginning with the slaughtering process through to transport of the final sale product. If human pathogens are present in the intestinal tract of chickens, forming part of the faecal micro-flora, the potential is there for contamination of carcasses during slaughter and processing. The extent of this will depend on the prevalence of the organisms in the birds as well as the hygienic standards

employed during processing. Such contamination can be described in two ways (Gill, 1999), first vertical contamination resulting from colonisation of the live bird, secondly horizontal or cross contamination which results from a source other than the bird/carcass, for example, the processing equipment or another bird/carcass. Horizontal contamination may augment vertical contamination and is especially important when considering uncontaminated carcasses that are being processed alongside contaminated carcasses.

Under the implementation of Hazard Analysis Critical Control Point (HACCP) systems in poultry processing, for each operation which effects the safety of the product, critical control points are identified and can be controlled to contain or eliminate an identified hazard (Gill, 1999). The identification of such control points within chicken processing requires an understanding of the process itself and the behaviour of the microbial hazard within that environment. There are four main points for consideration when investigating organisms in a processing environment. These are the temperature range for growth, the range of water activity over which the bacteria can grow, nutritional requirements, and resistance to heat and other stressing environmental factors. These factors will determine the behaviour of a given organism within this environment.

For most bacterial species found in the food chain, for example salmonella and staphylococcus, these characteristics facilitate amplification through processing as a result of growth and establishment within the environment. Consequently their total elimination from the food chain may only be possible when the bacteria are eliminated from the livestock. Any intervention strategies at later stages of the food chain may reduce the magnitude of the problem and should certainly be taken for that reason, but the main problem should be attacked at the origin. This is not the case for campylobacters as they are thermophillic and strictly microaerophillic, having an optimal growth temperature of 42°C with a permissible growth range of 32-35°C and an optimum oxygen concentration of as little as 5% along with 10% carbon dioxide. Unless these conditions are met, they are not able to propagate in the processing environment or on the raw, processed product. The pattern of contamination is therefore different from many other bacteria the main problem being horizontal contamination.

To examine the possible impact on the levels of contamination of chicken products during processing it is necessary to first understand the process and the impact each of the stages has on the prevalence of contaminated products and the levels of contamination. Here a model is presented which describes the processing of chicken, considering each of the stages in turn and modelling the impact of the stage on the levels of contamination on the carcass. The outcome from this model is an estimate of the probability that a random chicken product will be contaminated with *Campylobacter* and the likely number of *Campylobacter* associated with a contaminated product. Furthermore, the model is used to investigate the areas of processing most likely to have an impact on the levels of contamination on the final sale product with the aim of identifying critical control points, which reduce the incidence and level of *Campylobacter* contaminated chicken products at retail.

3.3.2 The Stages of Chicken Processing

The processing of chicken consists of nine main stages beginning with the slaughter of the birds through to final grading and packaging of the sale product, which is then transported to the retail outlet for distribution. The overall process is illustrated in Figure 3.2. Each of these nine stages will now be described.

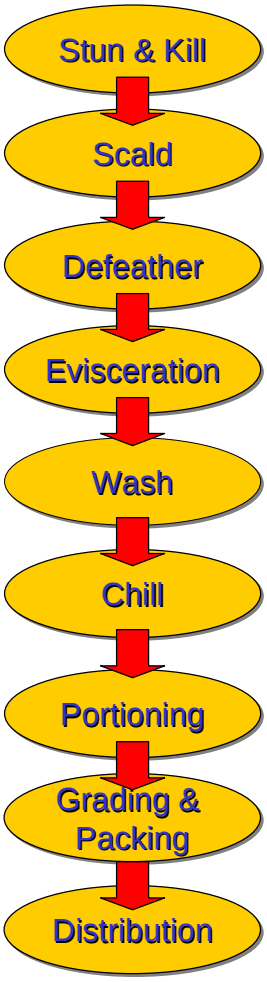

Figure 3.2 Diagram illustrating the ten main stages of the processing of chicken from stunning to retail and distribution.

3.3.2.1 Stun and Kill

The first stage involves the stun and subsequent kill of the live birds. Upon arrival at the slaughter facility, the birds are removed from their crates and put onto the killing line, where they are hung upside down by their feet in shackles. It is a legal requirement that there is physical separation between this and the rest of the slaughterhouse to prevent the spread of the dust and dirt generated by this process. From here, a conveyor moves the birds towards the stunning equipment. Commonly electrical water-bath stunning is used however other methods are available such as gassing. After stunning, the birds are bled for up to two minutes before processing begins. Due to the high intensity of the slaughter process, birds are hung in close proximity and will be in contact with each other, as well as with machinery, throughout stun and kill. Despite this, these stages have few microbiological implications; although electrical, water-bath stunning may lead to both inhalation of contaminated water by the birds and microbial contamination of the carcass tissues (Lillard, 1973). Consequently, the effect of stun and kill is assumed to be negligible.

3.3.2.2 Scald

Once birds have been slaughtered the carcasses are immersed in a scald tank. This process loosens the feathers and facilitates plucking. As birds enter the scald tank there may be involuntary defecation, leading to accumulation of faecal matter in the tank. In the case of birds colonised with *Campylobacter* this results in contamination of the scald water.

The process of scalding depends upon whether the carcass is destined for fresh or frozen sale. Carcasses used for fresh products undergo soft scald where the water is at a temperature of 50-52°C for up to 3.5 minutes, those used for frozen products undergo hard scald, and in this case the water is at 56-58°C for 2-2.5 minutes. The different scalding methods are used as soft scalding avoids damage to the cuticle and hence prevents skin discolouration, an undesirable quality in fresh sale chickens but not a large concern for frozen products. The slaughtering and scalding processes interact in influencing microbial contamination of the internal organs. If the birds are not given long enough to stop breathing or gasping before scalding, there is a danger that they will inhale the scald water. This may result in the trachea, oesophagus, lungs, crop, gizzard and air sacs becoming contaminated with scald water during the scalding procedure (Lillard, 1973). This would not assume any importance if the contamination was restricted to the inedible offal, but Lillard (1973) showed that low level internal contamination could occur. Further, scalding may lead to external contamination if an uncontaminated carcass is passed through contaminated scald water.

3.3.2.3 De-feathering

De-feathering is a mechanical process, which occurs immediately after scalding. This process is carried out by a series of in-line plucking machines that comprise banks of counter rotating stainless steel domes, or discs, with mounted rubber fingers. These machines incorporate continuous water sprays that flush out the removed feathers. Any remaining feathers are removed by hand. These machines are major sites of potential cross-contamination in primary processing. Rubber fingers can scour the carcass and can also harbour contamination, following contact with a contaminated carcass, in the 'cobweb' of tiny cracks that form when the rubber becomes brittle. This has the potential to contaminate a previously uncontaminated carcass. However, this will only result in low-level contamination. Significant contamination results due to the spinning action of the plucker heads. In particular this action results in the formation of aerosols, which spread contamination (Hinton *et al.,* 1996). The process of defeathering has been demonstrated to generally increase the number of carcasses contaminated with organisms (Oosterom *et al.,* 1983a; Izat *et al.,* 1988). This is due to re-distribution of the organisms and therefore has a large inpact on previously ‘clean’ carcasses. This is due to the aerosol spray and contamination of machinery.

3.3.2.4 Evisceration

Following plucking the head and feet are removed and the birds are eviscerated, that is the internal organs are removed. In some plants, carcasses are detached from the hanging hooks and transferred to the evisceration area to be re-hung. This handling activity provides the opportunity for cross-contamination to spread. For the majority of production, evisceration is carried out mechanically, but manual evisceration is still practised. On automated lines, a cut is made around the vent of the carcass, a spoon-shaped device is inserted into the opening and the viscera are withdrawn. The intestines etc. remain attached for inspection, hanging over the back of the carcass connected by their natural tissues, and gross contamination of the carcass may result if they are damaged. This is not an uncommon occurrence because the machinery used is not able to allow for natural variation in the size of the carcasses being processed. It has been shown that even when the viscera remain in-tact the levels of enteric bacteria, including *Campylobacter*, increase on the exterior of the carcass (Oosterom *et al.,* 1983b; Izat *et al.,* 1988). If a carcass originates from a classified negative bird then damage to the viscera can be

ignored. For birds colonised with campylobacters gross contamination may result if damage occurs to the viscera during this process.

Partial evisceration is carried out in Great Britian. The intestines are removed but the remaining viscera are left inside the carcass. Delayed evisceration is also permissible, where un-eviscerated birds are held for up to 15 days under refrigeration at no more than 4°C. With regards to *Campylobacter* this will have few microbiological implications. Due to the thermophillic nature of the organism it can be assumed no growth will occur. Despite these methods being employed only complete evisceration is considered within this model as the frequency and microbial implications of partial and delayed evisceration are currently unknown.

3.3.2.5 Washing

After post-mortem inspection the viscera are separated into edible and inedible offal. The eviscerated carcass is spray washed internally and externally. It is EU regulation that following evisceration there is a carcass wash. The mandatory use of inside-outside carcass washes removes visible faecal contamination, but does not eliminate bacteria attached to the surface. Attachment is a time-dependent process; therefore washing the carcasses at different stages may remove bacteria before they become attached to the carcass. It has been demonstrated (Cudjoe *et al.,* 1991) that the washing procedure typically reduces the numbers of *Campylobacter* on a carcass by 90% percent.

3.3.2.6 Chilling

The poultry meat hygiene regulations (Doyle and Roman, 1981) require that poultry meat be chilled to 4°C or less as soon as possible after evisceration. Within the EU, three types of chilling process are used. These are air-chill, water immersion and spray chilling. All three methods may lead to cross-contamination however the problem is greatest in systems that use water (EU concerted action, 1997). Air chillers are generally used where carcasses are for fresh sale and methods employing water are mainly used for frozen products. With regards to campylobacter, despite the potential for cross-contamination to occur, water chilling reduces the levels of contamination on a carcass as they move through a counter-flow current (Laisney *et al.,* 1991). Further it has been demonstrated that the addition of chlorine to the chill water prevents the cross contamination of organisms which have been washed-off into the water. Despite campylobacters being able to survive levels of chlorine likely to be present in poultry processing water, the chemical hinders the attachment of the organisms to a carcass. Air chilling has been shown to have no effect on the levels of *Campylobacter* due to their ability to survive under these conditions. (Cudjoe *et al.,* 1991). Spray chillers are rarely employed as they require large volumes of water but this technique avoids cross contamination. Addition of chlorine to the chill water may further reduce the levels of carcass contamination during the chilling procedure.

3.3.2.7 Portioning

There is a growing trend towards production of poultry meat for the retail and catering sectors as portions rather than whole carcasses. In 1998, 41% of all chicken sold in Great Britian was portioned and amounted to approximately 148 thousand tonnes (British poultry meat federation, 1998). The jointing of the carcass is increasingly being carried out using mechanical or semi-mechanical methods, which allow faster line speeds and higher through puts required by the industry. However the increased contact with machinery and/or human hands and tools during portioning could result in higher numbers of pathogens and spoilage organisms contaminating the product due to cross-contamination. This occurs as a result of either redistribution of the organisms contaminating the carcasses, which have been processes that day or carry-over of contamination from the day before that has persisted through cleaning procedures. A variety of cuts are marketed, and the principal ones have been defined by a working party on standardisation of perishable produce:

(i) Half: half the carcass obtained by a longitudinal cut in plane through the sternum and the backbone;
(ii) Quarter: a half divided by a transversal cut, by which the leg and breast quarters are obtained;
(iii) Breast: sternum and the ribs distributed on both sides of it, together with the surrounding musculature;
(iv) Leg: femur, tibia, and fibula, together with the surrounding musculature;
(v) Thigh : femur together with the surrounding musculature;
(vi) Drumstick: tibia, and fibula together with the surrounding musculature.

Few reports have been found in the scientific literature regarding contamination of poultry meat during portioning but automatic portioning equipment is likely to be a potential source of contamination (Gill, 1999). In addition, other surfaces with which the portions come into contact, such as conveyor belts, cutting boards, and packaging material, may add to the microbial load of the final product. Hands and clothing of factory personnel and utensils such as knives are also likely to contribute to microbiological contamination. The degree of microbial contamination on cut portions reflects their degree and duration of exposure to the processing environment.

3.3.2.8 Carcass de-boning and mechanically recovered meat

The growth in the sale of further-processed poultry has placed a heavy demand on the production of de-boned poultry meat. Mechanically recovered meat of good quality has found a ready market and is widely used in a variety of white and red meat products such as frankfurters, sausages and burgers. Mechanically recovered meat can be held chilled at 2°C for use within 48 h or frozen in shallow layers in a plate freezer.

Due to the absence of data with regards to the microbial implications of carcass de-boning and mechanical recovery any effect is assumed to be negligible, however this assumption can easily be modified should such information become available.

3.3.2.9 Grading and packaging, and distribution

Once carcasses have been portioned they are weighed and graded. This can result in the cross-contamination of organisms from the equipment to the carcasses. The carcasses are then packed. Packing is governed by the scald and chill system used. If carcasses are water chilled they may be trussed with pre-packed giblets inserted into the body cavity and then packed in a polythene bag. Air chilled carcasses are usually packed without giblets on polystyrene trays and wrapped in cling film. Alternatively they may be bulk packed. Again there is potential for cross-contamination here. Despite the opportunities for contamination there is no information in the literature on the effect of grading and packaging on contamination levels. Therefore it is assumed that grading and packing have no effect on carcass contamination levels. The packaged carcasses are then distributed appropriately.

3.4 Simulation model describing the slaughter and processing of chicken

3.4.1 Introduction

The processing of poultry is a sequential process that provides a number of opportunities for contamination of a carcass with food poisoning organisms such as *Campylobacter* spp. Each of the stages of processing have been described in detail in section 3.3.

However, given that chicken processing is highly controlled by governing bodies such as the European Union (EU), this presents the opportunity for the application of mitigation strategies,

which have the ability to reduce current contamination levels. To be able to consider control of potential contamination the process and the factors contributing to contamination must be understood. In this section a simulation model is presented which describes the processing of chickens in a random plant within Great Britain (GB). Stochastic in nature, the model mimics the uncertainty and variability present in such an intensive but highly regulated process.

3.4.2 The slaughter and processing model

A model describing the processing of chicken has been presented by Hartnett *et al.* (c). This model is as follows.

The model considers the stages of processing which may have an impact upon the level of *Campylobacter* contaminating a carcass. As detailed in section 3.3, these stages are scald, de-feathering, evisceration, washing, and chilling.

In the first instance, the simulation model considers a group of 100 birds from a random flock at the point of slaughter in a randomly selected processing plant in Great Britian. Based on the outputs from the models describing the rearing and transport stages of broiler production, each bird in this group is assigned a history. More specifically the group is assigned a flock status and each bird within the group is assigned a *Campylobacter* status, a level of contamination and a level of colonisation.

The status of the flock the group originates from is defined as θ_f where $\theta_f \in \{0,1\}$ such that θ_f =1 means that the flock was positive for *Campylobacter* and θ_f = 0 means that the flock was negative for *Campylobacter*. The condition θ_f=1 occurs with probability P_{pf}, the probability that a random flock is *Campylobacter* positive, therefore θ_f = 0 occurs with probability 1-P_{pf}. Further, the colonisation status of a bird in the group is defined as C_x, where $C_x \in \{0,1\}$ such that $C_x = 1$ means that the bird was colonised with *Campylobacter*, and $C_x = 0$ means that the bird was not colonised with *Campylobacter*. The condition $C_x = 1$ occurs with probability P_{wfp}, the probable within-flock prevalence of a positive flock, therefore $C_x = 0$ occurs with probability $1 - P_{wfp}$. If $C_x = 1$ the bird is colonised with Λ_s organisms. It follows that if $\theta_f = 1$, then $C_x \in \{0,1\}$, however if $\theta_f = 0$ then $C_x = 0$. Next the contamination status is considered. This is θ_c where $\theta_c \in \{0,1\}$ so that θ_c=1 means that a given bird has organisms contaminating the exterior and θ_c=0 means that the bird has no external contamination with *Campylobacter* spp. The condition $\theta_c = 1$ is dependent upon the contamination status of the bird on the farm, (CD), and the occurrence of cross-contamination during transport, (CT) as described in section 3.2. If θ_c=1 a given bird is assigned η_{ext} contaminating organisms. The variables P_{pf}, P_{wfp}, C_x, CD, CT, Λ_s and η_{ext} are generated from the model described in section 3.1 and section 3.2.

Once carcass history has been designated the position of the flock in the flocks to be processed that day is allocated. On any given day, 4 to 6 flocks may be dealt with in a processing facility. It is assumed that a plant processes five flocks. The position of the selected flock in the processing day is given by θ_p, where θ_p is a uniform random integer variable between 1 and 5. Here, θ_p = 1 means that the flock is the first in the day to be processed, θ_p = 2 means that the flock is the second to be processed, up until θ_p = 5 the fifth flock to be processed.

Following characterisation of history, the product type of the group of carcasses at the point of sale is determined. Product types are defined as (i) fresh and whole, (ii) fresh and portioned,

(iii) frozen and whole, and (iv) frozen and portioned. However portioning is reported to have little impact on the contamination on a carcass (Holder *et al.,* 1997). Further, there are limited data therefore it is currently assumed that the process of portioning has no effect on the microbial load. Hence, at the point of sale, a random carcass is product type θ_s, where $\theta_s \in \{\alpha, \beta\}$ such that α represents a fresh product, and β a frozen product.

Given characterization of history, flock position and product type the model follows the group of carcasses through the first stages of processing, that is stun and kill, scald and de-feathering. Subsequent to these steps a random bird is selected from the group and followed through the remaining stages of processing, that is evisceration, wash and chill.

The model estimates the stochastic effect of each of the processing stages on the contamination levels on the carcass(es). Multiple runs of the model reflect the processing of multiple birds from multiple flocks and hence a probability distribution for the number of campylobacters contaminating a product and the probability that a product is contaminated at the point of sale are generated. Derivation and estimation of these distributions is now described.

3.4.2.1 Level of Contamination

The model considers what happens during all stages of processing. From the description of chicken processing presented in section 3.3, it can be seen that scalding results in a proportion of organisms to be washed off the carcass. De-feathering causes both a proportion to be washed off /removed with the feathers, and a number of organisms to be added from cross-contamination. Evisceration allows a number of organisms to contaminate a carcass from both cross- and vertical-contamination but may also result in a proportional reduction. During washing a proportion of organisms will be washed off. Finally chilling results in either no effect (air chilling) or a proportional wash-off (water-chilling). The final number of organisms that are on any carcass is a result of the effect of all stages of processing. Hence, it is necessary to estimate the changes afforded by each of the processing stages. The cumulative effect of these changes results in the number of organism contaminating a random carcass.

This effect is quantified in equation (3.3) where the contamination level on a selected carcass *i*, defined as η_{p_i} is given by

$$\eta_{p_i} = \tau_i \nu_i \left(\mu_i \eta_{ext\,i} + \varphi_i + \xi_i\right) \qquad (3.3)$$

where $\eta_{ext\,i}$ is the number of *Campylobacter* contaminating bird *i* at the point of slaughter that is the level of contamination on entry into the processing plant, μ_i is the change in numbers due to scalding, φ_i is the change in numbers due to de-feathering, ξ_i is the change in numbers due to evisceration, ν_i is the proportion remaining in the numbers of *Campylobacter* achieved by washing and τ_i is the proportion remaining on a carcass after chilling.

The distribution for each of these parameters is estimated by use of available sample data, which measures the levels of contamination on a carcass before and then after a given process. There are several methods available to make such measurements such as counting levels of contamination on the neck skin, estimating levels by swabbing a particular section of the carcass of a fixed size, or enumerating the contamination on the whole carcass via a carcass rinse. Given the parameters of equation (3.3) are measures of proportion change in organisms it is assumed that on any given carcass the measured proportion reduction on one site of the carcass will be consistent across the whole carcass. Therefore, all data that measures levels of contamination before and after sampling in a consistent manner can be utilised to estimate

model parameters. As such, throughout this model measures of contamination on a carcass in a data set is referred to as mean log cfu per unit as each study will have used a different sampling strategy and hence measured a different unit.

Due to the use of sample data, there is associated uncertainty with regards the true distribution of the variability in these parameters. This is dealt with by the use of second-order non-parametric distributions (Vose, 2000). Each of these variables is estimated as follows.

Estimation of changes in number of organisms after scalding, μ_i

The probable proportion of organisms remaining after the scalding process, that is μ_i, is dependent upon whether a carcass undergoes hard or soft scald, this is governed by product type, θ_s under the following condition:

$$\mu_i = \begin{cases} SS & \theta_s = \alpha \\ HS & \theta_s = \beta \end{cases}$$

Here *SS* and *HS* are distributions describing the variability in the proportion of organisms remaining after the processes of soft and hard scald respectively. Sample data consisting of the mean microbial counts of *n* carcasses selected at random before and after scalding were used to estimate the distribution for the variables *HS* and *SS.* In particular, for each scald type proportions remaining were calculated for each data point. The data points and calculated proportions are given in Table 3.5. The variability distributions were then derived as follows.

Table 3.5 Measured mean log cfu *Campylobacter* on a carcass before and after soft and hard scald and the calculated proportion remaining as a result of the scalding process.

Number of carcasses sampled	Type of scald	Mean log cfu per unit before scald	Mean log cfu per unit after scald	Calculated proportion of organisms remaining post scald	Reference
8	Soft	3.99	1.37	0.002	Oosterom et al., 1983b
8	Soft	3.30	1.68	0.020	Oosterom et al., 1983b
8	Soft	2.18	2.40	1.66	Oosterom et al., 1983b
8	Soft	3.74	<1.26	0.003	Izat et al., 1988
8	Soft	3.56	1.26	0.005	Izat et al., 1988
8	Soft	3.03	1.19	0.014	Izat et al., 1988
5	Soft	2.9.0	1.00	0.012	Berrang & Dickens, 2000
5	Soft	5.00	2.00	0.001	Berrang & Dickens, 2000
5	Soft	5.00	1.70	0.001	Berrang & Dickens, 2000
5	Soft	3.10	2.40	0.199	Berrang & Dickens, 2000
5	Soft	5.80	2.40	0.0003	Berrang & Dickens, 2000
5	Soft	4.60	1.50	0.001	Berrang & Dickens, 2000
8	Hard	2.39	0.61	0.016	Oosterom et al., 1983b
8	Hard	3.42	1.25	0.007	Oosterom et al., 1983b
8	Hard	3.44	1.26	0.007	Oosterom et al., 1983b

For soft scald, the calculated proportions were used to derive a non-parametric second-order distribution (see Vose, 2000) for the variability in the proportion remaining.

For hard scald, there are only three data points available. Therefore, the variability in the effect of this process on the contamination level of a carcass is assumed to be a uniform random variable between zero and 10% above the maximum value observed for proportion remaining (Table 3.5). The maximum proportion remaining observed is 0.16 therefore this translates to a Uniform(0,0.0176). Further, due to limited data an assumption is made that there is no associated uncertainty with this maximum value. This assumption can be modified should more information become available.

Estimating change in contamination after feathering, φ_i

The change in contamination due to de-feathering, defined as φ_i, is estimated by considering the cross-contamination effects of de-feathering. Experimental work based on the use of a 'seeder' carcass artificially contaminated with a marker organism has demonstrated that contamination with the marker can be detected as far as 200 carcasses away from the 'seeder' carcass after the de-feathering procedure (Mead *et al.,* 1994). Further, the level of contamination was shown to be an inverse function of the number of birds between the nearest contaminated carcass and any given carcass.

When carcasses originate from a positive flock, the nearest positive carcass will most likely be the one next to it. In contrast, consider a group of carcasses that come from a flock previously classified as *Campylobacter* negative. Within such a group there will be a proportion of carcasses that are contaminated. This can be accounted for in two main ways. First the crates in which the birds are transported are cleaned between flocks. However, this process has been demonstrated as ineffective at removing the *Campylobacter* contamination resulting from the transport of a positive flock. Second, when the birds are caught, the hands of the catchers can cause contamination. (T. J. Humphrey, *Pers. Comm*.). In this situation, only low-level contamination may occur and the nearest contaminated carcass may be several carcasses away. However, it is important to consider the effect of contamination caused by de-feathering in negative flocks as such contamination may persist to the final sale product.

From the above description, it is apparent that the effect of de-feathering on any given carcass is dependent upon the place of the carcass in the de-feathering line with respect to any contaminated carcasses in the line. If there are no contaminated carcasses preceding a selected carcass then the numbers contaminating the carcass, if there are any, decrease due to the removal of feathers. It has been demonstrated that the de-feathering process can reduce numbers by 1000-fold (Hinton *et al.,* 1996) but there is no indication of the variability surrounding this decrease for different carcasses or indeed no suggestion of the uncertainty surrounding this point value. If there are contaminated carcasses in front of a given carcass, the numbers on the selected carcass may increase due to the aerosol spread and machinery contamination.

As previously mentioned, the increase in contamination is related to the number of carcasses between a selected carcass and the nearest contaminated carcass. Therefore, the model simulates the sequential de-feathering of the group of 100 birds and estimates the random effect of the de-feathering process on all 100 birds with respect to each de-feathering event within the group. This is shown schematically in Figure 3.3.

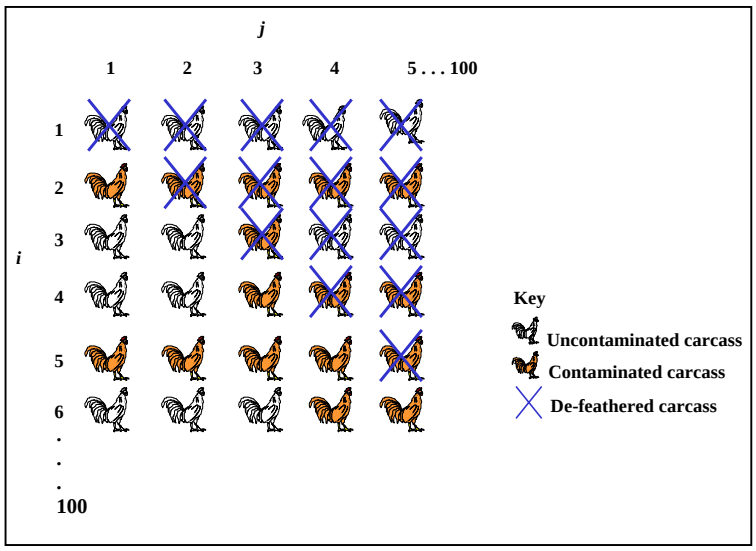

Figure3.3. Schematic representation of the effect of position in the de-feathering process on cross-contamination of carcasses

Figure 3.3 illustrates the 100 carcasses at the 100 different positions in the de-feathering line. Consider a random carcass, the position of the selected carcass is given by i (i=1,…,100) and the position of the carcass being de-feathered at the selected step is given by j (j=1,…,100). Thus when $i = j$ a selected carcass is being de-feathered. It therefore follows that for a selected carcass if $i < j$, the carcass is still to be de-feathered and for $i > j$ the carcass has been de-feathered. It can be seen that if a carcass being de-feathered is uncontaminated, $\theta_c = 0$, the contamination status of the birds behind that carcass does not change. However if a bird is contaminated, the result is a reduction on the level of contamination on the carcass being de-feathered but an increase in organisms on the carcasses following due to cross-contamination. This is illustrated by un-contaminated carcasses becoming contaminated. For example, consider the carcass in position $i = 3$ in Figure 3.3. It can be seen that when $j = 2$ the carcass in position $i = 3$ is uncontaminated, however in the next de-feathering step, that is $j = 3$, the carcass in position $i = 3$ has become contaminated as a result of the de-feathering of a contaminated carcass.

The extent of cross-contamination is related to the number of shackles, that is the number of birds, how far away a given carcass is from the carcass being de-feathered. Sample data (Hinton *et al.,* 1996) was used to estimate the effect of de-feathering on a series of carcasses. These data are shown in Table 3.6.

Table 3.6. Experimental data showing the spread of organisms from a contaminated seeder carcass to subsequent uncontaminated carcasses (after Hinton *et al.* 1996)

Carcass Number	Mean log cfu per carcass before de-feathering (n=4)	Mean log cfu per carcass after all carcasses de-feathered (n=4)	Calculated mean log cfu per carcass after first carcass de-feathered	Proportion of seeder contamination received
'Seeder'	9	7.9	7.9	N/A
1	0	5.9	7.1	0.0125
2	0	5.3	6.4	0.0025
3	0	5.2	6.3	0.0029
4	0	4.5	5.7	0.0005
5	0	4.8	5.9	0.0008
6	0	4.3	5.5	0.0003

A seeder carcass was artificially contaminated with 9 log cfu of a marker organism. A further six carcasses were then set in the shackle line proceeding the seeder bird. These six carcasses were known to be uncontaminated with respect to the marker organism. Microbial counts were then taken after all six birds had been through the de-feathering process. Using this data (Table 3.6) the effect of de-feathering the seeder carcass on the six proceeding carcasses was estimated. Taking this information and making two assumptions,

(i) the effect of the process on the carcass being de-feathered does not vary from carcass to carcass,
(ii) only the seeder carcass contributes to the contamination of the following carcasses;

the proportion of contamination a carcass receives from the carcass being de-feathered, given the distance between them, is estimated. A regression model was fitted to the experimental data using least squares to quantify the relationship between the proportion of seeder contamination received by a carcass and shackle position in relation to the seeder carcass using the data in Table 3.6. The predicted points along with a plot of the data are shown in Figure 3.4. The regression equation is $y = 0.0114(i-j)^{-1.8679}$ where *y* is the proportion of contamination a selected carcass receives from the carcass being de-feathered and (*i-j*) is the shackle position of the selected carcass, and the R^2 value is 0.91.

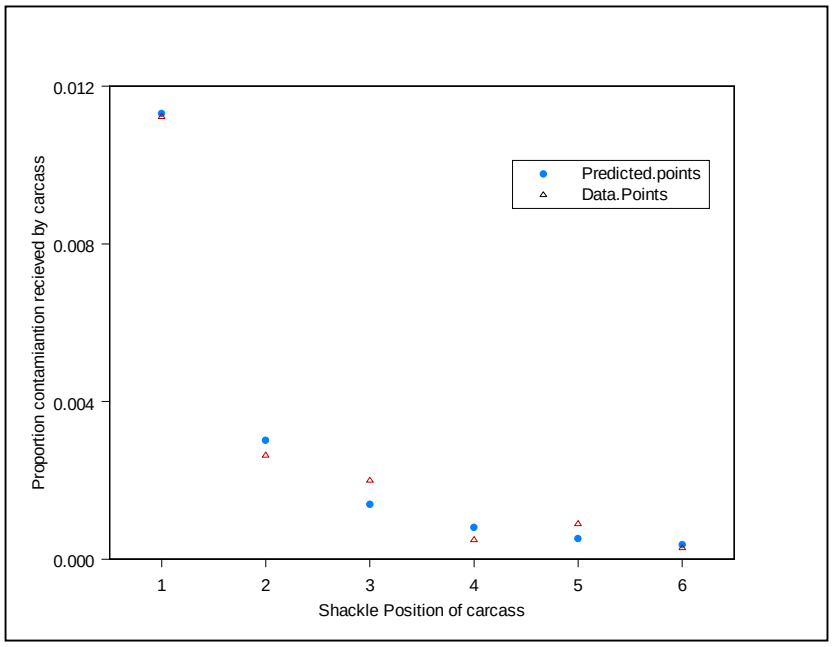

Figure 3.4. Graph showing the experimental data in comparison with the corresponding points predicted from the regression equation $y = 0.0113(i-j)^{-1.9067}$ to estimate the proportion of contamination received form a carcass being de-feathered given the number of shackles away a carcass is from the carcass undergoing the process, $(i-j)$. Here *i* is the position of the selected carcass and *j* is the position of the carcass being de-feathered.

For a given carcass the change in contamination resulting from the de-feathering process is the sum of the number of organisms gained from the de-feathering of the preceding birds, minus the sum of the number of organisms lost to the birds proceeding the carcass as a result of de-feathering of the carcass, and the reduction that results from de-feathering due to organisms being lost via the removal of feathers and flushing action of the water. Therefore the change in contamination due to de-feathering for the carcass in position *i* in the group given the carcass being de-feathered is in position *j*, that is φ_i, is given by equation 3.4.

$$\varphi_i = \left(\sum_{j=1}^{j=i-1} \eta_{c,d_{(i-j)}} \left(0.0113(i-j)^{-1.9067}\right) \right) - \left(\sum_{j=i+1}^{j=100} \eta_{c,d_i} \left(0.0113 j^{-1.9067}\right) + r_i \right) \qquad (3.4)$$

Here *r* is the reduction in the level of contamination on the carcass being de-feathered as a result of the removal of feathers and washing action of the water, and $\eta_{c,d(i-j)}$ is the level of contamination on the carcass being de-feathered which is given by $\mu_{(i-j)}\eta_{ext(i-j)}$ and η_{c,d_i} is the level of contamination on carcass *i* at the point of de-feathering given by $\mu_i \eta_{ext_i}$. To illustrate how Equation 3.4 works consider a group of 10 carcasses to be de-feathered. Table 3.7 shows how φ_i is calculated for the carcass which is fifth in this group, that is $i = 5$.

Table 3.7. Figure to illustrates how the calculation of the variable φ_i, the change in contamination levels as a result of de-feathering, is carried out by use of equation 3.4.

j	Number on carcass $i = j$	Number gained from preceding carcasses on carcass $i = 5$	Number lost to proceeding carcasses from carcass $i = 5$
1	100000	804	0
2	31623	440	0
3	125893	3794	0
4	251	28	0
5	N/A	N/A	N/A
6	0	0	226
7	0	0	60
8	0	0	28
9	0	0	16
10	0	0	11

$$\text{Number on carcass } i = 5 : \ 2000\text{cfu}$$

$$r_{i=5} = 1200$$

$$\phi_i = \sum \text{number gained from preceeding carcasses} - \left(\sum \text{number lost to proceding carcsses} + r_{i=5} \right)$$

The model described above solely describes the cross contamination which occurs during the process of defeathering as a result of the external contamination of the birds. However, given the nature of the process, it is likely that there may be some leakage of ceacal contents hence increasing the number of organisms available to contaminate the surface of the birds. At present there are no data sets available which allow the quantification of this effect.

Estimation of changes in numbers of *Campylobacter* due to evisceration, ξ_i

The data available for evisceration are shown in Table 3.8. These data are varied in nature with increases, decreases and no change in contamination all recorded. It is difficult to justify such observations when the biological process occurring is considered. Therefore, within the current model framework, evisceration is assumed to have no effect on contamination levels on a carcass. That is $\xi_i = 0$. This assumption can be modified should more data become available. During the expert consultation it was suggested that the assumption that evisceration has no effect should be modified. This will be investigated at a future date.

Estimation of change in number of Campylobacter after washing, v_l

Washing reduces the level of contamination on a carcass. This can be seen in the data shown in Table 3.9. This table consists of measurements of the level of carcass contamination taken before and after the washing process. Given the data in Table 3.9 the proportion of organisms remaining after a wash of the carcass, defined as $v_{i,}$ is estimated. A distribution describing the variability in the remaining proportion is then estimated. As the sample data set is small there is associated uncertainty with regards the true variability of this parameter. Therefore ν_i, the proportion remaining after washing, is described by a second-order non-parametric distribution estimated using methods previously described (Vose, 2000).

Table 3.8. Sample data measuring the levels of *Campylobacter* contamination on a carcass before and after evisceration.

Number of carcasses sampled	Log cfu per unit before evisceration	Log cfu per unit after evisceration	Change observed	Change in contamination level	Reference
				Increase log cfu per carcass	
8	1.99	2.44	inc	1.979019	Oosterom et al., 1983b
8	1.07	2.58	inc	0.962321	Oosterom et al., 1983b
8	2.09	2.62	inc	2.080651	Oosterom et al., 1983b
8	2.18	2.5	inc	2.172767	Oosterom et al., 1983b
8	2.37	3.12	inc	2.364181	Izat et al., 1988
8	2.82	3.49	inc	2.8177	Izat et al., 1988
8	2.82	3.49	inc	2.8177	Izat et al., 1988
5	3.2	3.2	none	0	Berrang & Dickens, 2000
5	3.7	3.7	none	0	Berrang & Dickens, 2000
				Proportion of organisms remaining post evisceration	
5	4.5	3.7	dec	0.158489	Berrang & Dickens, 2000
5	3.1	2.53	dec	0.269153	Berrang & Dickens, 2000
5	4.1	4	dec	0.794328	Berrang & Dickens, 2000
5	<3	1.6	dec	0.039811	Berrang & Dickens, 2000
11	5.75	5.7	dec	0.891251	Abu-Ruwaida 1994
8	3.68	3.49	dec	0.645654	Izat et al., 1988
8	2.46	2.24	dec	0.60256	Oosterom et al., 1983b
8	2.85	2.6	dec	0.562341	Oosterom et al., 1983b

inc = increase; dec = decrease; none = no change

Table 3.9 Sample data showing measures of *Campylobacter* before and after carcass washes.

Number of carcasses sampled	Mean log cfu before washing	Mean log cfu after washing	Proportion of organisms remaining	Reference
5	1.60	1.00	0.2511	Berrang & Dickens (2000)
5	3.20	2.10	0.0794	Berrang & Dickens (2000)
5	3.70	3.30	0.3981	Berrang & Dickens (2000)
5	2.53	2.00	0.2951	Berrang & Dickens (2000)
5	4.00	1.60	0.0039	Berrang & Dickens (2000)
5	3.70	2.70	0.1000	Berrang & Dickens (2000)
8	2.83	1.71	0.0758	Berrang & Dickens (2000)
8	2.94	2.39	0.2818	Berrang & Dickens (2000)
8	3.50	3.04	0.3467	Berrang & Dickens (2000)
11	5.70	5.10	0.2511	Berrang & Dickens (2000)

Estimation of changes in *Campylobacter* after chilling, τ_l

Within the EC, only air chilling is used for carcasses to be sold as fresh products. Air chilling is assumed to have no effect on the organism levels on the carcass (Cudjoe *et al.,* 1991). However if a carcass is to be sold as frozen products it is assumed that water chilling is used. This has been shown to have an impact on contamination levels. Further, when water chilling is used chlorine may be used as an additive. This affects the carcass contamination as can be seen in Table 3.10. Here carcass contamination was measured before and after chilling with water. Procedures both with and without chlorine are included in this data set.

Table 3.10 Data measuring the levels of *Campylobacter* contamination on a carcass before and after water chilling with and without chlorine added to the chill water.

Number of carcasses sampled	Chlorine added	Mean log cfu before chilling	Mean log cfu after chilling	Proportion remaining	Reference
8	-	1.71	1.43		Izat et al, 1988
8	-	2.39	1.85		Izat et al, 1988
8	-	3.04	1.18		Izat et al, 1988
15	-	2.92	1.74		Wempe et al, 1983
15	-	2.62	1.38		Wempe et al, 1983
15	-	3.32	2.33		Wempe et al, 1983
15	-	2.50	1.76		Wempe et al, 1983
5	+	2.10	1.20		Berrang & Dickens (2000)
5	+	3.30	1.10		Berrang & Dickens (2000)
5	+	2.00	0.90		Berrang & Dickens (2000)
5	+	1.60	3.20		Berrang & Dickens (2000)
5	+	2.70	1.10		Berrang & Dickens (2000)
90	+	5.35	3.86		Cason, 1997

The frequency with which chlorine is used in Great Britian is currently unknown. This frequency is defined as P_{Cl} and is assumed to be a uniform random variable with a minimum value of zero and a maximum of one. Based on this, the use of chlorine in the chilling of a given carcass is defined as Cl where $Cl \in \{0,1\}$ such that $Cl = 1$ means that chlorine is used as an additive to the chill water; this occurs with probability P_{Cl}. Further, $Cl = 0$ means that chlorine was not used as an additive to the chill water. This condition therefore occurs with probability $1 - P_{Cl}$.

It can therefore be seen that the probable reduction achieved by chilling the carcass is dependent upon the status of the product, either fresh or frozen. If the product is to be sold as fresh, $\theta_s = \alpha$, there is assumed to be no change in contamination levels and $\tau_i = 1$. In contrast if the product is to be sold as frozen, $\theta_s = \beta$, water chilling will be used and this may have an impact on microbial levels on the carcass. This impact depends on the use of chlorine in the water. More specifically the proportion of organisms remaining following chilling is given under the following condition

$$\tau_i = \begin{cases} 1 & \theta_s = \alpha \\ \tau_{cl} & \theta_s = \beta; \quad Cl = 1 \\ \tau_{ncl} & \theta_s = \alpha; \quad Cl = 0 \end{cases}$$

Here τ_{cl} is the proportion of carcass contamination remaining following water chill without chlorine, and τ_{ncl} is proportion of contamination remaining after a water chill which has chlorine added to the water. The variables τ_{cl} and are τ_{ncl} estimated, first by calculating the reduction in contamination in the samples shown in Table 3.10 with and without chlorine addition to the water. These data are then combined to give a second-order non-parametric distribution using previously described methods.

In summary, a description of a simulation model, which predicts the number of *Campylobacter* that will contaminate a carcass post-processing in a random plant in Great Britian has been provided. This model looks at the major stages of processing and involves several parameters.

3.4.2.2 Estimating the prevalence of contaminated products, P_{pp}

The model described above follows initially a group of 100 birds from a randomly selected flock and subsequently a random bird from within this group through the processing plant. At each of the processing stages modelled the number of contaminating organisms on the carcass is calculated. This calculation mimics the changes in numbers as a result of the particular step.

At the end of processing, a carcass can be defined as contaminated if it carries at least organism. By means of a conditional statement, the model can state whether a selected product is contaminated or not. The conditional statement is

$$\psi_i = \begin{cases} 1 \text{ if } \eta_p \rangle 1 \\ 0 \text{ if } \eta_p \langle 1 \end{cases}$$

where ψ_i is whether or not the product is contaminated at retail. Within a given simulation distributions are sampled *n* times, on each time the result is either a contaminated or uncontaminated product. Multiple samplings of the distributions represent the production of multiple products. Therefore running the model allowing for *n* samplings of each distribution the probability that a product is contaminated can be calculated by use of Equation 3.5.

$$P_{pp} = \frac{\sum_{i=1}^{i=n} \psi_i}{n} \qquad (3.5)$$

where P_{pp} is the probability that a product is contaminated based upon *n* samplings within a simulation of the model.

The Expert consultation recommended the drafting group to consider transport and retail storage. These subjects will probably be included at a later stage.

3.5 Home preparation and handling of chicken

In the home, during meal preparation, individuals can be exposed to *Campylobacter* from fresh chicken through a large number of pathways. These pathways could include: direct contamination from the chicken to any food commodities not undergoing a subsequent cooking step before ingestion, indirect contamination of surfaces upon which cooked products or ready-to eat food are placed, contamination directly onto hands and subsequent ingestion, insufficient cooking and many other potential contamination events.

The FAO/WHO *Campylobacter* Risk Assessment model assumes that liquid from a broiler or chicken, which contains *Campylobacter*, may cross-contaminate the already cooked chicken or other foods prepared and consumed during the same meal or potentially ingested directly from contaminated hands for instance. Transfer can be facilitated by liquid carried on hands, utensils and cutting boards and these mechanisms may be a significant contributor to exposure and foodborne illness. Unsafe food handling procedures in private kitchens are assumed to be responsible for a large number of cases of food-borne diseases in most countries (Zhao *et al.,* 1998; Worsfold *et al.,* 1997b). In USA it was estimated that 21% of 7219 cases of food-borne diseases were related to private households in the period from 1973 to 1987 (Williamson *et al.,* 1992). Furthermore, in England it was estimated that 35% and 28%, respectively, of 101 outbreaks of food-borne diseases were related to insufficient heat treatment and cross-contamination of foods during preparation of meals in private households (Ryan *et al.,* 1996). In Sweden, the authorities have estimated that half of the number of food-borne cases was acquired in private households (Anderson *et al.,* 1994).

In the present FAO/WHO Risk Assessment modeling of consumer handling and preparation in private kitchens has been divided into two parts: (1) Cross-contamination of a meal due to unsafe food handling procedures, and (2) The survival of *Campylobacter* due to undercooking of the chicken. (On request of the Expert consultation, food preparation at restaurants, etc. might be included in future).

In the following data from studies on food handling procedures in private kitchens are reviewed. Since *Campylobacter jejuni* is assumed not to grow below 30°C factors influencing growth during storage are not included. The areas described in this section are therefore restricted to food handling procedures in private kitchens assumed to have the greatest impact on the exposure to consumers. Differences and the potential influence of age and sex on food preparation hygiene are also discussed.

3.5.1 Cross contamination

Several investigations have been carried out to elucidate consumer habits during food handling in relation to cross contamination due to unsafe versus safe food behaviour. In the following, studies related to cross contamination by hands and utensils are summarized.

3.5.1.1 Cross contamination by hands

The outcome of interviews and observations of consumer habits regarding washing hands is summarised in Table 3.11. Washing hands after having handled raw meat and poultry is one essential factor for minimising cross-contamination. Brown *et al.* (1988) found that *Campylobacter* spp. were detected on hands before, but not after washing hands during a handling procedure involving raw chicken. When washing hands was not performed, other food items became cross-contaminated with *Campylobacter* spp. from the chicken in 2 of 5 cases. The fact that hands will become contaminated during handling of *Campylobacter* positive chickens was demonstrated by De Boer and Hahne (1990). In this study *Campylobacter* spp. were isolated from hands in 42 of 58 trials (73%), in which raw poultry was handled. After 3 minutes *Campylobacter* spp. could still be detected in 30 of 54 trials (55%). Another study (Coates *et al.,* 1987) showed that *Campylobacter* spp. suspended in chicken meat juice and introduced on fingers could survive up to one hour. The same study revealed that *Campylobacter* was not detected after washing hands with water or water and soap followed by drying. If drying was not performed, *Campylobacter* was not eliminated from the fingers. Estimates on the number of *Campylobacter* on hands during handling of chickens in private households has not been generated, only for workers at the dressing and portioning step at a chicken slaughterhouse (Oosterom *et al.,* 1983b). On 6 of the 11 hands

examined *Campylobacter* was detected at a level of $\log_{10}$ 0.48-1.24 cfu/hand (mean; $\log_{10}$ 0.9 cfu/hand). *Campylobacter* was not detected on 5 hands (< $\log_{10}$ 0.35 cfu/hand).

Table 3.11 Data on consumer habits related to washing hands after having handled raw meat and poultry.

Statement	**Respondents agreeing with the statement (%)**	**Study performed in**	**Reference**
Washing hands not performed after handling raw meat and poultry	34% of 1620 persons	US 1992-1993	Altekruse *et al.,* 1995
	18.6% of 19356 persons	US 1995-1996	Yang *et al.,* 1998
	55.8% of 1203 persons	Australia 1997	Jay *et al.,* 1999
	36% of 15 households	Denmark 1998	CASA, 1999
Washing hands not important in relation to food hygiene	18.4% of 1203 persons	Australia 1997	Jay *et al.,* 1999
Personal hygiene (inc. washing hands) not important for prevention of food-borne disease	62% of the 990 persons	Denmark 1996	AIM Nielsen & Levnedsmiddelstyrelsen, 1997
Drying of hands performed after hand wash	70% of 15 households	Denmark 1998	CASA, 1999
Observation	**Households where the observation was done (%)**	**Study performed in**	**Reference**
Washing hands not performed after handling raw meat and poultry	58% of 108 persons	UK 1996	Worsfold *et al.,* 1997a; Griffith *et al.,* 1998
	57% of 106 households	US and Canada	Daniels, 1998

3.5.1.2 Cross-contamination by utensils

Exposure to food borne pathogens in the private kitchen due to cross-contamination by utensils such as cutting boards, knives, etc. is assumed to pose a considerable risk. The outcome of interviews and observations of consumer habits regarding procedures that could lead to cross-contamination through utensils are summarised in Table 3.12.

In a Dutch investigation *Campylobacter* spp. were found on cutting boards in 38 of 76 trials (50%) after handling raw chicken. On plates where raw chicken was stored for 5 minutes, *Campylobacter* spp. could be detected 25 of 54 trials (46%). In the same study *Campylobacter* spp. could be detected in 5 of 54 samples of vegetables (9%) handled on a cutting board previous used for raw chicken. Further on, *Campylobacter* spp. could be detected in 2 of 21 samples of heat-treated chicken handled on a cutting board previous used for raw chicken (De Boer & Hahne, 1990). Martin *et al.* (1999) found that it was possible to recover *Campylobacter* spp. from naturally contaminated domestic kitchen surfaces 50 minutes after the area was observably dry. In addition, Bolton *et al.* (1999b) isolated *Campylobacter* spp. from 3% to 8% of outer packaging of chicken products sold at retail level and from 4.5% of outer packaging of offal products sold at retail level. These results indicate that cross-contamination may take place

not only from the meat products but also from packaging material brought into the kitchen along with the meat products.

Table 3.12 Data on consumer habits related to cross-contamination by utensils.

Statement	Respondents agreeing with the statement (%)	Study performed in	Reference
Knifes and cutting boards not cleaned in warm water + soap after handling raw meat and poultry and before cutting vegetables and salads	46% of 865 responses	US 1990-1991	Williamson *et al.,* 1992
Cutting board not washed after handling raw meat and poultry	33% of 1620 persons	US 1992-1993	Altekruse *et al.,* 1995
	19.5% of 19356 persons	US 1995-1996	Yang *et al.,* 1998
The kitchen facilities not sufficiently cleaned to avoid cross-contamination	11.6% of 1203 persons	Australia 1997	Jay *et al.,* 1999
Food items handled on not sufficiently cleaned cutting boards	25% of 108 persons	UK 1996	Worsfold *et al.,* 1997a; Griffith *et al.,* 1998
Meat and poultry packing material stored in the food handling area	18% of 108 persons	UK 1996	Worsfold *et al.,* 1997a; Griffith *et al.,* 1998
Food items handled in a way that could lead to cross-contamination	76% of 106 households	US and Canada	Daniels, 1998

3.5.2 Modelling cross contamination

As indicated above there is a large degree of uncertainty and variability associated with the food handling procedures in the kitchen. Estimating the risk of infection via cross-contamination is a difficult exercise and the data available are limited. The studies that currently exist primarily report on the presence of contamination on various surfaces following preparation activities, but little information is provided on the degree of contamination. These studies thus provide insight on the possibility of contamination but do not provide a quantitative estimate of the transfer of *Campylobacter* from a contaminated chicken.

We assume that during the preparation of a chicken meal a single event will occur that will result in the transfer of *Campylobacter* from the chicken to a utensil or cutting board and also a subsequent transfer from the kitchen surface to a food that is consumed during the meal such as cooked chicken or salad or vegetables. Although we acknowledge that during the preparation of a chicken meal an individual may cross-contaminate several surfaces and foods with *Campylobacter*, there are insufficient data to estimate the frequency of multiple cross-contamination events.

Therefore, at present we do not believe that it is possible to separate kitchen processes in different contamination routes and to quantify to which extend each of these routes contribute to the overall risk. Instead two models “the drip fluid model’ and “the contact transfer model’

have been considered, which in two different ways try to describe the overall cross-contamination process from a raw chicken to exposure.

"The drip fluid model" is based on a model previously described in a Canadian risk assessment (Fazil *et al.,* 2000) and the "the contact transfer model" is developed combining the models of a Danish and English risk assessment.

3.5.2.1 Description of the drip fluid model

The drip fluid model is a mechanistic approach related to the water a chicken gains through processing. The model is based on the fact that when water is applied to chickens during processing such as in the immersion chiller, a proportion of the load which is loosely attached is diluted in the volume of water, some of which may be absorbed by the chicken or form a thin layer around the carcass. A certain portion of the fluid subsequently drips off the chicken during processing, however the concentration of loosely attached organisms in the fluid that remains on the carcass is the same, provided that additional cells do not subsequently go into suspension in the fluid. For example, if the concentration on a chicken is estimated to be 3 log CFU/carcass, and 5% of the load are assumed to be loosely attached. Further it is assumed that the loosely attached cells are diluted in 200ml of fluid as a result of immersion chilling and that after the chill tank, approximately 75 ml of fluid remain on the carcass and the rest drips of at the processing plant, then the concentration in the fluid contained on the chicken can be crudely estimated as follows:

Concentration on Chicken = 3 log CFU/Chicken = 1000 CFU/Chicken
Fraction of loosely attached = 0.05
Number of cells loosely attached = 1000 x 0.05 = 50 CFU/Chicken
Volume of fluid diluting loosely attached cells = 200ml
Number of cells in 200 ml of fluid = 50 CFU
Concentration in fluid = 50CFU / 200ml = 0.25 CFU/ml

If only 75 ml of the fluid remain on the carcass when it is packaged, the concentration in this volume of fluid would still be 0.25 CFU/ml. There would be approximately 19 CFU in the fluid on the chicken (0.25 CFU/ml x 75 ml) and approximately 31 CFU would have 'dripped' off in the fluid that did not remain on the carcass.

Figure 3.6 summarizes the steps taken to estimate the number of *C. jejuni* in chicken drip fluid ingested by a random person in the home, based on the concentration on the chicken at process.

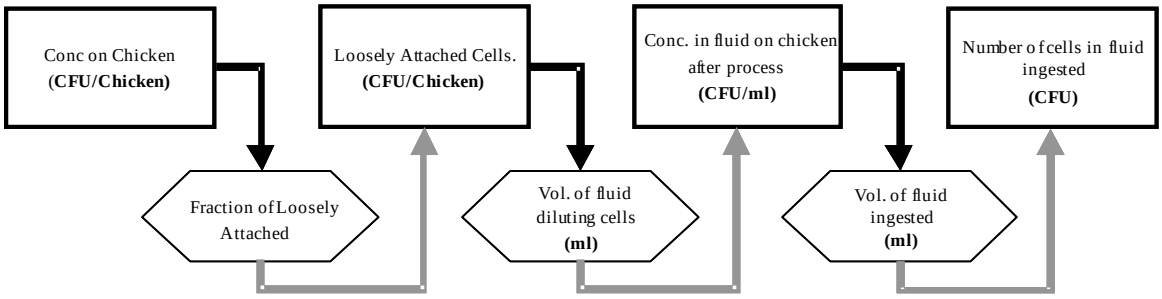

Figure 3.6 Steps in estimating the number of *C. jejuni* in chicken drip fluid that a random person gets exposed to.

The Canadian Food Inspection Agency (CFIA) has regulations in place on the permissible weight increase for chicken from the addition of water during processing of up to 8% for chickens under 2.3 kg. In the US, similar guidelines exist with chickens 4.5 lb. (2 kg) or less allowed to absorb no more than 8% of their weight in water during processing (FSIS, 1996). A broiler of 1400 grams could thus have approximately 100 ml of water contributing to its weight. The volume of water on a chicken could be quite substantial and the assumptions for the dilution volume are quite feasible. However, research looking at issues such as the amount of fluid on a chicken and the concentration in the fluid in relation to the concentration on the carcass would provide valuable information towards a better estimation of the risk associated with cross-contamination. This current assessment provides an estimate of the potential risk from exposure to drip fluid from chicken. The assumptions used to estimate the concentration in the fluid may be reasonable estimates, however it should be noted that there is no 'hard' data to support the assumptions. As stated earlier, there exists a substantial data gap in this area of the process that can be well served by research activities.

The risk estimated from the drip fluid module is based on the consumer being exposed to between 0.5 and 1.5 ml of drip fluid. Exposure in the assessment refers to the ingestion of this volume of fluid without further reduction in the concentration (through cooking for instance). The route of exposure to this volume of drip fluid is not explicitly specified, however the volume of fluid used in the estimate is relatively small and could be transferred to the consumer along many pathways during meal preparation (some of which were highlighted earlier in the section).

The distributions used to describe the fraction of the load on processed chickens ($\mathbf{F_{loose}}$) that is loosely attached, the volume of fluid that is assumed to dilute the loosely attached cells ($\mathbf{V_{dilute}}$) and the volume of fluid to which the consumer may be exposed ($\mathbf{V_{drip}}$) are summarized in Table 3.13.

Table 3.13 Model parameters in drip fluid model

Parameter	Description	Unit	Distribution/expression
F_{loose}	Fraction of loosely attached *Campylobacter* cells		Uniform(0.01,0.1)[a]
V_{Drip}	Volume of drip fluid ingested	ml	Uniform(0.5,1.5)[a]
V_{Dilute}	Volume of fluid assumed to dilute loose cells	ml	Uniform(150,250)[a]

[a]Uniform(min,max)

3.5.2.2 Description of contact transfer model

In the contact transfer model it is assumed that the transfer of *Campylobacter* cells to a meal or direct ingestion via e.g. licking on fingers is a process of two steps (Figure 3.7).

In the first step the organisms are transferred from the raw chicken to preparation surfaces (cutting board, utensils etc.) or hands. In the second step the organisms are transferred from the preparation surface to a prepared meal or the organisms are directly ingested by e.g. licking on fingers.

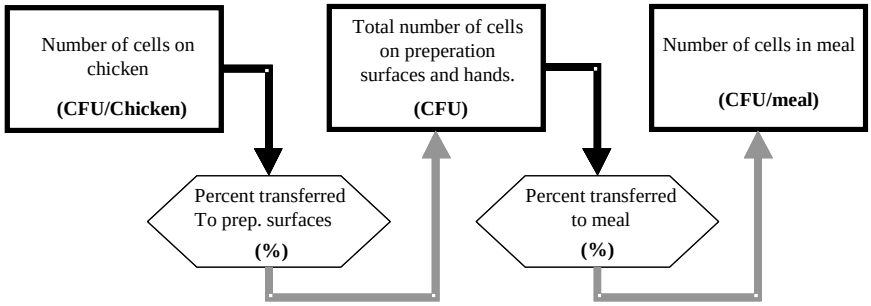

Figure 3.7 Steps in the contact transfer model.

In a study by Zhao *et al.* (1998) the fraction of organisms transferred from a contaminated raw chicken to a cutting board, and further from the cutting board to salad was reported. Although data where based on another organism*, E. aerogenes,* the study gave good indication of the possible maximum transfer of an organism from one surface to another.

In the study the bacteria were added to the raw chicken, which following was placed with the skin side down on a cutting board and then cut into very small pieces. Approximately 10% of the organisms were transferred from the chicken to the cutting board. Subsequently, the salad was chopped carefully on the contaminated cutting board, which again resulted in transfer of approximately 10% from the cutting board to the salad. A number of $\log_{10}$ 3.0 cfu of *E. aerogenes* could be detected on vegetables handled on a cutting board previously contaminated with $\log_{10}$ 5.0 cfu/cm^2. Yuhuan and colleagues (Yuhuan *et al.,* 2001) recently showed varying levels ofof transfer could be accomplished starting with $\log_{10}$ 8.0 cfu of *E. aerogenes* onto 150g pieces of chicken by transfer of organisms from the surface of chicken onto hands, utensils and vegetables during preparation of a meal.

Because of the extreme careful chopping of the chicken and the salad, we concluded that the data represent the optimal transfer of bacteria from the raw chicken to salad, rather than the average transfer of organisms in a random household.

In the present model the distributions, which describe the transfer of *Campylobacter* from a contaminated raw chicken to a meal, should represent all levels of transfer that might occur during preparation of a random meal and not only the maximum transfer. When *Campylobacter* is transferred from a raw chicken to preparation surfaces or hands, a certain fraction of the bacteria may be hidden in the feather follicles and not all parts of the skin may touch the preparation surfaces or hands. Further, on parts of the chicken containing *Campylobacter,* cells may have dried out leading to a reduction in the number of living cells on the chicken. Therefore, in most cases transfer of organisms from the raw chicken to the preparation surfaces or hands in a "real household' is lower than in the study presented by Zhao *et al.* (1998). In the present risk assessment we chose to model the variation in the parameter, $\mathbf{F_{chic\_prep}}$ (Table 3.14), describing the fraction of *Campylobacter* transferred from the raw chicken to preparation surfaces or hands by a LogPert distribution with a minimum of –6 (0.0001% transferred), a mode of -2 (1% transferred) and a maximum of -1 (10% transferred) (the maximum is given by Zhao *et al.,* 1998).

With respect to transfer from the preparation surfaces or hands to the meal, there may be some delay between the preparation of the raw chicken and subsequent preparation of other food items such as salad, bread or the prepared chicken. This delay may result in a reduction in the number of living organisms on the preparation surfaces, e.g. because of drying of the surfaces. In addition, probably very often only small parts of the preparation surfaces or hands which have

been in contact with the raw chicken, will also come into contact with the prepared food items that do not undergo any further cooking step. Therefore, also in this situation the transfer of organisms from the preparation surfaces or hands to a meal will be lower than in the study presented by Zhao *et al.* (1998). We chose to employ a LogPert distribution for the parameter $\mathbf{F_{prep\_meal}}$ (Table 3.14), describing this part of the process with a minimum of -6, a mode of -2 and a maximum of -1.

Table 3.14 Model parameters in contact transfer model

Parameter	Description	Unit	Distribution/expression
F_{chic_prep}	Fraction of cells transferred from raw chicken to preparation surfaces and hands		$10^{Pert(-6,-2,-1)\ a}$
F_{prep_meal}	Fraction of cells transferred from preparation surfaces and hands to meal		$10^{Pert(-6,-2,-1)\ a}$

[a]Pert(min,median,max)

3.5.2.3 Comparison of drip fluid model and contact transfer model

As the assumptions and data input for the two models are different, it was of interest to analyze and compare the outcome of the two models.
For each of the two models the variation in fraction of cells transferred from the raw chicken to an exposure dose can be converted into a single distribution, which is described as:

Drip fluid model:

$$F_{transfer} = \frac{F_{loose} \cdot V_{Drip}}{V_{Dilute}} = \frac{Uniform(0.1,0.01) \cdot Uniform(0.5ml,1.5ml)}{Uniform(150ml,250ml)}$$

Contact transfer model:

$$F_{transfer} = F_{chic\_prep} \cdot F_{prep\_chic} = 10^{pert(-6,-2,-1)} \cdot 10^{pert(-6,-2,-1)}$$

The resulting distributions for the fraction of cells transferred to a meal for each of the models are presented in Figure 3.8. Clearly, the contact transfer model incorporates a much broader distribution for the fraction of cells being transferred than the drip fluid model.

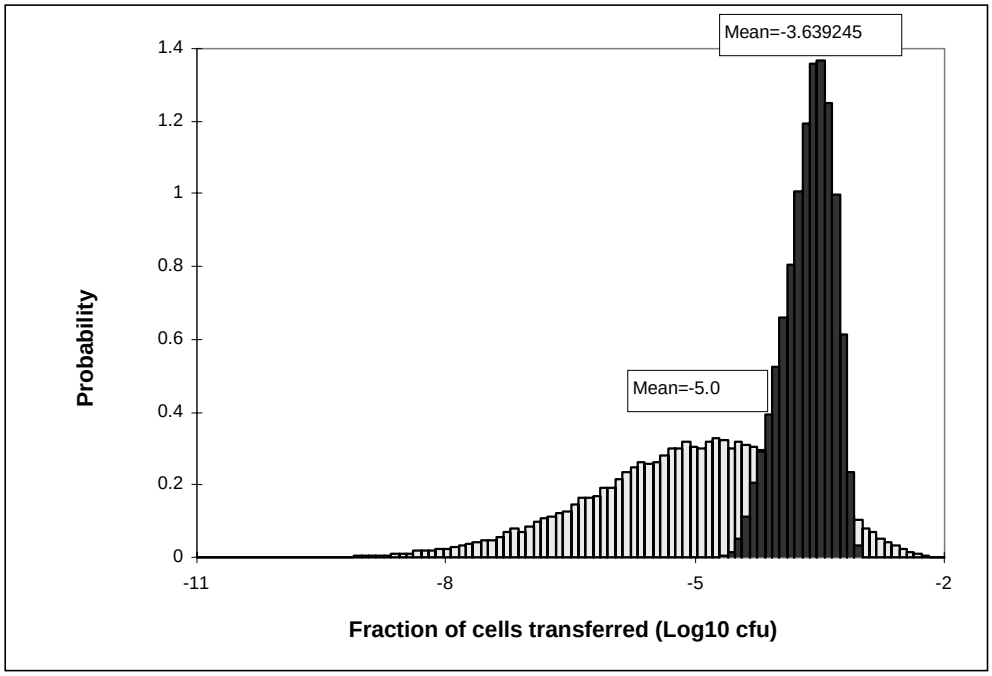

Figure 3.8 Distributions for the overall transfer of *Campylobacter* cells in "drip fluid model' (dark grey bars) and the contact transfer model (light grey bars).

For both models the resulting transfer from a chicken to an exposure dose can be described with the same single overall mathematical parameter, $F_{transfer}$.

$$N_{meal} = Binomial(N_{chicken}, F_{transfer})$$

where N_{meal} and $N_{chicken}$ indicate the number of cells ingested or on a chicken, respectively.

Despite the fact that the assumptions behind the models are different the models are mathematically equal. The only difference is the different resulting transfer distribution, $F_{transfer}$.

In order to compare the outcome of the two models given the transfer parameters in Table 3.13 and Table 3.14 we used a distribution for the *Campylobacter* concentration on the chickens from the Danish risk assessment (see scenario "0' in Figure 3.10 below).

Simulations carried out for each of the models showed that for the contact transfer model the probability of being exposed to *Campylobacter* in a meal was approximately half the probability of being exposed in the drip fluid model (Table 3.15).

Table 3.15 Probability of transfer of *Campylobacter* by cross contamination from a positive chicken, assuming the concentration on the chicken follows the "0' scenario (See Figure 3.10) obtained from the Danish risk assessment (Christensen *et al.,* 2001).

Model	Probability
Drip fluid	0.667
Contact transfer	0.328

Next, the two models were compared on the level of the dose of exposure, i.e. the number of cells in a meal ingested. Figure 3.9 shows the cumulative probability distribution of the exposure

for both models. Apparently the level of exposure predicted by the drip fluid model is higher than that predicted by the contact model. This is the case both when all meals are considered Figure 3.9a, as when contaminated meals only are considered Figure 3.9b. Thus, the model that predicts the highest probability of exposure also predicts a larger level of exposure. For a better comparison of the shapes of the distributions of the transfer rates as depicted in Figure 3.9c, d, one of the models was adapted to get two models predicting the same probability of exposure to Campylobacter: the drip fluid model is changed by adding a factor φ:

$$F_{transfer} = \varphi \frac{F_{loose} \cdot V_{Drip}}{V_{Dilute}} = \varphi \frac{Uniform(0.1,0.01) \cdot Uniform(0.5ml,1.5ml)}{Uniform(150ml,250ml)}$$

This parameter φ can be interpreted as a change in the assumptions on one or some of the distributions used in the model (e.g. a smaller drip volume). It was found that by putting φ=1/12, the predicted probability of exposure in both models is about 33%. A comparison of the two models then shows that the contact transfer model predicts a higher level of exposure. This implies that if also the dose response model were taken into account it would be difficult to say which of the two models would produce most illnesses. This would be dependent on shape of the dose response curve (See section 4.3).

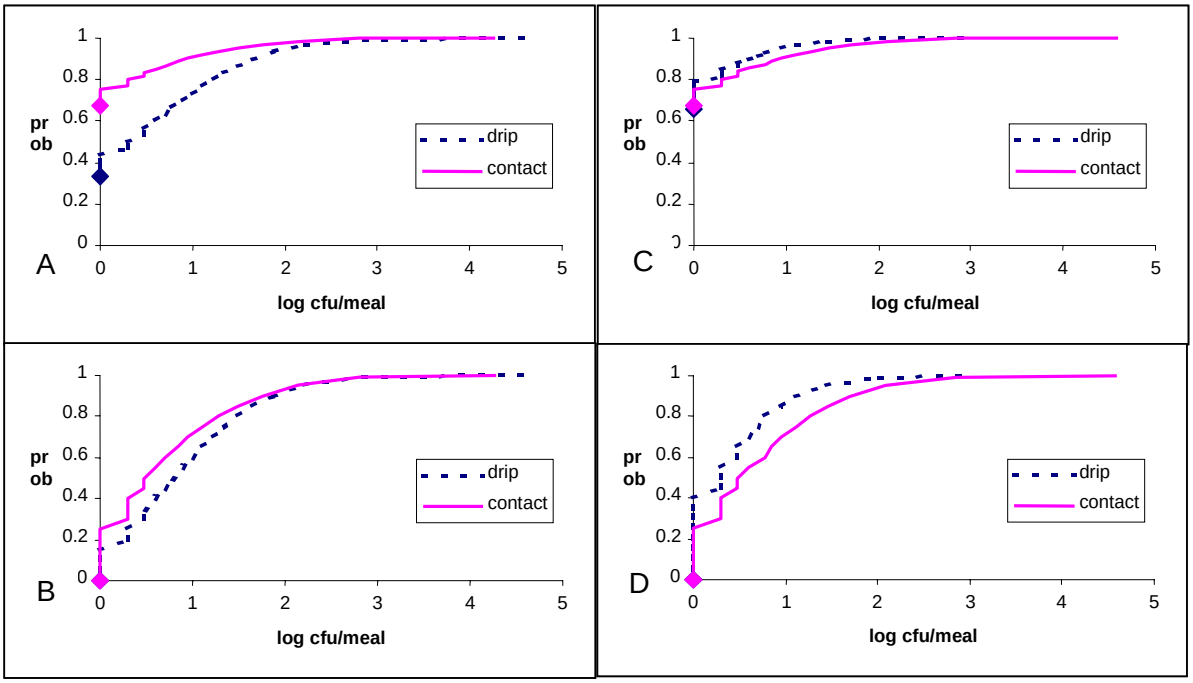

Figure 3.9 Accumulated probabilty distributions for the number of *Campylobacter* cells in a meal in the drip fluid model (dotted line) and the contact transfer model (straight line), respectively, compared for the orginal models ,φ =1 ,(A and B) and the adapted models which predict a equal probability of exposure to Campylobacter contaminated meals, φ =1/12 ,(C and D). A and C shows the level of exposure in all meals, B and D the level of exposure in contaminated meals only.

The two models represent two different ways of describing cross-contamination in private kitchens. Both models are based on a number of rough estimates. As such, it might seem at little inadequate to compare the outcome of the two models. However, the result clearly shows that independently of the assumptions made in development of the models, they both yield risk estimates that appear to be at least within the same order of magnitude.

Apart from the mathematical structure of a model it might also be important to consider the models conceptually, i.e. one model might be easier to understand in some cases and facilitate the communication aspect of the risk assessment. This could be especially important when it comes to visualizing the effect of a mitigation strategy for instance.

As an example, it could be relevant to compare the use of an air chiller to a spin chiller. In the air chiller, the volume of fluid attached to chicken will be lower than for the spin chiller. Thus, the volume in which *Campylobacter* is diluted is lower, giving rise to a higher concentration in one ml of drip fluid. However, at the same time the total volume of drip fluid will be lower and thus it might be less possible to be exposed to one ml of fluid. Mechanistically the drip fluid seems to allow for a relatively good explanation of the actual difference between the two different chilling processes. In the contact transfer model, in order to account for differences in the processes more interpretation would be required to adjust the transfer fraction, such as: air chilling will result in a dryer chicken; the dryer chicken will result in a lower likelihood of survival on the surface which subsequently justifies an adjustment to the transfer fraction.

Each of the two models have advantages and disadvantages in the mechanistic descriptions of different parts of a particular mitigation strategy, and in total it might be considered whether a mix of the two model approaches is the most appropriate way of modeling the over all cross contamination in kitchens.

3.5.2.4 Effect of mitigation and intervention strategies

We compared the two models given the introduction some kind of mitigation strategy in the slaughter process, which reduces the load of *Campylobacter* on the chicken. As input, we used data from the Danish risk assessment where the load on the chickens was reduced on the carcasses in slaughter process due to implementation of a not defined mitigation strategy. The effect of such a strategy is a reduction in the number of positive chickens, and a reduction in the concentration on the remaining positive chickens (Figure 3.10).

In relation to the probability of being exposed to *Campylobacter*, the effect of changing the load on the chickens entering the kitchen is different in the two models (Figure 3.11). The number of positive exposures decreases more in the contact transfer model as compared to the drip fluid model. The difference is due to differences in the resulting transfer distributions (see Figure 3.8). Not only is the shape of the distributions different, but also the mean number transferred. *Not surprisingly this has an impact on reducing the number of positive exposures that occur.*

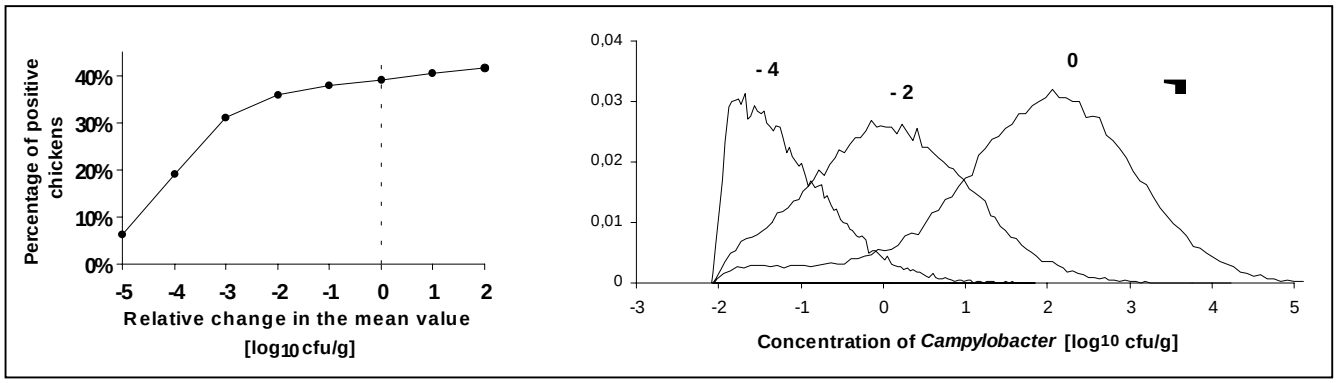

Figure 3.10 Data from the Danish risk assessment representing the fraction of positive chickens (A), and distribution of concentration on carcasses (B) as function of changes in the slaughter process, which reduces the load on the chicken. For the concentration of *Campylobacter* on chickens (B) only distributions for relative change in the mean value of 0, -2 and –4 (indicated above distributions) are presented.

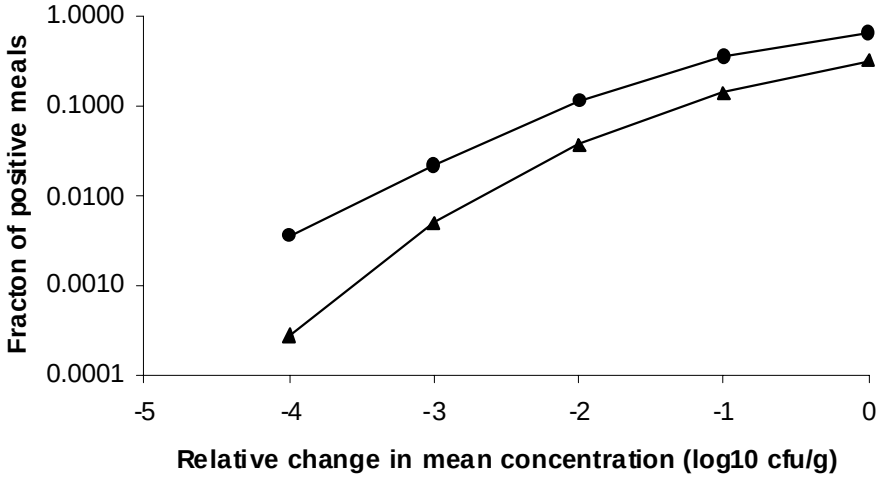

Figure 3.11 Change in the fraction of positive meals as function of change in the mean load of *Campylobacter* on chickens entering the kitchen in the drip fluid model (✯), and the contact transfer model (π).

In order to monitor the difference between the two models we determined which part of the transfer distributions (Figure 3.8) generates positive exposures. From the accumulated distributions (Figure 3.12), it is shown that as the load on the chickens is reduced in the contact transfer model, there is a significant shift in the resulting distributions relating to the positive meals. In other words, a positive meal is only produced in cases where high fractions of organisms are transferred from the raw chicken to the meal.

In the drip fluid model it seems to be slightly different. We suggest that this can be explained by the much narrower distribution describing the transfer from the chicken to a meal, which simply leaves the resulting distributions responsible for the positive meals relatively unchanged.
The analysis indicates that with a broad transfer distribution, as in the contact transfer model, the effect of changing the load on a chicken is more pronounced in those cases where low numbers are transferred from the chicken to the meal. In reality, this could be converted into hygiene levels of individuals. When people have extremely low hygiene practice, the effect on

the probability of obtaining a meal with *Campylobacter* is more or less unaffected upon changing the load on the chicken, whereas for people with a better hygiene standard the effect is much more ponounced.

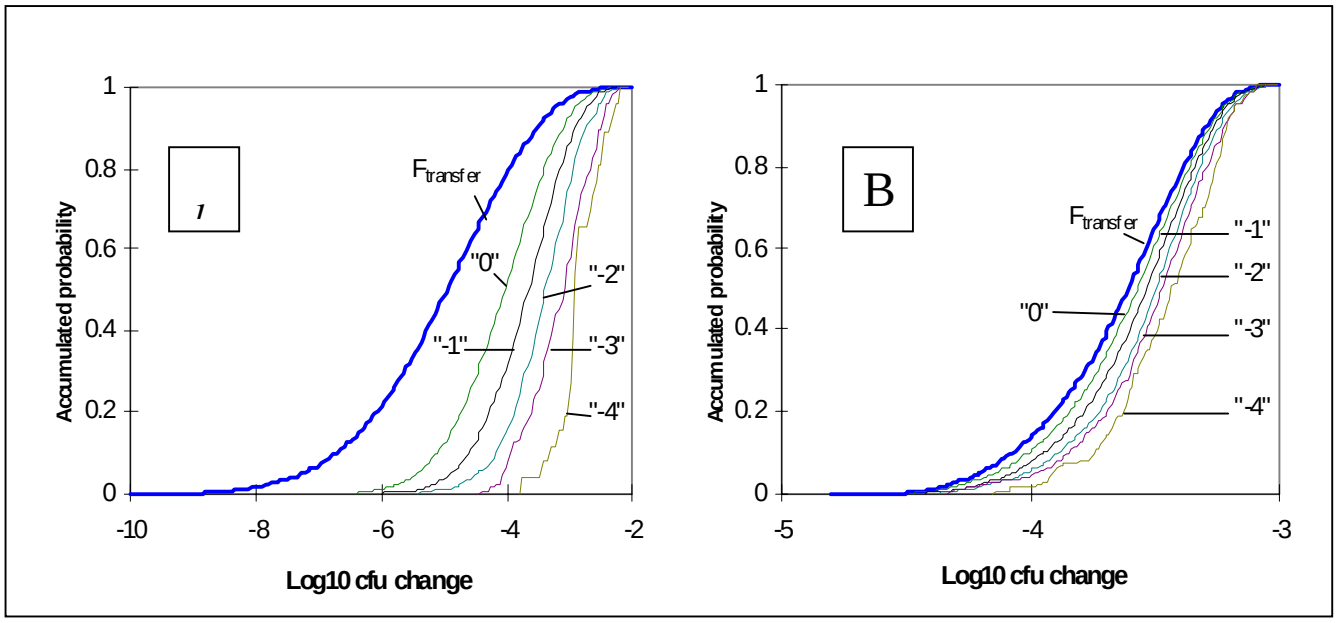

Figure 3.12 Accumulated distributions for the resulting part of the distribution, $F_{transfer}$ (bold line) that give rise to a meal containing one or more *Campylobacter* cells. The resulting distributions are presented for each of the mean reductions levels as indicated with '0' ,'-1','…'-4'. (A) represents the results of the contact transfer model and (B) the results of the drip fluid model.

3.5.2.5 Additional considerations: Hygiene practices of food preparer

Although the exposure assessment portion does not address the variation in preparation practices among food preparers this could be incorporated as an additional consideration in the future. Such an approach was used in the Denmark Risk Assessment on Campylobacter in Chicken (2000) because they had access to data on the food preparer in the home.

The contact transfer model and the drip fluid model assume that any prepared meal has a chance of getting contaminated with *Campylobacter* due some kind of unsafe food handling. But the models do account for the large fraction of people take strong precautions (hygiene barriers) to prevent the transfer of any organism from the raw chicken to a meal. For example by using separate cutting board for handling raw meat, or by washing cutting board, washing hands, utensils etc. Later, it might be of interest to introduce a parameter describing the ratio between the fraction of people with safe relative to unsafe food handling procedures, because this parameter might be important in relation to estimating the effect of different mitigation strategies.

Several studies have shown that the ratio between people with safe and unsafe food handling depends on age and sex of the person who prepares the food (see Table 3.16). Therefore, it might be important to consider the age and sex of the person who prepares a meal, because this is the person responsible for the hygiene level during preparation of the food and therefore also the degree to which the final meal will be contaminated with *Campylobacter*.

By dividing a given population into a defined number of different age and sex groups, it is possible to determine to which extent each group contributes to the preparation of meal. For

example, in families with two adults (male and female) the male person will, in most countries, prepare the meal less often than the female person. Therefore, in total, despite their lower hygiene standards the contribution from males to the contamination of meals may be lower than the contribution from females.

Table 3.16 Data on food handling procedures related to age and sex.

Statement	**Respondents agreeing with the statement (%)** MALE	FEMALE	**Related to different age groups**	**Reference**
Washing hands not performed after handling raw meat and poultry	47%	25%	18-29: 42% 30-64: 32% > 65: 29%	Altekruse *et al.,* 1995
Cutting board not changed or washed after handling raw meat and poultry	47%	28%	18-29: 47% 30-64: 29% > 65: 24%	Altekruse *et al.,* 1995
Cutting board not sufficiently washed			17-35: 45% 36-45: 38% > 46: 33%	Jay *et al.,* 1999
Utensils not sufficiently washed			17-35: 32% 36-45: 28% > 46: 27%	Jay *et al.,* 1999
Clean utensils and change of cutting boards are not important issues in preventing food borne disease	51%	46%	< 24: 63% 25-34: 47% 35-54: 41%	AIM Nielsen & Levnedsmiddelstyrelsen, 1997
Sufficient heat treatment not recognized as a preventive option to food borne disease	51%	57%	< 24: 55% 25-34: 52% 35-54: 50%	AIM Nielsen & Levnedsmiddelstyrelsen, 1997

Furthermore, if a person prepares a meal, which contains *Campylobacter*, not only will the person who prepares the meal be exposed to *Campylobacter*, but also potentially every person who ingests that meal. In this way the age and sex of the person preparing the meal become important in relation to the average number of persons being exposed to a contaminated meal. If for example the person is in the age group of 18-29, the fraction of persons living as pairs and the fraction of children per person is lower than for people in the 30-65 year age group. Consequently, because of a smaller average family size fewer people will eat the same meal and, therefore, fewer people will (in average) become exposed to a meal prepared by a young person (18-29 years) compared to a middle aged person (30-65 years).

In a Danish Dietary Survey from 1995, people were asked about age and sex of the person who in general prepared the food in the household. In addition, data from Statistics Denmark provides information about the number of households with one and two adults, respectively, and the average family size, given the age of the adults in the family. By combining these data it is possible to determine the relationship between age and sex of people ingesting a meal and age and sex of people preparing the meal (Table 3.17). For example, from the data it is seen that the female group 30-65 years is the dominant meal preparing group. Compared with the male group (30-65) they prepare almost 3 times as many meals for the male group 30-65 than the male group it self. Therefore, despite lower hygiene level of the male group the probability of

getting ill is relatively low because in most cases it is the female group with good hygiene practices that prepares the food.

Table 3.17 Percentage of servings divided into people ingesting a meal in each age and sex group and age and sex groups for the person who prepares the meal.

		Age and sex of person preparing the meal (ASPM)					
		Male 18-29	Female 18-29	Male 30-65	Female 30-65	Male > 65	Female > 65
Age and sex of person ingesting the meal (ASIS)	Male < 18	0.208%	0.560%	0.989%	8.953%	0.001%	0.007%
	Male 18-29	3.033%	1.651%	0.204%	1.767%	0.005%	0.102%
	Male 30-65	0.000%	0.000%	6.726%	18.226%	0.000%	0.000%
	Male >65	0.000%	0.000%	0.000%	0.000%	1.288%	4.488%
	Female < 18	0.208%	0.560%	0.989%	8.953%	0.001%	0.007%
	Female 18-29	1.106%	3.554%	0.204%	1.767%	0.007%	0.102%
	Female 30-65	0.000%	0.000%	1.949%	23.680%	0.000%	0.000%
	Female >65	0.000%	0.000%	0.000%	0.000%	0.241%	8.464%

3.5.3 Exposure via Cooked Chicken

This section addresses the exposure pathway of ingestion of *Campylobacter* that survive a roasting process applied to a whole carcass in a domestic kitchen. The goal of this analysis is to determine the frequency with which carcasses will remain contaminated following cooking and the extent of that contamination in terms of the number of surviving cells. In order to generate a model which would fully characterize this exposure, the following information is required:

a) the number of cells on the carcass;
b) the distribution of those cells throughout the carcass surface and mass;
c) the extent to which there are areas of the carcass that provide protection of the cells from the heat of the oven ('protected areas');
d) the time-temperature profile on the surface of the chicken and within the mass of the chicken;
e) the time-temperature profile within any protected areas during the cooking process;
f) the rate of deactivation of cells as a function of time and temperature;
g) variability of each of the above phenomena across carcasses, consumers, ovens and strains.

Several modelling approaches have been pursued with alternate assumptions and varying levels of complexity. Three approaches are described briefly below and in the following sections. Further work is required to compare and contrast the approaches and to provide support for conclusions on the overall efficacy of domestic oven roasting processes.

3.5.3.1 Approaches to Modelling of Cooking – Overview

3.5.3.1.1 General Issues

Campylobacter is sensitive to the effects of thermal processing, and cooking is likely to result in substantial log reductions. One of the reasons for this expectation is the fact that cooking

regimens for poultry are based on deactivation of Salmonella spp. which are more tolerant of heat. Due to this somewhat 'over-designed' heat treatment with respect to *Campylobacter*, there is a tendency to forego calculation of the reduction of *Campylobacter* which are on the surface of the carcass, since the sustained high surface temperatures (>70C) will generate more than enough log-reductions to eliminate them. Instead, the focus of the modelling described here is to discover the conditions whereby some *Campylobacter* might survive and to attempt to characterize this exposure. The goal is to provide a basis for reasoned attribution of the home exposure risk between the 'undercooked' and 'cross-contamination' pathways. This type of analysis is intended to complement the results of epidemiological approaches which attempt to attribute risk between undercooking and food handling practices.

Given the list of information requirements listed above, it is clear that modelling of cooking retains a number of imposed shortcomings. However, modelling assists in the management of this risk by providing a mechanistic explanation of the relatively large log reductions associated with cooking, and by describing alternate explanations of the means by which *Campylobacter* might survive an otherwise thorough heat treatment (e.g. in thermally protected areas such as cracks, air pockets or deep within the carcass mass).

3.5.3.2 Internal Temperature Approach (Summary)

In this approach, a representative point is chosen in the mass of the chicken and it is assumed that this represents an area of the chicken that can be expected to receive the mildest heat treatment. In the approach described below, a point within the mass of the drumstick portion of the chicken is selected. This selection is partially based on the existence of a cooking temperature survey which measured the internal temperature of chicken drumsticks. The reduction in the number of cells at this point is then calculated by calculating a step-wise time-temperature profile and applying reductions at each time-step. This approach also includes the possibility of an increase in cell numbers in the period where the chicken is being heated through the range of temperatures which might allow growth of *Campylobacter*. The variability in consumer practices is implemented in this approach by varying the stopping point (i.e. the final temperature) in the simulated time-temperature profile. The variation in the stopping point is based on survey data measuring this final temperature.

3.5.3.3 Protected Areas Approach (Summary)

This approach employs four main assumptions. 1) Assume that only some fraction of the carcasses will experience the type of undercooking describe in this approach. 2) Assume that the only cells which have any possibility of survival are within an area which is relatively protected (or insulated) from the heat of the oven. 3) Assume some fraction of the cells to be located in the protected areas. 4) Assume a maximum temperature reached within this protected area and the time for which this maximum temperature is applied.

With these assumptions, a reduction is calculated based on the assumed final temperature stage within the protected area. Uncertainty is characterized for each of the numerical assumptions.

3.5.3.4 The Heat Transfer Approach (Summary)

This approach attempts to calculate the internal time-temperature profile at a number of different depths into the meat of the carcass. This requires use of transient heat transfer models and parameters of thermal properties of chicken which are generally available in food engineering texts. This approach requires the following assumptions: 1) the proportion of the bacterial load found at the surface and at various depths into the carcass, 2) a simplified characterisation of the roasting of a carcass with respect to specifying heat transfer assumptions, and 3) the oven temperature and the time at which the chicken is removed from the oven. The reduction in cell numbers can be characterized at each depth into the chicken meat by considering the reduction in each simulated time-step.

3.5.3.5 Internal Temperature Approach (Detail)

3.5.3.5.1 Model description

The size of the bacterial population after exposure for a given amount of time, t at temperature T is given by the following equation (3.6).

$$\ln(N) = \begin{cases} \ln(N_0) + \mu t & T < T_c \\ \ln(N_0) - \left(\frac{t}{D}\right) & T => T_c \end{cases} \quad (3.6)$$

Here N_0 is the population size at $t = 0$, prior to exposure to the temperature for time t, μ is the specific growth rate constant and D is the D-value, that is the time required for a 1 log reduction in the size of the bacterial population at a given temperature. The above equation is a composite of the exponential models for both growth and inactivation. As such, the equation describes the growth of campylobacters in the exponential phase. The parameter T_c is the temperature above which growth does not occur and the numbers of *Campylobacter* begin to decline. T_c is assumed to be 46°C (Roman & Doyle, 1984).

The specific growth rate, (μ), and D-value, (D), are related to temperature. To quantify this relationship a least squares regression model is fitted to the data in Table (3.18). In the specific implementation of this approach, care was exercised to use data from a single strain of *Campylobacter* in characterizing both the growth and D-value relationships. The resulting equations for D-values are not the same as the equations employed in the subsequent approaches discussed below. The regression models are:

$$\mu = 1.25\ln(T) - 5.996$$

$$D = 16026e^{-0.171T}$$

Table 3.18 Growth and D-value Data applied in Internal Temperature Approach

Temperature	Value	Parameter	Reference
32	11.1	Doubling time	Doyle & Roman (1981)
35	2.96	Doubling time	Doyle & Roman (1981)
37	2.16	Doubling time	Doyle & Roman (1981)
42	1.24	Doubling time	Doyle & Roman (1981)
45	1.28	Doubling time	Doyle & Roman (1981)
48	7.7	D-value	Doyle & Roman (1981)
50	3.3	D-value	Doyle & Roman (1981)
53	1.85	D-value	Doyle & Roman (1981)
55	0.93	D-value	Doyle & Roman (1981)
60	0.345	D-value	Gill & Harris (1982)
65	0.22	D-value	Gill & Harris (1982)
70	0.185	D-value	Gill & Harris (1982)

The temperature of the chicken product over time is estimated from experimental data. Kelly *et al.* (WHO report) report temperature profiles for the internal and external temperature of chicken drumsticks when oven roasted. Similar profiles are given by Bryan *et al.* (1971), Bryan and McKinley (1974), Lyon *et al.* (1975), Chang *et al.* (1998). The internal temperature of the product during cooking is calculated from a regression model fitted to this data set (Figure 3.13, below). Specifically,

$$T(t) = 50.789 \ln(t) - 106.2 \,.$$

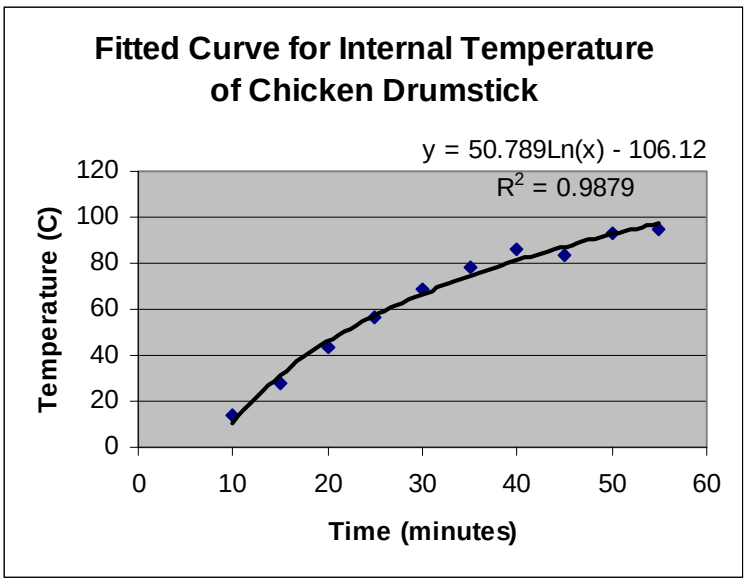

Figure 3.13 Fitted Curve for Internal Temperature of a Chicken Drumstick.

Given that the temperature changes with time and this process is described by $T(t)$, the amount of growth or inactivation can be estimated by considering each time step and re-evaluating the growth and inactivation in each time interval until cooking stops. This usually requires knowledge of how long individuals choose to cook chicken meals. An alternative approach is

to consider the final temperature actually achieved and use this to infer the duration of cooking. This alternate approach can take advantage of a large survey where individuals were asked to measure the temperature of the chicken product immediately after they had cooked the item. This measurement incorporates the combined effect of both the temperature at which the product was cooked and the amount of time during which the item was cooked. This can be incorporated into the model where the temperature of the product and the predicted temperature to which the individual will cook the chicken determine the time for which the item is cooked. This temperature is defined as T_{stop}. Figure 3.14 is a schematic representation of the modelling approach to estimate the number of organisms remaining in the product immediately post-cooking. As there is uncertainty about the manner in which different strains of *Campylobacter* respond to temperature and given the limited size of the data set there is uncertainty associated with the regression line for the D-value. To incorporate this, the regression lines relating to growth rate and D-value is first linearized and the distributions of uncertainty associated with the residuals are generated.

Advantages of Approach

This approach has a number of advantages:

This approach benefits from the use of actual measured temperature-time profiles in oven-roasted chicken. As such, it does not rely on a purely mathematical model to infer the internal temperature of chicken during roasting.

It includes the possibility of growth during the heating phase as opposed to the relying on the final temperature alone to estimate the total lethality (see next approach).

Limitations of Approach

The approach assumes that the entire load of *Campylobacter* in the carcass is subject to the temperature profile associated with the center of a drumstick cooked under the same conditions as in the experimental data. This might be expected to provide a very conservative notion of the overall effectiveness of heat treatment (i.e., it would underestimate the reduction in the total population on the carcass).

An alternate assessment might suggest that this approach is not at all certain to be conservative. If there are organisms occupying parts of the carcass which are more insulated from heat than the centre of a drumstick, then the model might underestimate the number of surviving organisms.

In summary, this approach relies on a significant simplification of the heat treatment efficacy in order to maintain a numerical connection with an actual measured temperature profile within a chicken carcass. It is not entirely clear whether the result is likely to be conservative or non-conservative with respect to the overall heat treatment efficacy.

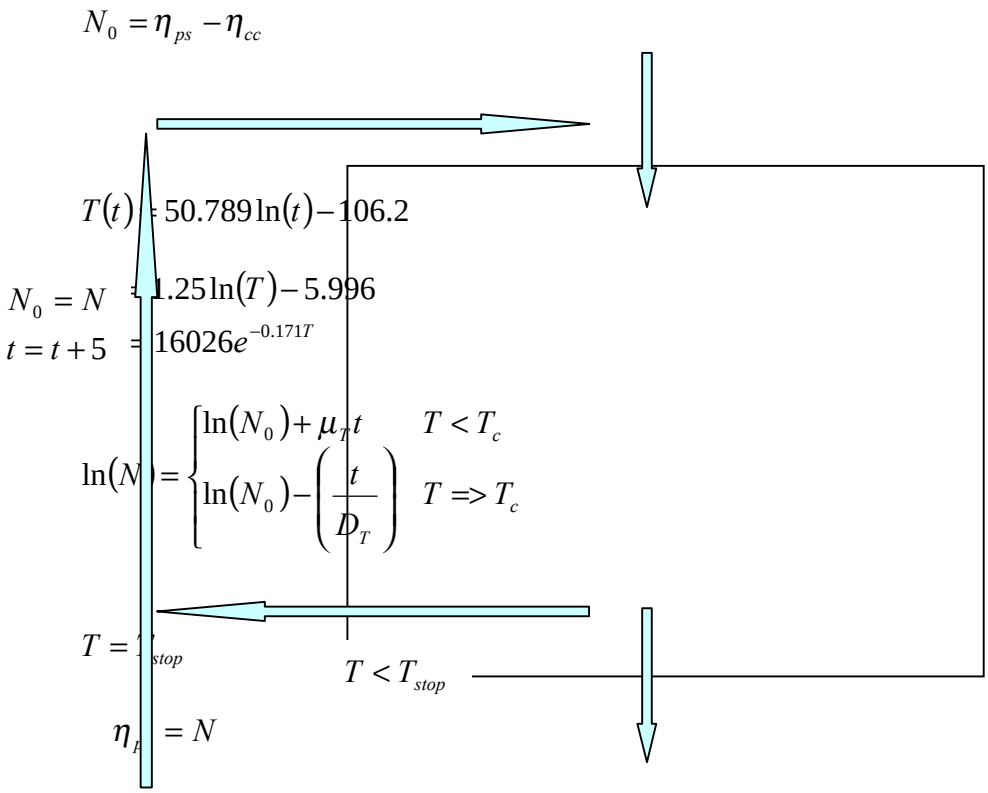

Figure 3.14 Schematic representation of the modelling approach to estimate the number of organisms remaining in the product immediately post-cooking.

3.5.3.6 Protected Areas Approach (Detail)

3.5.3.6.1 Estimation of D-value and Log Reductions during Cooking

(used in Protected Areas and Heat Transfer Approaches)

In order to estimate the log reductions from cooking, the effects of temperature on the organism were modelled using experimentally determined D and z values. The D-value is the time required at a specific temperature to destroy 90% (1 log decrease) of the population. The z-value is the temperature increase required to reduce the D-value by 90%, or a factor of 10.

Blankenship and Craven (1982) studied the thermal sensitivity of C. jejuni in poultry meat. The thermal death times for a five-strain composite and strain H-840 in autoclaved ground chicken were determined (Table 3.19). The z-values for the five-strain composite and strain H-840 were reported as 6.35°C and 5.91°C respectively. It is evident from these data that large log reductions can be expected during normal cooking (e.g. greater than a 5 log reduction if temperature exceeds 57°C for 5 minutes).

Table 3.19 Thermal death times for 5 strain *C. jejuni* composite (Blankenship and Craven, 1982).

	H-840 z-value = 5.91 C	**5 strain composite z-value = 6.35 C**
Temperature (deg C)	**D-value (min)**	**D-value (min)**
49	20.5	ND
51	8.77	9.27
53	4.85	4.89
55	2.12	2.25
57	0.79	0.98

To estimate the log reductions at different times and temperatures, a linear regression was performed on the data. The regression used the log transformed D values, using an equation of the form shown in Equation 3.7:

$$Log(D) = (-a \times Temp) + b \qquad (3.7)$$

"a" and "b" are constants that are estimated through the regression procedure. However, within this equation the term "a" is equivalent to the inverse of the z-value. Therefore, the published z-value for the study was used and fixed while adjusting the "b" coefficient in order to provide a “least squares” fit to the data. In the current analysis only the data for the 5-strain composite in chicken meat was used in the linear regression, however the data could be pooled and a linear regression performed on this data set as well. The results of both analysis (composite and pooled) are shown in Figure 3.15 and Figure 3.16.

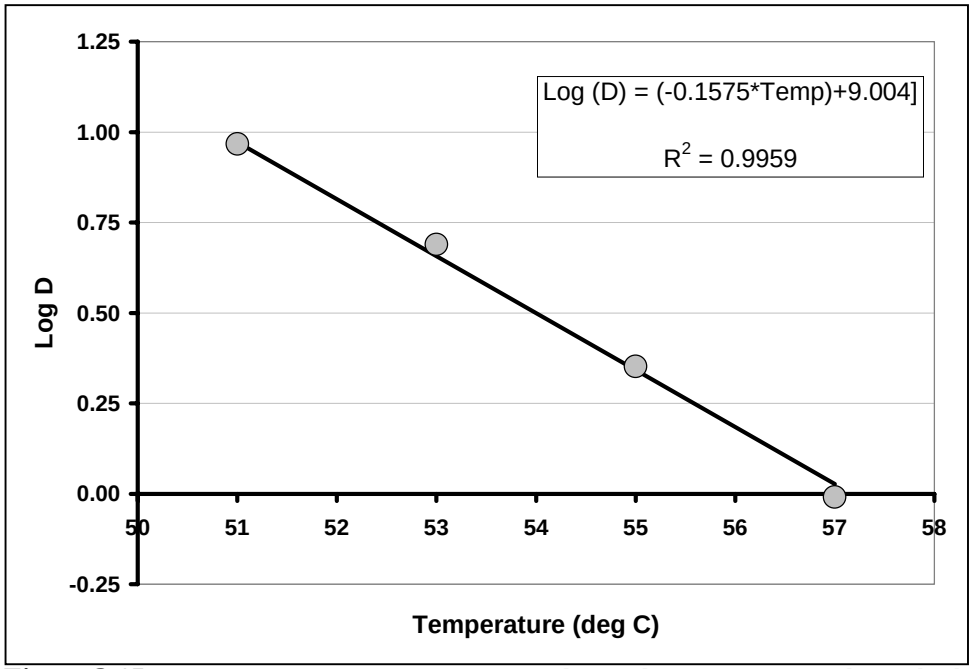

Figure 3.15 Linear regression using given Z-value and composite sample in chicken. Data from Blankenship and Craven (1982)

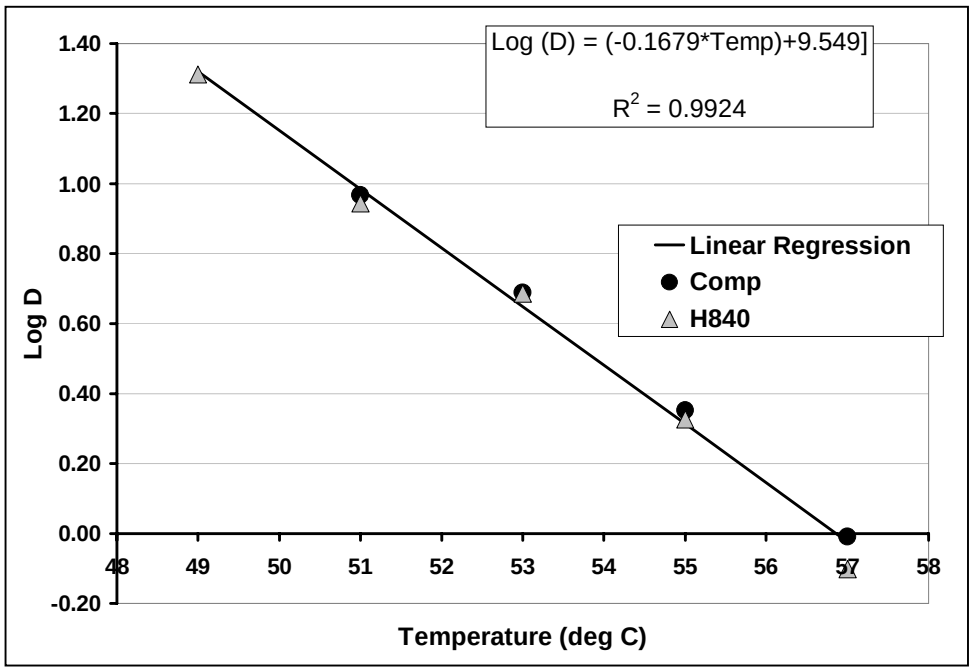

Figure3.16 Linear regression using composite and H-840 in chicken meat. Data from Blankenship and Craven (1982)

The equation used in both the Protected Areas approach and the Heat Transfer approach is:

$$Log(D) = (-0.1575 \times Temp) + 9.004 \qquad (3.8)$$

The D-value at that temperature is then simply the log transform of this value:

$$D = 10^{(-0.1575 \times Temp)+9.004} \qquad (3.9)$$

Finally, given the D-value, and recalling the definition of the D-value given earlier, the log reduction that would occur at that temperature for a given period of time (t), was estimated using Equation 3.10.

$$LogR = \frac{t}{10^{(-0.1575 \times Temp)+9.004}} \qquad (3.10)$$

The model assumes that only a certain proportion of the consuming population will undercook chicken and thus allow *Campylobacter* spp. to survive. The effects of thermal inactivation are only applied to these chickens. The rest are assumed to be thoroughly cooked and thus free of any contamination. Worsfold and Griffith (1997a) conducted a study to assess consumer food safety behaviour. The study reported approximately 15% of the individuals did not cook food to the correct internal temperature. Daniels (1998) using restaurant auditing techniques in the home, reported that 24% of the households studied had cooked products whose internal temperature was too low. In addition to these consumer level studies, epidemiological

investigations looking at the factors that contribute to outbreaks of poultry-borne disease attribute 27% of the cases to inadequate cooking or thermal processing (Bryan and Doyle, 1995). Consumer awareness about undercooked chicken is relatively high and since the consumer behaviour studies listed above were related to undercooking in general, it was estimated that the rate of undercooking for chicken would be lower than for other meats or foods. It was assumed that approximately 5 to 15% of the chickens prepared in the home would be prepared in such a manner as to allow a certain portion of the *C. jejuni* cells present a chance of survival (Table 3.20).

The sensitivity of *C. jejuni* to temperature effects suggests that organisms exposed to the heat without significant protection are unlikely to survive cooking. Those cells that are present on the surface of chicken are likely to be inactivated with even moderate heat, and unless the chicken is grossly undercooked. Thus, it may only be those cells that are in an area of the bird that affords them some level of protection from direct heat that will actually survive. These areas may include visceral cavities, crevices, and areas around joints or in cut and bruised tissues. It was assumed that 10 to 20% of the organisms would be located in these "protected" areas (Table 3.21).

Bryan and Doyle (1995) referring to studies conducted by Bryan & McKinley (1974) and Roberts (1972) state that during typical roasting, frying and grilling, surfaces of poultry usually reach temperatures of 74°C. In order to estimate the log reductions that could occur in areas of the chicken that are "protected", it was assumed that during cooking these "protected" areas would be exposed to a temperature of between 60°C to 65°C (Table 3.23), for a period of time ranging from 0.5 to 1.5 minutes (Table 3.22). There are no data indicating what the true time and temperature combinations might be in cracks and crevices within the chicken during moderate cooking scenarios. This parameter is likely to be an important determinant of risk and additional experimentation into this area may be valuable.

In summary, of the 5 to 15% of chickens that are undercooked, 10 to 20% of the cells on those chickens have a possibility of survival. However, the possibility of survival for the 10 to 20% is dictated by the thermal death times as a result of time and temperature combinations to which the "protected" areas is exposed.

Table 3.20 Distribution for proportion of chickens not totally cooked

Distribution	Min	Mode	Max	Expected Value
TRIANGULAR	0.05	0.10	0.15	0.10

Table 3.21 Distribution for proportion of cells in "protected" areas of chicken carcass

Distribution	Min	Mode	Max	Expected Value
TRIANGULAR	0.10	0.15	0.20	0.15

Table 3.22 Distribution for time “protected” area is exposed to maximum heat

Distribution	Min	Mode	Max	Expected Value
TRIANGULAR	0.50	1.00	1.50	1.00

Table 3.23 Distribution for maximum temperature in “protected” area

Distribution	Min	Mode	Max	Expected Value
TRIANGULAR	60.0	64.0	65.0	63.0

Advantages

This approach has the advantage of providing a conceptual basis on which to discuss and explore the potential survival of pathogens within chicken carcasses (*Campylobacter* or otherwise). Without this notion of very heterogeneous levels of thermal insulation, it is difficult to explain the survival of *Campylobacter* without alternate assumptions of, for example, a) significant loads within the carcass tissue as opposed to on (or just under) the skin, or b) extremely mild overall heat treatments which would likely leave the product significantly undercooked from an organoleptic perspective.

An additional advantage is the relative simplicity and flexibility. The model is captured by one log reduction calculation and one calculation based on the percentage of protected cells.

Limitations

This approach has the following potential limitations:

It does not include log reductions other than those at the end-stage of heat treatment (i.e. ignores log-reductions during the course of warming;

It does not include the effect of post-cooking reductions (e.g. during cooling). Presumably the protected areas would be equally insulated from the change to room temperature, they would continue to draw heat from the warmer parts of the carcass and they would slowly return to room temperature in keeping with their level of insulation.

It excludes survival of *Campylobacter* in ‘unprotected areas’ though this is not so much a limitation as a fundamental assumption in the approach.

It relies on a somewhat abstract notion of protected areas which are ill-defined. While conceptually it may be quite valid, it is difficult to validate the approach (either experimentally or thermodynamically) since the physical representation of these areas and the material basis for thermal protection is not specified or explored. In addition, without specification of the protected areas themselves, it is difficult to speculate on the proportion of cells which would reside there. In contrast to the Internal Temperature Approach, the assumed temperature profile is not ‘grounded’ in experimental or survey data. Since the temperature profile effectively determines the efficacy, the overall effectiveness of cooking is dependent upon an assumption of the final temperature in an unspecified part of the carcass.

3.5.3.7 Heat Transfer Approach (Detail)

This approach constitutes somewhat of a compromise between the first two approaches. It departs from the notion of specifying a particular volume of protected areas, but by applying a heat transfer model, it includes the notion of insulation of the internal parts of the carcass tissue from the heat source as a function of depth from the carcass surface. It is conceptually very similar to the Internal Temperature approach in that it includes the potential for thermal deactivation during the entire warming phase and predicts a similar type of logarithmic internal temperature profile curve as that fitted to the experimental data on chicken drumsticks. In somewhat of a compromise between the two earlier approaches, it includes the ability to specify proportions of the organism population which reside at the surface and at various depths below the surface.

The approach and the required assumptions can be summarized as follows:

- assume a physical model of the chicken carcass to allow for simplifications in the thermodynamic model (e.g. represent the carcass as a sphere, or a folded-over slab).
- specify the dimensions of the carcass with respect to the physical model (e.g. the thickness of the slab of meat, and whether it is heated from one-side of two-sides).
- assume a temperature for the surface of the carcass during cooking. This assumes that the surface temperature will be reached fairly rapidly and will be relatively constant through the heat treatment.
- assume a starting temperature for the product. This starting temperature is assumed to have reached steady-state in the product and to be uniform.
- specify the exponential rate constant for surface heating and the final surface temperature.
- calculate the parameters of the Fourier equation which predicts heat transfer through the mass as a function of surface temperature, distance from the surface and time (in discrete time steps).
- calculate the temperature at each time step using the Fourier equation.
- calculate the D-value (in minutes) for the temperature at each time step.
- calculate the Log Reduction achieved during each time step as the duration of the time step divided by the D-value.
- 9a) calculate the cumulative log reductions as a function of time for each distance from the service.
- 9b) or, calculate the cumulative log reduction associated with some final internal temperature,
- assume a distribution for the total bacterial load at each distance from the surface of the meat (e.g. surface, 1mm, 2mm, 5mm, etc.).
- calculate the number of surviving *Camplyobacter* at each distance (or depth) from the surface.
- total the number of *Campylobacter* remaining on the carcass as a sum across the different depths.

3.5.3.7.1 Examples of Assumptions and Results for Heat Transfer Approach

Table 3.24, Table 3.25 and Figure 3.17 below demonstrate one set of calculations carried out in the Heat Transfer Approach (subject to the set of assumptions required). One set of curves

(monotonically increasing) shows the temperature as a function of time for different depths below the surface of the carcass. The other set of curves (monotonically decreasing) demonstrates the corresponding decrease in the population size (on a log scale) at the same set of depths below the surface of the carcass.

For example, the surface temperature increases most rapidly (upper curve) while the internal temperature increases most slowly and is still increasing at the end of the time series. Accordingly, the population at the surface (starting at ~6 log CFU) declines to 1 cell (0 log CFU) within the first 10 minutes of this cooking process. The population at a depth of 50mm (starting at ~1 log CFU) declines to 1 cell (0 log CFU) only after 120 minutes. Note that this is just one example calculation which is dependent upon the choice of parameters (such as starting populations at various depths below the surface, cooking temperatures, etc.).

Table 3.24 Initial Population Distribution

	cells	**log cells**	
Total	1E+06	6.0	
	(mm)	**cells**	**log cells**	**% of load**
Surface	0	800000	5.9	80.00%
Under skin	2	100000	5.0	10.00%
Flesh	10	48000	4.7	6.00%
Flesh	20	2000	3.3	2.00%
Flesh	30	720	2.9	1.50%
Flesh	50	10	1.0	0.50%
				100%

Table 3.25 Time to less than 0 log CFU

Region	**Depth (mm)**	**minutes**
Surface	0	7
Under skin	2	9
Flesh	10	21
Flesh	20	54
Flesh	30	95
Flesh	50	119

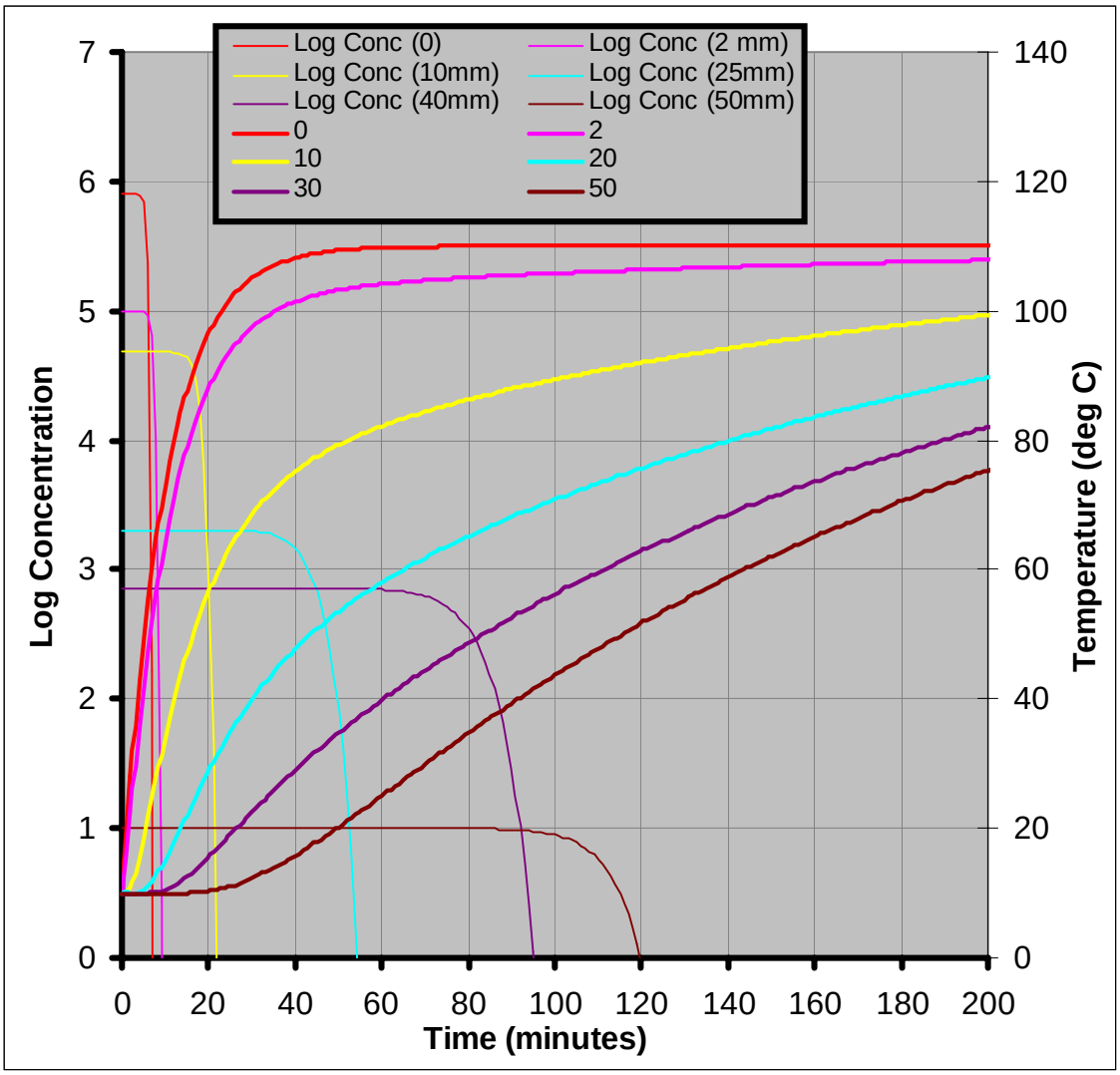

Figure 3. 17 One set of calculations carried out in the Heat Transfer Approach

Advantages

This model has the following advantages:

it provides a mechanistic explanation of the most important elements of the heat treatment process;
it is flexible enough to describe the fate of organisms from the surface and within the carcass tissue.
its predictions with respect to temperature profile can be validated by measurement of carcasses during heating
it can be used to simulate the log-reductions during the cooling period as well as during the heating period (though this is not currently implemented)
it captures the significant log-reductions that can occur at the surface while the internal temperatures are still be quite cool.
it may be useful to extend to other types of cooking, such as frying or barbecuing (Whiting, 2001).

Limitations

This approach has the following limitations:

it requires that a carcass be represented as a simple physical shape which may not be appropriate.
it requires specification
any gains in accuracy in the thermodynamic modelling may be largely wasted effort given that there is uncertainty in the distribution of pathogen load through the surface and mass of the carcass.
the final estimate of the number of surviving model remains highly sensitive to the final temperature achieved deep in the tissue and the likelihood and quantity of pathogens residing there.
as currently implemented, the log reductions associated with cooling are not calculated.

3.5.3.8 Discussion (All Approaches)

1. The various definitions of 'undercooking' defined for poultry refers to cooking temperature standards which are recommended to address the risk posed by Salmonella spp. Cooking which is insufficient to achieve, for example, 5 log-reductions for Salmonella may be quite sufficient for *Campylobacter*.

2. Caution should be exercised in separating the normative (or behavioural) definition of undercooking (that is, failure to cook to recommended internal temperature for the product) and a more technical definition of undercooking (that is, a process that allows a particular pathogen to survive, with some given likelihood, in the product given an initial load of pathogens and a particular distribution of those pathogens on and within the product mass). There is a tendency to blur the two definitions in converting behavioural definitions into technical definitions which may not be appropriate.

As an implication of the above, the term 'undercooking' should not be taken at face value to imply that pathogens will necessarily survive. Without evidence as to the temperature profile and its impact on a given pathogen, the assignment of the label 'undercooked' is of little value in assessing risk. This further implies that the prevalence of the behaviour which is labelled 'undercooking' and the importance of this phenomenon with respect to attributing risk of *Campylobacter*iosis should be reconsidered in light of the significantly higher heat sensitivity relative to other pathogens of concern.

3. Significant deactivation can take place during the cooling phase between the time when a roast is removed from the oven and the time when it is ultimately consumed (obviously this refers to cooling through the higher temperature range and not once the product reaches growth temperatures). This post-cooking deactivation can be comparable to the deactivation achieved during the time when the temperature is increasing. In this sense, each of the thermal deactivation models described above will underestimate the deactivation that would be predicted if the cooling phase was included. The importance of the cooling phase will likely be most important in parts of the carcass which are insulated, given that the temperature will significantly lag the ambient temperature and may still be increasing in temperature due to continuing heat transfer from warmer mass in the carcass, even after the chicken is removed from the oven.

4. The implications of this analysis is to suggest that insufficient deactivation of *Campylobacter* from poultry carcasses during roasting is highly unlikely unless there are significant numbers of bacteria in thermally protected areas of the carcass. This proposition begs the question of whether there can be any level of thermal treatment that can be considered completely sufficient in light of protection of *Campylobacter* from heating. Without this assumption of 'protected' or

'deeply-embedded' organisms, large log-reductions resulting in carcasses free of *Campylobacter* are predicted for even mild heat treatments of the surface of the carcass.

5. Each of the modelling approaches described above is dependent upon a number of important assumptions. These assumptions are quite diverse in their underlying basis:
abstract (e.g. supposition of thermally protected areas);
simplifying (e.g. assumption that all *Campylobacter* experience heating equivalent to the centre of a drumstick; characterization of a carcass as a sphere or a folded-over slab);
material properties (e.g. air-to-surface thermal conductivity during roasting);
uncertain assumptions (e.g. proportion of cells which occupy different places in the carcass such as protected areas, or at varying levels of depth in the carcass mass).

It is not immediately clear how the reliance on such assumptions can be resolved. Given the value of knowing the relative importance of consumer behaviour variables in food safety (cooking temperature profiles and cross-contamination processes), the ultimate risk characterization (in both the absolute and comparative senses) will be highly dependent upon several 'ungrounded' assumptions. It is possible that a carefully directed research effort could elucidate some of these issues. In the near term, however, risk characterization as it relates to consumer risk factors of cooking and cross-contamination will continue to be largely a matter of mathematical combinations of unvalidated assumptions. These may be useful for conceptualising the risk factors in support of food safety decisions, but are not likely to be sufficient to stand-alone as providing reliable numerical estimates of risk.

3.6 Interventions/mitigation strategies

(On request of the Expert Consultation, a section on possible interventions/mitigation strategies will be included).

4. Hazard characterization

Hazard characterization describes the adverse health effects of a substance, organism or other entity. This component of the risk assessment usually includes a dose-response relationship, which is represented as a probability percentage of a population that will become infected or ill after exposure to a specific quantity of *Campylobacter* organisms. The types of data that can be used to establish dose-response relationships include animal and human feeding studies, and epidemiological data such as data from outbreak investigations.

4.1 Pathogen, host and food matrix factors

4.1.1 Infectivity, virulence and pathogenicity of the organism

The dose which may cause infection (infection = colonization without signs of illness) depends upon a number of factors including the virulence of the strain, the vehicle with which it is ingested and the susceptibility of the individual.

The pathogenesis of *Campylobacter* has been reviewed (Ketley, 1995; 1997; Wooldridge & Ketley, 1997; Smith, 1996). In general, the mechanisms involved in the pathogenesis of *Campylobacter* are rather poorly understood. Motility, chemotaxis and the flagella are known to be important factors in the virulence as they are required for attachment and colonization of the intestinal epithelium (Ketley, 1997). Once colonization has occurred, *Campylobacter* bacteria may perturb the normal absorptive capacity of the intestine by damaging epithelial cell function either directly, by cell invasion and/or production of toxin(s), or indirectly, following the initiation of an inflammatory response (Wooldridge & Ketley, 1997). Several virulence determinants have been described to be involved in the induction of diarrhoea; adhesion and invasion molecules, outer membrane proteins, lipopolysaccharides, stress proteins, flagella and motility, M cells, iron acquiring mechanisms, and cytotonic and cytotoxic factors (Smith, 1996). However, their relative role and importance for development of diarrhoea is not quite clear. The ability of *Campylobacter* to invade host cells in vitro is well established and cytotoxin production is consistently reported (Ketley, 1997). Early reports of enterotoxin production have not been confirmed and thus the opinion that *Campylobacter* produce an enterotoxin is no longer widely held (Allos & Blaser, 1995; Wooldridge & Ketley, 1997). Not all strains involved in human enteritis produce toxins, and no correlation has been found between serotype and toxin production (Fricker & Park, 1989).

4.1.2 Host characteristics

4.1.2.1 Susceptibility

Regarding the infectious diseases populations at risk often include the elderly, children and individuals suffering from illnesses that compromise their immune systems (e.g. AIDS and cancer patients). As regards campylobacteriosis, young adults (around 15-25 years old) appear to be more frequently exposed or more susceptible than other age groups (Blaser *et al.,* 1983; Engberg & Nielsen, 1998; Kapperud & Aasen, 1992).

4.1.2.1.1 Age

In developed countries all age groups may become infected with *Campylobacter*. However, in most countries the reporting rate of campylobacteriosis is higher in young children (0-4 years old) and young adults. i.e. Norway (Kapperud & Aasen, 1992; Kruse, 2001), Denmark (Anon. 2001b), Iceland (Thorkelsson *et al.,* 2001), Finland (Anon., 2001d), New Zealand (Brieseman, 1990; Perks, 2001), England and Wales (PHLS, 2001) and the USA (CDC-FoodNet; Friedman *et al.,* 2000a). The high incidence rate in children may be a result of a higher susceptibility, a more frequent exposure to pets for example, or a higher notification rate in this age group as compared to adults, reflecting that parents more frequently seek medical care for their children.

The high incidence rate in young adults has been suggested to be due to a higher travel activity in this age group compared to other age groups (Kapperud & Aasen, 1992), a higher recreational activity including participation in water sports (Skirrow, 1987), and an increased exposure to high risk food items (Engberg & Nielsen, 1998). The higher incidence may also be a result of unsafe food handling practices in a population that has left the parents and still has to learn how to prepare food.

In developing countries, illness is more common among infants and children and it is presumed that young adults and adults have acquired immunity following repeated exposure (WHO, 2000).

4.1.2.1.2 Sex

In general, males seem to have a higher incidence rate of *Campylobacter* infections than females. In the USA in 1998 (CDC-FoodNet) 30% of the infected persons were women and 70% were men. It has been reported that young boys have a higher incidence rate than young girls (Skirrow, 1987; Kapperud & Aasen, 1992; Statens Seruminstitut, unpublished results). With regard to the young people, the sex differences seem to vary between countries. In Denmark, the incidence rate is higher for young females than young males (Statens Seruminstitut, unpublished results). In USA, New Zealand, and Norway young males are more frequently getting infected (Skirrow, 1987; Brieseman, 1990; Kapperud & Aasen, 1992). The reason for this sex difference has not been explained.

4.1.2.2 Demographic and socio-economic factors

4.1.2.2.1 Ethnicity

Differences in infection rates between different ethnicity groups have been observed. In New Zealand, the rate of infection has been calculated for people belonging to different ethnicity groups. Pacific people had a lower rate (50.8) as compared to Europeans (245.0) and other ethnicities (216.2). The reason for the difference was not explained (NZ Ministry of Health, 2001).

4.1.2.2.2 Area/environmental factors

The incidence of campylobacteriosis seems to be area-dependent i.e. some areas in for example Denmark, Norway, UK, and New Zealand have a much higher incidence than the rest of the country (Engberg & Nielsen, 1998; Brieseman, 1990; Kapperud, 1994; Jones *et al.,* 1990). In UK and New Zealand *Campylobacter* infections have occurred at a higher incidence in rural than urban areas (Skirrow, 1987; Brieseman, 1990). In Norway the opposite has been observed (Kapperud & Aasen, 1992). The higher incidence in urban areas was explained by a higher proportion of imported cases in these areas as compared to rural areas (Kapperud & Aasen, 1992).

4.1.2.2.3 Poultry slaughterhouse workers

Several investigations have revealed that new workers, which have recently been employed at slaughterhouses, are a part of the population with an increased risk of getting infected by *Campylobacter* spp. (Jones & Robinson, 1981; Christenson *et al.,* 1983; Mancinelli *et al.,* 1988; Berndtson *et al.,* 1996). This is presumably due to the heavily contaminated environment at the slaughterhouse. The presence of *C. jejuni* in the air at broiler slaughterhouses has been investigated. Berndtson *et al.* (1996) demonstrated that 40% to 75% of air samples from the surroundings of a processing line were contaminated with *C. jejuni*, and Oosterom *et al.* (1983a) found that the number of *C. jejuni* per m^3 air was in the range $\log_{10}$ 1.70 - $\log_{10}$ 4.20. The contents of *C. jejuni* in the air along the processing line could pose a risk to the workers through contact with contaminated aerosols. Further, contamination of the hands of processing line workers by *C. jejuni* at levels up to $\log_{10}$ 4.26 *C. jejuni* per hand has been demonstrated

(Oosterom *et al.,* 1983a); Ono & Yamamoto, 1999). This may pose a risk to the health of the exposed person and may enhance the possibilities of cross-contamination of the products.

4.1.2.2.4 Season

A seasonal variation in the number of human cases has been noticed in several countries including Denmark, Sweden, Norway, Finland, Iceland, The Netherlands, UK, the USA, and New Zealand with a more than doubling of the incidences in late summer (Statens Seruminstitut, unpublished results; Brieseman, 1990; Kapperud & Aasen, 1992; Skirrow, 1991; Newell *et al.,* 1999; Friedman *et al.,* 2000a; Anon. 2001d; Kruse, 2001; Thorkelsson, 2001; Waagenar, 2001). The significance of seasonality seems to increase with increasing latitude (Kapperud & Aasen, 1992). The late summer peak coincides with seasonal habits of travelling abroad, but domestically acquired infections also increase in number during this period (Kapperud, 1994; Engberg & Nielsen, 1998).

In countries such as Denmark, Norway, Sweden, the UK and the Netherlands, the seasonality in the human cases coincident with the seasonality in the number of infected broiler flocks (Danish Veterinary Laboratory unpublished data; Kapperud *et al.,* 1993; Berndtson, 1996; Newell *et al.,* 1999, Waagenar, 2001). This may indicate that humans aquire *Campylobacter* from eating chickens or that humans and broilers are becoming infected from the same 'unknown' source.

4.1.2.3 Health factors

Different health factors may affect the susceptibility of the host, e.g. immunity, concurrent infections and medication and underlying disease.

4.1.2.3.1 Acquired immunity

Patients suffering from campylobacteriosis may develop acquired immunity for the causative *Campylobacter* strain (for a period of time). This was demonstrated by Black *et al.* (1988), where the volunteers, who became ill, developed a serum antigen response to the *Campylobacter* strain they had ingested and hence were protected from subsequent illness but not infection with the same strain. Acquired immunity may explain why employees in broiler slaughterhouse get campylobacteriosis in the beginning of an employment, but not after a while (Christenson *et al.,* 1983). In addition, a higher rate of poultry and meat process workers than the normal population have been found to have complement fixing antibody against *Campylobacter* (Jones & Robinson, 1981). This was not observed in a Danish study where Lings *et al.* (1994) found no significant differences in the prevalence of serum antibodies against *C. jejuni* between a group of 217 Danish slaughterhouse workers and a group of 113 Danish greenhouse workers.

4.1.2.3.3 Underlying disease

Underlying disease has been described as a predisposing factor for acquiring enteric infections. In addition, underlying disease seems to enhance the severity of such infections. In a study carried out in Spain, 93% of 58 patients with bacteraemia caused by *Campylobacter* spp. had an underlying disorder, including liver cirrhosis, neoplasia, immunosuppressive therapy and human immunodeficiency virus infection (Pigrau *et al.,* 1997). In a similar study carried out in Denmark, Schonheyder *et al.* (1995) described 15 cases of bacteraemia caused by *Campylobacter* spp.. Eleven of the 15 patients in this investigation had underlying disorders, including immunological, neoplastic and vascular disease. Neimann (2001) has in a Danish case-control study described that underlying disease like kidney-, vascular- and intestinal disorders were dominating among patients with campylobacteriosis. The disease diabetes melitus is also recognised as a factor increasing the risk related to infections by enteric pathogens (Neal & Slack, 1997).

Persons infected with Human Immunodeficiency Virus (HIV) are also at increased risk of acquiring *Campylobacter* infections. A study in Los Angeles, USA, 1983-1987 showed that the reported incidence of laboratory confirmed campylobacteriosis in persons with AIDS was 519 per 100,000, much higher than the reported rate in the general population (Sorvillo *et al.,* 1991).

4.1.2.3.3 Concurrent medication

Medication with antisecretory drugs like omeprazole and H_2 and H_2-antagonists has been showed to increase the risk for acquiring campylobacteriosis, presumably due to a raise in pH of the stomach contents (Neal *et al.,* 1996; Neal & Slack, 1997). Further on, results of case-control studies suggest that the use of antibiotics and hormones will increase the risk of acquiring infection by *Campylobacter* spp. (Neal *et al.,* 1996; Effler *et al.,* 2001; Neimann, 2001).

4.1.3 Factors related to the matrix/conditions of ingestion

The vehicle with which *Campylobacter* bacteria are ingested is important for development of illness. In a volunteer feeding experiment, the illness rate was higher in volunteers given the organisms in bicarbonate as compared to milk (Black *et al.,* 1988). This can be explained by the barrier effect of the gastric acid, which is reduced when *Campylobacter* bacteria are ingested with a buffering vehicle.

4.2. Adverse health effects

4.2.1 Acute gastrointestinal manifestations

Enteropathogenic *Campylobacter* can cause an acute enterocolitis, which is not easily distinguished from illness caused by other enteric pathogens. The incubation period may vary from 1 to 11 days, typically 1-3 days. The main symptoms are malaise, fever, severe abdominal pain and diarrhoea. Vomiting is not common. The diarrhoea may produce stools that can vary from profuse and watery to bloody and dysenteric. In most cases the diarrhoea is self-limiting and may persist for up to a week, although mild relapses often occur. In 20% of the cases symptoms may last from one to three weeks (Allos & Blaser, 1995). Excretion of the organism may continue for up to 2-3 weeks.

4.2.2 Non-gastrointestinal sequelae

Campylobacter infections may be followed by rare but severe non-gastrointestinal sequelae which may hit the normal population. There are reactive arthritis, the Guillain-Barré syndrome and the Miller Fisher Syndrome. These complications show different pictures of symptoms or disorders.

Reactive arthritis (incomplete Reiters Syndrome) has been estimated to occur in approximately 1% of patients with campylobacteriosis. Reactive arthritis is a sterile post infectious process, which may affect multiple joints, particularly the knee joint. The symptoms occur seven to ten days after onset of diarrhoea (Peterson, 1994). Pain and incapacitation can last for months or become chronic. Reactive arthritis is often associated with the tissue phenotype HLA-B27 and cannot be separated from the affectation of the joints that may follow from a *Yersinia*, *Salmonella* or *Shigella* infection (Peterson, 1994; Allos & Blaser, 1995). The condition is immunological and cannot be treated with antibiotics. The medical treatment may consist of a non-steroid anti-inflammatory drug (NSAID). The pathogenesis of this entity is unknown (Allos & Blaser, 1995).

Evidence suggests an association between *Campylobacter* illness and a rare but serious paralytic condition, Guillain-Barré syndrome (GBS), a demyelating disorder of the peripheral nervous system resulting in weakness, usually symmetrical, of the limbs, weakness of the respiratory muscles and loss of reflexes (areflexia). Early symptoms of GBS include burning sensations and numbness that can progress to flaccid paralysis. GBS has been estimated to

occur about once in every 1000 cases of campylobacteriosis, i.e. up to 40% of all GBS cases in the US occur after *Campylobacter* infections (Mishu & Blaser, 1993; Mishu *et al.,* 1993; Allos, 1997). GBS seems to be more common in males than females (Mishu *et al.,* 1993). Although most GBS patients recover (about 70%), chronic complications and death may occur (Blaser *et al.,* 1997). There is no relation between the severity of the gastrointestinal symptoms and the likelihood of developing GBS after infection with *C. jejuni*; in fact, even asymptomatic *Campylobacter* infections can trigger GBS (Allos & Blaser, 1995). The pathogenesis of GBS is only partly known. GBS is presumably caused by an immunological cross-reaction between *Campylobacter* anti-genes (lipopolysaccharides) and glycolipids or myelin proteins in the peripheral nervous system. The serotype O:19 seems to be more often involved in this condition than other *Campylobacter* serotypes (Allos & Blaser, 1995; Allos, 1997).

In some cases, campylobacteriosis have also been associated with the Miller Fisher Syndrome, which is considered to be a variant of the Guillain-Barré syndrome. The Miller Fisher syndrome is characterized by opthalmoplegia, ataxia and areflexia (Othsuka *et al.,* 1998).

4.2.3 Mortality

In general, very few deaths are related to *Campylobacter* infections and these deaths do usually occur among infants, elderly and immuno-suppressed individuals (Tauxe, 1992; Altekruse *et al.,* 1999). In England and Wales fewer than 10 deaths of approx. 280.000 cases has been reported from 1981 to 1991 (<0.0036%) (Philips, 1995). In 1999 in the USA, 2 of 4025 registered patients died (0.05%) (CDC-FoodNet). The average annual number of deaths related to *Campylobacter* in the USA has been estimated to be 124 of 2,453,926 estimated campylobacteriosis cases (0.005%) (Mead *et al.,* 1999). A recent Danish analysis of mortality at 30-days post infection suggests that in Denmark the case-fatality rate may be 4 per 100.000 (0.004%). HIV infection may contribute to this mortality (WHO, 2000). In New Zealand, 2 deaths were reported in 1997 giving a case-fatality rate of 0.02% (NZ Ministry of Health, 2001).

4.2.4 Effect of antimicrobial resistance

Development of antimicrobial resistance may compromise treatment of patients with severe diarrhoea and bacteremia. In the beginning of the 1990s, fluoroquinolone-resistant *C. jejuni* and *C. coli* emerged in human populations in Europe as reported in the UK, Austria, Finland, and the Netherlands (Piddock, 1995, Entz *et al.,* 1991). This resistance has been linked to the approval of enrofloxacin for treatment of diseases of broiler chickens as investigations have shown that fluoroquinolone-sensitive *C. jejuni* strains were able to convert to resistant forms when fluoroquinolone was added to broiler chicken feed (Jacobs-Reitsma *et al.,* 1994b). In general, most human *Campylobacter* infections are self-limiting and do not need antimicrobial therapy. However, in severe cases medication may be necessary. In such cases the drug choice is usually erytromycin, though fluoroquinolones such as ciprofloxacin and norfloxacin are also used (Blaser *et al.,* 1983).

Fluoroquinolone-resistant Campylobacter from chicken and other poultry is an emerging public health problem. A study by Smith and colleagues (Smith *et al.*, 1999) found that patient with resistant *C. jejuni* infections had a longer duration of diarrhoea than patients infected with susceptible strains. Although lower frequencies of resistance are reported in many countries, the problem of fluoroquinolone-resistant *Campylobacter* is particularly acute in Taiwan, Thailand and Spain where resistance levels are 56.9%, 84%, and 88% respectively (Gallardo *et al.,* 1998, Hoge *et al.,* 1998, and Li *et al.,* 1998.) Treatment can also be complicated by the emergence of multidrug resistance. In Thailand Hoge *et al.* (1998) found 100% resistance to both fluoroquinolones ciprofloxacin and azithromycin. Although resistance to many drugs is mounting in *Campylobacter*, many bacteria classified as resistant to a certain antibiotic in laboratory tests may nevertheless be amenable to antimicrobial therapy. This has been shown by Piddock

(1999), who noted that only 1 of 39 patients with ciprofloxacin-resistant *Campylobacter* enteritis did not respond to ciprofloxacin therapy.
(On request of the Expert Consultation the Campy fluoroquinolone Risk Assessments will be mentioned)

4.3 Campylobacter Dose-Response Analysis

The probability of illness is dependent on the occurrence of three conditional probabilities: 1) the probability that the organism is ingested, 2) the probability that the organism is able to survive and infect the host once it is ingested, and 3) the probability of the host becoming ill once infected. The environment, the pathogen and the host are all variables that play an important role in the probability of illness. Environmental influences include the food vehicle and the stability of the GI tract ecosystem. Pathogen influences include the dose, virulence, and the colonization potential in the host GI tract. Host influences include immune status, age and stomach contents (Coleman and Marks, 1998).

The dose-response analysis translates the number of organisms to which an individual is exposed, into an estimate of the individual's probability of infection. In developing a relationship for the quantitative dose-response analysis there are two types of data that can be used if they are available: 1) epidemiological, outbreak data, and 2) feeding trials with human subjects. Epidemiological data, if collected well and if information such as attack rate and ingested dose are provided, can be an ideal data set. These data would essentially provide "real-world' response using subjects that are representative of the population at large. Although there are an abundant number of epidemiological studies, the investigations rarely analyse the foods to enumerate the disease causing doses. The second type of data, feeding trials using human subjects, can provide useful dose-response analysis data, however the doses applied in these studies are usually high, and the subjects are predominantly healthy individuals. Furthermore, these studies often use one or a limited number of strains that may not represent all the virulence characteristics.

There is insufficient information in the epidemiological literature, that we have been able to review, to allow a dose-response relationship to be derived using this type of data. There is one human feeding trial study that has been conducted Black *et al.* (1988). This study used healthy young adult volunteers from the Baltimore community. The challenge dose was administered in milk, and the volunteers fasted for 90 minutes before and after ingesting the organism. This study involved the use of two strains of *C. jejuni* (A3249 and 81-176). Strain A3249 was isolated from a 16-year old boy with a sporadic infection after an outbreak at a camp in Connecticut. Strain 81-176 was isolated from an ill nine-year old girl in an outbreak in Minnesota. The results of the feeding trial study are presented in Table 4.1 and in Figure 4.1 and 4.2.

Table 4.1 Human feeding trial data (Black *et al.,* 1988)

Strain	Dose	Log Dose	Total Number	Positive Stool (infection)	Prop (infection)	Diarrhea or Fever (illness)	Prop (illness)
A3249	8.00E+02	2.90	10	5	0.50	1	0.10
A3249	8.00E+03	3.90	10	6	0.60	1	0.10
A3249	9.00E+04	4.95	13	11	0.85	6	0.46
A3249	8.00E+05	5.90	11	8	0.73	1	0.09
A3249	1.00E+06	6.00	19	15	0.79	2	0.11
A3249	1.00E+08	8.00	5	5	1.00	0	0.00
81-176	1.00E+06	6.00	7	7	1.00	3	0.43
81-176	2.00E+08	8.30	10	10	1.00	6	0.60
81-176	2.00E+09	9.30	22	22	1.00	9	0.41

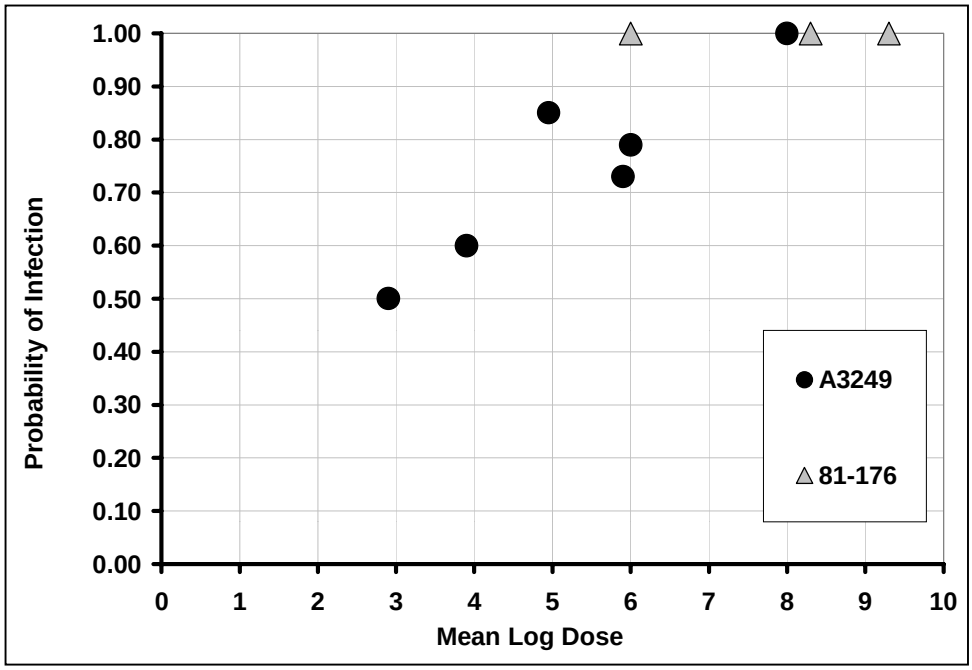

Figure 4.1 Human feeding trial data, probability of infection

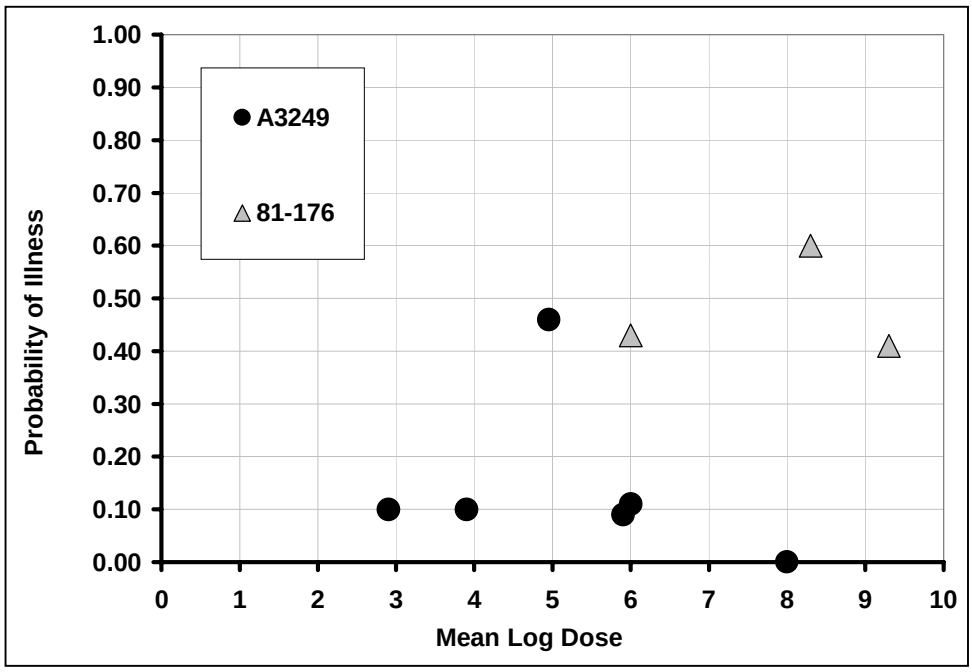

Figure 4. 2 Human feeding trial data, probability of illness

Two distinct hypotheses have been proposed for the nature of the dose-response relationship for foodborne pathogens. The first is based on an historical notion that there is a threshold number of organisms, or minimum infectious dose, that must be ingested before any infection or adverse effects occur. The second hypothesis is that a single pathogen cell has the ability to initiate an infection or illness (Haas, 1983, Rubin, 1987, Rubin and Moxon, 1984). Thus there is no threshold number, and the probability of causing infection increases as the levels of the biological agent increases.

Several investigators have examined the available data and proposed non-threshold models for a number of pathogens (Haas, 1983, Teunis *et al.,* 1996). The sufficiency of these models to describe the data and more importantly the acceptance of the theory underpinning the models has resulted in non-threshold dose-response models being the currently accepted models for describing the dose-response relationship. In addition, with low dose linearity, they are recommended by WHO/FAO for use in hazard characterization (MRA 00/06,2000). The primary non-threshold, single-hit models currently used in microbial risk assessment are the exponential and beta-poisson dose-response models.

In the exponential model it is assumed that all of the ingested organisms have the same probability, r, of causing an infection. The dose ingested is assumed to be poisson distributed with a mean of N organisms per portion (Haas, 1983).

$$P_{inf} = 1- \exp(-r \times N) \quad (4.1)$$

Where:

Pinf is the probability of infection
r is the probability of one cell initiating an infection
N is the dose

In the beta-poisson model, heterogeneity in the organism/host interaction is introduced and r, the probability of an organism initiating an infection given a successful introduction in the host, is assumed to follow a Beta-distribution (Furomoto & Mickey, 1967; Haas, 1983). In the derivation of this model a complex function results. However, under the assumption that β is much larger than both α and 1 the following approximation can be used:

$$Pinf = 1- (1 + N/\beta)^{-\alpha} \quad (4.2)$$

Where:

P_{INF} is the probability of infection
D is the dose ingested
α and β are the dose response parameters

Some of the human feeding trial data of Black *et al.* (1988) has been fit to dose-response models. The dose-response data for infection for strain A3249 have been fit to the dose-response models presented using maximum likelihood techniques. The beta poisson model has been reported to provide a statistically significant fit to the data with parameters alpha = 0.145 and beta = 7.59 (Medema *et al.,* 1996; Teunis *et al.* 1996). The value of these parameters, estimated using the approximate form of the beta-poisson model, which can be considered questionable with regard to the conditions for the approximated solution (beta much larger than both alpha and 1), motivated Teunis and Havelaar (2000) to estimate the parameters using the exact solution to the beta-poisson. The parameters generated using the exact approach are alpha = 0.145 and beta = 8.007. These parameters and the exact form of the beta-poisson equation do not deviate very much from the approximate solution for the best fitting curve, however the confidence limits for the two approaches have been shown to be very different. The approximate solution with this set of parameters produces a likelihood-based confidence limit that is greater than should be theoretically possible. The approximate beta-poisson dose response model fit to the A3249 infection data is shown in Figure 4.3, from which the overestimation in the upper confidence limit can be seen. The limiting curve represents the probability of infection with a single hit hypothesis and the probability of one cell initiating an infection set at 1. For comparison, the exact beta-poisson dose-response model are presented in Figure 4.4.

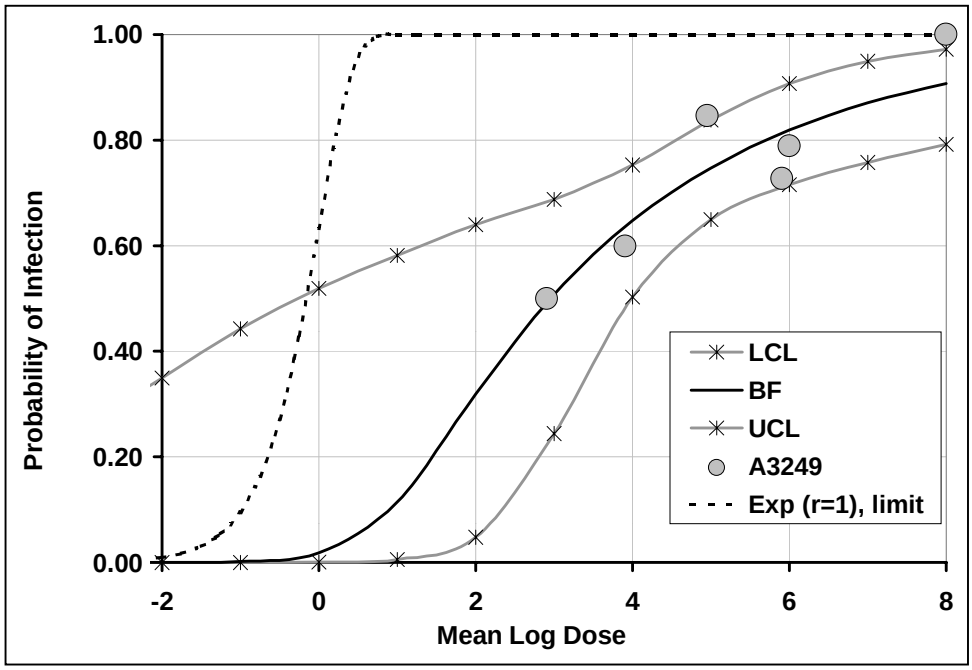

Figure 4.3 Approximate beta-poisson dose-response model fit to infection data for *C. jejuni* A3249.

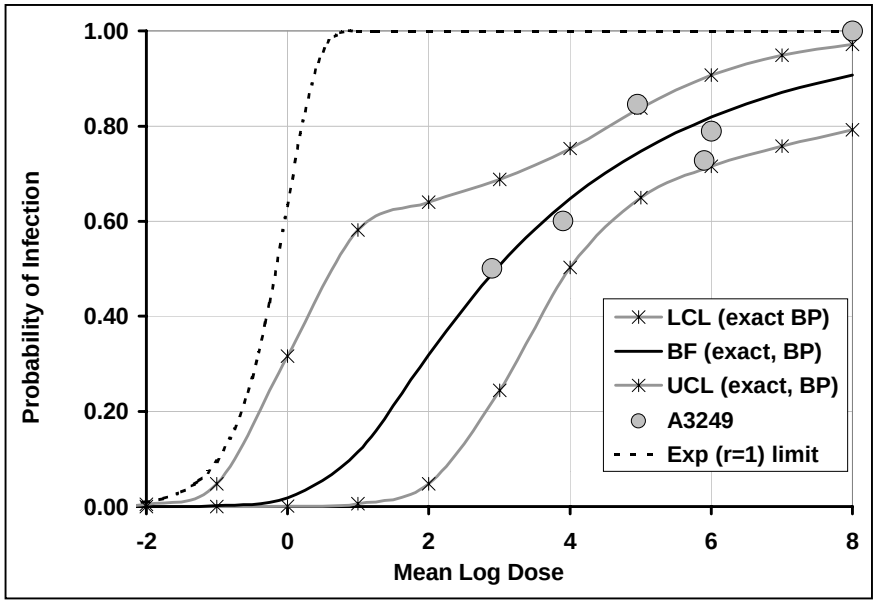

Figure 4.4 Exact beta-poisson dose-response model fit to infection data for *C. jejuni* A3249.

In addition to human feeding trial data for A3249, Black *et al.,* (1988) also studied the dose-response relationship for *C. jejuni* 81-176. The response, at the three doses tested for this strain, were 100%. As a result, it is not possible to derive a dose response relationship for 81-176 by itself. However, if our goal is to generate a dose-response relationship for *C. jejuni*, it may be reasonable to combine the data from the two strains and generate a dose-response relationship for this new data set. Since no distinction is made in the risk assessment for different strains, it would seem illogical to distinguish -between two strains at the dose-response

level. Furthermore, a visual comparison of the probability of infection for the two strains (Figure 4.1) does not indicate that one strain is more or less infective than the other.

In the current exercise, the data for *C. jejuni* A3249 and 81-176 were pooled and fit to the beta-poisson dose response model. The fit to the data was found to be statistically significant at the 95% confidence level (2 log likelihood = 4.67, with 6 degrees of freedom). The parameters of the beta-poisson dose response model were estimated to be alpha = 0.21, and beta = 59.95. The values of the parameters, when the data for both strains is pooled, allow the approximate and simpler form of the beta poisson equation to be used. Furthermore, the upper confidence limit in this case does not exceed the theoretical maximum, as was the case when the approximate solution was used with the data for A3249 alone. The best fitting dose-response curve and the 95% confidence limits generated for the two strain pooled data are shown in Figure 4.5. The confidence region, on a log scale, for the parameters of the dose-response model are shown in Figure 4.6.

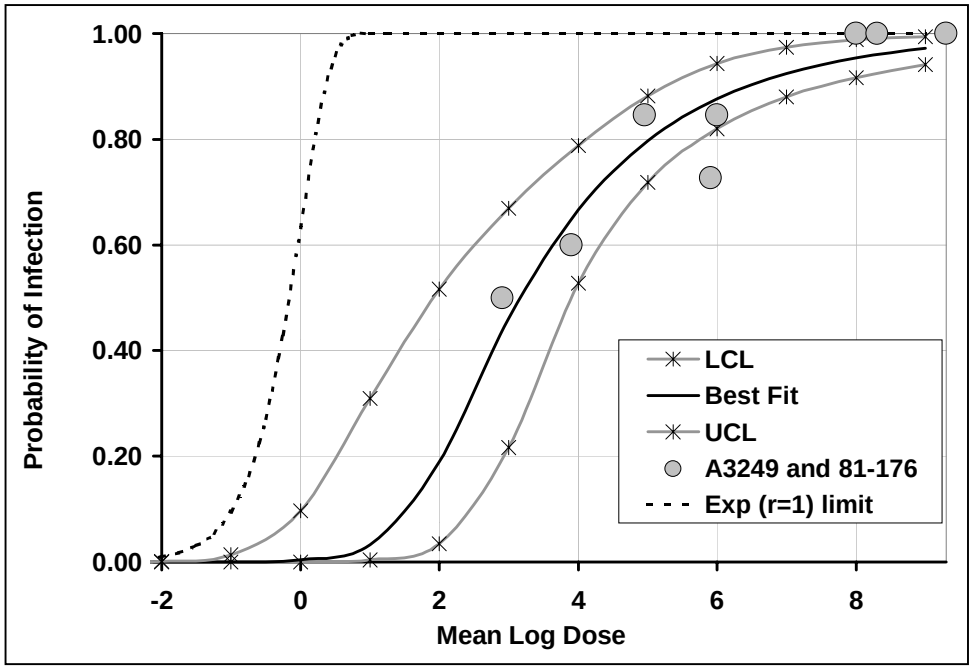

Figure 4.5. Beta-poisson model fit to human feeding trial data for *C. jejuni*, strains A3249 and 81-176

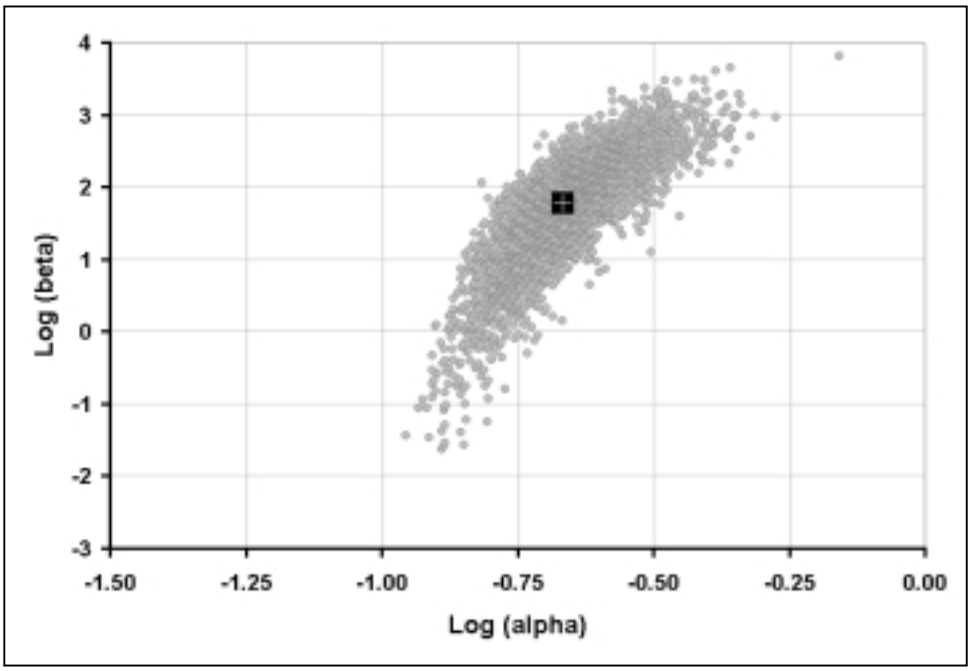

Figure 4.6 Confidence region for parameters (alpha and beta) of the beta-poisson dose response model.fit to feeding trial data for *C. jejuni* A3249 and 81-176**.**

The beta-poisson model in the form expressed in Equation 4.2, estimates the average risk to a population following the ingestion of an average dose. In order to estimate the probability of infection for an individual consuming a meal with a specific dose, the beta-poisson model needs to be expressed in a format that will allow it to be simulated in a similar manner to the exposure assessment. Equation 4.3, reflects the same assumptions as the original beta-poisson model, however variability for the probability of infection from a particular dose is incorporated within the simulations so that the model estimates the risk of infection for an individual consuming a specific dose.

The simulated beta-poisson model, samples the beta distribution, using the parameters generated (e.g alpha = 0.21, and beta = 59.95) to estimate the probability of infection from one organism. The dose ingested is estimated using a Poisson sample, which assumes the organisms on the chicken with some mean concentration are randomly distributed. Finally, the probability of infection from the dose ingested is estimated assuming a binomial process with the number of trials equal to the dose ingested and the probability of 'success' at each trial equal to the value returned from the beta distribution.

$$P_{INF} = \mathbf{1} - \left[\mathbf{1} - P_{INF}(\mathbf{1})\right]^{D} \qquad (4.3)$$

Where:

P_{INF} is the probability of infection from the dose

$P_{INF}(1)$ is the probability of infection from one organism (Beta Distribution)

D is the number of organisms estimated to be in the meal (Dose).

The simulated beta-poisson model can be interpreted as estimating, during a simulation, the probability of infection for different individuals at every iteration. The host-pathogen relationship, or the probability of infection for an individual from one cell, is assumed to vary according to a beta distribution. Some host-pathogen combinations may have a high probability of infection

and some may have a low probability of infection. The range and frequency with which the various probability of infections upon exposure to one cell occur during a simulation is dictated by the parameters of the beta distribution.

The probability of illness upon exposure to a dose of a pathogen is conditional upon the probability of infection. Stated another way, in order for an individual to become sick, the individual has to first become infected. The dose-response relationships described so far have estimated the probability of infection upon exposure to a dose. In order to estimate the probability of illness, the conditional probability of illness following infection is required.

The human feeding trial data does not indicate a clear dose-response relationship for the conditional probability of illness following infection. For strain A3249, the data in the human feeding trials actually shows a decreasing trend for the conditional probability of illness with increasing dose. This observation has motivated some researchers (Teunis *et al.,* 1999) to postulate that perhaps upon exposure to a larger dose of some pathogens, the elicited host defenses are stronger, therefore reducing the probability of illness upon exposure to a very large dose compared to a moderate dose. The other alternatives that exist for the relationship of the conditional probability of illness following infection are that the probability increases with increasing dose or the probability is independent of dose. The three alternatives, using hypothetical illustrative data are shown in Figure 4.7.

In the case of the feeding trial data for *C. jejuni* A3249 the probability of illness decreases with increasing dose and as such a decreasing hazard function has been estimated (Teunis *et al.* 1999). However, when the data for both strains are pooled the conditional probability of illness following infection does not exhibit a dose relationship but rather is randomly distributed (Figure 4.8).

It may be appropriate in this case to use a dose independent ratio to estimate the conditional probability of illness. The conditional probability can be estimated from the feeding trial data. For A3249, out of 50 people that got infected at various doses, 11 got sick (22%), while for 81-176, out of 39 people that got infected at different doses, 18 got sick (46%). Overall, pooling all the data, a total of 29 people got sick out of 89 individuals that were infected (33%). In order to account for the uncertainty in the conditional probability, it is possible to use a beta distribution, with parameters that are based on the observations (alpha = 30 {number ill +1}and beta = 61 {number infected – number ill +1}). This distribution is shown in Figure 4.9.

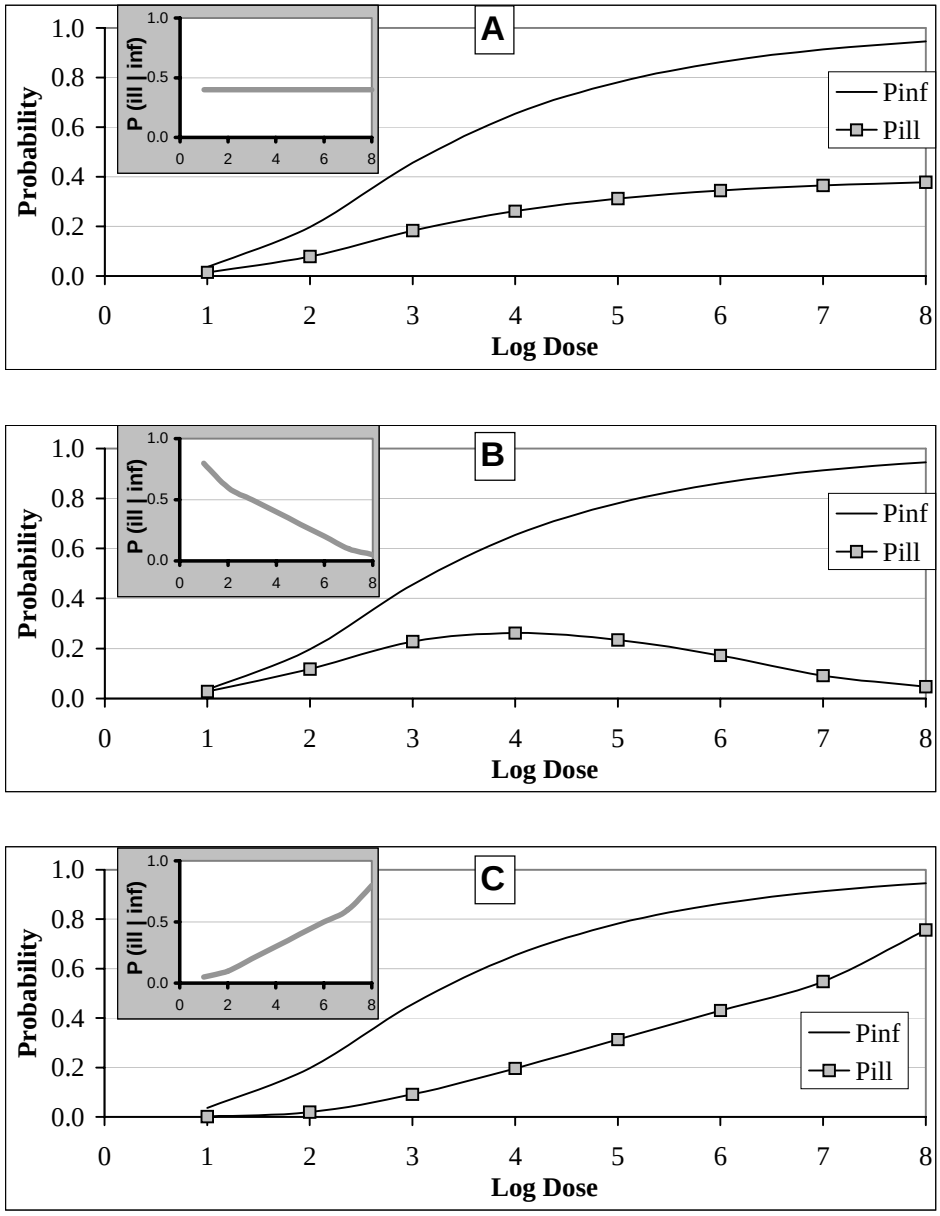

Figure 4.7 Hypothetical probability of illness curves, influenced by three alternative conditional probabilities. Conditional probability assumption shown in inset curves. (A) Probability independent of dose; (B) Probability decreasing with dose; (C) Probability increasing with dose.

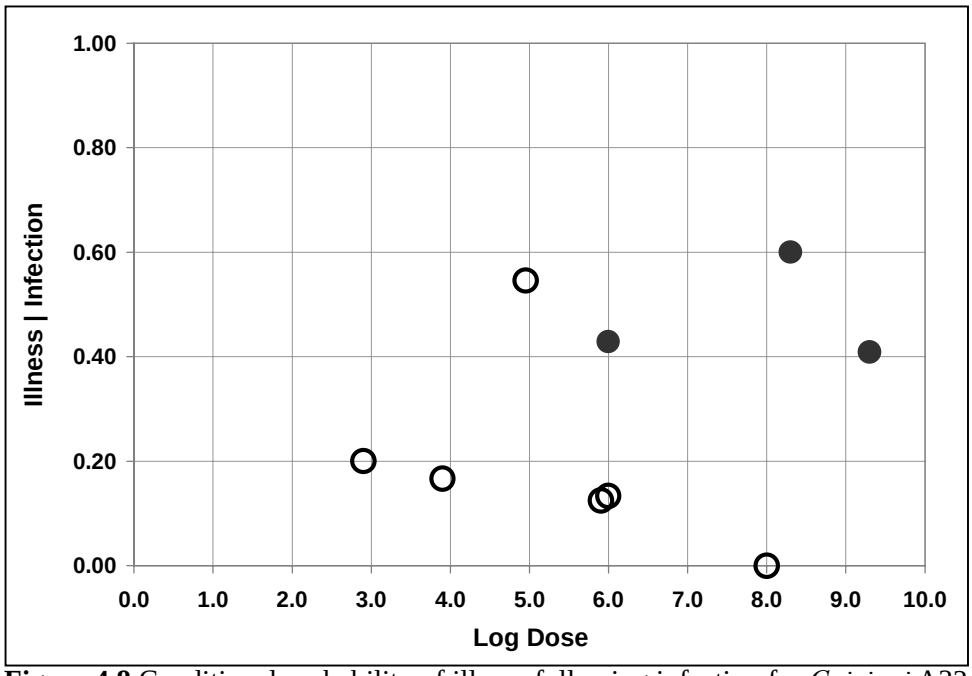

Figure 4.8 Conditional probability of illness following infection for *C. jejuni* A3249 and 81-176.

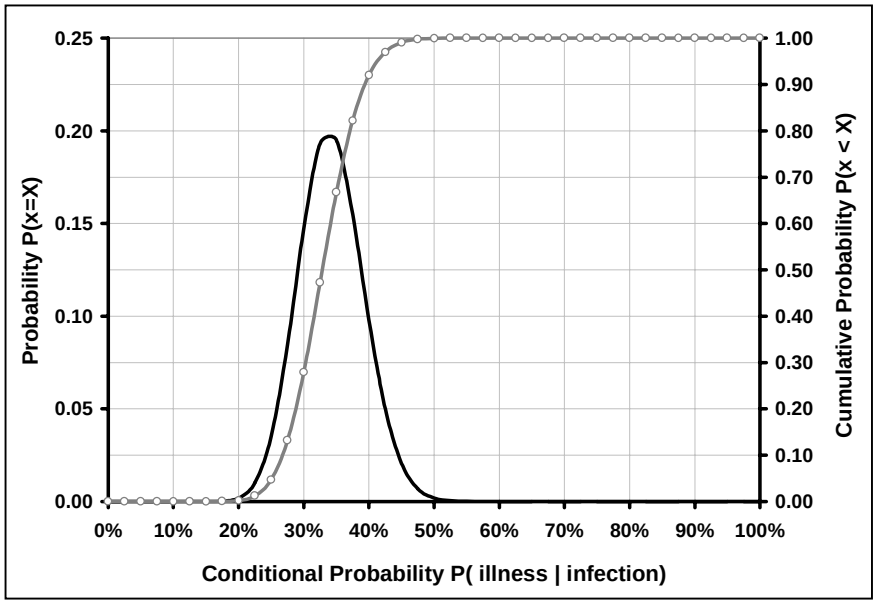

Figure 4.9 Distribution for conditional probability of illness following infection.

In addition to the feeding trial data, Havelaar *et al.* (2000) report on an outbreak investigation conducted by Bremell *et al.,* (1991) in which 35 individuals became sick out of a total of 66 that were infected (53%). This estimate would place the conditional probability at the tail of the distribution shown in Figure 4.9.

In conclusion, several issues need to be finalized for application of the dose-response model to the risk assessment.
The acceptability of pooling data from the two strains in order to estimate the probability of infection.
Use of the approximate and exact beta-poisson equation if only data from *C, jejuni* A3249 is used. This should not be an issue for the pooled data set, since the approximate solution is sufficient in that case.
The appropriate conditional probability for infection following illness, including assumptions on the dose relationship and the value and range of the probability.

(On request of the Expert Consultation the limitations of this dose-response model should be included).

5. Gaps in data

An important role of risk assessment is that it can identify areas where data are lacking or very limited. Lack of data or the availability of limited data contribute to the uncertainty of exposure and risk estimates. Thus, collection of additional data and new research directed at key parameters of a risk assessment can vastly reduce uncertainty. Combined with sensitivity analysis, which identifies the influence of and the importance of key parameters on the final risk estimate, research priorities can more efficiently and effectively target areas that improve the accuracy of the risk estimate. We identify a number of data gaps where additional data may be used in a Campylobacter in broilers risk assessment and enhance the estimates of adverse outcomes.

5.1 Hazard Identification

National surveillance data on the number of *Campylobacter* infections per 100.000 inhabitants in developing countries.
Survey data on the load of *Campylobacter* in chicken products in developing countries.
The risk factors for campylobacteriosis in developed and developing countries.

5.2 Exposure assessment

On-Farm
Data on the routes of *Campylobacter* infection of broilers.
Survey data on the prevalence of slaughtered flocks and within flocks.
Data on the probability of contamination of a bird during transport.
Studies on the dynamics of within flock transmission.

Processing
Prevalence and enumeration data for poultry before and after various processing steps such as scalding, defeathering, evisceration, washing and chilling
Prevalence and enumeration data comparing various methods of chilling –air chilling, water chilling, water chilling with chlorine, etc.
Data describing the actual cross-contamination between positive and negative flocks and within positive flocks during the different slaughter processes.
Prevalence and enumeration data comparing different scalding temperatures and different packaging methods[1].
Data on the relationship between the concentration on neck skin samples and the concentration on the whole chicken in order to calculate a conversion factor.
Data on the microbial implications of carcass de-boning.

Post-processing and Consumer Handling
Survey data and direct observational data on consumer practices in preparation and handling of chicken that especially detail the number of times transfer of *Campylobacter* could occur during handling and preparation.
Research data detailing amounts of *Campylobacter* that are transferred to and from surfaces during preparation of chicken and the meal.
Survey data and direct observational data on preparation and handling practices of chicken in restaurants and other retail establishments.

[1] The survival of *Campylobacter* may be increased/favoured by packaging in a modified gas atmosphere (Blankenship & Craven, 1982). This may be important as more and more chicken parts are packed in this way. Data, which elucidate the effect of MAP-packaging on the prevalence and concentration in these products, need to be generated

Additional data on cooking of chicken that addresses areas of the chicken where *Campylobacter* may be protected from heat.
Survey data on the dietary habits of consumers.

5.3 Hazard characterization

Data on strain variability regarding virulence/pathogenicity.
Studies on the mechanisms of infectivity, virulence/pathogenicity
Additional human volunteer studies with other strains of *Campylobacter* jejuni ranging from 10^1 to 10^9 organisms.
Additional human volunteer studies that focus on lower doses of the organisms from 1 to 10^3 organisms.
Epidemiological data available from outbreak studies that have enumerated the number of *Campylobacter* in suspected food items and includes information on attack rates, illnesses, etc.
Additional epidemiological data on susceptible sub-populations including immuno-compromised, children under the age of 5, elderly, etc
Additional epidemiological data on susceptibility of children under 1 year of age.
Data describing the impact of immunity.
Studies on the true number of human infections caused by *Campylobacter*, incl. GBS etc.
Studies on the true number of human infections caused by *Campylobacter* from different sources e.g. chicken products.
In order to utilize an alternative approach to dose-response that can be used for management purposes in a country that lacks enumeration data in their system, several pieces of information are required for a specific country. Data is needed on the number of *Campylobacter* illnesses attributed to chicken in that country in a time interval (e.g. per year). Coupled with this, the prevalence of contaminated chickens at a point in the farm-to-fork chain is also required. The further up stream the prevalence estimation (i.e. closer to the consumer) the more utility in the approach for risk mitigation strategies.

6. Conclusions

6.1 Exposure Assessment

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6.2 Hazard Characterization

The current document has attempted to synthesise and summarise the information that was available either in the literature, or through the call for data to describe the factors that influence the likelihood of an individual becoming infected, ill, and developing sequelae. The quantification of the importance of most of these factors requires a substantial amount of additional research. Nevertheless, the information and analysis conducted does allow some advances to be made in estimating the risk from *Campylobacter* and putting in context the apparent importance of other factors.

The probability of any pathogen initiating an infection is influenced to various degrees by three factors. These three factors include the pathogen characteristics, host characteristics and the matrix/conditions of ingestion. The influence of specific components within these three factors were expanded upon and included a qualitative description of the current thinking in relation to the influence of these factors. Unfortunately, there is currently insufficient information from which a detailed analysis can be performed, that would allow fine distinctions to be made on, for instance the probability of illness upon ingestion of a certain strain vs. another, ingested in milk vs. water by an individual who is taking medication.

The adverse effects that can occur upon infection with *C. jejuni* were also summarized, and included acute gastro-enteritis and non-gastrointestinal sequelae. The sequelae that can result from *Campylobacter* infections were identified as Reactive arthritis, Guillain-Barre syndrome, and Fisher syndrome. Reactive arthritis has been estimated to occur in approximately 1% of patients with campylobacteriosis. Guillain-Barre syndrome is a serious paralytic condition, which has been estimated to occur once in every 1000 cases. Finally, Fisher syndrome, which is considered to be a variant of Guillain-Barre syndrome is also reported to occur, however there are no estimates on the frequency of the occurrence of this condition following campylobacteriosis.

The hazard characterization was also able to present existing dose-response models and derive a new dose-response model that can be used to mathematically describe and estimate the probability of infection following the ingestion of a dose of *C. jejuni*. The dose-response equations used were based upon the single-hit hypothesis, fit to human feeding trial data conducted using healthy volunteers and two strains of *C. jejuni*. It was proposed in the current report that pooling the infectivity data for the two strains from the feeding trial study may be appropriate, however this is an issue that needs to be finalized. It was illustrated that the probability of illness is conditional upon the probability of infection. Using the data from the human feeding trial study, there does not appear to be a clear trend for the behaviour of this conditional probability. When the data for both strains are pooled, the conditional probability tends to exhibit a dose independent relationship, however for one of the strains some researchers have proposed a decreasing probability with dose.

In conclusion, the probability of infection upon ingestion of a dose of *C. jejuni* can be estimated with the caveat that the data is for healthy volunteers and the impact of susceptibility or other factors cannot at this time be quantified from the data. The probability of illness following

infection can also be estimated using either a dose independent probability, or, if only one strain is considered some researchers have proposed a decreasing with dose hazard function. Again, the impact of other factors, such as susceptibility, on the probability of illness cannot be quantified due to a lack of data and resolution to this level. Finally, the progression of the illness to more serious outcomes and the development of some sequelae can be crudely estimated from the approximate proportions reported in the literature.

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Appendix 1

A 1.1 Campylobacter on the farm

Estimating P_{fp}

Sample data obtained from two fully-integrated poultry companies, an epidemiological study (Evans, 1996) and a published source (Humphrey *et al.,* 1993) were used to obtain an estimate of P_{fp}. More specifically, individual estimates of flock prevalence were derived for each source using beta distributions as follows

$$P1_{fp} = Beta(r_1 + 1, s_1 - r_1 + 1)$$
$$P2_{fp} = Beta(r_2 + 1, s_2 - r_2 + 1)$$
$$P3_{fp} = Beta(r_3 + 1, s_3 - r_3 + 1)$$
$$P4_{fp} = Beta(r_4 + 1, s_4 - r_4 + 1)$$

where $P1_{fp}$ and $P2_{fp}$ are estimates of flock prevalence derived from data from the two leading Great Britian poultry producers which together account for 35% of national chicken production; $P3_{fp}$ is an estimate of flock prevalence based on the epidemiological study (Evans, 1996) which involved 5 separate poultry producers, together responsible for 50% of the national flock, and $P4_{fp}$ estimates flock prevalence from a published study (Humphrey *et al.,* 1993). In each case, *r* denotes the number of positive flocks and *s* the number of flocks sampled. The beta distribution is used to characterize the uncertainty in the sample data and assumes a random sample and that the sample size is smaller than the total population. It also assumes that each positive flock is equally likely to be detected (Evans, 1996).

The prevalence of positive flocks based on each source, $P1_{fp}$, $P2_{fp}$, $P3_{fp}$, and $P4_{fp,}$ are weighted according to market share to give the overall flock prevalence, that is

$$P_{fp} = (P1_{fp}\, w1) + (P2_{fp}\, w2) + (P3_{fp}\, w3) + (P4_{fp}\, w4)$$

where *w*1, *w*2, *w*3 and *w*4 are the associated weights. The values for *w*1, *w*2, *w*3 and *w*4 are based on the companies market share using denominator data derived from MAFF statistics (Ministry of Agriculture Fisheries and Food (MAFF), 1999).

Estimating P_{wfp}

Stage 1: Chain Binomial

In 1982 Reed & Frost developed chain-binomial models of epidemic spread (Jacquez, 1987). Although this work was not published, the theory was popularised by Bailey (1975) and these models have frequently appeared in the literature, for example to study HIV epidemics (Ng and Orav, 1990). The initial transmission of *Campylobacter* within a flock is described using such a model (Bailey, 1975; Jacquez, 1987). Such a model is deemed appropriate when the data available for parameter estimation are measured in discrete time (Bailey, 1975) as in the occurrence of colonised birds within the cluster containing the first positive bird.

In the situation presented here the basic chain binomial model describes the colonisation of a random susceptible bird which becomes colonised after a fixed constant time. The colonised bird is then removed from the susceptible population. New cases occur within the cluster in distinct groups at each time point, as described by the recurrence equation (2)

$$I_c(t+1) = I_c(t) + NI_c(t+1) \qquad (2)$$

where $I_c(t)$ is the number of colonised birds in the cluster at *t*, and $NI_c(t+1)$ is the number of newly colonised birds in the period (*t, t+1*] where (*t, t+1*] is defined as one day. The number of newly colonised birds at each time point will follow a binomial distribution which depends upon the probability that any susceptible bird in the cluster becomes infected in time (*t, t+1*], that is *p(t)*. Following on from this, the binomial likelihood for $NI_c(t+1)$ can be written as:

$$P\left[NI_c(t+1) = x_{t+1}, NI_c(t) = x_t, ..., NI_c(1) = x_i \middle| I_c(0) = x_0\right] = \prod_i P\left[NI_c(i) = x_i \middle| H(i-1)\right]$$

where this binomial likelihood is given by the binomial probabilities dependent on *p(t)*, the probability that a susceptible bird becomes colonised in the period (*t, t+1*], and *H(t)* can be described as the history of the epidemic up to that point. More specifically

$$P\left[NI_c(t+1) = x_{t+1} \middle| H(t)\right] = \binom{S_c(t)}{x_{t+1}} p(t)^{x_{t+1}} \left[1 - p(t)\right]^{S_c(t) - x_{t+1}}$$

$$H(t) = \left\{NI_c(t) = x_t, NI_c(t-1) = x_{t-1}, ..., NI_c(1) = x_1, I(0) = x_0\right\}$$

where $S_c(t)$ is the number of susceptible birds in the cluster at time *t.*

When considering transmission of *Campylobacter* within a flock, the probability that a bird becomes colonised is dependent upon the transmission rate, the social need to make contact with other birds, and the probability of contact with a colonised bird. The generic form of the chain binomial model assumes a randomly mixing population, that is, a given bird would be equally likely to make a contact with every infected bird (Jacquez, 1987). In reality, commercial flocks can be many thousands in size, hence random mixing is not a reasonable assumption. However, by assuming a bird moves around a limited number of birds, defined as a cluster, and by considering the number of birds a given bird comes into contact with, and the number of times contact is made, we are able to model the spread of infection in a

small neighbourhood. The basic chain binomial model described above is then modified to include these factors. Such a modified chain binomial model has been used previously by Ng & Orav (1990) to describe the transmission of HIV within a male community. Within this work the number of sexual partners an individual had and the number of times sexual contact was made were considered. Within the present problem each sexual partner is analogous to the number of birds a given bird makes contact with, and each sexual contact is analogous to the number of times contact is made with each bird. Use of the modified model requires several assumptions (Ng and Orav, 1990):

i) The total cluster size remains constant i.e. $S_c(t)+I_c(t)=n_c$ for all values of t where n_c is the total cluster size;
ii) A bird, which becomes colonised at time t, cannot transmit the organism to another bird until time $t+1$, this allows for a fixed latent period of one day;
iii) Birds within the cluster act independently; and
iv) Each non-colonised bird has the same probability of being colonised at time t

Let b equal the probability of transmission given a single contact of a susceptible bird with a colonised bird, A equal the number of birds a given bird comes into contact with in one day, that is $(t, t+1]$ and R equal the number of times the bird is contacted by each of the A contacts in $(t, t+1]$. The parameters A and R are random variables which have probability density functions given by

$$P(A=a)=f(a)$$
$$P(R=r)=g(r)$$

Within the model probability generating functions are used for A and R as they are easier to manipulate (Jacquez, 1987). The associated probability generating functions are given by

$$\Phi_A(s)=E(s^A)=\sum_{a=0}^{\infty} f(a)s^a$$
$$\Phi_R(s)=E(s^R)=\sum_{r=0}^{\infty} g(r)s^r \qquad 0\le s\le 1$$

From the work of Ng and Orav (Ng and Orav, 1990) assuming independence of individual birds, the probability that a susceptible bird becomes colonised in the period $(t, t+1]$, $p(t)$, is derived as follows:

P(no transmission occurs $|$ contact with one colonised bird) $=(1-b)$

P(no transmission occurs $|$ R contacts with one colonised bird) $=\Phi_R(1-b)$

P(no transmission occurs $|$ R contacts with a random bird in cluster)

$$=1-\left\{\left[\frac{I_c(t)}{n_c(t)}\right]\left[1-\Phi_R(1-b)\right]\right\}$$

Therefore, the probability that a susceptible bird becomes colonised in the period $(t, t+1]$, $p(t)$, is given by equation (3)

$$p(t) = 1 - \Phi_A\left[1 - \left\{\left[\frac{I_c(t)}{n_c(t)}\right]\left[1 - \Phi_R(1-b)\right]\right\}\right] \qquad (3)$$

This can be written equivalently without the use of generating functions:

$$p(t) = 1 - \sum_a f(a)\left\{1 - \frac{I_c(t)}{n_c(t)}\left[1 - \sum_r g(r)(1-b)^r\right]\right\}^a$$

It is assumed that the variable *A*, the number of contacts a bird makes with an individual in one day follows a binomial distribution i.e. Binomial(n_c,P_c) where P_c is the probability that contact is made with another bird. Also it is assumed that the variable *R*, that is the number of times that a bird makes contact with a given bird follows a Poisson distribution, i.e. Poisson(*y*), where *y* is the mean number of times contact is made with each bird. In this way the number of contacts is limited to be equal to or less than the cluster size, but the number of times contact is made is theoretically unbounded.

The generating functions for the number of contacts made, Φ_A and the number of times contact is made with each bird, Φ_R are therefore given by

$$\Phi_A = (1 - P_c + P_c s)^{n_c}$$
$$\Phi_R = e^{(-y(1-s))}$$

Thus substituting these generating functions into equation (3), the probability that a non-colonised bird becomes colonised in one day, that is *p(t),* is given by:

$$p(t) = 1 - \left[1 - P_c\left(\frac{I_c(t)}{n_c(t)}\right)\left(\frac{1-\exp^{-yb}}{1-\exp^{-y}}\right)\right]^{n_c}$$

The mean number of newly colonised birds is then given by:

$$NI_c(t+1) = p(t)S_c(t)$$

Stage 2: Epidemic spread

As previously discussed during the process of *Campylobacter* colonisation within a flock, a threshold time is reached when the water and feed become contaminated. This threshold normally occurs 4 days after the first bird in the cluster becomes colonised and colonisation rapidly spreads throughout the remainder of the flock. Thereafter, stage 2 begins at time $t=t_5$.

In the second stage it is assumed that the number of newly colonised birds at any time point is dependent upon the initial number of colonised birds, that is, the number of birds colonised within the cluster, at the time when stage 2 begins ($I_c(t_4)$) and the transmission rate. Under this assumption, the colonisation process in stage 2 can be represented by a simple epidemic model.

It is assumed that in stage two, *n* is the total population size and $I_c(t_4)$ is the number of colonised birds in the cluster modelled in stage 1. The colonisation process begins with $I_c(t_4)$ colonised birds and $S_B(t_4)$ susceptible birds, where

$$S_B(t_4) = n - I_c(t_4)$$

In any time period, it is assumed that the number of newly colonised birds is proportional to both the numbers of colonised and susceptible birds. Therefore the process can be described by the differential equation (4)

$$\frac{dS_B}{dt'} = -b_B S_B(t')[n - S_B(t')] \qquad (4)$$

where $S_B(t)$ is the number of susceptible birds, b_B is the biological transmission and t' is equal to $(t-4)$ where the value 4 is the time in days until the second stage begins. By incorporating t' into the differential equation the result is a small lag in the overall epidemic curve at the point when the change occurs from the first to the second stages of the model. This is biologically consistent as the organism changes mode of transmission, from bird to bird to environmental transmission via feed and water. The transmission probability, b_B is assumed to be proportional to the transmission probability *b*. This assumption is made because in the second stage, transmission occurs both directly and indirectly from bird to bird. In the indirect case, colonised birds contaminate feed and water which then leads to exposure and subsequent colonisation of susceptible birds. Thus the probability of transmission in stage 2 is related to the probability of transmission in stage 1. The constant of proportionality is calculated as $\frac{1}{10n}$.

Solving (4) for the number of susceptibles gives equation (5)

$$S_B(t') = \frac{S_B(t_4)n}{S_B(t_4) + I_c(t_4)\exp^{[nb_B t']}} \qquad (5)$$

After completion of the first and second stages the total number of colonised birds within a flock *I(t)* is given by

$$I(t) = n - S_B(t)$$

Therefore the within-flock prevalence at time *t* since the time of exposure can be calculated directly from equation (6):

$$P_{wfp}(t) = \frac{I(t)}{n} \qquad (6)$$

A 1.2 Source of infection is contaminated feed and/or water

The differential equation to described the mode of transmission is a result of contaminated feed and/or water is given by

$$\frac{dS_B}{bt} = -b_B S_B(t)[N - S_B(t)]$$

which when solved for the number of susceptibles yields

$$S_B(t) = \frac{S_B(t_4)N}{S_B(t_4) + I_c(t_4)\exp^{[Nb_B t]}}$$

It can be seen that this is analogous to equation 5 except the equation is differentiated with respect to t as opposed to t'. This is because here the differential equation is used in isolation, there is no first stage of transmission to consider.

A 1.3 Source of infection is via vertical transmission

When the colonisation of a floc is the result of vertical transmission the model can be modified as follows.
Consider a flock that initially has i birds which become colonised at some time as a result of vertical transmission. This results in i clusters initiating the colonisation process of the flock. Assuming that each cluster acts independently, the probability that a bird will become colonised in one day in cluster i, that is $P(t)_i$, is given by

$$p(t)_i = 1 - \left[1 - P_i\left(\frac{I_i(t)}{n_i(t)}\right)\left(\frac{1-\exp^{-y_i b}}{1-\exp^{-y_i}}\right)\right]^{n_i}$$

where $I_i(t)$ is the number of colonised birds in cluster i, n_i is the total number of birds in cluster i, P_i is the probability that contact is made with another bird in cluster i, and y_i is the mean number of times contact is made with each bird in cluster i. The number of colonised birds at time t is then given by

$$NI_c(t) = \sum_{i=1}^{n} p(t)_i S_i(t)$$

where $NI_c(t)$ is the total number of newly colonised birds to appear in *t* in all the clusters in the flock, and S_i is the number of susceptibles in cluster i. Following on from this stage 2 therefore begins with $NI_c(t_4)$ colonised birds. Stage 2 is then as described in previously.

Appendix 2

FAO/WHO have received information from several countries in relation to the *Campylobacter* risk assessment work. In this appendix some of this information is listed.

A2.1 Farm Data

Country	Num Pos	Num	Prevalence	Sample	Prev Type	Species
Denmark			95	Cloacal	Flock	Duck
Denmark			60	Cloacal	Flock	Chicken
Denmark			45	Cloacal	Flock	Broiler
Finland	33	1132	2.9	Fecal	Flock	Chicken
Ireland	82	100	82		Bird	Broiler
Netherlands			29.8		Flock	Broiler
Netherlands	405	936	43.27		Flock	Chicken
New Zealand	7	33	21.2		Flock	Chicken

A 2.2 Processing Data

Country	Number Positive	Number Tested	Prevalence	Sample Type	Prev Type	Species	Processing Step
Brazil	15	30	50		Bird	Chicken	End Product
Brazil	17	30	36.6		Bird	Chicken	After slaughter
Brazil		34	73.5		Bird	Chicken	
Brazil	14	40	35		Bird	Porcine	
Brazil	19	40	47.5		Bird	Broiler	Final Rinsing
Brazil		45	62.2	Surface Swab	Bird	Broiler	Chilling
Brazil		45	51.1	Intestinal	Bird	Broiler	
Cuba	15	30	50	Intestinal	Bird	Broiler	
Cuba	24	30	80	Carcass	Bird	Broiler	Post Evisceration
Cuba	20	30	66.7	Carcass	Bird	Broiler	Chilling
Cyprus	11	27	41		Bird	Broiler	End of Processing Line
Cyprus	17	57	30		Bird	Broiler	End of Processing Line
Cyprus	80	118	68		Bird	Broiler	End of Processing Line
Cyprus	22	51	43		Bird	Broiler	End of Processing Line
Cyprus	128	288	44		Bird	Broiler	End of Processing Line
Cyprus	23	41	56		Bird	Quail	End of Processing Line
Cyprus	52	64	81		Bird	Quail	End of Processing Line
Cyprus	85	93	91		Bird	Quail	End of Processing Line
Cyprus	32	42	76		Bird	Broiler	End of Processing Line
Finland	606000	1970000	3	Fecal	Bird	Broiler	
Ireland	384	400	87.3	Neck	Bird	Broiler	
Ireland					Bird	Broiler	Post Wash - Pre Chill
Ireland					Bird	Broiler	Post Evisceration
Ireland					Bird	Broiler	After Defeathering
Ireland					Bird	Broiler	Post Stun & Bleed
New	15	15	100	Misc Giblets	Bird	Chicken	
New	15	15	100	Carcass	Bird	Chicken	

A 2.3 Retail Data

Country	Number Positive	Number Tested	Prevalence	Sample Type	Prev Type	Species	Sample Treatment
Brazil	4	27	14.8	Heart	Bird	Chicken	
Brazil		200	13.5	Parts	Bird	Chicken	Raw,
Brazil	1	5	20	Carcass	Bird	Chicken	
Brazil	15	82	18.3	Parts	Bird	Chicken	
Brazil	0	32	0	Liver	Bird	Chicken	
Brazil	1	4	25	Feet	Bird	Chicken	
Brazil	40	64	62.5		Bird	Chicken	Fresh
Brazil	30	64	46.9		Bird	Chicken	
Brazil		62	42.1	Carcass	Bird	Chicken	
Brazil	34	50	68	Carcass	Bird	Chicken	
Brazil	6	50	12	Gizzard	Bird	Chicken	
Denmark			34		Bird	Broiler	
Finland	16	174	9.2		Bird	Chicken	
Finland	7	145	15.6		Bird	Chicken	
Netherlands			34	Carcass	Bird	Broiler	Fresh
Netherlands	1	35	2.9		Bird	Guinea-	
Netherlands	1	27	3.7		Bird	Pheasant	
Netherlands	3	52	5.8		Bird	Duck	
Netherlands	1	145	0.7		Bird	Turkey	
Netherlands	1381	4574	30.2		Bird	Chicken	
Netherlands			71	Carcass	Bird	Broiler	Frozen
Netherlands			38	Carcass	Bird	Broiler	Fresh
Netherlands			9	Carcass	Bird	Broiler	Frozen
New	20	50	40	Wings	Bird	Chicken	
Norway	1	255	.4		Bird	Broiler	
Norway			10.2		Bird	Poultry	Fresh
Norway			2.3		Bird	Poultry	Frozen
Norway			4.5		Bird	Poultry	Fresh
Norway	9	101	8.9	Parts	Bird	Poultry	Fresh
Norway	0	4	0		Bird	Poultry	
Norway	17	133	12.8	Parts	Bird	Poultry	Fresh
Scotland	18	51	35.3	Parts	Bird	Chicken	
Switzerland		302	33		Bird		
Switzerland	22	144	16	Liver	Bird	Poultry	Frozen
Switzerland	43	139	31	Liver	Bird	Poultry	Fresh
Switzerland		302	33		Bird		

A 2.4 Human Surveillance Data by Country

Country	Austria			
Population	**8,100,000**			
Date of Population				
Date of Data	*1996*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1131	13.9	June 1 - Dec 31
Date of Data	*1997*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1667	20.6	
Date of Data	*1998*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		2454	30.3	

Country	Bangui (Central Africa)			
Population				
Date of Population				
Date of Data				
Age	***Season***	***Cases***	***Incidence***	***Other info***
				Res. Study

Country	Brazil			
Population	**169,000,000**			
Date of Population				
Date of Data				
Age	***Season***	***Cases***	***Incidence***	***Other info***
children				Loureiro, E.C.B & Lins; Res. Study

Country Canada

Population

Date of Population

Date of Data *1998-1999*

Age	*Season*	*Cases*	*Incidence*	*Other info*
				Res. Study

Country Cuba

Population

Date of Population

Date of Data

Age	*Season*	*Cases*	*Incidence*	*Other info*
				2.8% of positive samples of *Campylobacter* were found in pediatric hospital

Country Czech Republic

Population

Date of Population

Date of Data *1993*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		2243		
	Fall	631		
	Spring	392		
	Summer	999		
	Winter	221		
<1 yr		262		
1-4 yr		788		
10-14 yr		199		
15-19 yr		181		
20-24 yr		115		
25-34 yr		106		
35-44 yr		88		
45-54 yr		76		
5-9 yr		326		
55-64 yr		36		
65+		66		

Date of Data *1994*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		2262		
	Fall	649		
	Spring	458		
	Summer	913		
	Winter	250		
<1		193		
1-4 yr		781		
10-14 yr		215		
15-19 yr		196		
20-24 yr		135		
25-34 yr		129		
35-44 yr		104		
45-54 yr		89		
5-9 yr		304		
55-64 yr		43		
65+		78		

Date of Data *1995*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		3030		
	Fall	880		
	Spring	493		
	Summer	1349		
	Winter	308		
<1 yr		237		
1-4 yr		1036		
10-14 yr		251		
15-19 yr		248		
20-24 yr		211		
25-34 yr		219		
35-44 yr		143		
45-54 yr		100		
5-9 yr		419		
55-64 yr		72		
65+		94		

Date of Data *1996*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		2545		
	Fall	876		
	Spring	266		
	Summer	889		
	Winter	247		
<1		178		
1-4 yr		804		
10-14 yr		214		
15-19 yr		209		
20-24 yr		197		
25-34 yr		182		
35--44 yr		129		
45-54 yr		97		
5-9 yr		380		
55-64 yr		51		
65+		104		

Date of Data *1997*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		3811		
	Fall	1151		
	Spring	501		
	Summer	1579		
	Winter	392		
<1 yr		221		
1-4 yr		161		
5-9 yr		572		
10-14 yr		355		
15-19 yr		326		
20-24 yr		356		
25-34 yr		320		
35-44 yr		192		
45-54 yr		173		
55-64 yr		81		
65+		154		

Date of Data *1998*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		5542		
	Fall	1910		
	Spring	850		
	Summer	2204		
	Winter	578		
<1 yr		281		
1-4 yr		1504		
10-14 yr		495		
15-19 yr		466		
20-24 yr		536		
25-34 yr		495		
35-44 yr		281		
45-54 yr		269		
5-9 yr		858		
55-64 yr		159		
65+		198		

Date of Data *1999*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		9843		
	Fall	3281		
	Spring	1709		
	Summer	3748		
	Winter	1105		
<1 yr		454		
1-4 yr		2324		
10-14 yr		884		
15-19 yr		908		
20-24 yr		1115		
25-34 yr		1072		
35-44 yr		508		
45-54 yr		515		
5-9 yr		1330		
55-64 yr		327		
65+ yr		406		

Date of Data *2000*

Age	Season	Cases	Incidence	Other info
		16916		
	Fall	5778		
	Spring	3508		
	Summer	5654		
	Winter	1976		
<1		722		
1-4 yr		3485		
10-14 yr		1636		
15-19 yr		1571		
20-24 yr		1842		
25-34 yr		2122		
35-44 yr		1051		
45-54 yr		939		
5-9 yr		2239		
55-64 yr		601		
65+ yr		708		

Population **10,300,000**

Date of Population

Date of Data *1993-1998*

Age	Season	Cases	Incidence	Other info
		19438		
<1 yr		1372		
1-4 yr		5974		
10-14 yr		1729		
15-19 yr		1626		
20-24 yr		1550		
25-34 yr		1451		
35-44 yr		937		
45-54 yr		804		
5-9 yr		2859		
55-64 yr		442		
65-74 yr		433		
75+ yr		261		

Population

Date of Population

Date of Data *1998*

Age	Season	Cases	Incidence	Other info

		5000	5	
Date of Data	*1999*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
			131	Brno region
		10000	10	

Country Denmark

Population

Date of Population

Date of Data	*1999*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		4161		820 hospitalizations

Population **5,300,000**

Date of Population **1994**

Date of Data	*1988*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1276		
Date of Data	*1989*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1432		
Date of Data	*1990*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1367		
Date of Data	*1991*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1261		
Date of Data	*1992*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1129		
Date of Data	*1993*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1776		
		1767		
Date of Data	*1994*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		2196		
		2177		
Date of Data	*1995*			
Age	***Season***	***Cases***	***Incidence***	***Other info***

		2601		
Date of Data	*1996*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		2973		
Date of Data	*1997*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		2666		
Date of Data	*1998*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		3372		

Country England

Population
Date of Population
Date of Data

Age	***Season***	***Cases***	***Incidence***	***Other info***
				Res. Study

Population
Date of Population
Date of Data

Age	***Season***	***Cases***	***Incidence***	***Other info***
				Res. Study

Country England & Wales

Population
Date of Population
Date of Data

Age	***Season***	***Cases***	***Incidence***	***Other info***
				Res. Study

Population
Date of Population

Date of Data	*1980*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		227		Norway
		1273		United Kingdom, Scotland
Date of Data	*1981*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1887		United Kingdom, Scotland
		266		Norway

Date of Data	*1982*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		343		Norway
		1922		United Kingdom, Scotland
Date of Data	*1983*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1895		United Kingdom, Scotland
		360		Norway
Date of Data	*1984*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1462		Denmark
		2181		United Kingdom, Scotland
		393		Norway
Date of Data	*1985*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		804	16.4	Finland
		2563		United Kingdom, Scotland
		1457		Denmark
		403		Norway
Date of Data	*1986*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1201		Denmark
		441		Norway
		2372		United Kingdom, Scotland
		848	17.3	Finland
Date of Data	*1987*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1411		Denmark
		2740		United Kingdom, Scotland
		1203	24.6	Finland
		491		Norway
Date of Data	*1988*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		1276		Denmark
		543		Norway
		2906		United Kingdom, Scotland
		1452	24.6	Finland
Date of Data	*1989*			

Age	*Season*	*Cases*	*Incidence*	*Other info*
		1432		Denmark
		3080		United Kingdom, Scotland
		1627	29.6	Finland
		474		Norway

Country European Union

Population

Date of Population

Date of Data *1995*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		43902		England & Wales
		6600		Germany (6 Lander)
		4879		Belgium
		5580		Sweden
		644		Ireland
		3225		Spain
		4377		Scotland
		1046		Norway
		2871		The Netherlands
		2273		Finland
		2601		Denmark
		557		Northern Ireland

Date of Data *1996*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		2629		Finland
		5081		Sweden
		2973		Denmark
		5218		Scotland
		653		Northern Ireland
		43240		England & Wales
		1131		Austria
		10124		Germany (7 Lander)
		4991		Belgium
		16		Greece
		646		Ireland
		1137		Norway

		3557		Spain
		3737		The Netherlands
		129		Luxembourg
Date of Data	*1997*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		943		Ireland
		106		Luxembourg
		3711		Spain
		1667		Austria
		5528		Scotland
		26		Greece
		50201		England & Wales
		5617		Belgium
		1178		Norway
		5306		Sweden
		13095		Germany (7 Lander)
		2666		Denmark
		778		Northern Ireland
		3661		The Netherlands
		2404		Finland
Date of Data	*1998*			
Age	***Season***	***Cases***	***Incidence***	***Other info***
		33235		Germany (10 Lander)
		136		Greece
		4328		Spain
		1700		Norway
		6544		Sweden
		1318		Ireland
		2454		Austria
		6610		Belgium
		58058		England & Wales
		3372		Denmark
		774		Northern Ireland
		6375		Scotland
		3489		The Netherlands
		2851		Finland
Date of Data	*1999*			
Age	***Season***	***Cases***	***Incidence***	***Other info***

5101	Spain
4164	Denmark
171	Luxembourg
2085	Ireland
3252	Austria
15	Greece
2027	Norway
3305	Finland
7137	Sweden
28882	Germany (11 Lander)
56451	England & Wales
861	Northern Ireland
3175	The Netherlands
5861	Scotland
6521	Belgium

Country Finland

Population

Date of Population

Date of Data *1995*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		2198	44.6	
	Fall	598		
	Spring	305		
	Summer	1024		
	Winter	270		

Date of Data *1996*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		2629	52.3	
	Fall	658		
	Spring	505		
	Summer	1106		
	Winter	360		

Date of Data *1997*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		2402	47	
	Fall	560		

	Spring	493		
	Summer	985		
	Winter	366		

Date of Data *1998*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		2851	56	
	Fall	816		
	Spring	448		
	Summer	1172		
	Winter	415		

Date of Data *1999*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		3303	364	
	Fall	778		
	Spring	514		
	Summer	1454		
	Winter	557		
< 1yr		19		
1-4 yrs		98		
15-24 yrs		447		
25-44 yrs		1452		
45-64 yrs		883		
5-14 yrs		156		
65+		250		

Date of Data *2000*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		3527	68.2	
	Fall	806		
	Spring	680		
	Summer	1418		
	Winter	623		

Country Greece

Population **10,500,000**
Date of Population **1998**

Date of Data *1998*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		136	1.3	

Country Hungary

Population
Date of Population

Date of Data *1998*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		7941		

Population **10,100,000**
Date of Population **1998**

Date of Data *1993-1998*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		373		

Date of Data *1996*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		41		

Date of Data *1997*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		75		

Date of Data *1998*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		205		

Country Netherlands

Population

Date of Population

Date of Data *1996-1999*

Age	***Season***	***Cases***	***Incidence***	***Other info***
	Summer	5700 per year		36 incidence higher in 0-4 yrs & 15-29 yrs; incidence in rural areas half that of urban.

Population **15,800,000**

Date of Population **1999**

Date of Data *1995*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		2871	18.2	start surveillance in April

Date of Data *1996*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		3741	23.7	

Date of Data *1997*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		3646	23.1	

Date of Data *1998*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		3398	21.5	

Country New Zealand

Population

Date of Population

Date of Data *1996*

Age	***Season***	***Cases***	***Incidence***	***Other info***
			210.8	

Date of Data *1997*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		8848	244.5	case-fatality ratio = 0.02% (2/8848); hospitalization rate = 5.0% (319/6440)
<1 yr		251	458.8	
1-4 yrs		1004	446.5	
10-14 yrs		400	151.4	
15-19 yrs		568	215.9	

20-29 yrs		2182	400.4	
30-39 yrs		1437	248.3	
40-49 yrs		1041	209.8	
5-9 yrs		394	136.7	
50-59 yrs		758	219.5	
60-69 yrs		421	156.9	
70+ yrs		376	129.8	

Date of Data *1999*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		7147		

Date of Data *Jan-Nov 2000*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		7497	235.3	

Date of Data *Nov 1999*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		818		

Date of Data *Nov 2000*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		770		also # & incidence by health district

Country Norway

Population

Date of Population

Date of Data *1997*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		1174		

Date of Data *1998*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		1700		

Date of Data *1999*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		2027	45.2	1099 cases (54% of total cases) were known to be imported
<1 yr		33		
1-4 yrs		197		
15-24 yrs		326		
15-24 yrs		139		

	25-44 yrs	739		
	45-64 yrs	458		
	65+ yrs	135		

Population **4,400,000**

Date of Population **1998**

Date of Data *1993*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		877	20.4	

Date of Data *1994*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		1050	24.4	

Date of Data *1995*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		1046	24.3	

Date of Data *1996*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		1145	26.6	

Date of Data *1997*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		1178	27.4	

Date of Data *1998*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		1700	39.5	

Population

Date of Population

Date of Data

Age	***Season***	***Cases***	***Incidence***	***Other info***
				Res. Study

Country Sweden

Population **8,861,000**

Date of Population **1999**

Date of Data *1993*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		2		waterborne outbreak cases
		39		foodborne outbreak cases

Date of Data *1993-1998*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		5941		waterborne outbreak cases
		360		foodborne outbreak cases

Date of Data *1994*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		2900		waterborne outbreak cases
		36		foodborne outbreak cases

Date of Data *1995*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		3011		waterborne outbreak cases
		19		foodborne outbreak cases

Date of Data *1996*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		2		waterborne outbreak cases
		143		foodborne outbreak cases

Date of Data *1997*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		19		waterborne outbreak cases
		96		foodborne outbreak cases

Date of Data *1998*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		27		foodborne outbreak cases
		7		waterborne outbreak cases

Population

Date of Population

Date of Data

Age	*Season*	*Cases*	*Incidence*	*Other info*
				Res. Study

Population

Date of Population

Date of Data

Age	*Season*	*Cases*	*Incidence*	*Other info*
				Res. Study

Country Switzerland

Population

Date of Population

Date of Data *Feb -Dec 1991*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		167		risk factors listed

Population

Date of Population

Date of Data *1993*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		5001		

Population **7,130,000**

Date of Population **1994**

Date of Data *1988*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		3171		

Date of Data *1989*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		3904		

Date of Data *1990*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		4137		

Date of Data *1991*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		3605		

Date of Data *1992*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		4098		

Date of Data *1993*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		5058	72.4	

Date of Data *1994*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		4931	70.1	

Date of Data *1995*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		5044	71.2	

Date of Data *1996*

Age	***Season***	***Cases***	***Incidence***	***Other info***
		5656	79.6	

Date of Data *1997*

Age	***Season***	***Cases***	***Incidence***	***Other info***

		5955	83.7	

Date of Data *1998*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		5455	76.5	

Country USA

Population

Date of Population

Date of Data

Age	*Season*	*Cases*	*Incidence*	*Other info*
				Res. Study

Population

Date of Population

Date of Data *1978-1985*

Age	*Season*	*Cases*	*Incidence*	*Other info*
		4983		waterborne outbreak cases
		1308		foodborne outbreak cases
		150		travel associated outbreak cases
		6441		all outbreak cases

Population

Date of Population

Date of Data

Age	*Season*	*Cases*	*Incidence*	*Other info*
				Res. Study

Population

Date of Population

Date of Data

Age	*Season*	*Cases*	*Incidence*	*Other info*
				Res. Study

Population

Date of Population

Date of Data

Age	*Season*	*Cases*	*Incidence*	*Other info*
				Res. Study

Population

Date of Population

Date of Data

Age	Season	Cases	Incidence	Other info
				Res. Study

Population
Date of Population
Date of Data

Age	Season	Cases	Incidence	Other info
				Res. Study

Population
Date of Population
Date of Data

Age	Season	Cases	Incidence	Other info
				Res. Study

Population
Date of Population
Date of Data

Age	Season	Cases	Incidence	Other info
				Res. Study

Population
Date of Population
Date of Data

Age	Season	Cases	Incidence	Other info
				Res. Study

Country Wales

Population
Date of Population
Date of Data

Age	Season	Cases	Incidence	Other info
				Res. Study

Country World

Population
Date of Population
Date of Data

Age	Season	Cases	Incidence	Other info
				Res. Study

Population
Date of Population
Date of Data

Age	*Season*	*Cases*	*Incidence*	*Other info*
				Res. Study

Population

Date of Population

Date of Data *1999*

Age	*Season*	*Cases*	*Incidence*	*Other info*
			26	Ireland
			70	Germany (parts of)
			23	The Netherlands
			47	Finland
			50	Denmark
			21	Austria
			108	Scotland
			96	England & Wales
			47	Northern Ireland
			60	Sweden