参考資料7

RISK ASSESSMENT ON *CAMPYLOBACTER JEJUNI* IN CHICKEN PRODUCTS

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SUMMARY

A first draft of a quantitative risk assessment on *Campylobacter jejuni* in chicken products in Denmark has been prepared. The risk assessment was ordered by the Danish Veterinary and Food Administration as part of a strategy to control pathogenic micro-organisms after the principles for Food Safety Risk Analysis. The risk assessment comprises the elements hazard identification, exposure assessment, hazard characterization, and risk characterization. To quantify the risk, two models have been developed, one describing the transfer and spread of *Campylobacter* through a chicken slaughterhouse and another dealing with the transfer and spread of *Campylobacter* during food handling in private kitchens. In areas where no Danish data were available, data from investigations in other countries have been used to generate input distributions.

The work has shown that it is realistic to expect that at least a fraction of the human exposure to *Campylobacter* originate from *Campylobacter* in chickens. The outcome of the risk modelling reveals that important factors for human exposure to *Campylobacter* are the broiler flock prevalence and hence, the prevalence in retail chickens, the *Campylobacter* concentration on positive products, and the extent of cross-contamination in private kitchens during food handling. Further, the model indicates that the prevalence of the *Campylobacter* positive chickens is not changed significantly during the slaughter processes assuming that the degree of cross-contamination is relatively low. However, the concentration of *Campylobacter* on the positive chickens will decrease. The concentration of *Campylobacter* on the positive chickens is important in relation to the risk of becoming infected.

In order to outline risk management options, three distinct ways of reducing the probability of exposure and illness were analysed: i) by reducing the prevalence of *Campylobacter* positive flocks; ii) by reducing the concentration of *Campylobacter* on the contaminated chickens; or iii) by improving the relative level of hygiene during food handling in private kitchens. The simulations showed that altering the broiler prevalence, the *Campylobacter* concentration on positive chickens, and the level of food hygiene could reduce the probability of getting ill from *Campylobacter* in chickens. To obtain a reduction in human cases by, for example, a factor 25, the flock prevalence should be reduced by a factor 25. A similar reduction in the number of human cases could be obtained by reducing the concentration of *Campylobacter* on the contaminated chickens by a factor 100 (2 log cfu pr g), or by improving the level of food hygiene in private kitchens by a factor 25.

As this report is the first draft of a quantitative risk assessment on *Campylobacter jejuni* in chicken products of Danish origin, it should be kept in mind that the quality and quantity of the data and the modelling tool used is a subject to ongoing improvement. Likewise it should be stressed that the quantitative risk assessment procedure is not a static document as data, assumptions and the models actually used may be changed as new informations are available.

DANSK SAMMENDRAG (DANISH SUMMARY)

Denne rapport er første udgave af en kvantitativ risikovurdering vedrørende *Campylobacter jejuni* i slagtekyllinger i Danmark. Risikovurderingen er bestilt af Fødevaredirektoratet som led i en risikoanalyse vedrørende sygdomsfremkaldende *Campylobacter* i fødevarer udført efter principperne skitseret i WHO/FAO's rapport: "Application of Risk Analysis to Food Standard Issues" fra 1995. Risikovurderingen indeholder elementerne i) identificering af sundhedsfare, ii) eksponerings vurdering, iii) karakteristik af sundhedsfare og iv) risiko karakteristik. Med henblik på at kvantificere sundhedsfaren er der udviklet to modeller, der beskriver dels overførsel og spredning af *Campylobacter* gennem et kyllingeslagteri og dels overførsel og spredning af *Campylobacter* gennem håndtering af fødevarer i private køkkener. På områder, hvor danske data ikke har været tilgængelige, er der anvendt data fra udenlandske undersøgelser til generering af fordelinger brugt i den matematiske model.

Resultaterne viser, at det er realistisk at antage, at i det mindste en del af den humane eksponering for *Campylobacter* kan tilskrives *Campylobacter* i slagtekyllinger. Resultaterne fra risikovurderingen viser endvidere, at vigtige faktorer for human eksponering for *Campylobacter* fra kyllinger kan være prævalensen af kyllingeflokke og dermed også prævalensen af kyllingeprodukter solgt i detailleddet, koncentrationen af *Campylobacter* på kontaminerede produkter samt graden af krydskontamination i private køkkener. Resultaterne viser ligeledes, at prævalensen af *Campylobacter* kontaminerede kyllinger ikke synes at ændres væsentligt gennem slagteprocessen, forudsat at graden af krydskontamination er relativt lav. Derimod tyder resultaterne på, at koncentrationen af *Campylobacter* på de enkelte kyllinger vil reduceres under slagteprocessen.

Med henblik på at udpege håndteringsmuligheder er tre forskellige måder at reducere sandsynligheden for eksponering og sygdom blevet analyseret nemlig; i) ved at reducere prævalensen af *Campylobacter* positive flokke; ii) ved at reducere *Campylobacter* koncentrationen på kyllinger og iii) ved at forbedre niveauet af køkkenhygiejne i private køkkener. Simuleringerne viser, at ved at ændre prævalensen blandt kyllingeflokkene, koncentrationen på kontaminerede kyllingeprodukter eller niveauet af køkkenhygiejne kan sandsynligheden for at blive inficeret med *Campylobacter* fra kyllinger reduceres. For at opnå en reduktion i sandsynligheden for sygdom som følge af kyllinger på f.eks. 25 gange skal flokprævalensen reduceres med en faktor 25. En tilsvarende reduktion kan opnås, hvis koncentrationen af *Campylobacter* på de kontaminerede kyllinger reduceres med en faktor 100 (2 log cfu pr g) eller hvis hygiejneniveauet ved håndtering af kylling i private hjem forbedres med en faktor 25.

Da denne rapport er et første udkast af en kvantitativ risikovurdering for *Campylobacter jejuni* i danske slagtekyllinger skal opmærksomheden henledes på, at kvaliteten og mængden af data samt de anvendte modeller er under stadig udvikling. Det er ligeledes vigtigt at understrege, at den kvantitative risikovurdering ikke er et statisk papir, idet data, forudsætninger og anvendte modeller kan ændres når ny viden bliver tilgængelig.

CONTENTS

INTRODUCTION

IMPLEMENTING THE FOOD SAFETY RISK ANALYSIS IN DENMARK

During the 1990s many countries, including Denmark, have experienced an increase in the number of registered cases of human enteric infections caused mainly by *Salmonella* and *Campylobacter* spp. (Anon., 1998b). In addition, several countries in Europe have experienced an increase in the number of cases caused by *E. coli* O157 (WHO, 1997b). Due to this development the Danish Veterinary and Food Administration decided to initiate a strategy for the control of pathogenic microorganisms in foods in 1997 (Anon., 1999b). In this paper it is established that significant pathogenic microorganisms in foods in Denmark should be handled by the principles for Food Safety Risk Analyses currently being established in different international fora e.g. the World Health Organization, the Food and Agriculture Organization, the Codex Alimentarius Commission and the European Commission.

Ranking Hazards

As stated by the Codex Alimentarius Commission the Risk Management part includes - at a regional or national level - a ranking of the most important hazards (CAC, 1999a). The ranking procedure carried out by the Danish Veterinary and Food Administration states that special attention should be given to *Campylobacter* species, *E. coli* O157 and *Salmonella* as a consequence of the actual number of registered cases of *Campylobacter* and *Salmonella* infections and the potential threat by *E. coli* O157 to human health.

Risk Profile

In 1998 it was decided to initiate the Risk Management procedure on *Campylobacter* and *E. coli* O157 by elaborating Risk Profiles describing the food safety problems related to these organisms (CAC, 1999a). The Risk Profile on *Campylobacter* was prepared by the Danish Veterinary and Food Administration in corporation with the Danish Zoonosis Center, the Danish Veterinary Laboratory, Statens Serum Institut, the Danish Meat Research Institute, Danpo A/S, the Danish Environment Protection Agency, and the Danish Consumer Council in order to ensure transparency and communication between stakeholders (CAC, 1999a). The Risk Profile on *Campylobacter* was finished in September 1998 and published on the Internet (www.fdir.dk/publikationer).

Risk Assessment

The Risk Profile regarding *Campylobacter* recommends the Risk Management procedure to be continued by ordering a formal Risk Assessment carried out according to the principles stated by the Codex Alimentarius Commission (CAC, 1999b). In commissioning the Risk Assessment (CAC, 1999a), risk managers and risk assessors

agreed that the initial phase of the Risk Assessment should focus on *Campylobacter jejuni* in chicken products. This was because the prevalence of *Campylobacter* in retail chicken products is high as compared to other food items, and because case-control studies have revealed that consuming and handling chicken seems to be an important risk factor. Additionally, chickens are the most extensively described food item through data from literature and ongoing surveillance programs. It was decided to include other food and environmental items when sufficient data related to these areas have been generated. The responsibility for the Risk Assessment procedure regarding *Campylobacter* in Denmark has been placed in The Division of Microbiological Safety at the Institute of Food Safety and Toxicology, the Danish Veterinary and Food Administration.

Objective

The objective of the present report is to describe a quantitative risk assessment model for *Campylobacter jejuni* in chicken products based on the principles stated by the Codex Alimentarius Commission (CAC, 1999b). The report provides the risk managers with information on the spread of *Campylobacter* from 'slaughterhouse to consumer' and the relative importance of different critical control points at the production, retail and consumer level. The quantitative risk assessment model also reveals areas where it will be necessary to improve and optimise sampling plans and analytical methods in the future. The report is structured as recommended by the Codex Committee on Food Hygiene (CAC, 1999b) and the data included are based on surveillance programs established in Denmark as well as information from the international literature in areas where no Danish data are available.

The present report is the first draft version of a risk assessment on *Campylobacter jejuni* in chicken products. The report will be reviewed when additional information is generated.

THE FOOD SAFETY RISK ANALYSIS

The Food Safety Risk Analysis used as a tool for control of biological hazards in foods is becoming internationally accepted. The context was described in a report from the FAO/WHO in 1995 (FAO/WHO, 1995). This report states that 'It should be the role of official bodies to use risk analysis to determine realistic and achievable risk levels for food-borne hazards and to base food safety policies on the practical application of the results of these analyses'. Further on, with the implementation of the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS agreement) an international trade agreement for the first time explicitly recognises that for establishment of rational harmonised regulations and standards for food in international trade a rigorous scientific process is required. Finally, elements of the Food Safety Risk Analysis e.g. Risk Assessment and Risk Management should form the basis when governmental agencies and the industry are establishing Food Safety Objectives (ICMSF, 1999).

As stated by the FAO/WHO the Risk Analysis procedure consists of three components, namely Risk Management, Risk Assessment and Risk Communication (FAO/WHO,

1995; FAO/WHO 1997). According to the Codex Committee on Food Hygiene, the Risk Management procedure is defined as ‘a process, distinct from risk assessment, of weighing policy alternatives in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options’ (CAC, 1999a).

The procedure for Risk Assessment is described by the Codex Committee on Food Hygiene as ‘a scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterisation, (iii) exposure assessment, and (iv) risk characterisation (CAC, 1999b). Finally, the definition of Risk Communication has been discussed at a FAO/WHO expert consultation in 1998 (FAO/WHO, 1998). This FAO/WHO expert consultation recommends that the definition should be ‘Risk communication is the exchange of information and opinions concerning risk and risk related factors among risk assessors, risk managers, consumers and other interested parties’.

The implementation of Food Safety Risk Analysis in relation to biological hazards is still in its infancy and existing definitions and procedures covering the elements of risk management, risk assessment and risk communication still have to prove useful and sufficient in practice and could be the subject for further development.

HAZARD IDENTIFICATION

In the 1970s, with the development of suitable selective media, it was established that *Campylobacter jejuni* and to a lesser extent *Campylobacter coli* were a major cause of diarrhoeal illness (Skirrow, 1977). *Campylobacter* is now rivalling and even surpassing Salmonella in importance in many countries. In 1997 the incidence rate of *Campylobacter* had exceeded that of *Salmonella* in Spain, Sweden, The Netherlands, Scotland, Northern Ireland, and England and Wales (Anon., 1999c). In Denmark, *Campylobacter* became the most frequent cause of human enteric infections in 1999 with more than 4000 registered cases (Fig. 1).

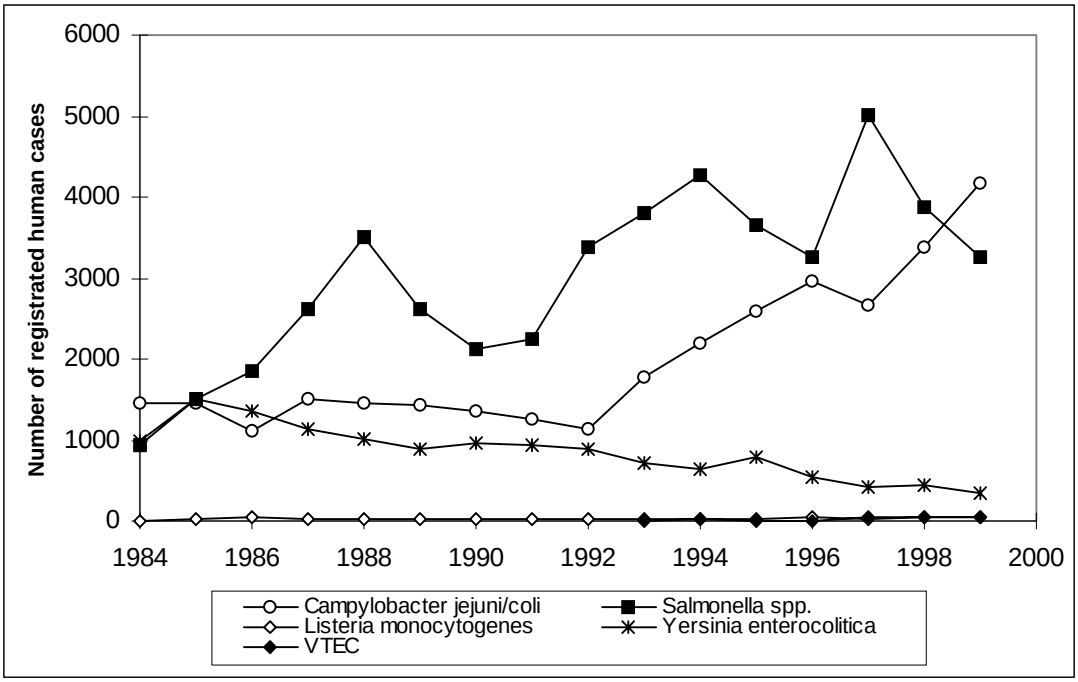

Figure 1. The number of registered human cases in Denmark caused by the enteric pathogens *Campylobacter jejuni/coli*, *Salmonella* spp., *Yersinia enterocolitica*, *Listeria monocytogenes* and verotoxin producing *Escherichia coli* (VTEC) (Anon., 1994; 1995; 1996; 1997a; 1998b; Anon., 1999a)

The incidence rates of *Campylobacter* infections in EU Member States vary widely (from 9.5 in Spain up to 108 per 100,000 inhabitants in Scotland in 1997) (Fig.2). This is probably due to differences in surveillance systems, diagnostic methods and way of reporting. Therefore, the data from the Member States should not be compared directly.

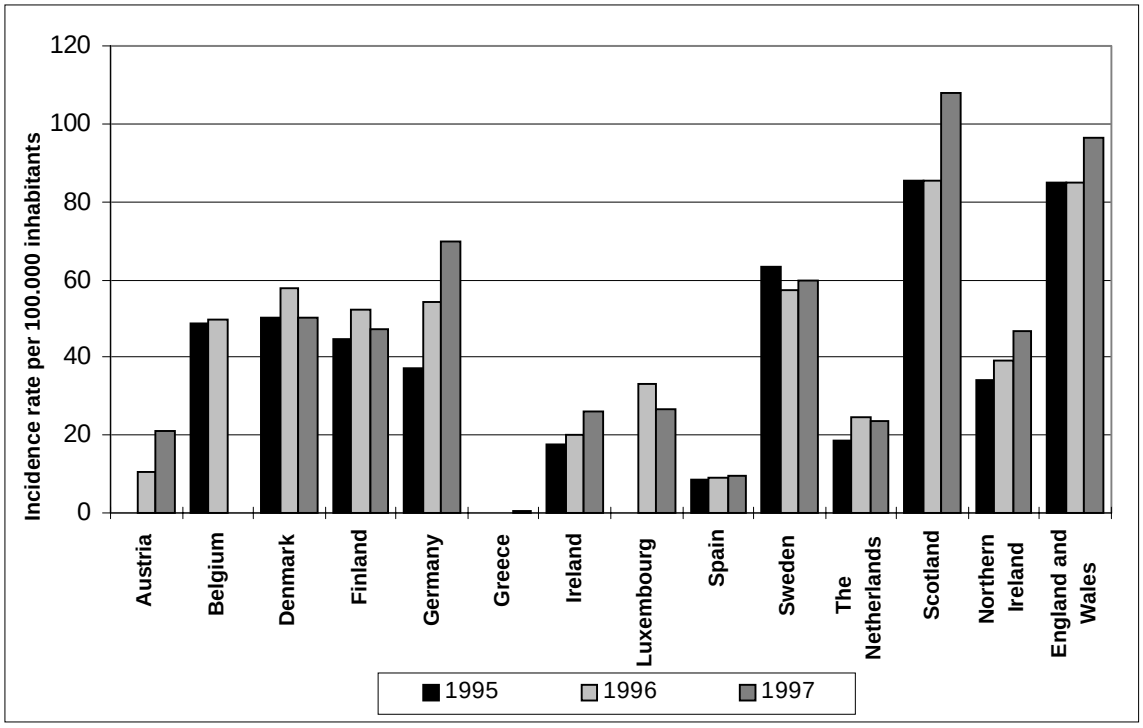

Figure 2. Campylobacteriosis in humans in twelve EU member states. Incidence rate per 100,000 inhabitants (Anon., 1999c)

Fig. 1 and Fig. 2 reflect the laboratory confirmed cases of *Campylobacter* infections, cases where the patients have consulted a general practising 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). This means that 30,000-400,000 people in Denmark may have had a *Campylobacter* infection in 1999 corresponding to a 'true' incidence rate of 566-7550 cases per 100,000 inhabitants.

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, lysed 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 to a lesser extent *C. coli* are the species most often encountered in medical laboratories as causes of acute enterocolitis in man (Nielsen *et al.*, 1997; Wooldridge & Ketley, 1997; Anon., 1999c). They 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).

RESERVOIR

The principal reservoir of pathogenic *Campylobacter* spp. is the alimentary tract of wild and domesticated mammalians and birds. The prevalence of *Campylobacter* in these animals and birds as reported for 1997 by the Member States (Anon., 1999c) is listed in Table 1. From these data it is evident that *Campylobacter* is commonly found in broilers, cattle, pigs, sheep, wild animals and birds, and in dogs. 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*.

The prevalences of *Campylobacter* in Danish broilers and ducks are seen in Fig. 3. The data represent all flocks slaughtered in Denmark in 1998 and 1999. A distinct seasonal variation is seen for broilers, with around 30% positive flocks in winter and around 70% positive flocks in summer. Turkey flocks have not been examined until September 1999. In this month the prevalence was 70%. In December the prevalence had decreased to 50% (Danish Veterinary Laboratory, unpublished data). These data may indicate that the prevalence in turkey flocks is dependent on season like the prevalence in broiler flocks.

In 1998 the prevalences of *Campylobacter* in Danish cattle and pigs were 51% and 59%, respectively (Table 1). In 1999 the prevalences were 50% in cattle and 54% in pigs (Anon., 1999a). The prevalences in cattle and pigs are estimated on basis of one faecal sample (from one animal) per herd at slaughtering.

Table 1 Prevalence of *Campylobacter* in domesticated and wild animals and birds in 1997 in EU (mod. after Anon., 1999c)

Source	Country	Prevalence ** (%)	No. of units investigated	Unit	Dominating serotypes
POULTRY, FOWL					
fowl, all	D	47.1	17	farms	
fowl, all	D	5.0	334	animals	jejuni (41%), coli (18%)
poultry, all	I	9.9	71	animals	
BROILER					
broiler	D	< 0.3	343	animals	
broiler, at slaughter	DK	37.0	1037	samples	jejuni (76%), coli (14%)
broiler	NL	44.7*	47	animals	
broiler, at slaughter	S	9.8	3641	farms	
CATTLE					
cattle	D	10.2	10051	animals	
cattle, dairy	D	< 1.4	74	farms	
cattle, dairy	D	< 0.5	217	animals	
cattle	D	0.3	287	farms	
cattle, at slaughter	DK	51.0	96	1 animal/herd	jejuni (96%), coli (2%)
cattle, bulls	FIN	< 0.3	367	animals	
cattle	I	52.7*	55	animals	
cattle	I	< 6	17	animals	
dairy	I	< 0.4*	269	animals	
cattle	L	50.0	40	animals	
cattle	NL	1.4	141	animals	
cattle	P	1.1*	91	animals	
PIGS					
pigs	D	0.5*	196	farms	
pigs	D	8.0	1629	animals	coli (40%), jejuni (1%)
pigs, at slaughter	DK	59.0	319	1 animal/herd	coli (95%), jejuni (3%)
pigs	I	13.1*	61	farms	
SHEEP AND GOATS					
goats	D	< 4	28	animals	
sheep	D	6.0	117	animals	jejuni (14%), coli (14%)
sheep	FIN	< 0.8	125	animals	
sheep	I	0.9*	891	animals	jejuni (38%)
goats	I	< 7	16	animals	
sheep	NL	< 2	41	animals	
sheep	P	< 7	15	samples	
SOLIPEDS					
solipeds	D	1.0	1488	animals	
solipeds	NL	< 0.1	823	animals	
WILDLIFE					
wildlife	DK	8	232	animals	
deer	DK	< 4	24	animals	
european hare	DK	3	38	animals	
red fox	DK	14	29	animals	
birds, other	DK	12	25	animals	
water birds	DK	14	16	animals	
marine mammals	DK	55	11	animals	
mammals	DK	6	180	animals	
OTHER ANIMALS					
dogs	D	2.2	1472	animals	jejuni (73%)
dogs	FIN	12.0	100	animals	
dogs	I	4.4	46	animals	
dogs	NL	17.1	82	animals	
cats	D	0.4	751	animals	jejuni (100%)
cats	NL	0.4	533	animals	
reptiles	NL	< 3	30	animals	
birds	NL	< 0.2	468	animals	

* thermophilic *Campylobacter*, ** <p, no positive samples were found, p = prevalence of positive samples

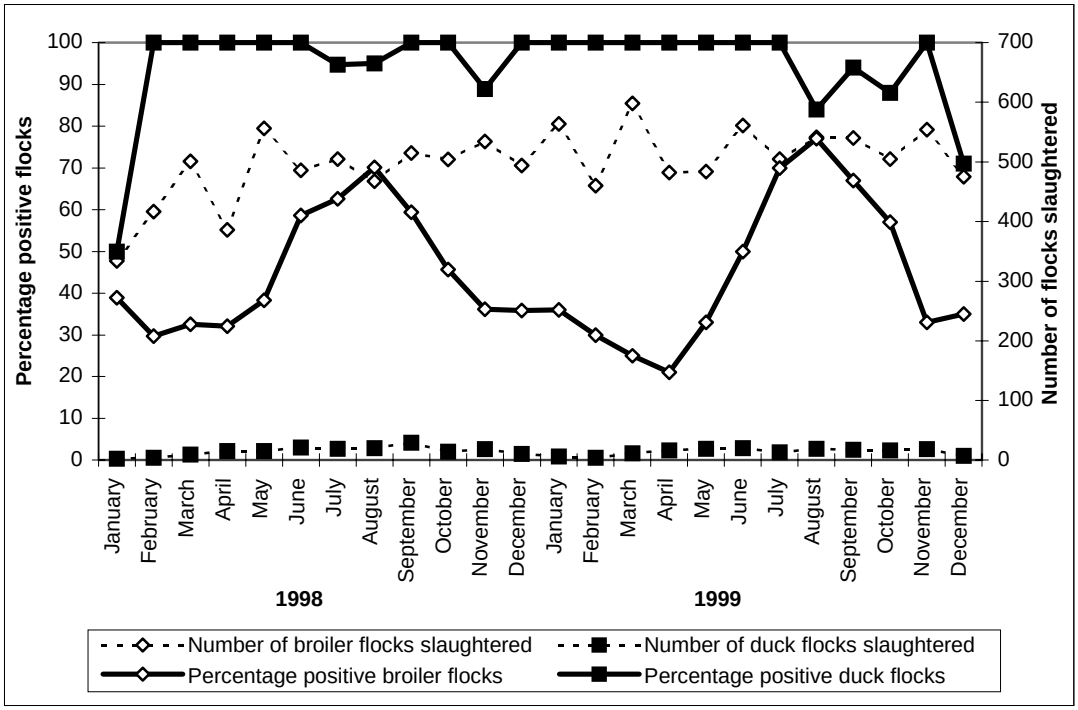

Figure 3. The prevalences of *Campylobacter* in Danish broilers and ducks. The data are generated on the basis of one pooled sample comprising 10 cloacal svabs taken from each flock at the entrance to the slaughterhouse. The data represent all slaughtered flocks in Denmark in 1998 and 1999 (Danish Veterinary Laboratory, unpublished data).

C. jejuni and *C. coli* seem to have a favoured reservoir. *C. jejuni* is predominantly associated with poultry (Tauxe, 1992), but have also been isolated from cattle, sheep, goats, dogs and cats (Nielsen *et al.*, 1997; Anon., 1999c). *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., 1999c). In a Norwegian survey, 100 percent of the pigs examined were contaminated with *C. coli* (Rosef *et al.*, 1983). In a Danish investigation of faeces from 600 pigs, 94.7% of the animals were contaminated with *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.*, 1999). This means that *Campylobacter* may be present in untreated drinking water and bathing water. Unfortunately, the occurrence of *Campylobacter* in water in Denmark has not yet been surveyed. *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 nonculturable 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 nonculturable state'. The importance of this 'state' in transmission of *Campylobacter* to animals and man is not agreed upon. The question is if the viable nonculturable organisms are still virulent or if they can reverse into a culturable, virulent state after passage through a host. In some studies 'viable but nonculturable' *Campylobacter* organisms have shown to regain culturability after passage through for example chicks (Stern *et al.*, 1994), mice (Jones *et al.*, 1991), 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 nonculturable' *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 nonculturable' *Campylobacter* on human health is not dealt with in the present risk assessment, as their role in the food chain is unknown.

EXPOSURE ASSESSMENT

MICROBIAL ECOLOGY

Behaviour during processing

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. This decline is primary a consequence of the dehydration that takes place during cooling with forced ventilation procedures (Oosterom *et al.*, 1983b). In 1995 a Danish investigation of 600 pig carcasses showed that the chilling procedure reduced the prevalence of *Campylobacter* on the carcass surfaces from 43-85% to 11-18%) (Sørensen & Christensen, 1996).
Contrary to the processing of cattle and pigs, broiler processing does not tend to reduce the *Campylobacter* prevalence significantly. Scalding, plugging, cooling, freezing and subsequent storage do not eliminate the organism only reduce the concentration (Oosterom *et al.*, 1983a; 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. The occurrence of *Campylobacter* in broiler processing is described in more details later in this report (see the section on page 43).

Generel growth and survival characteristics

The general growth characteristics of *Campylobacter* are seen in Table 2. From this it appears that *Campylobacter* grows at 37°C, but not below 32°C, i.e. in general *Campylobacter* does 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 3). Though *Campylobacter* may persist for prolonged periods in chilled and frozen products, a reduction in the concentration (Table 3) and a decline in the viability are observed during storage. *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 pasteurization temperatures (D-values are 0.21-2.25 minutes at 55-60°C) (ICMSF, 1996) (see also Table 4).

Exposed to chemical or physical stress conditions *Campylobacter* has shown to revert to a ‘viable but nonculturably’ state where the organism cannot be isolated by cultural methods but remains active (infective). Evidence for this is conflicting. Some studies have shown that viable not-culturable strains can revert to a culturable state by passage through an animal host. Other studies have not been able to confirm this finding (see

also the section on page 6). The occurrence and influence on human infection of viable not-culturable *Campylobacter* in food is not known and has to be investigated.

Table 2. 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 3. 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.*, 1983b
Chicken liver	-20	-1/'few'	-1.6/84	NF	Oosterom *et al.*, 1983b
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.*, 1983b
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 4. 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	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*	3	RT	Blankenship & Kraven, 1982
Cooked chicken	55	2.12-2.25	-	6*	3	RT	Blankenship & Kraven, 1982
Cooked chicken	57	0.79-0.98	-	6*	3	RT	Blankenship & Kraven, 1982

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

CAMPYLOBACTER IN FOOD

The incidence of *Campylobacter* in food at retail in the EU in 1997 is seen in Table 5. This table shows that especially poultry meat is contaminated with *Campylobacter* (prevalences up to 85.7%). At low frequencies, *Campylobacter* has also been found in beef, pork, other meat products, raw milk and milk products, and in fish and fish products. In 1996, also oysters and mussels were found to contain *Campylobacter* at a prevalence of 11% and 58%, respectively (Anon., 1998a). 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).

Prevalence in retail products

In Denmark the prevalences of thermophilic *Campylobacter* in retail poultry products have been surveyed since 1995. The results concerning chicken and turkey products are presented in Fig. 4. From 1995 to 1999 the prevalences of thermophilic *Campylobacter* have decreased in both Danish and imported products. As regards chicken products, the prevalences seem to be slightly higher in imported products than in Danish products. In Danish and imported turkey products the prevalences are similar. The influence by season on the prevalence of *Campylobacter*, which is seen in Danish broiler flocks, is reflected in chilled retail chicken products of Danish origin (Fig. 5), the prevalences being higher in summer than in winter (see also Rosenquist & Nielsen, 1999). The prevalence of thermophilic *Campylobacter* tends to be higher in chilled chicken products than in frozen products, and this is the case for both Danish and imported products except from Danish products sampled in the low prevalent winter period. The difference in prevalence between chilled and frozen Danish products was 7% in the summer period 1998 and 38% in the summer period 1999. As regards imported chicken products the difference was 24% in 1998 and 20% in 1999.

Table 5. Prevalence of *Campylobacter* in food in EU in 1997 (mod. after Anon., 1999c)

Food item	Country	Prevalence ** (%)	Number of samples	Dominating serotypes
MEAT				
meat except poultry meat	D	< 0.3	286	
Meat	I	< 5	22	
BEEF				
Beef	I	< 7	15	
at retail, not heat treated	DK	0.7	516	
Beef	S	< 1	100	
Beef	UK (N.IR.)	15.0*	320	jejuni (60%), coli (19%)
PORK				
Pork	D	< 0.6	165	
at retail, not heat treated	DK	1.0	433	
Pork	I	< 8	13	
Pork	S	< 1	97	
OTHER MEAT				
wild game	D	< 10	10	
different types of food; beef, pork and broiler	S	1.51	529	
MINCED MEAT AND PREPARATIONS				
minced meat and meat preparations	A	< 3	37	
meat preparation, raw material	D	< 0.4	254	
meat preparation	I	< 1	99	
MEAT PRODUCTS				
meat products, heat treated	D	< 1	103	
meat products, treated other than heat	D	< 2	61	
meat products	P	6.0*	67	coli
meat products, dried and fermented	UK (E&W)	< 0.2	455	
POULTRY MEAT				
poultry meat	A	10.5*	19	
poultry meat ready for consumption	A	14.3	14	jejuni
poultry meat	D	20.1	812	jejuni (75%), coli (21%)
poultry meat products	D	2.5	40	jejuni
poultry meat, at retail, not heat treated	DK	33.0	676	
broiler cuts, at retail	F	10.5	114	
poultry meat	I	1.9*	52	
poultry meat, at retail	I	< 8	12	
poultry meat, chilled, fresh, at retail	NL	31.7	1314	
poultry meat ready for consumption	P	85.7*	28	jejuni (50%), coli (50%)
swabs of poultry carcasses	P	73.3*	60	jejuni (52%), coli (48%)
poultry meat at retail	P	84.2*	19	jejuni (38%), coli (62%)
EGGS				
eggs	A	< 8	12	
MILK				
raw	A	< 1.4	73	
raw, at farm	D	1	257	jejuni
raw, certified	D	< 0.2	542	
raw	I	< 5	19	
pasteurized	D	< 4	23	
UHT/sterilized	D	< 8	12	
MILK PRODUCTS				
milk products	A	< 2	49	
milk products	D	1	89	jejuni
raw milk products	D	< 1.4	74	
FISH AND PRODUCTS				
fish and products	D	1.1	90	jejuni

* thermophilic *Campylobacter;* N.IR. = Northern Ireland, E&W = England and Wales; ** < p, no positive samples were found, p = prevalence of positive samples

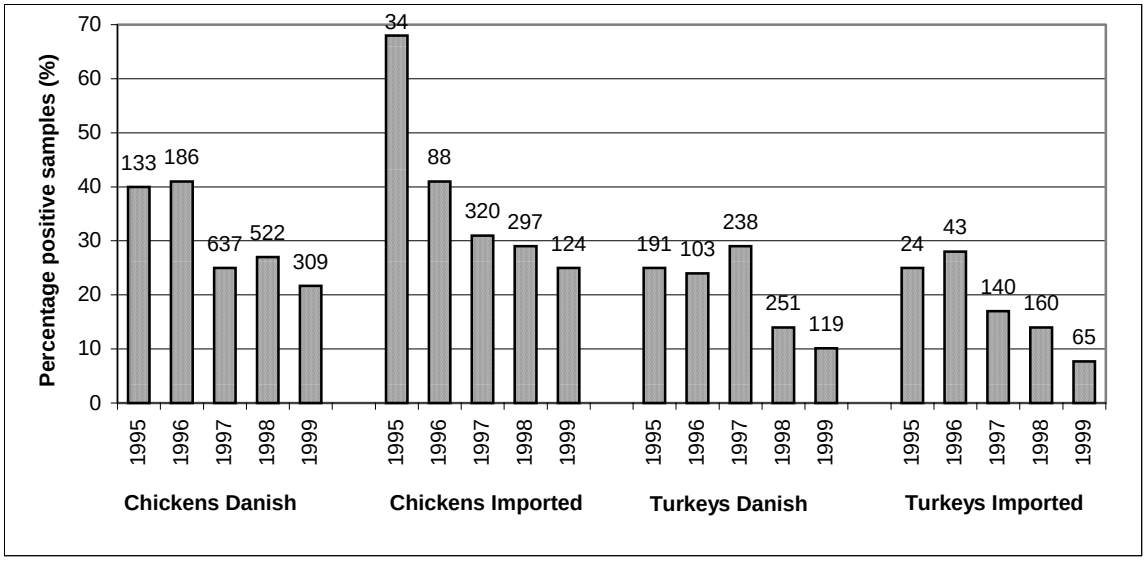

Figure 4. The prevalence of thermophilic *Campylobacter* in Danish and imported chicken and turkey products sampled at retail level 1995-1999. The numbers above the bars are the numbers of samples examined (Danish Veterinary and Food Administration, unpublished data).

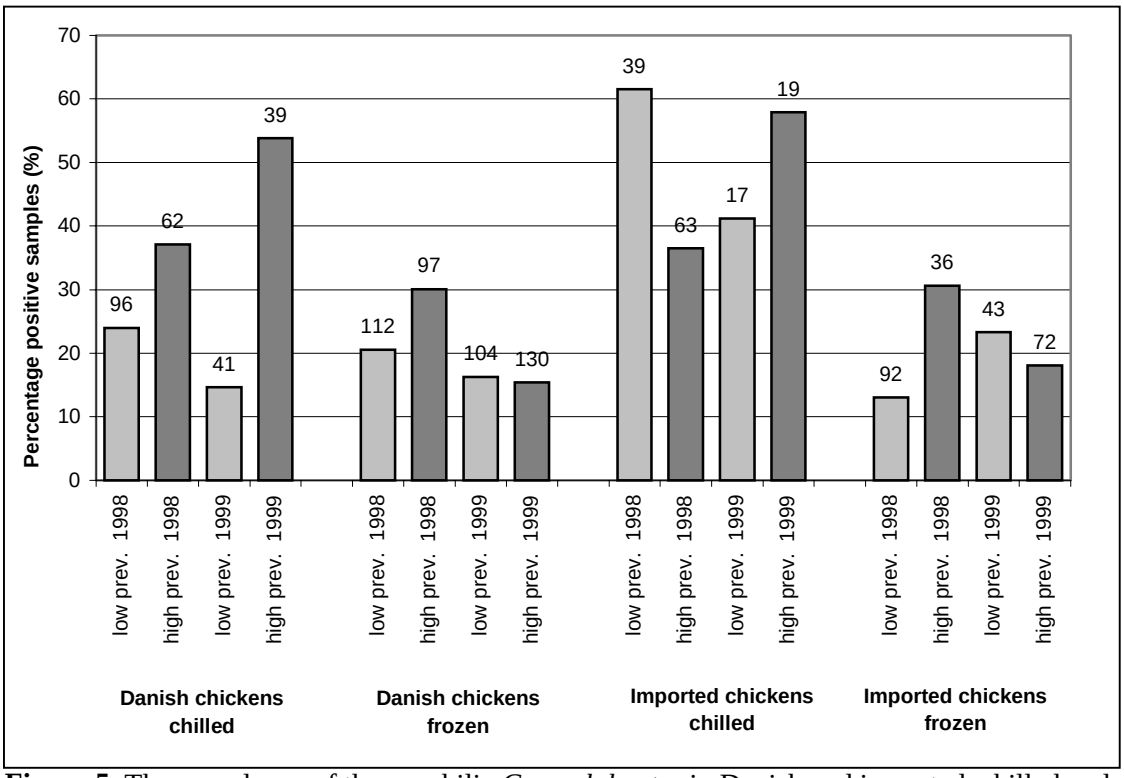

Figure 5. The prevalence of thermophilic *Campylobacter* in Danish and imported, chilled and frozen chicken products sampled at retail level 1998-1999 (Danish Veterinary and Food Administration, unpublished data). The numbers above the bars are the numbers of samples examined. *High prev* and *low prev* refer to the high prevalent period (June, July, August, September and October) and low prevalent period (January, February, March, April, May, November and December) of *Campylobacter* in broiler flocks.

A survey in 1995-1997 showed that the *Campylobacter* prevalences in raw beef, pork and game products were 1% (N=1166), 1.2% (N=1080), and 3% (N=202), respectively. *Campylobacter* was not found in vegetables (N=154), fruit (N=138), and shellfish (N=186) (Danish Veterinary and Food Administration, unpublished data).

Concentration in retail products

In 1999, 183 samples of poultry products, mainly chilled, from the retail level were examined for the presence of thermophilic *Campylobacter* spp. according to a semi-quantitative method developed by the Danish Veterinary and Food Administration[1]. The results are illustrated in Fig. 6. Of the 183 samples 27% were found to contain less than 0.04 cfu/g, 20% contained 0.04-10 cfu/g, 18% contained 10-100 cfu/g, 23% contained 100-1000 cfu/g, 9% contained 1000-10000 cfu/g, and 3% contained more than 10000 cfu/g.

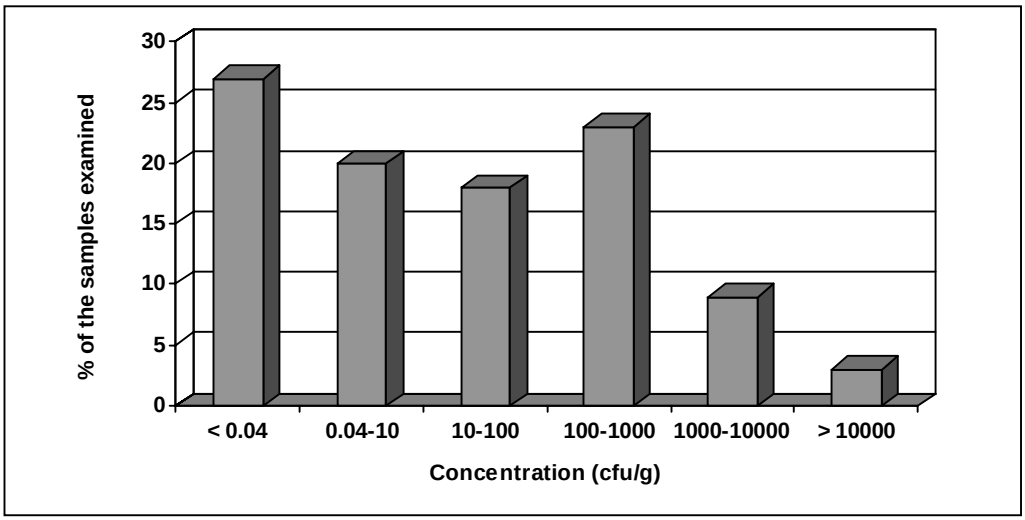

Figure 6. The concentrations of *Campylobacter* in 183 samples of poultry products sampled at retail level. The samples were analysed according to NMKL method no. 119 (1990) and a semi-quantitative method using Mueller-Hinton broth as preenrichment media and mCCDA as isolation media (The Danish Veterinary and Food Administration, unpublished results).

[1] In brief, 5 – 10 g sample material are mixed 1:9 with physiological saline solution added peptone and stomachated. Dilutions 1:10 are prepared. 1 ml from each dilution is enriched under microaerophilic conditions for 24 hours at 42 ºC in 9 ml of Mueller-Hinton bouillon supplemented with sodium pyrovate 0.25 mg/l, sodium metabisulphite 0.25 mg/l, ferro sulphate 0.25 mg/l, cefaperazone 30 mg/l, and trimethoprim lactate 50 mg/l. After preenrichment 10 µl is striked on mCCDA and further incubation is carried out under microaerophilic conditions for 24-48 hours at 42 ºC. mCCDA plates are examined for the presence of *Campylobacter*-like colonies. Suspected colonies are verified by phase-contrast microscopy, positive oxidase reaction, and hydrolysis of hippurate- and indoxyl acetate.

CONSUMPTION DATA

Consumption data are needed when estimates for the exposure of *Campylobacter* in a given food item are to be calculated.

In 1995 a survey was conducted to estimate the Danish dietary habits (Andersen *et al.*, 1996). A total of 3098 persons from 1 to 80 years old registered their daily intake for one week. The estimated average consumption per day of different food items in Denmark in 1995 appears from Table 6. This table may be used to compare daily intake between sex and age groups. In risk assessment, however, the frequency of consumption of a given product is preferred to the average consumption per day. Consumption frequencies related to chicken meals have been estimated. These are presented in the consumer model (see the section on page 81).

Table 6. Estimated average consumption per day of different food items in Denmark in 1995 (Andersen *et al.*, 1996; The Danish Veterinary and Food Administration, unpublished data)

	Male aged (years)			Female aged (years)		
Food item (g/day)	1-6	7-14	15-80	1-6	7-14	15-80
Beef meat	3.3	12.7	24.1	3.0	9.5	16.2
Pork meat	6.7	13.9	27.3	6.4	14.3	21.4
Minced meat	12.7	15.9	21.0	10.2	13.9	14.4
Lamb meat	0.6	1.4	3.3	0.7	1.1	2.1
Chicken	4.9	11.9	10.3	5.6	11.4	9.9
Turkey	1.5	2.8	3.5	1.5	1.7	3.6
Duck and geese	0.2	0.2	0.8	0.1	0.2	0.4
Fish and fish products	13	17	24	12	13	23
Milk and milk products	514	601	368	506	500	333
Cheese	14	25	33	13	23	31
Eggs	10	16	21	11	15	19
Bread and other cereals	175	234	239	155	193	186
Vegetables	126	202	255	117	198	205
Fruit	174	199	152	177	179	179

The consumption of different food items can also be calculated on basis of the registered retail sale. The sale of Danish and imported poultry products is seen in Fig. 7. From this figure it is evident, that chicken products dominate the sale of poultry and that the Danish products account for a large percentage of this sale. From 1996 to 1998 the sale of chicken parts has increased considerably.

Table 7 and Table 8 show the retail sale of Danish produced and imported chickens products from 1985 to 1999. During this period the sale of frozen whole chickens has decreased significantly, whereas the sale of chilled whole chickens has increased. Moreover, the sale of both chilled and frozen chicken parts has increased dramatically since 1996. As regards the imported frozen chicken products a peak was seen in the middle of the nineties.

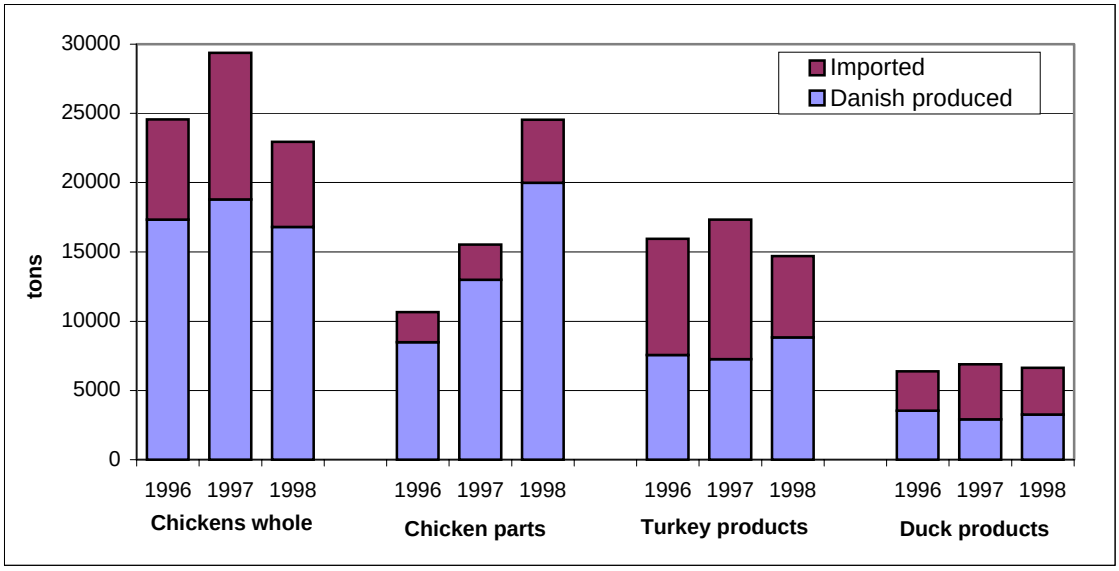

Figure 7. Development in the sale of poultry products in Denmark 1996-1998 (Statistics Denmark and the Danish Poultry Counsel, unpublished data)

Table 7. Retail sale of **Danish** produced chilled and frozen chicken products 1985-1999 (Statistics Denmark and the Danish Poultry Counsel, unpublished data)

	Retail sale of Danish produced chicken products in 1000 tons						
	1985	1989	1992	1996	1997	1998	1999
Chickens whole – chilled	2.9	4.2	3.3	4.4	4.8	4.6	5.4
Chicken parts – chilled	na	na	na	3.4	5.0	5.6	7.5
Chickens total – chilled	*na*	*na*	*na*	*7.8*	*9.8*	*10.2*	*12.9*
Chickens whole – frozen	25.9	22.2	20.6	13.0	14.0	12.2	15.0
Chicken parts – frozen	na	na	na	5.1	8.0	14.4	16.7
Chickens total – frozen	*na*	*na*	*na*	*18.1*	*22.0*	*28.6*	*31.7*
Chicken – processed	na	na	na	9.2	8.6	9.4	9.2

na = Data not available.

Table 8. Retail sale of **imported** chilled and frozen chicken products 1985-1999 (Statistics Denmark and the Danish Poultry Counsel, unpublished data)

	Retail sale of imported chicken products in 1000 tons						
	1985	1989	1992	1996	1997	1998	1999
Chickens total – chilled	-	0.06	0.3	3.6	1.8	2.9	1.7
Chickens total – frozen	0.5	1.1	1.6	6.9	12.5	9.5	4.7

RISK FACTORS AND RISK BEHAVIOUR

In this section the term 'risk factor' is related to the specific sources of infection (like different food items and water) or specific host factors (like underlying disease or

medication) revealed by epidemiological investigations. The term 'risk behaviour' is in this context used to describe different patterns of behaviour assumed to increase the risk of acquiring an infection by *Campylobacter* spp. Typically, the term risk behaviour is related to areas like travelling, contact with animals, profession, recreational activities and food handling procedures.

The impact of different risk factors and patterns of behaviour on human cases of campylobacteriosis are not fully described. The information available on these topics is mainly based on epidemiological investigations of outbreaks, case control investigations and general studies on food handling procedures in private households. Although a number of potential risk factors and risk behaviours have been described it is still not possible to explain all the infections caused by *Campylobacter* spp.. Therefore, more work has to be directed into elucidating the epidemiology of *Campylobacter* in order to point out the most important sources and ways of infection and thereby provide a basis for a more specific control strategy.

Risk factors

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 *et al.*, 1998).

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; Deming *et al.*, 1987; Harris *et al.*, 1986; 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; Neimann *et al.*, 1998). The major risk factors described in these studies have been

- handling raw poultry
- eating poultry
- eating other meat types
- eating undercooked or barbecued meat
- drinking untreated surface water
- drinking unpasteurized milk or dairy products
- contact with farm animals and pets

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). Person to person transmission is considered to be infrequent (Altekruse *et al.*, 1999; Tauxe, personal communication).

The relative importance of the potential sources of *C. jejuni* for human cases of campylobacteriosis has been investigated 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) has 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 milkborne 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, poultry products and - to a lesser extent – cattle, with serotype O:2 being the most dominant type (Table 9) (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 (Table 10). However, the frequency by which *C. coli* is isolated from humans and from retail poultry products in Denmark is low compared to *C. jejuni*.

Table 9. Serotype* distribution (%) of *Campylobacter jejuni* from human patients, animals and poultry products at retail level in 1998 (Anon., 1998b)

Serotype	Human	Broilers	Cattle	Pigs	Retail poultry products
1,44	9	18	8	0	8
2	36	26	30	9	18
3	2	5	0	0	4
4-complex**	19	7	22	18	14
5	4	4	0	0	8
6,7	4	6	3	0	8
11	6	1	0	9	1
12	3	2	3	0	3
19	2	1	6	0	11
21	2	1	0	0	1
23,36	0	1	14	27	1
27	0	4	0	0	1
29	1	1	6	0	0
31	3	4	0	0	3
35	2	0	8	27	0
57	0	5	0	0	3
Others	4	9	0	8	7
Not typeable	3	5	0	0	9
Number typed	128	82	36	11	74

* The Penner serotyping scheme (heat stable antigens) was used for serotyping
** 4-complex:Reaction with one or more of the following antisera: 4, 13, 16, 43, 50, 64, 65

Table 10. Serotype* distribution (%) of *Campylobacter coli* from human patients, animals and poultry products at retail level in 1998 (Anon., 1998b)

Serotype	Human	Broilers	Pigs	Retail poultry products
5	17	7	15	0
24	17	7	9	0
30	33	13	16	33
34	17	0	8	8
46	0	20	20	0
54	0	7	1	17
59	0	20	6	33
Others	17	20	19	8
Not typeable	0	6	6	0
Number typed	6	15	101	12

The Penner serotyping scheme (heat stable antigens) was used for serotyping

In Denmark the anti-microbial resistance of *Campylobacter* strains from broilers, cattle, poultry meat and humans is routinely being surveyed. Table 11 shows the results from 1997 to 1999. Percentages from 1997 and 1998 cannot be compared to 1999 due to changes in sampling scheme and breakpoints for some anti-microbials. From the limited number of data, no relationship seems to be evident between anti-microbial resistance in human isolates and isolates from broilers and cattle. However, resistance to nalidixic acid, tetracycline and ciprofloxacin is seen for both human isolates and isolates from broiler meat and other poultry meat (Table 11). In 1999, resistance patterns were determined in Danish and imported broiler meat and it was seen that isolates from imported broiler meat showed a more frequent resistance to tetracycline and ciprofloxacin compared to Danish produced broiler meat.

Table 11. Anti-microbial resistance (%) among *Campylobacter jejuni* isolates from food-producing animals, poultry meat and humans (Anon., 1997b; 1998c; 1999d).

			Antimicrobial drug									
	N	År	APR	CHO	COL	ENR	ERY	GEN	NAL	STR	TET	CIP
Cattle	46	1997	0	0	0	1	4	0	2	9	0	-
Cattle	32	1998	0	0	0	3	0	0	3	0	0	-
Cattle	40	1999	-	0	0	-	0	0	3	5	3	3
Broilers	75	1997	0	0	0	1	1	0	3	7	3	-
Broilers	71	1998	0	0	1	3	1	0	4	0	1	-
Broilers	69	1999	-	0	0	-	0	0	4	4	1	3
Broiler meat*	40	1997	0	0	0	-	3	0	5	3	8	5**
Broiler meat*	93	1998	-	4	-	-	5	0	16	5	11	16
Broiler meat*	93	1999	-	0	-	-	0	0	20/21¤	4	5/38¤	8/17¤
Other poulty meat*	46	1998	-	1	-	-	3	0	10	7	8	7
Other poulty meat*	31	1999	-	0	-	-	0	0	42	13	55	39
Humans	111	1997	0	2	2	12	2	0	14	7	9	12
Humans	117	1998	0	0	1	-	0	0	11	1	7	11
Humans	98	1999	-	0	-	-	0	0	21	2	10	20

APR, apramycin; CHO, chloramphenicol; COL, colistin; ENR, enrofloxacin; ERY, erythromycin; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; TET, tetracyclin; CIP, ciprofloxacin; -, not determined; *, Danish produced and imported retail products; ** both CIP and ENR; ¤, percentage of isolates from Danish produced broiler meat/imported broiler meat.

Underlying disease has been described as a predisposing factor for acquiring intestinal 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 bacteremia caused by *Campylobacter* spp. had an underlying disorder, including liver cirrhosis, neoplasia, immunosuppresive 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 bacteremia caused by *Campylobacter* spp.. Eleven of the 15 patients in this investigation had underlying disorders, including immunological, neoplastic and vascular disease. Neimann (1999) 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).

Medication with antisecretory drugs like omeprazole and H_2 and H_2-antagonists has also 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; Neimann, 1999).

So far it has not been possible to quantify the number of *Campylobacter* cases related to each of the different risk factors described. This is mainly due to the fact that only a minor part of the human cases is registered and verified by analysis of stool specimen, the causative agent is seldom found, and isolates are not routinely sub-typed. The latter is mainly due to the lack of a sufficiently discriminatory and reliable sub-typing method. Sub-typing of isolates from patients, food, animals, and environment is essential for the elucidation of causal relations between human campylobacteriosis and the potential risk factors.

Risk behaviour

Travelling

Travel abroad seems to be a common cause 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; Neimann *et al.*, 1998; Mølbak *et al.*, 1999).

Contact with pets

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, 1999). 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.*

Professional occupation and residential area

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.. Other investigations have revealed a higher incidence among the urban population than in the population living in rural areas (Kapperud & Aasen, 1992; Stafford *et al.*, 1996). Danish results (Neimann, 1999) do not indicate an increased risk of acquiring campylobacteriosis for people handling production animals at farm level. In addition, the incidence of campylobacteriosis in the rural areas of Denmark seems to be equal to or lower than the incidence in the Copenhagen area (Anon., 1999a) – se also the section on page 32.

Several investigations have revealed that workers 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.* (1983b) 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.*, 1983b); 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. In spite of the risk described in association with working at slaughterhouses 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.

Recreational activities

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 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 Bathing Water Directive standard (Bolton *et al.*, 1999).

Unsafe food handling procedures in private households

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). Further on, 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 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 is therefore restricted to food handling procedures in private kitchens assumed to have the greatest impact on the exposure to consumers. These are for example cross-contamination, insufficient heat treatment, barbecuing and differences in handling procedures due to age and sex.

Cross-contamination by hands
Several investigations have been carried out to elucidate consumer habits during food handling. The outcome of interviews and observations of consumer habits regarding washing hands are summarised in Table 12.

Washing hands after having handled raw meat and poultry is essential 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 *et al.* (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.*, 1983a). On 6 of the 11 hands examined *Campylobacter* was detected at a level of log10 0.48-1.24 cfu/hand (mean; log10 0.9 cfu/hand). *Campylobacter* was not detected on 5 hands (< log10 0.35 cfu/hand).

Table 12. 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 & Levnedsmiddel-styrelsen, 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

Cross-contamination by utensils

Exposure to food borne pathogens in the private kitchen due to cross-contamination by utensils 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 13.

Table 13. 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

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 *et al.*, 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.* (1999) 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.

Insufficient heat treatment

Consumers could be exposed to *Campylobacter* through contaminated food items which are not sufficiently heat-treated. Table 14 summarises studies related to insufficient heat treatment in private kitchens.

In England, it was showed that insufficiently heat treatment was the course in 35 out of 101 food-borne outbreaks (Ryan *et al.*, 1996). In USA it was revealed that insufficiently heat treatment of meals was the second most important course (31.3%) of 345 food-borne outbreaks related to private households (Knabel, 1995). In 1996 to 1997 a case control study was performed by the Danish Zoonosis Centre (Neimann *et al.*, 1998). This study showed that 16 of 168 cases (10%) and 5 of 189 controls (3%) had ingested insufficiently heat-treated chicken.

Table 14. Data on consumer habits related to insufficient heat treatment of meals

Statement	**Respondents agreeing with the statement (%)**	**Study performed in**	**Reference**
Sufficient heat treatment not recognised as a preventive option to food borne disease	33% of 1620 persons	US 1992-1993	Altekruse *et al.*, 1995
	54% of 990 persons	Denmark 1996	AIM Nielsen & Levnedsmiddelstyrelsen, 1997
	62% of 15 households	Denmark 1998	CASA, 1999
Meals not heated to a core-temperature of 74 °C	15% of 108 persons	UK 1996	Worsfold *et al.*, 1997a; Griffith *et al.*, 1998
Meals not heated sufficiently	24% of 106 households	US and Canada	Daniels, 1998

Age and sex

Several investigations have shown that demographic factors like age and sex have impact on risk behaviour related to food safety in the private kitchen. Table 15 summarises data on food handling procedures related to age and sex.

The American study performed in 1995 and 1996 showed that high-risk food handling procedures like insufficient wash of hands and cutting boards were more prevalent (approx. 10%) among men than women. Further, the high-risk food handling procedures were more prevalent among younger people than among middle age and elderly people (Yang *et al.*, 1998).

The study performed by CASA in 1998 showed that males and females in general had the same standard of hygiene regarding washing hands. Related to age, it seems that elderly people more often will use a separate cutting board for handling raw meat and poultry. In general, the study showed that males and single persons without children and young people in education are less focused on the use of clean utensils (CASA, 1999).

Several investigations on the distribution of *C. jejuni* infections among different age groups and between sex have showed that the incidence is relatively high in children 0 - 4 years of age and in young people 15 – 30 years of age. Further, the incidence is generally higher among males than in females (Skirrow, 1987; Brieseman, 1990; Kapperud & Aasen, 1992). The higher incidence of campylobacteriosis in the group of people between 15 and 30 years of age could for example be explained by less attention to safe ways of handling food in this population group. Se also the section on page 32.

Table 15. Data on food handling procedures related to age and sex.

Statement	Respondents agreeing with the statement (%)		Related to different age groups	Reference
	MALE	FEMALE		
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 & Levnedsmiddel-styrelsen, 1997
Sufficient heat treatment not recognised as a preventive option to food borne disease	51%	57%	< 24: 55% 25-34: 52% 35-54: 50%	AIM Nielsen & Levnedsmiddel-styrelsen, 1997

Number of bacteria transferred

At present, no data are available describing the actual number of *Campylobacter* transferred from surfaces and utensils to foods in private kitchens. However, data on this subject generated for other bacteria may be useful in estimating frequencies and number of bacteria transferred through cross-contamination.

In 1982 a study performed in American households showed that high numbers of bacteria, mainly enterobacteria, were isolated from wet areas in private kitchens. Pathogenic bacteria could be isolated in low numbers from 49% of the surfaces with food contact (Scott *et al.*, 1982). Another study revealed that $\log_{10}$ 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. In addition, the study showed that *E. aerogenes* introduced onto cutting boards at a level of $\log_{10}$ 5.0 cfu/cm^2 was reduced to $\log_{10}$ 3.0 – 4.0 cfu/cm^2 after one hour and to $\log_{10}$ 2.0-3.0 cfu/cm^2 after four hours (Zhao *et al.*, 1998).

Barbecuing

Preparing meals at barbecue has in several investigations been shown to increase the risk of acquiring infection by *Campylobacter* spp. (Kapperud, 1994; Ikram *et al.*, 1994; Adak *et al.*, 1995; Neimann *et al.*, 1998). The increased risk associated with barbecue may be explained by the increased risk of cross-contamination and insufficient heat treatment related to this way of handling food.

HAZARD CHARACTERIZATION

DISEASE

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.

Late complications

Late complications may follow gastrointestinal infections caused by various food-borne pathogens including infections with *Campylobacter*. The late complications associated with *Campylobacter* infections 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 postinfectious 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).

In rare cases, *Campylobacter* has shown to cause the serious disease, 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 (Blaser & Allos, 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 (Ohtsuka *et al.*, 1998).

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 the US the average annual number of deaths related to *Campylobacter* has been estimated to be 124 of 2,453,926 estimated campylobacteriosis cases (0.005%) (Mead *et al.*, 1999).

Antimicrobial resistance

Development of antimicrobial resistance may compromise treatment of patients with bacteremia. In the beginning of the 1990-ties, fluoroquinolone-resistant *C. jejuni* emerged in human populations in Europe as reported in the UK, Austria, Finland, and the Netherlands (Piddock, 1995). 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.*, 1994). 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). Hence, fluoroquinolone resistance may cause severe problems in cases where drug treatment is required.

VIRULENCE / PATHOGENITY

The pathogenesis of *Campylobacter* has been reviewed by several authors (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).

DOSE-RESPONSE

The infective dose 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.

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 susceptible or more frequently exposed than other age groups (Blaser *et al.*, 1983; Engberg & Nielsen, 1998; Kapperud & Aasen, 1992; Stafford *et al.*, 1996) (see also the section on page 32).

Vehicle

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.

Dose-response investigations

The infective dose of *C. jejuni* has been investigated in a few experiments involving volunteers. In one experiment a dose of 500 organisms ingested with milk caused illness in one volunteer (Robinson, 1981). In another experiment involving 111 healthy young adults from Baltimore, doses ranging from 800 to 20,000,000 organisms caused diarrhoeal illness (Black *et al.*, 1988). Rates of infection increased with dose, but development of illness did not show a clear dose relation. In an outbreak at a restaurant, the number of *C. jejuni* in the causative chicken meal ranged from 53 to 750 *Campylobacter* per g (Rosenfield *et al.*, 1985).

These few investigations indicate that the infective dose of *C. jejuni* may be relatively low. This also seems to be the case in two Norwegian outbreaks, where the only possible route of infection was through the water splash from bicycle wheels. Both outbreaks were related to a cycle race in a specific agricultural area. It rained during

both races and the farmland had recently been manured (Kapperud, personal communication).

The mathematical relationship between the ingested dose and the probability of infection (or illness) can be applied to quantify the risk of acquiring an infection by exposure to known numbers of *Campylobacter* via a certain vehicle. This is further described later in this report.

Immunity

Patients suffering from campylobacteriosis may develop immunity for the causative *Campylobacter* strain (for a period of time). This was demonstrated in the investigation 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. Required 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).

RISK CHARACTERIZATION

INCIDENCE IN HUMAN MEDICINE

Most human *Campylobacter* infections occur as sporadic single cases or as part of small family related outbreaks, but larger outbreaks have been described. 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).

Age and sex distribution

All age groups may become infected with *Campylobacter*. However, the reporting rate of campylobacteriosis is higher in young children and young adults) (Table 16). This has also been observed in other countries (Blaser *et al.*, 1983, Skirrow, 1987, Brieseman, 1990, Kapperud & Aasen, 1992, Stafford *et al.*, 1996). The high incidence rate in children may be a result of 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.

Table 16. Incidence of infections with *Campylobacter* by age and sex in Denmark 1999 (Statens Serum Institut, unpublished results).

Age group	Number of cases				Cases per 100,000		
(years)	Female	Male	Unknown	Total	Female	Male	Total
<1	33	39	6	78	103	115	118
1-4	180	258	42	480	133	181	172
5-9	63	118	21	202	39	69	61
10-19	190	218	37	445	67	74	77
20-29	584	419	117	1120	162	113	153
30-39	277	341	79	697	69	82	85
40-49	188	190	35	413	51	51	56
50-59	169	165	18	352	47	45	49
>60	156	181	40	377	26	40	36
Total	1840	1929	395	4164	68	73	78

Table 16 shows that the incidence rate seems to be higher in young boys than young girls, but higher in females than males in the 20-29 year group. Surveys in other countries have also found a higher incidence rate in young boys (Skirrow, 1987; Kapperud & Aasen, 1992; Stafford *et al.*, 1996). But with reference to the young adults

the surveys report a higher incidence rate in males (Skirrow, 1987; Brieseman, 1990; Kapperud & Aasen, 1992) or an equal rate in males and females (Stafford *et al.*, 1996). The reason for this sex difference has not been explained.

Area distribution

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). The *Campylobacter* incidence per 100.000 inhabitants in different counties in Denmark in 1999 appears from Fig. 8. In UK and New Zealand *Campylobacter* infections have occurred at a higher incidence in rural than urban areas (Skirrow, 1987; Brieseman, 1990). In Norway and Australia the opposite has been observed (Stafford *et al.*, 1996; Kapperud & Aasen, 1992). In Norway, 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).

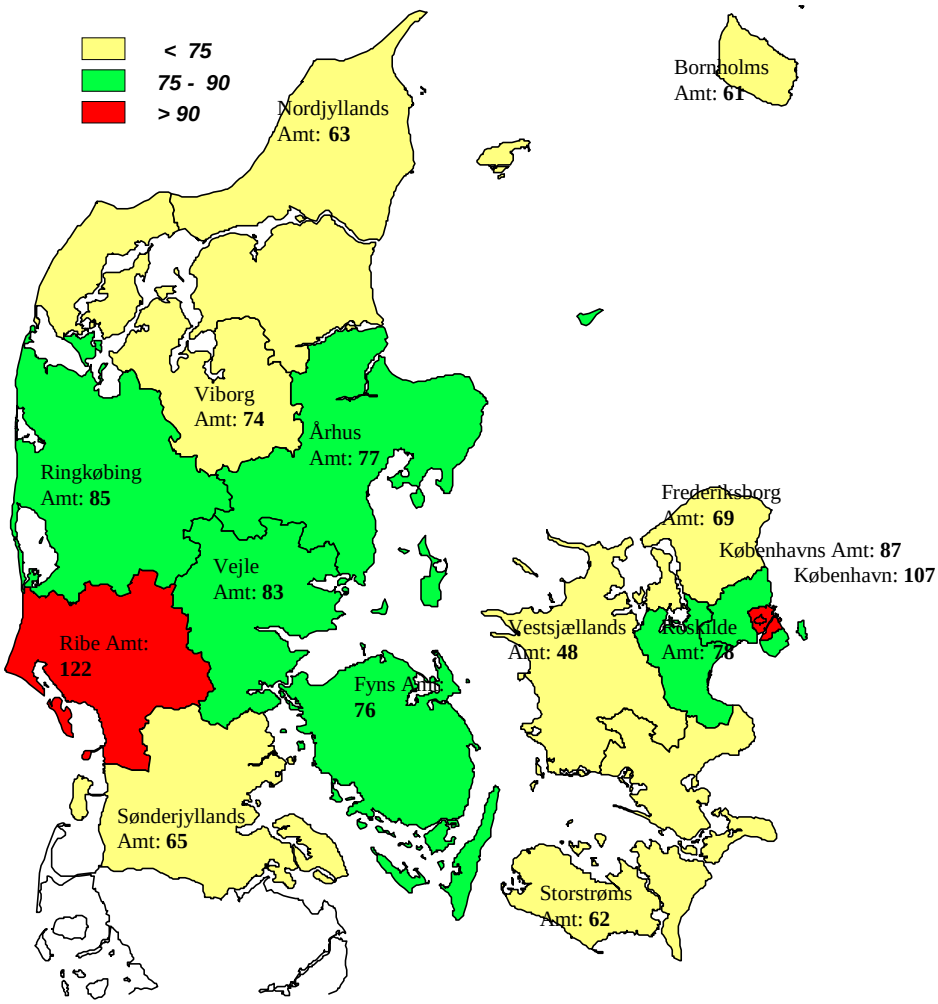

Figure 8. The *Campylobacter* incidence per 100.000 inhabitants in Denmark in 1999 (Statens Serum Institut, unpublished data).

Seasonal variation in the number of human cases

A seasonal variation in the number of human cases has been noticed in Denmark (Fig. 9) and in several countries including Sweden, Norway, UK and New Zealand with a more than doubling of the incidences in late summer (Brieseman, 1990; Kapperud & Aasen, 1992; Skirrow, 1991; Newell *et al.*, 1999). 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). The prevalence of *Campylobacter* in broilers shows a similar seasonality. It has been stated that the broiler flocks tend to peak after the human cases (Kapperud *et al.*, 1993; Berndtson, 1996; Newell *et al.*, 1999). This tendency is also seen in Denmark (Fig. 9). However, broilers seem to be infected before humans in 1998 and vice versa in 1999. If poultry are the primary source of human infection, it should be expected that the broilers peak before or coincident with the humans and not the other way around.

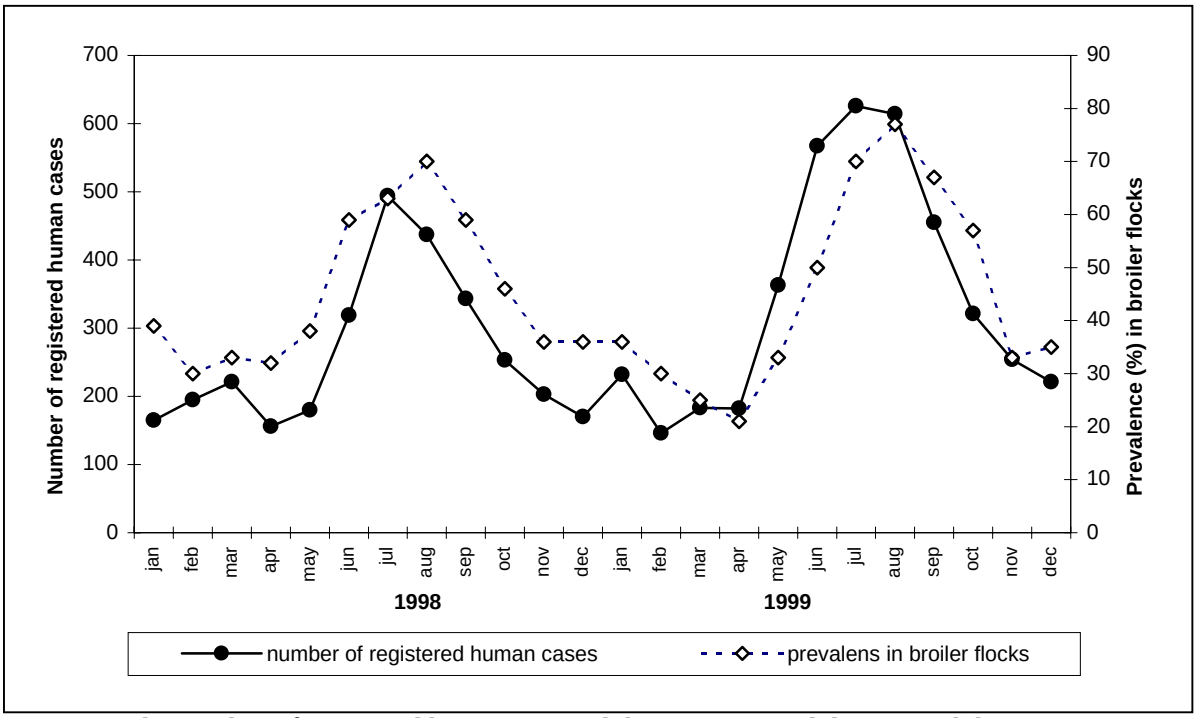

Figure 9. The number of registered human *Campylobacter* cases and the *Campylobacter* prevalence in broiler flocks in 1998 and 1999 (Danish Veterinary Laboratory and Statens Serum Institut, unpublished data).

Using Penner serotyping and pulsed-field gel electrophoresis of restriction enzyme-produced DNA fragments on isolates obtained from human and veterinary cases, raw milk, chicken and untreated water (from a restricted geographical area), Hudson *et al.* (1999) found that some *Campylobacter jejuni* subtypes dominated in summer while other subtypes dominated in winter. This finding may reflect different survival patterns among *Campylobacter* strains.

A QUANTITATIVE RISK ASSESSMENT MODEL ON *CAMPYLOBACTER JEJUNI* IN CHICKEN PRODUCTS

Quantitative risk assessment (QRA) is a possible approach for designing programs to estimate the risk of illness caused by a given hazard, e.g. *Campylobacter*. Such programs includes mathematical modelling of all relevant processes involved in the spread of the hazard, e.g. from farm to fork. QRA models are typically based on the technique of probabilistic scenario analysis, which makes it possible to estimate the probability of occurrence of an event in light of the inherent uncertainty within the steps of the model. The output of a QRA model is for example an estimate of the probability of exposure and the probability of illness per meal served. Besides giving a risk estimate, QRA modelling also contributes to the understanding of the spread of given hazard as all processes e.g. from farm to fork are systematically gone through, including the existence of relevant data. The latter may help to point out where additional data has to be generated. Another and perhaps the most important outcome of QRA models are the identification of the steps in the process that have most influence on the risk estimate. This information may assist risk managers to make decisions geared towards reducing the risk to the consumer.

Selected parts of a quantitative risk assessment model for *C. jejuni* on chicken are available on www.who.int/fsf/mbriskassess/studycourse/annac/index. Another risk assessment on broilers is being carried out (Hartnett *et al.*, 1999). Finally, a risk assessment dealing with the human health impact of fluoroquinolone resistant *Campylobacter* associated with the consumption of chicken is available on www.fda.gov/cvm/fda/mappgs/ra/risk.

A quantitative risk assessment model on *Campylobacter jejuni* in chicken products carried out at the Danish Veterinary and Food Administration is described in the following. As mentioned in the introduction to this report, risk assessors and risk managers at the Danish Veterinary and Food Administration agreed that the initial phase of the risk assessment on *Campylobacter* should focus on *Campylobacter jejuni* in chicken products. This decision was based on 1) the frequent outcome in case-control studies of chicken as a risk factor for campylobacteriosis, 2) the high prevalence of *Campylobacter* in retail chicken products as compared to other food items, and 3) the availability of relevant data. Therefore, a quantitative risk assessment model describing the transmission and spread of *Campylobacter* from broilers at the entrance to the slaughterhouse to consumption by consumers was developed.

The objective of the present model was to generate output, which could supply us with information about

- the relative importance of different steps that have influence on the risk estimate at production, retail, and consumer level
- the spread of *Campylobacter* in chickens from slaughterhouse to consumer
- the areas where it is most benefit to improve and optimise sampling plans and analytical methods
- an estimate of the potential risk of getting campylobacteriosis after having consumed a meal with chicken

Introduction to the steps included in the QRA model

In order to develop a quantitative risk assessment model describing the spread of *Campylobacter* from broilers at the slaughterhouse to consumer, a number of steps in broiler processing and chicken handling must be taken into consideration. Fig. 10 shows a flow sheet describing the most important steps from the living broilers in the broiler houses through the processing at slaughterhouse, post processing either in private kitchens or catering kitchens to the final exposure to humans. The slaughterhouse described in the model is a fictive "average slaughterhouse" representing most chicken slaughterhouses in Denmark.

Steps included in the model

The steps included in the QRA model have been split into two parts: One part that deals with the slaughterhouse from entrance of the broiler flocks to chilled or frozen retail chicken products (= **slaughterhouse model**). And another part that describes handling and consumption of chicken products in private households (= **consumer model**). This latter model also includes a dose-response relationship. The two models are described in details in the following sections.

Steps not included in the model

Some steps showed in the flow sheet (Fig. 10) are not included in the QRA model. This is mainly because relevant data are limited or missing. The excluded steps are broiler house, transport, catering, further processing, import and export. Each step will be commented in the following.

Concentration and prevalence of Campylobacter in the broiler house and during transport
Although some quantitative as well as qualitative data describing the *Campylobacter* status of broilers in the broiler house and during transport are available, we have decided to exclude these steps in the model.

The process by which *Campylobacter* gets into in the broiler houses is poorly understood. Very little is known about how and when the first broilers in a broiler house become contaminated by *Campylobacter*, but as *Campylobacter* has entered a broiler house, it takes less than 3-4 days before all the broilers are *Campylobacter* positive. This means that in most cases either all or none of the broilers in a broiler flock will be positive at the day of slaughter - with respect to the fraction of positive broilers. Therefore, in the model we have assumed that either 0% or 100% of the broilers in a flock are contaminated upon arrival to the slaughterhouse (see also the section on page 45).

With respect to the quantitative levels of *Campylobacter* most data from broilers in the broiler houses are based on measuring concentrations in faecal or caecum samples. Unfortunately, the *Campylobacter* status on the chicken skin surface rather than the concentrations in the faeces/caecum is important in relation to human exposure.

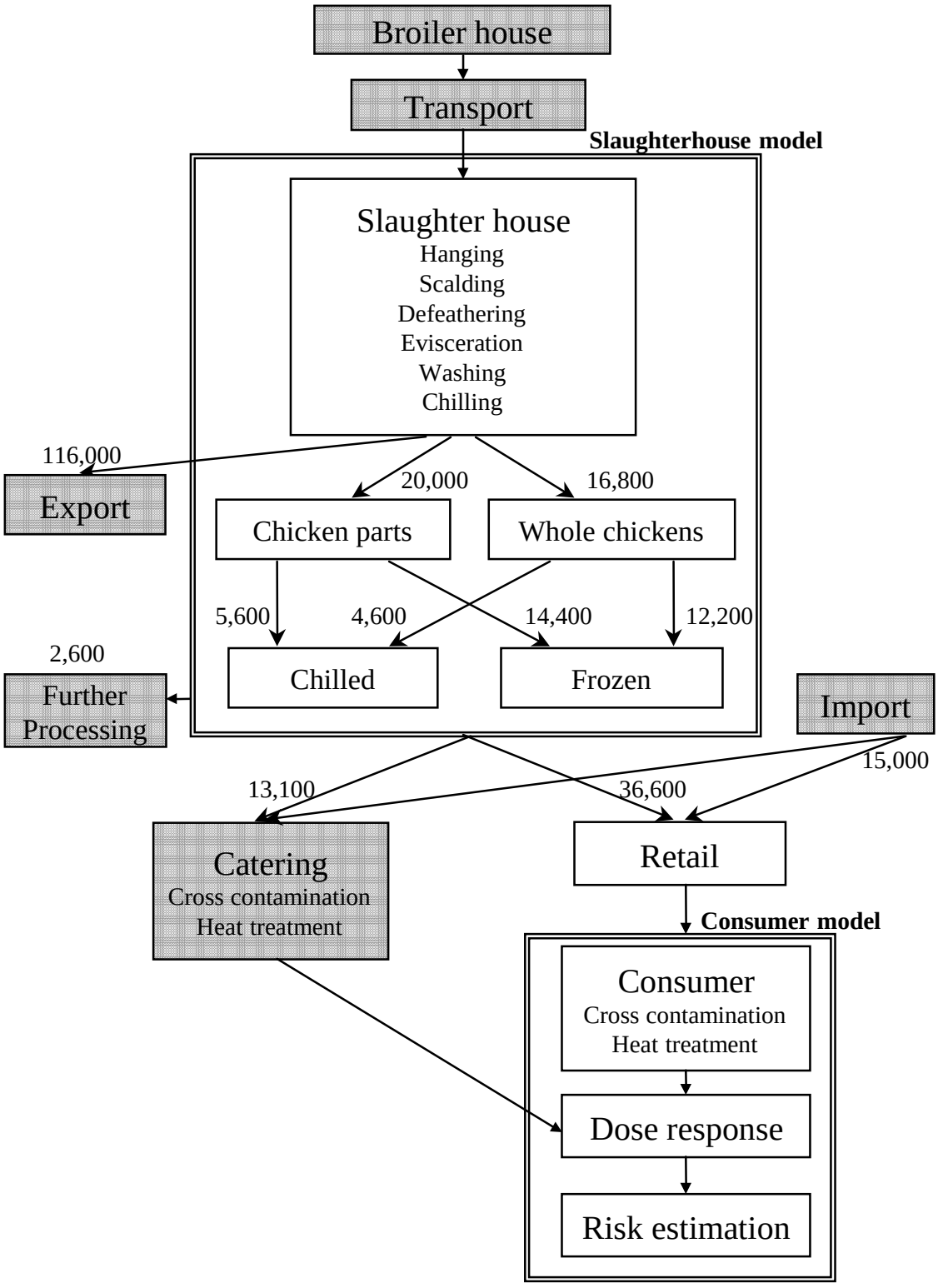

Figure 10. Schematic overview of the steps describing the flow of broilers/chickens from farm to fork. Shaded areas are not included in the QRA model. The numbers are the amount of whole chickens in tons in 1998.

The relation between the level of *Campylobacter* on the skin surface and the level in faeces is dependent on the spread of *Campylobacter* from the interior to the exterior of the broiler, which is mainly due to spread of faeces to the outer surface of the chicken. The process by which *Campylobacter* is spread from the interior to the exterior is not known. Some contamination of the chicken skin surfaces occurs in the broiler houses, but also transport from the broiler house to the slaughterhouse may result in spread of *Campylobacter* to the skin surface. Instead of trying to model this relatively complex process by which *Campylobacter* in the faeces spread to the exterior of the chickens we have decided to use data describing the concentration of *Campylobacter* on the chicken skin measured shortly after arrival to the slaughterhouse.

The transportation step is also not dealt with in the model, though is it known, that transportation of broilers from farm to slaughterhouse may contribute to the spread of *Campylobacter* between broilers. In particular, cross-contamination within a broiler flock may occur, allowing faeces to spread from one crate to another. Also cross-contamination between flocks could occur, for example due to insufficient cleaning of the transport vehicle and crates between transports. Stern *et al.* (1995) and Mead *et al.* (1995) showed that broilers with a *Campylobacter* negative caecum status at the broiler house all had low concentrations of *Campylobacter* on the skin surfaces after transportation. This may indicate that, at least in some countries, cross-contamination between flocks does occur during transport. As the status of a broiler flock in the present model is based on data, which are actually sampled at the slaughterhouse, it could be speculated that possible cross-contamination during transport could lead to an overestimation of the *Campylobacter* status of the broiler flocks in the present model. However, due to the rather short transportation time of broilers in Denmark, an eventual cross-contamination will only contaminate the exterior of the broilers. Colonization of the chicken gut is less likely to occur, at least not above the detection level. Furthermore, in Denmark all crates and trucks are carefully cleaned between each transport. Therefore, cross-contamination between flocks is not considered to be a significant problem in the broiler transport system in Denmark. In conclusion, the *Campylobacter* concentration on broiler carcasses may increase in positive flocks during transport, due to spread of faeces to the exterior of the broilers, but transport is not expected to result in significant cross-contamination between flocks. Therefore, we assume that the prevalence in Danish broiler flocks remains unaffected during transport.

Export and Import

A rather large part of the chicken products produced in Denmark are exported to other countries, mainly to The Middle East, Germany and the UK (Statistics Denmark). Exported chicken products are not included in the model as the overall objective was to estimate the potential risk to Danish consumers from *Campylobacter* in chicken products. Imported chickens are also not dealt with in this version of the model due to insufficient quantitative data. However, we are in the process of generating semi-quantitative data for imported retail products. Hence, in the next version of the risk assessment these data will be included. Whole chickens are mainly imported from Sweden, Germany and France (Statistics Denmark).

Further processing and catering
Some chicken products are further processed to for example sausages, prepared dishes, etc. Due to the Danish legislation most plants, which produce these products, have implemented a HACCP-based quality assurance program to ensure microbiological 'safe' products. Moreover, the further processing often includes heat treatment, drying or smoking, which should eliminate the *Campylobacter* bacteria. We therefore assume that these products do not contain *Campylobacter*. This in combination with the lack of data is the basis for the exclusion of the further processing step from the model. Chicken meals prepared in catering kitchens are not dealt with in the present model, as absolutely no data describing this area are available.

General comments

The risk assessment modelling has been carried out after the 'farm to fork' approach in order to systematically describe the transfer and spread of *Campylobacter* through the different processing steps at the slaughterhouse and in private kitchens until the final exposure to humans.

The present quantitative model deals with data describing the *Campylobacter* situation in broilers/chickens and humans in 1998-1999. The Danish dietary habits included in the model are from 1995. The model will be updated when more recent data are generated.

The present model deals with the prevalence and concentration of *Campylobacter* on the skin surface of Danish whole chilled and frozen chickens, which have been spin-chilled. The input data describing the prevalence in broilers at the entrance to the slaughterhouse, the flock sizes and the slaughter order is from a Danish slaughterhouse and part of the input data describing the different slaughterhouse processes is based on foreign data. We are aware of the fact that there may be differences between slaughterhouses, that the main fraction of chilled Danish chickens is air chilled, and that the foreign data may not exactly describe the Danish situation. These factors may have influence on the outcome of the models. When additional data describing the Campylobacter status on Danish chickens are generated, they will be included in the model.

The consumer model deals with households with only one family. Exposure due to food handling in the private kitchen has been modelled and cross-contamination via cutting boards represents the cross-contamination during food preparation, though other routes of cross-contamination may also be important with reference to human exposure to *Campylobacter*. The input data regarding households and consumption patterns are from Statistics Denmark and a Danish dietary survey from 1995. The data describing the risk behaviour and the dose-response relationship are from the other countries. However, we assume that these data represent the behaviour and susceptibility of Danish consumers. If Danish data are generated, these will be included in the model.

Several assumptions have been made during the model building. The validity of the outcome of the model is dependent on the validity of these assumptions.

The statistical methods used to generate input distributions

In quantitative risk assessment modelling the input as well as the output data are described as distributions. However, the data, which should form the basis of the input distributions in the slaughterhouse model, are often insufficient to produce a smooth histogram (Fig. 11). In order to overcome this problem, we assume that each of the data points (D) measured at a given site are normal distributed with the mean and standard deviation given in Table 22-26. In this way we make use of the knowledge of both the D data points and their standard deviations. Before developing an input distribution of the D data points, the data material was analysed (according to Technique I, see below) in order to be able to determine (if significantly different) if parts of the data material should be excluded due to certain factors or conditions. The factor or condition could for example be different measurement methods, including or excluding chlorine in the water, scalding at different temperatures, etc. Data material was only excluded if it was significantly different ($\alpha<0.05$) from the rest of the data material and if there was a logical explanation/reason for excluding the data (for example if they were different from the kind of data needed in our model). Technique II describes how an input distribution of the concentration of *Campylobacter* is developed by creating a 'sum distribution'. Technique III describes the development of an input distribution of the changes in concentration of *Campylobacter* throughout the different processes at the slaughterhouse.

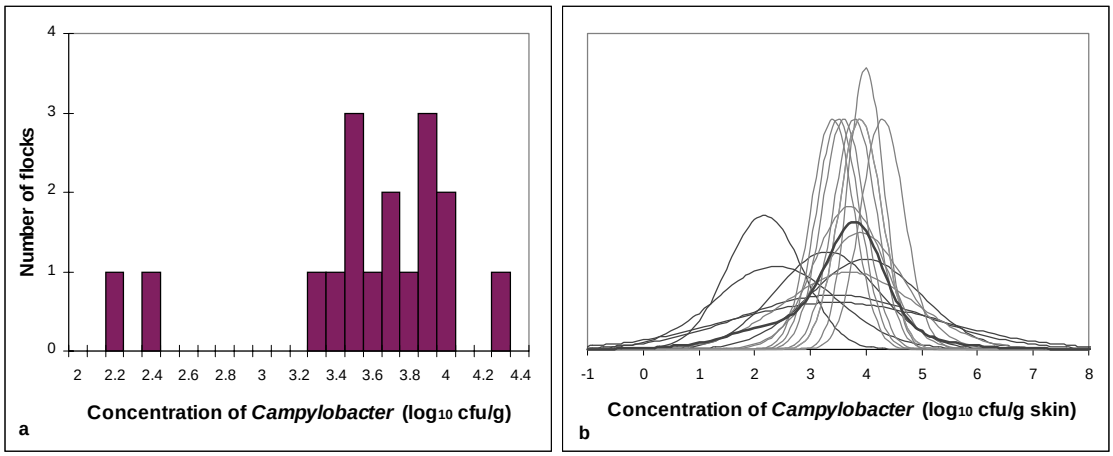

Figure 11. An example of available input data and how these can be used in developing an input distribution. a) A histogram of a given available data set. b) Distributions for each of the data points in the data set and the sum distribution (thick line).

Technique I): This technique was employed in order to determine if parts of the data material should be excluded before developing an input distribution. A test for equality of variances (Bartlett's test, see Table 17) was carried out prior to a one-way variance analysis, since equality of variances is a prerequisite for the variance analysis.

Bartlett's test for equality of variances is calculated as the following:

Hypothesis: H_0 : $\sigma_1^2 = \sigma_2^2 = \cdots = \sigma_k^2$ against H_1 : $\exists$i, j($\sigma_i^2 \neq \sigma_j^2$)

The hypothesis H_0 is rejected if the following equation is true:

$$f \log_e s^2 - \sum_{i=1}^{k} f_i \log_e s_{1i}^2 > \chi^2(k-1)_{1-\alpha}$$

Bartlett's test is sensitive toward deviations from the assumption that the observations are normal distributed. Hence, a possible rejecting of the hypothesis can be due to such a deviation and not to the variances being significantly different. Other tests can be employed, e.g. the Kendall-Bartlett's test, which is not sensitive toward smaller deviations from the assumption that the observations are normal distributed. Neither the variance analysis is sensitive toward small deviations from the assumption of normality (but very sensitive toward inhomogeneous variances).

Table 17. Bartlett's test

Bartlett's test	Formula
Test value	$f \log_e s^2 - \sum_{i=1}^{k} f_i \log_e s_{1i}^2$
Chi-square distribution	$\chi^2(k-1)_{0.95}$
Degree of freedom within groups	$f_i = n_i - 1$
Degree of freedom total	$f = N - k$
Variance for each groups	$s_{1i}^2 = \frac{1}{n_i - 1} \sum_{j=1}^{n_i} (x_{ij} - \bar{x}_i)^2$
Variance 'total'	$s^2 = \frac{1}{f} \Sigma\, f_i s_{1i}^2$
Significant different variances?	?

n_i is the number of samples in the i^{th} group, s_i is the standard deviation for the i^{th} group, N is the total number of samples, and k is the number of groups. If the test value is larger than the table value from the chi-square-distribution, then there is a significant difference between the variances.

One-way variance analysis (Table 18) is carried out in order to test whether there is significant difference between flocks. If the difference is significant, the reason for the difference should be discussed, before selected data are excluded. The difference could for example be an effect of the variability within the system.

Table 18. One-way variance analysis

Variance source	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	Test Value (z)	$F(df_1, df_2)_{0.95}$
Between flocks (bf)	$\sum_{i=1}^{k}(n_i * \overline{X}_{i.}^2 - N\overline{X}_{..}^2)$	k-1	SS_{bf} / df_1	MS_{bf}/MS_{wf}	Table value
Within flocks (wf)	$\sum_{i=1}^{k}(s_i^2 * (n_i - 1))$	N-k	SS_{wf} / df_2		
Total	$SS_{bf} + SS_{wf}$	N-1			

n_i is the number of samples in the i^{th} flock, s_i is the standard deviation for the i^{th} flock, N is the total number of samples, and k is the number of groups. If the test value, z, is larger than the table value from the F-distribution, then there is a significant difference between the flocks.

Technique II): This technique was used to produce an input distribution for the *Campylobacter* concentration on broilers at the entrance to the slaughterhouse. The D normal distributions were summed up to give a new distribution (a sum distribution), which is normalised. In Fig. 11b a sum distribution is shown together with the D normal distributions each representing a data point. The sum distribution is not necessarily a normal distribution – the D distributions "talk for themselves". However, for large numbers of data points the sum distribution will approximate a normal distribution. A normal distribution for all the data could also have been estimated using the results from a variance analysis, which could provide us with an estimate of the total uncertainty. The sum distribution, however, was calculated by summarising the probability for each value of x (concentration of *Campylobacter*), multiplying it with a weight, ω_i, and an interval, Δx.

$$\sum_{i}^{D} \text{NORMDIST}(x, \mu_i, \sigma_i, 0) * \omega_i * \Delta x$$

where NORMDIST is an Excel-function, μ_i is the mean, σ_i is the standard deviation, ω_i is the weight function, and Δx is a step interval (set to 0.1 in the present model). The weight function is given by equally weighting the D distributions, $\omega_i = 1/\mathrm{D}$ (though with one exception as described later). The weight function could also have been determined by the relative number of samples taken at each broiler flock ($\omega_i = n_i / N$) or by how well the mean values were determined. We have chosen to weigh the flocks equally as the number of samples taken from each flock were approximately the same, and because we did not wish to emphasise a particular flock, which incidentally comprised a few more samples or showed a slightly smaller standard deviation.

Technique III): This technique was mainly used to generate distributions for the change in *Campylobacter* concentration throughout the different processes along the slaughter line. A given distribution of change in concentration is assumed to be normal distributed, and the mean is estimated by subtracting the data after a given process with the data before the same process. The variance of the distribution is given by the estimate of the variance component (σ_0^2), since we are only interested in describing the variation of the mean change, also named the variability. The estimation of the variance

component is shown in Table 19. Some of the elements in the table are calculated from a variance analysis as described in Technique I.

Table 19. Estimation of the variance component

	Formula
Variance component (variability)	$\sigma_0^2 = (MS_{bf} - MS_{wf})/n_0$
Variance of residual (uncertainty)	$\sigma_\varepsilon^2 = MS_{wf}$
Weighted group average	$n_0 = (N - \frac{\Sigma_i n_i^2}{N}) /(k-1)$
Variance of a random sample (total uncertainty)	$\sigma_t^2 = \sigma_\varepsilon^2 + \sigma_0^2$

The distribution for the D 'change in concentration' data is thus given by N(μ, σ_t^2), where $\mu = (\mu_1 + \mu_2 + \ldots \mu_D) / D$ and $\sigma_t^2 = \sigma_\varepsilon^2 + \sigma_0^2$. μ_i is the mean change for flock *i*, and σ_ε^2 and σ_0^2 are the variances between chickens in a flock and between the different flocks, respectively.

Slaughterhouse model – data input

In the following the different steps in the slaughterhouse will be discussed, in particular the data, which are used as input data in the QRA model.

In order to estimate the spread and changes in the *Campylobacter* prevalence and concentration on the broiler/chicken carcasses the following data were needed as input data in the slaughterhouse model:

1. The flock prevalence at the entrance to the slaughterhouse.
2. The concentration of *Campylobacter* on the broiler carcasses at the entrance to the slaughterhouse.
3. The changes in *Campylobacter* concentration through different plant processes.
4. The cross-contamination between flocks during slaughter.

In the slaughterhouse model multiplication of *Campylobacter* during processing is not included. This is because *Campylobacter jejuni* is assumed not to grow at temperatures below 30°C, which in principle means that they only grow and proliferate in a host. At least this will be true in Denmark where the temperature is below 30°C most of the year.

Table 20 gives a summery of the availability of data related to the slaughterhouse model, from the entrance of the broilers to the packed chicken product. Danish data are preferred in the model, but as it appears from Table 20, only few Danish data exist. Therefore, published data from other countries are used in the model. An overview of these data is seen in Table 21.

Table 20. A summary of data availability regarding *Campylobacter* prevalences and concentrations in broilers/chickens from the entrance to the slaughterhouse to packed retail product

Campylobacter on broilers/chickens	Danish		Other countries	
	Qualitative data	Quantitative data	Qualitative data	Quantitative data
at entrance to the slaughterhouse	+	-	+	-
before/after bleeding	-	-	+	+
before/after scalding	-	-	+	+
before/after defeathering	-	-	+	+
before/after washing and chilling	-	-	+	+
packed chicken product	-	-	+	+
retail chicken product – chilled	+	+	-	-
retail chicken product – frozen	+	+	-	-

Table 21. An overview of the sampling locations described in the non-Danish data. Some locations are merged to make the studies comparable.

Slaughterhouse process included in the model	Izat *et al.* (1988)	Oosterom *et al.* (1983b)	Mead *et al.* (1995)	Cason *et al.* (1997)
After bleeding	Prescald	After bleeding	After exsanguination	
After scalding	Postscald	After scalding		
After defeathering	Postpick	After defeathering		
After evisceration	Postvicera removal/Prewash	After evisceration		Prechill*
After washing + chilling	Postchill/Prepackage	After washing + chilling		Postchill

* before the washing location

Mead *et al.* (1995) studied the effect of improving the hygiene at a slaughterhouse, mainly by increasing the concentration of chlorine in the processing water at different locations in the plant. A total of 15 flocks were examined, 5 flocks before and 10 flocks after the changes. For each flock neck skin from 15 birds were sampled except for two flocks, where 10 samples were collected. Of the 15 flocks 11 were positive in 97% of the caecal samples indicating that the broilers sampled in these flocks came from positive flocks. Only data from these 11 flocks are included in the model, because we are only interested in the concentration on the carcasses of positive flocks. Although chlorine was used in the killing machine after changing the process (and not before), we have not differentiated between data sampled before and after the changes, since these had no effect on the neck skin concentrations ‘after bleeding’.

Oosterom *et al.* (1983b) investigated different broiler plant processes at two different slaughterhouses. At each plant three independent flocks were examined. For each flock pericloacal skin pieces were collected from 8 birds and pooled 2 in each pool (4 x 2 samples at each location). Data were presented as the number of cfu per gram skin.

Izat *et al.* (1988) investigated different broiler processes at three different broiler-processing plants. Samples were collected from each plant on two independent days. At each sampling location the right site of four broilers were swabbed and pooled and a second sample was obtained by making a pooled swab sample of the left site of the same four broilers (2 x 4 samples at each location). 50 cm^2 skin of each bird was swabbed at plant A, and 100 cm^2 at plant B and C. Data were presented in log cfu per 1000 cm^2.

Cason *et al.* (1997) analysed relationships between aerobic bacteria, *Salmonella* and *Campylobacter* on broiler carcasses before and after the washing/chilling process at one slaughterhouse. In that study 90 birds were sampled before the carcass washer and 90 birds after the chiller. Data were presented as the number of *Campylobacter* per carcass.

The flock prevalence at the entrance to the slaughterhouse

The available data on the *Campylobacter* status of Danish broilers are based on a single pooled faecal sample of 10 broilers from each broiler house (see Fig. 3), sampled when the broiler flocks enter the slaughterhouse. These data cannot be used to conclude on the prevalence of *Campylobacter* within a broiler flock. However, as previously mentioned, the rate by which *Campylobacter* is spread in a broiler house is fast, i.e. the time from infection of the first chicken to a full-blown infection of all chickens happens in most cases in less than a few days (Berndtson, 1996). Therefore, in the presented model all broilers belonging to the same flock are declared either contaminated or not contaminated, based on a single pooled sample of 10 broilers. Thus, the within flock prevalence is either 100 % or 0 %. Furthermore, since there is no correlation between the *Campylobacter* status of the flock and the size of the same flock (Fig. 12), the broiler prevalence equals the flock prevalence.

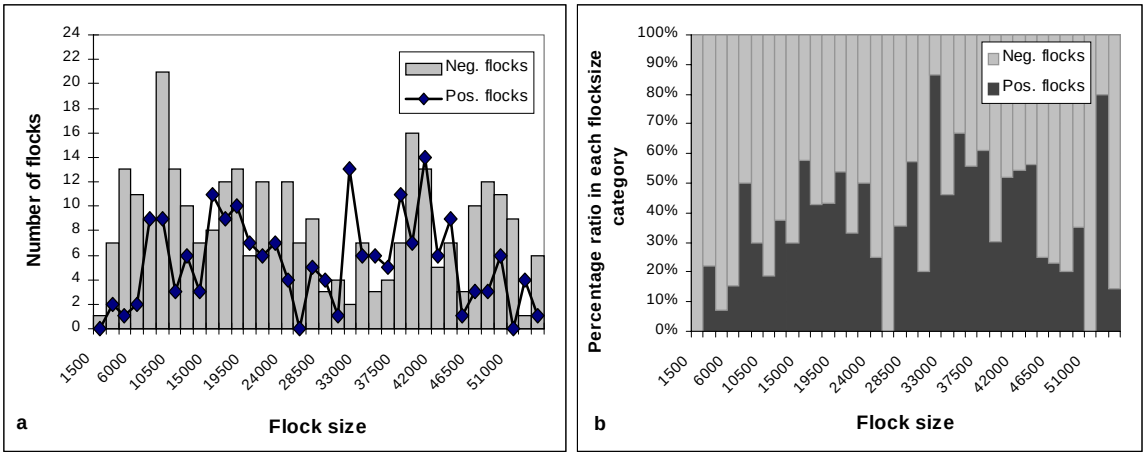

Figure 12. *Campylobacter* flock status as a function of flock size. a) The relationship between the actual number of positive/negative flocks for a given flock size. b) The same figures just as percentage of the total number of flocks.

There may be a little chance that the broilers are brought to the slaughterhouse during the 3 days infection period. Hence, only a fraction of the broilers are contaminated when they reach the slaughterhouse. Furthermore, in some broiler houses (probably few houses), areas in the house will contain sub populations of broilers that never, or only slowly, become contaminated (Tornøe, 1999). However, we believe that the error made by not taking these factors into account is rather limited.

Finally, the broilers are placed in houses, which often contain more than 40000 individuals without any separations. In such an environment the spread of faeces to the broiler surfaces is probably quite massive and as such also the spread of *Campylobacter*. We therefore assume that a *Campylobacter* positive status for a flock based on faecal samples (status of the interior) also applies for the exterior of the broilers. In other words, all broilers, who have been shown to have a positive *Campylobacter* status based on a faecal sample, are also assumed to be contaminated on the skin surface.

The prevalence data, used as input data in the model, are the actual flock prevalence for the broiler flocks slaughtered at a Danish slaughterhouse during the period February 1998 – October 1999. In fact not only the *Campylobacter* status is included, but also the exact slaughter order and flock size. This is important for example in relation to analysis of cross-contamination from a *Campylobacter* positive flock to a negative flock. The data also enables us to include seasonal variation. A disadvantage is that the input data (status, size and order of slaughtering) are fixed, and thus do not allow for analysis of the effect of e.g. changing flock size and order of flocks.

A few data from the slaughter program are presented in Fig. 13. As shown in Fig. 13b, the number of flocks slaughtered per day varies considerably, as do the number of broilers slaughtered per day (Fig. 13a). We believe that these variations are typical for most Danish slaughterhouses. Note also that the seasonal variation in the *Campylobacter* status of broiler flocks slaughtered at this particular slaughterhouse is in good agreement with the general seasonal variation of all Danish broiler flocks (compare Fig. 13c with Fig. 3). Thus, the broilers slaughtered at this particular slaughterhouse seem to be representatives for all broilers slaughtered in Denmark.

The concentration of *Campylobacter* on the broiler carcasses at the entrance to the slaughterhouse

At present no Danish data are available describing the concentration of *Campylobacter* on broilers at the entrance to the slaughterhouse. The entrance to the slaughterhouse is defined as the place, where the broilers are unloaded from the transportation vehicle or at the hanging station (see Fig. 16). A single report based on a study of a broiler house in the US determined *Campylobacter* levels in the caecum of broilers (Stern *et al.* 1995). However, at present there is no estimate of the relation between the distributions describing the *Campylobacter* concentrations in faeces/caecum and the distributions describing concentrations on the outer surface of the broilers. As an alternative, concentration data generated 'after bleeding' by Mead *et al.* (1995) and Oosterom *et al.* (1983b) are included in the present model (Table 22), representing the *Campylobacter* concentrations on the broilers at the entrance to the slaughterhouse. This is possible,

because we do not assume any significant changes in the *Campylobacter* concentrations from unloading of the broilers until after bleeding. We have chosen not to include the data published by Izat *et al.* (1988) as these data are swab-samples (log cfu/1000 cm^2) contrary to the data from Mead *et al.* (1995) and Oosterom *et al.* (1983b), which are skin samples (log cfu/g).

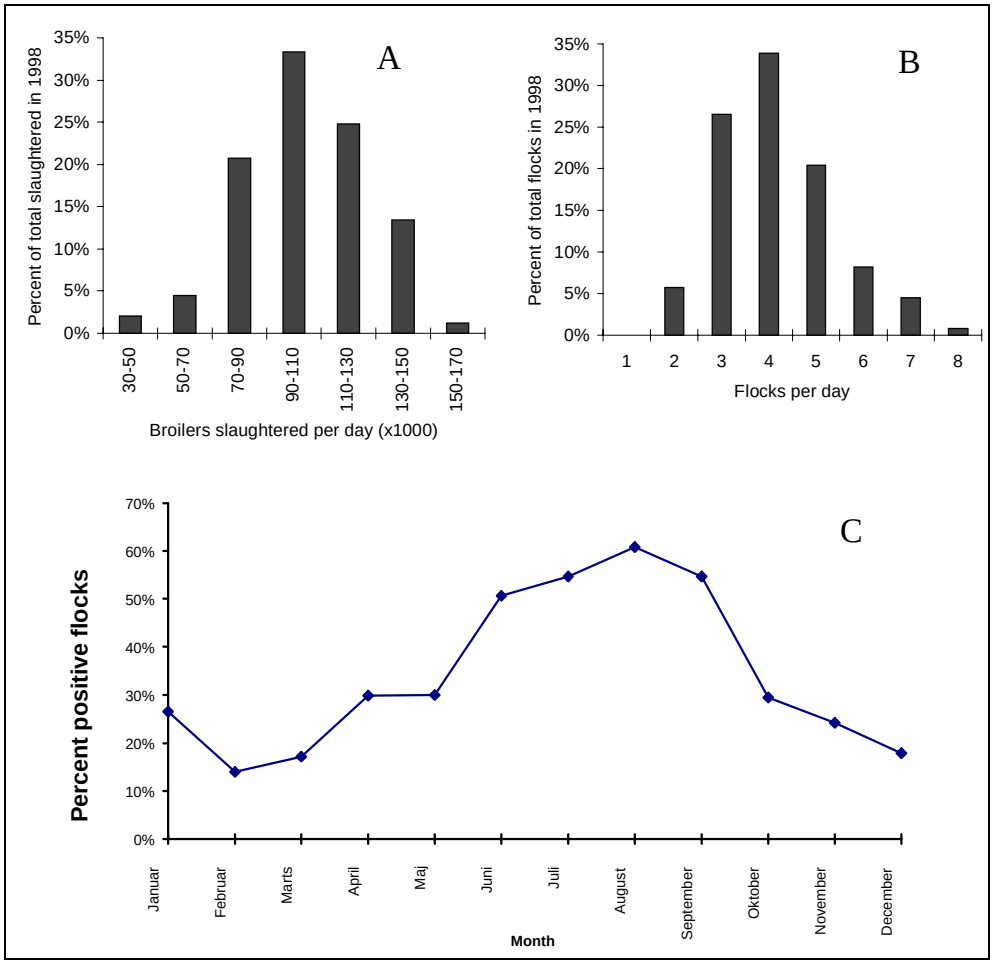

Figure 13. Key numbers from a slaughter program obtained from a Danish slaughterhouse for the period February 1998 – January 1999. A: Distribution of the number of broilers slaughtered per day in percent of total number of broilers slaughtered that year, B: Distribution of the numbers of flocks slaughtered per day in percent of total number of flocks slaughtered that year, C: Seasonal variation in the percentage of *Campylobacter* positive flocks.

For the data presented in Table 22 a pre-analysis was carried out using the statistical techniques described in Technique I (see section on page 40). Calculations and results are presented in Appendix 1 and the estimated distributions are presented in Fig. 14.

A one-way analysis of variances for all 17 data points was planned. However, when carrying out Bartlett's test for equality of variances (which is a prerequisite for the analysis of variances) we found that the variances differed. Thus, the 17 data points

could not be represented by one distribution. From Fig. 14 it appears that the variances of the data from Oosterom *et al.* (1983b) seem larger than those of Mead *et al.* (1995). This indicates that the data generated by Mead and co-workers and Oosterom and co-workers may be significantly different (either due to the variances or the means). Moreover, the sum distribution (Fig. 14) developed from Technique II is skewed (it has a thick tail to the left). This may be explained by a relatively large group of data having a mean less than the overall mean.

Table 22. Data used in developing a distribution of the *Campylobacter* concentration on the broiler carcasses at the entrance to the slaughterhouse (= 'after bleeding' data).

Reference	Sample type	No. of samples	Flock number	Slaughter plant	Log_{10} CFU/unit	Standard deviation	Unit
Mead *et al.* (1995)	Neck skin	10	M.1	A (UK)	3.7	0.60	g
Mead *et al.* (1995)	Neck skin	10	M.2	A (UK)	4	0.32	g
Mead *et al.* (1995)	Neck skin	15	M.3	A (UK)	3.9	0.39	g
Mead *et al.* (1995)	Neck skin	15	M.4	A (UK)	3.8	0.39	g
Mead *et al.* (1995)	Neck skin	15	M.5	A (UK)	3.4	0.39	g
Mead *et al.* (1995)	Neck skin	15	M.6	A (UK)	3.9	0.39	g
Mead *et al.* (1995)	Neck skin	15	M.7	A (UK)	3.6	0.39	g
Mead *et al.* (1995)	Neck skin	15	M.11	A (UK)	3.5	0.39	g
Mead *et al.* (1995)	Neck skin	15	M.12	A (UK)	4.3	0.39	g
Mead *et al.* (1995)	Neck skin	15	M.13	A (UK)	3.9	0.80	g
Mead *et al.* (1995)	Neck skin	14	M.15	A (UK)	3.7	1.12	g
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.1	A (NL)	2.39	1.08	g
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.2	A (NL)	3.42	1.65	g
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.3	A (NL)	3.44	1.92	g
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.4	B (NL)	3.99	1	g
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.5	B (NL)	3.3	0.92	g
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.6	B (NL)	2.18	0.67	g

The 'number of samples' for Oosterom *et al.* (1983b) was set to 4 due to the 4 pools each of 2 samples. The standard deviations for Mead *et al.* (1995) were calculated from the standard deviations of the mean values given in the article. The standard deviations were thus calculated by the squared root of the number of samples, multiplied by the standard deviations of the mean values ($SD = \sqrt{n_i} * SEM$).

The data from Oosterom *et al.* (1983b) and Mead *et al.* (1995) were examined separately. For each of the two groups Bartlett' test and an analysis of the variances (both described in Technique I) were carried out to examine if each of the two groups could be represented by a distribution. The results are given in Appendix 1.

No significant difference was observed between the variances and the means of the six flocks in the data of Oosterom *et al.* (1983b). Therefore, a distribution representing these six data points was created. As regards the data from Mead *et al.* (1995) different results of Bartlett's test were seen due to the inexact specification of the standard deviations of the means (SEM) (only one decimal was given in the article). We acted, however, as if there were no significant differences between the variances and carried

on with the variance analysis, which showed no significance between the means. Thus, a distribution representing these 11 data points of Mead and co-workers was created.

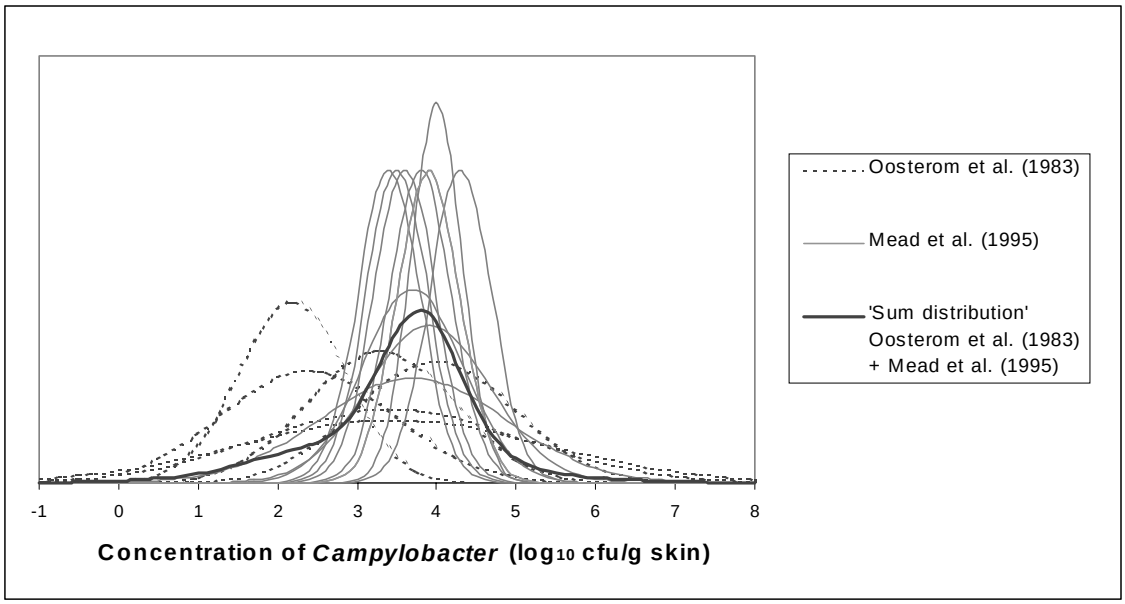

Figure 14. Estimated distributions of the *Campylobacter* concentration on chickens 'after bleeding' based on data published by Mead *et al.* (1995) and Oosterom *et al.* (1983b).

A t-test was carried out (Appendix 2) to test if the data published by Mead *et al.* (1995) and Oosterom *et al.* (1983b) were significantly different**.** The test showed that the mean concentration was significantly higher for the data published by Mead *et al.* (1995) than by Oosterom *et al.* (1983b). Also the variances of the two investigations differed. This may partly be explained by different sampling methods. Mead *et al.* (1995) sampled neck skin, and Oosterom et al (1983b) sampled pericloacal skin. However, different slaughter techniques, variations in microbiological methods, an actual difference in the *Campylobacter* concentration in the broiler flocks from the two countries may also have contributed to the data differences in the two investigations.

With reference to the outcome of the statistical tests, two estimated distributions describing the *Campylobacter* concentration on chickens 'after bleeding' were tended to be used as input in the model, one based on data from Mead *et al.* (1995) and one based on data from Oosterom *et al.* (1983b). The distributions are presented in Fig. 15a and 15b, respectively. The means and the variances for the two normal distributions are given by: N(3.79, 0.34) for the distribution based on the data of Mead *et al.*(1995) and N(3.12, 1.70) for the distribution based on the data of Oosterom *et al.* (1983b). The variances (0.34 and 1.70) represent the total uncertainty. These variances can be separated into uncertainty and variability. The separation of uncertainty and variability is important with regard to optimising the sampling strategy when further samples are to be collected. In these cases the uncertainty contributes most to the total uncertainty.

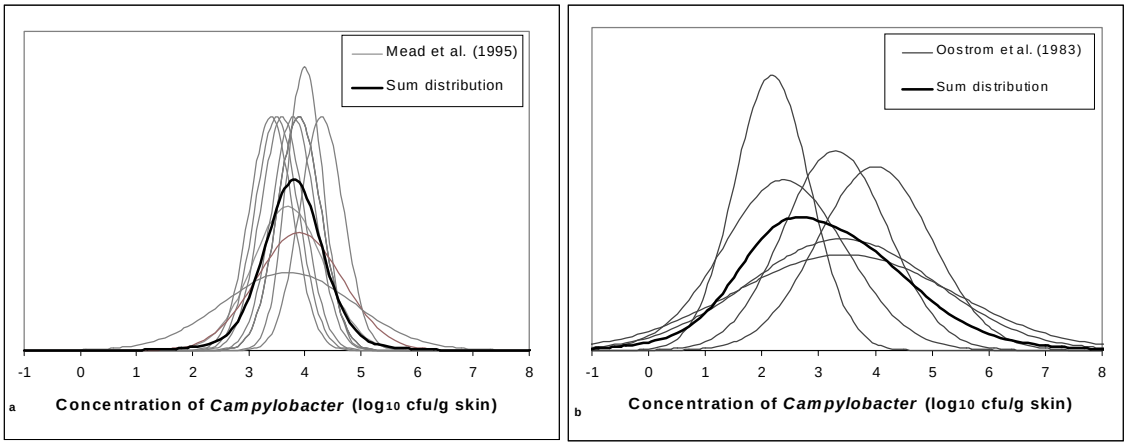

Figure 15. Estimated distributions of the *Campylobacter* concentration on chickens 'after bleeding' based on a) data published by Mead *et al.* (1995) and b) data published by Oosterom *et al.* (1983b). The 'sum distributions' were used as input distributions.

The changes in *Campylobacter* concentration through different plant processes

When the broilers have entered the slaughterhouse, the carcasses pass through series of slaughter processes before they end as packed products at the end of the slaughter line. The most important processes in relation to the present work are presented in Fig. 16.

A few papers describe, how *Campylobacter* concentrations on broiler carcasses change throughout the most important processing steps (see Table 21). In particular two groups (Izat *et al.*, 1988; Oosterom *et al.*, 1983b) have presented thorough studies. Data from these studies constitute the basis of the distributions that are implemented in the present model.

As we are interested in combining data from the different studies, only data obtained at comparable sampling locations at the slaughter line are taken into account (see Table 21). Some of the sampling locations published by Izat *et al.* (1988) are located at positions in the slaughter plant, where only minor changes in *Campylobacter* concentrations may occur. For example, between the sampling locations 'post viscerate removal' and 'pre wash' no processing occurs (see Fig. 16), which could alter the *Campylobacter* concentration on the broiler carcasses. In other words, some of the sampling points could be considered identical. We believe that the locations listed in Table 21, which are 'after bleeding', 'after scalding', 'after defeathering', 'after evisceration, and 'after washing and chilling, cover most of the important processes at the slaughter line, except from the carcass washer located before the chiller process. We would have preferred to include the washing process in the model. However, only Izat and co-workers have data for this process and 3 data points are insufficient to develop a distribution of the changes in the *Campylobacter* concentration. In the model we therefore consider the washing and chilling process as one process.

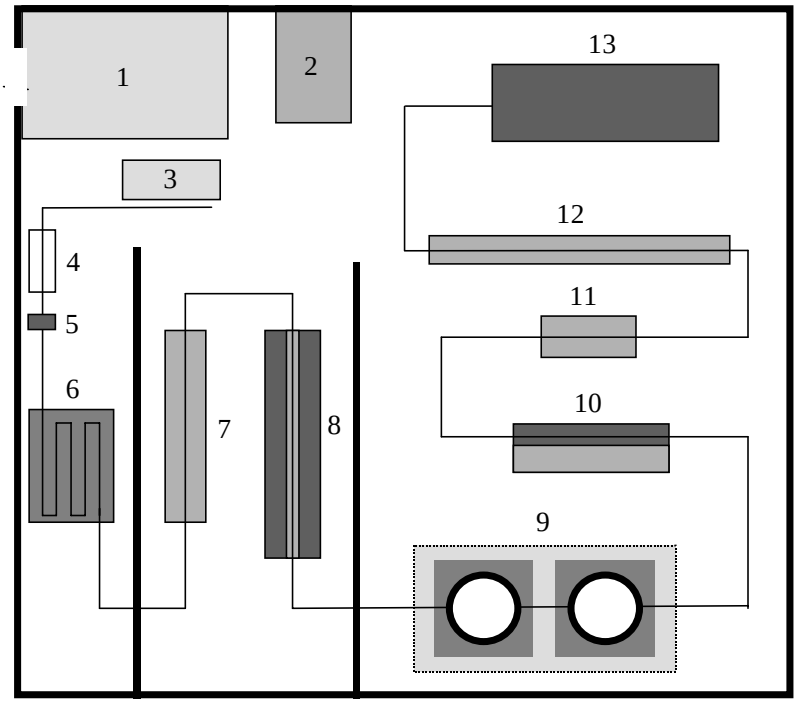

Figure 16: Overview of a broiler processing plant including the most important steps in relation to changes in the level of *Campylobacter* on the broiler carcasses. 1. Crate unloading, 2. Hanging station, 3. Crate washer, 4. Stunning, 5. Killing, 6. Bleeding, 7. Scalding, 8. Feather picker, 9. Evisceration, 10. Inspection, 11. Broiler washer, 12. Chiller process, 13. Package department.

We have chosen to process the data describing the different processes along the slaughter line as logarithmic changes in the *Campylobacter* concentration. Therefore, we can include the data from Izat *et al.* (1988). As mentioned previously, the data from Izat *et al.* (1988) are based on swab samples (cfu/1000cm^2), data from Cason *et al.* (1997) are based on whole carcass wash and the data from Oosterom *et al.* (1983b) are based on neck skin samples (cfu/g skin). However, when estimating logarithmic changes, the measuring unit is of less importance.

The data, which form the basis of the input distributions describing changes in concentration during 'scalding', 'defeathering', 'evisceration, and 'washing and chilling', are commented separately in the following.

Scalding

The data used in developing a distribution describing the scalding process are shown in Table 23. Oosterom *et al.* (1983b) investigated the effect of scalding at two different slaughterhouses. At one plant the scalding temperature was 58°C and at the other it was 52°C. The mean $\log_{10}$ reductions in the *Campylobacter* concentration at the two temperatures were 1.34 and 2.04, respectively. However, these were not significantly different (See Appendix 3). Due to the small sample sizes, sampling variation may overshadow a real effect. Therefore, in the present model we do not distinguish between different scalding temperatures.

Table 23. Data used in developing a model for the scalding process.

Reference	Sample type	No. of samples	Flock id.	Slaughter plant	Log_{10} a. b.	SD a. b.	Log_{10} a. s.	SD a. s.	Log_{10} change	SD change	Unit
Izat *et al.* (1988)	carcass swabbing	2	I.1 + I.2	A (US)	3.74		1.26		-2.48		1000 cm^2
Izat *et al.* (1988)	carcass swabbing	2	I.3 + I.4	B (US)	3.56		1.26		-2.30		1000 cm^2
Izat *et al.* (1988)	carcass swabbing	2	I.5 + I.6	C (US)	3.03		1.19		-1.84		1000 cm^2
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.1	A (NL)	3.99	1.00	1.37	1.44	-2.62	1.75	g
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.2	A (NL)	3.30	0.92	1.68	0.44	-1.62	1.02	g
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.3	A (NL)	2.18	0.67	2.40	0.80	0.22	1.04	g
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.4	B (NL)	2.39	1.08	0.61	0.06	-1.78	1.08	g
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.5	B (NL)	3.42	1.65	1.25	0.35	-2.17	1.69	g
Oosterom *et al.* (1983b)	Pericloacal skin	4	O.6	B (NL)	3.44	1.92	1.26	0.35	-2.18	1.95	g

Log_{10} = log_{10} cfu/unit. a.b. = 'after bleeding', a.s. = 'after scalding'.

The log_{10} change in concentration (in Table 23) is calculated by subtracting the data 'after scalding' from the data 'after bleeding'. The variances of the log_{10} changes (ch) were calculated by adding the variances of 'after bleeding' (ab) data with 'after scalding' (as) data:

$$\sigma^2_{ch,i} = \sigma^2_{as,i} + \sigma^2_{ab,i} \quad , \qquad i = 1, 2, \ldots , D$$

Since Izat *et al.* (1988) have not reported any standard deviations, only 6 out of the 9 estimates of the log_{10} change have an estimate of the variance ($\sigma^2_{ch,i}$). The mean change in concentration (μ_{ch}) and the variance between flocks $\sigma^2_{ch,0}$ (also called the variance component) are calculated from all 9 estimates of the log_{10} change, whereas the variance within the flocks $\sigma^2_{ch,\varepsilon}$ are calculated only from the 6 flock estimates. Thus, a mean of all 9 estimates of the change in concentration is estimated (μ_{ch}) and a variance for the individual observation of the change is calculated by ($\sigma^2_{ch} = \sigma^2_{ch,0} + \sigma^2_{ch.\varepsilon}$). The estimation of $\sigma^2_{ch,\varepsilon}$ and $\sigma^2_{ch,0}$ appears from Technique III (see section on page 40). The resulting normal distribution based on $\sigma^2_{ch,\varepsilon}$ and $\sigma^2_{ch,0}$ is very wide because it consists of both process variations and variations within the flock (the broilers sampled before scalding were for example not the same as the broilers sampled after scalding). However, we are only interested in describing the mean change in concentration (μ_{ch}) and it's variance for the process, which in this case is the variance component, $\sigma^2_{ch,0}$. To simulate the scalding process a normal distribution N(μ_{ch}, $\sigma^2_{ch,0}$) is employed (input distribution). In Fig. 17 the 9 distributions of the change in concentration are shown together with the distribution that was used as input in the model.

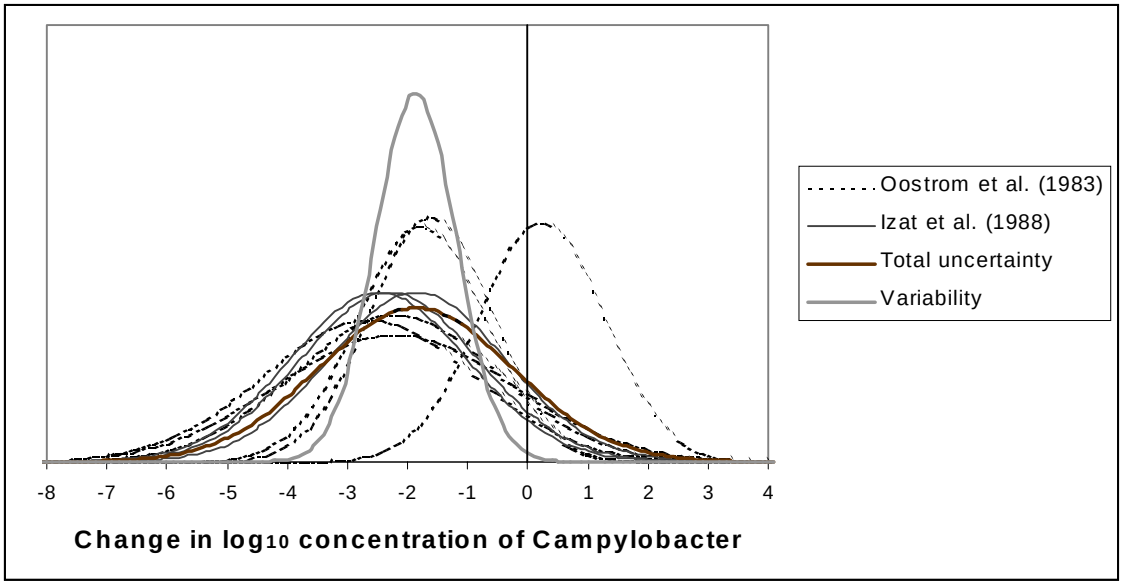

Figure 17. Estimated distributions of the change in the *Campylobacter* concentration on chicken carcasses during scalding. The distributions are based on data published by Izat *et al.* (1988) and Oosterom *et al.* (1983b). The 9 distributions are developed based on the assumption that the reported means are normal distributed. The input distribution is calculated from Technique III. The input distribution (referred to as 'variability' in the figure) is given by a normal distribution with a mean equal to the total mean of the 9 mean estimates and a variance equal to the variance component. Estimation of the variance component is given in Appendix 4.

To test if the mean change in concentration is significant for the input distribution $N(\mu_{ch}, \sigma^2_{ch,total})$ = N(-1.86, 0.46), a one-way variance analysis was carried out followed by a test hypothesis (Appendix 4). A parried t-test could also have been carried out, but then the variance of the individual samples would not have been accounted for. The results of the two tests were that scalding had a significant effect on the *Campylobacter* concentration on the carcasses.

Defeathering

In order to develop a distribution describing the defeathering process, data from Izat *et al.* (1988) and Oosterom et al (1983b) were used (Table 24). The change in concentration measured by Izat *et al.* (1988) seems slightly higher than the change measured by Oosterom *et al.* (1983b). It is not possible to test if this difference is significant, because the variances of the samples are not included in the paper of Izat *et al.* (1988). Therefore, we cannot exclude data from the material and all 9 data points are used in developing an input distribution.
The data included in the calculations of the effect of the defeathering process are sampled 'after scalding' and 'after defeathering'. The data are analysed as described for the scalding process (see the previous section). The distributions, describing the changes in the *Campylobacter* concentration on the carcasses, are shown in Fig. 18. The input distribution, which is used in the model, is given by a normal distribution with a mean calculated from the 9 estimates of the change (see Table 24) and a variance given by the variance component (see Appendix 5). The input distribution is, thus, given by N(1.03,

0.15). A test was carried out to examine if the mean change in concentration was significant (Appendix 5). The test results showed that the defeathering process leads to a significant increase in the *Campylobacter* concentration on the carcasses.

Table 24. Data used in developing a model for the defeathering process.

Reference	Sample type	No. of samples	Flock id.	Slaughter plant	Log_{10} a. s.	SD a. s.	Log_{10} a. d.	SD a. d.	Log_{10} change	SD change	Unit
Izat *et al.* (1988)	carcass swabbing	2	I.1 + I.2	A (US)	1.26	-	2.37	-	1.11		1000 cm^2
Izat *et al.* (1988)	carcass swabbing	2	I.3 + I.4	B (US)	1.26	-	3.68	-	2.42		1000 cm^2
Izat *et al.* (1988)	carcass swabbing	2	I.5 + I.6	C (US)	1.19	-	2.82	-	1.63		1000 cm^2
Oosterom et al. (1983b)	Pericloacal skin	4	O.1	A (NL)	1.37	1.44	2.46	0.81	1.09	1.652	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.2	A (NL)	1.68	0.44	2.09	0.44	0.41	0.622	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.3	A (NL)	2.40	0.80	2.18	0.35	-0.22	0.873	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.4	B (NL)	0.61	0.06	1.07	0.76	0.46	0.762	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.5	B (NL)	1.25	0.35	1.99	0.73	0.74	0.810	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.6	B (NL)	1.26	0.35	2.85	0.70	1.59	0.783	g

Log_{10} = log_{10} cfu/unit, a.s. = 'after scalding', a.d. = 'after defeathering'.

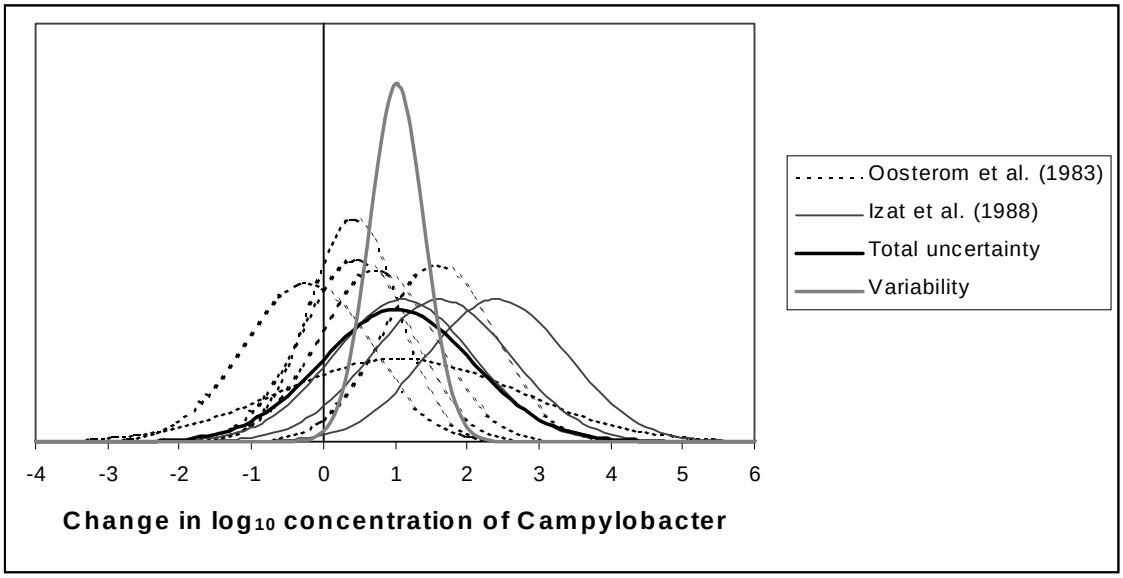

Figure 18. Estimated distributions of the change in the *Campylobacter* concentration on chicken carcasses during defeathering. The distributions are based on data published by Izat *et al.* (1988) and Oosterom *et al.* (1983b). The 9 distributions are developed based on the assumption that the reported means are normal distributed. The input distribution is calculated from Technique III. The input distribution (referred to as 'variability' in the figure) is given by a normal distribution with a mean equal the total mean of the 9 mean estimates and a variance equal to the variance component. Estimation of the variance component is given in Appendix 5.

Evisceration

The input data used to describe the evisceration process are listed in Table 25. Izat *et al.* (1988) examined more process locations than Oosterom *et al.* (1983b). Therefore, data from Izat *et al.* (1988) sampled at 'postvicera removal' and 'prewash' are merged (see also Table 21). Between these two sampling locations, only visual inspection occurs and this has likely no effect on the *Campylobacter* level on the carcasses. By merging the data, the number of samples was doubled resulting in a more precise estimation of the mean.

The data describing the change in the *Campylobacter* level on carcasses during evisceration are calculated and analysed (Appendix 6) as described for the scalding process (see above). The distributions describing the changes are shown in Fig.19. Statistical analysis showed that the *Campylobacter* concentrations before and after evisceration were not significantly different (see Appendix 6). Though the process seemed to have no influence on the *Campylobacter* concentration, it is still included in the model. The input distribution is given by N(0.35, 0.07).

Table 25. Data used in developing a model for the evisceration process.

Reference	Sample type	No. of samples	Flock id.	Slaughter plant	Log_{10} a. d.	SD a. d.	Log_{10} a. e.	SD a. e.	Log_{10} change	SD change	Unit
Izat *et al.* (1988)	carcass swabbing	2	I.1 + I.2	A (US)	2.37		2.98		0.61		1000 cm^2
Izat *et al.* (1988)	carcass swabbing	2	I.3+ I.4	B (US)	3.68		3.22		-0.46		1000 cm^2
Izat *et al.* (1988)	carcass swabbing	2	I.5+ I.6	C (US)	2.82		3.50		0.68		1000 cm^2
Oosterom et al. (1983b)	Pericloacal skin	4	O.1	A (NL)	2.46	0.81	2.24	1.18	-0.22	1.43	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.2	A (NL)	2.09	0.44	2.62	1.24	0.53	1.32	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.3	A (NL)	2.18	0.35	2.50	0.63	0.32	0.72	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.4	B (NL)	1.07	0.76	2.58	0.68	1.51	1.02	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.5	B (NL)	1.99	0.73	2.44	0.53	0.45	0.90	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.6	B (NL)	2.85	0.7	2.60	1.56	-0.25	1.71	g

Log_{10} = log_{10} cfu/unit, a.d. = 'after defeathering', a.e. = 'after evisceration'

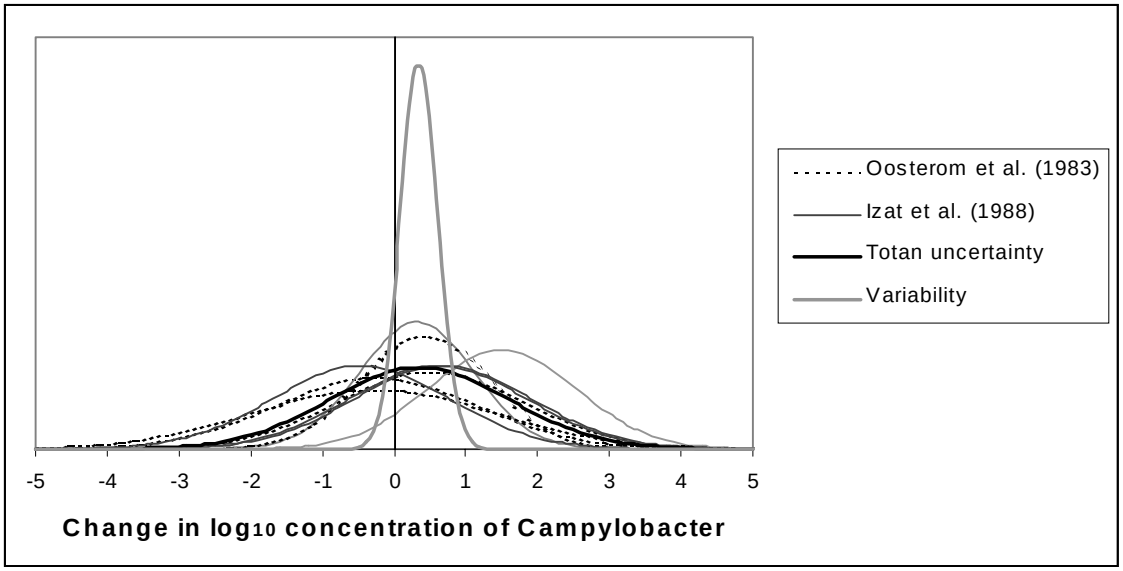

Figure 19. Estimated distributions of the change in the *Campylobacter* concentration on chicken carcasses during evisceration. The distributions are based on data published by Izat *et al.* (1988) and Oosterom *et al.* (1983b). The 9 distributions are developed based on the assumption that the reported means are normal distributed. The input distribution is calculated from Technique III (see text). The input distribution (referred to as 'variability' in the figure) for the defeathering process is given by a normal distribution with a mean equal to the total mean of the 9 mean estimates and a variance equal to the variance component. Estimation of the variance component is given in Appendix 6.

Washing and chilling

The data from Izat *et al.* (1988), Oosterom *et al.* (1983b) and Cason *et al.* (1997) constitute the major part of the available data (Table 26). In order to use these data we have merged the 'washing and chilling process' published by Izat *et al.* (1988), and the 'postchill' and 'prepackage' processes (see Table 21). By merging the data, the number of samples was doubled resulting in a more precise estimation of the mean.

Oosterom *et al.* (1983b) studied two different slaughter plants. At one plant the broilers were cooled by spin-chilling and at the other plant the broilers were cooled by air for 55 min. At each plant three independent flocks were examined. Unfortunately, the results varied and made it impossible to conclude anything about the effect of air cooling systems. Therefore, only data describing the water chilling have been included in the present model.

Cason *et al.* (1997) analysed relationships between aerobic bacteria, *Salmonella* and *Campylobacter* on broiler carcasses at a single broiler processing plant. In that study 90 birds were sampled before the carcass washer and 90 birds after the chiller. 30 birds were sampled at 6 different days and examined by a whole carcass rinse procedure. The 'whole carcass rinse' procedure gave rise to a much higher number of bacteria per unit (carcass) than the number of bacteria per g skin. However, as we focus on the change in log cfu, the units are of less importance. The study by Cason *et al.* (1997) differs from the other studies by sampling from different flocks before and after the washing and

chilling process, overlooking a flock effect. This means that a change in concentration over the washing and chilling process could be due to differences between flocks and not due to an actual change in the concentration caused by the process. Therefore, we have chosen to weight the data from Cason *et al.* (1997) less by setting the number of samples to 20 instead of 90.

Table 26. Data used in developing a model for the washing and chilling processes.

Reference	Sample type	No. of samples	Flock id.	Slaughter plant	Log_{10} a. e.	SD a. e.	Log_{10} a.w.+c	SD a.w.+c	Log_{10} change	SD change	Unit
Izat *et al.* (1988)	carcass swabbing	2	I.1+ I.2	A (US)	2.98		1.68		-1.3		1000 cm^2
Izat *et al.* (1988)	carcass swabbing	2	I.3+ I.4	B (US)	3.22		1.89		-1.33		1000 cm^2
Izat *et al.* (1988)	carcass swabbing	2	I.5+ I.6	C (US)	3.5		1.20		-2.3		1000 cm^2
Cason *et al.* (1997)	Whole carcass	90 (20)	C.1	D (US)	5.33	0.621	3.82	0.582	-1.51	0.851	carcass
Oosterom et al. (1983b)	Pericloacal skin	4	O.1	B (NL)	2.58	0.68	0.98	0.61	-1.6	0.914	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.2	B (NL)	2.44	0.53	1.24	0.89	-1.2	1.036	g
Oosterom et al. (1983b)	Pericloacal skin	4	O.3	B (NL)	2.60	1.56	1.83	0.21	-0.77	1.574	g

Log_{10} = log_{10} cfu/unit, a.e. = 'after evisceration', a. w.+c. = 'after washing and chilling'. The number of samples in the study of Cason *et al.* (1997) is 90, but in the statistical analysis we count it as 20.

The data, which describe the change in the *Campylobacter* level on carcasses during the washing and chilling processes, are calculated and analysed (Appendix 7) as described for the scalding process (see above). The distributions describing the changes are shown in Fig. 20. The distribution used in the model (input distribution) is given by a normal distribution with a mean calculated from the 7 estimates of the change and a variance given in Appendix 7, thus the input distribution is N(-1.46, 0.05). Statistical analysis showed that the *Campylobacter* concentration decreased significantly over the washing and chilling process (See Appendix 7).

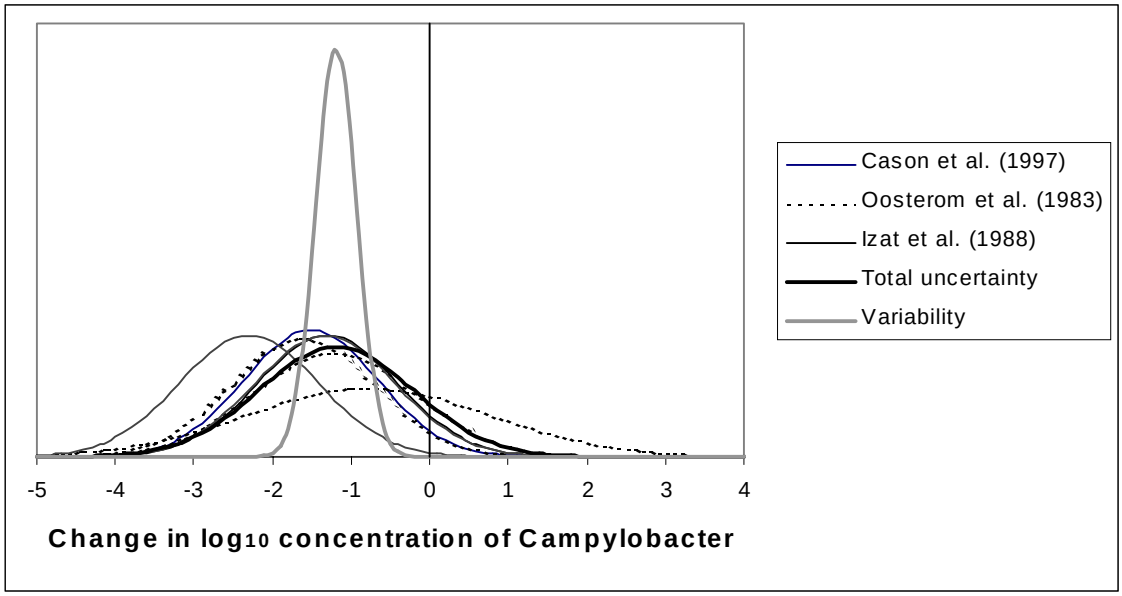

Figure 20. Estimated distributions of the change in the *Campylobacter* concentration on chicken carcasses during washing and chilling. The 7 distributions are based on data published by Cason *et al.* (1997), Izat *et al.* (1988), and Oosterom *et al.* (1983b) and are developed based on the assumption that the reported means are normal distributed. The input distribution is calculated from Technique III (see text). The input distribution (referred to as 'variability' in the figure) is given by a normal distribution with a mean equal the total mean of the 7 mean estimates of the change in concentration and a variance equal to the variance component. Estimation of the variance component is given in Appendix 7.

Overview of the effect of selected slaughter plant processes

The results of the studies referred to in this slaughterhouse model are quite alike, despite a possible difference in the *Campylobacter* concentration in the broiler flocks examined, different slaughter techniques, different sampling locations, variations in sampling methods (carcass swabbing, pericloacal skin, neck skin and whole carcass wash), etc. The concentration level does of course depend on these factors, whereas the change in concentration is more independent. In Fig. 21 the effect of the different processes are shown graphically. In Fig. 22 the mean effect related to the different processes are shown for an index concentration of zero. The estimated processes through the slaughter plant are additive. Hence, the estimated mean and variance at the exit of the slaughter plant (after washing + chilling) is calculated as:

$$Y_{mean} = \mu_{after\ bleeding} + \Delta\mu_{after\ scalding} + \Delta\mu_{after\ defeathering} + \Delta\mu_{after\ evisceration} + \Delta\mu_{after\ wash+chiller}$$
$$\sigma^2_{mean} = \sigma^2_{after\ bleeding} + \Delta\sigma^2_{after\ scalding} + \Delta\sigma^2_{after\ defeathering} + \Delta\sigma^2_{after\ evisceration} + \Delta\sigma^2_{after\ wash+chiller}$$

where $\Delta\mu_{after\ scalding}$ is the mean change over the scalding process and $\Delta\sigma^2_{after\ scalding}$ is the variance of the mean change (here the variance component).

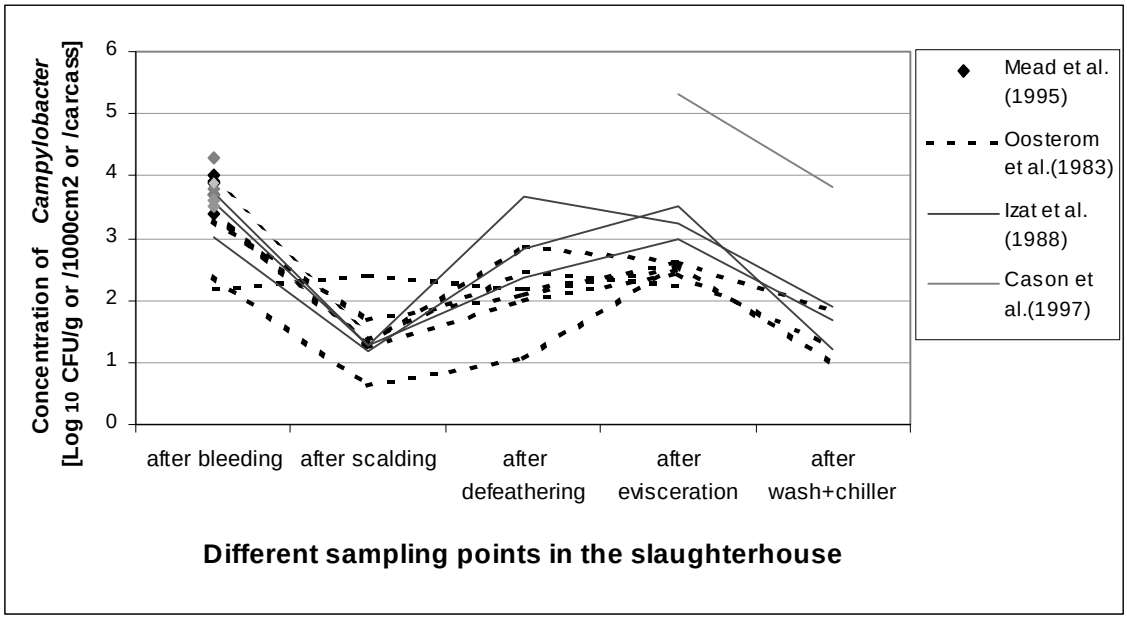

Figure 21. The influence of selected slaughterhouse processes on the *Campylobacter* concentration on chicken carcasses. Data are based on studies published by Mead *et al.* (1995), Izat *et al.* (1988), Oosterom *et al.* (1983b), and Cason *et al.* (1997).

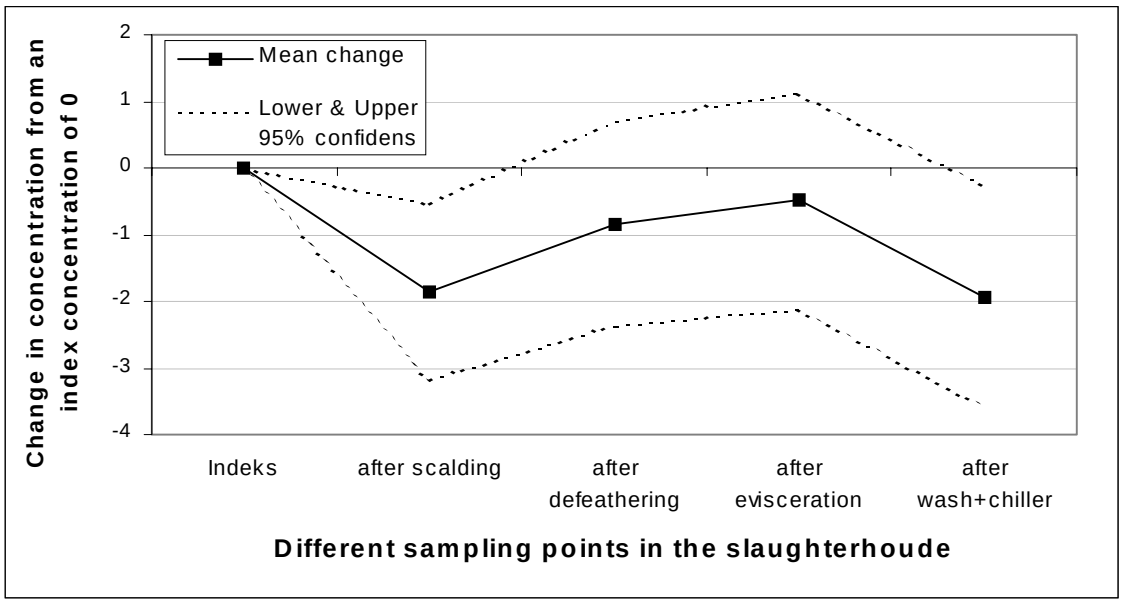

Figure 22. The mean influence of selected slaughterhouse processes on the *Campylobacter* level on chicken carcasses - for an index concentration of zero. Data are based on studies published by Izat *et al.* (1988), Oosterom *et al.* (1983b), and Cason *et al.* (1997).

The cross-contamination between flocks during slaughter

A lot of water is used in the broiler plant processes. This gives optimal conditions for survival and spread of *Campylobacter* from one broiler to another during processing. Therefore, in addition to the changes in *Campylobacter* concentration, the different

slaughter processes may also contribute to cross-contamination. An example is the spin chiller. Although this process seems to result in significant reductions in the *Campylobacter* concentration, it may have a large impact on *Campylobacter* cross-contamination between broilers. In the process a large amount of broilers are introduced into a flow of cooling water. The mixing of broilers and the continuous water flow may result in detachment of a large portion of *Campylobacter* into the cooling water, which may then spread to other broilers. A few studies have reported rather high concentrations of *Campylobacter* in the chiller water overflow, ~100 cfu/ml (Wempe *et al.* 1983) and 1000 cfu/ml (Oosterom *et al.* 1983b). Cross-contamination also seems to occur during other processes. High concentrations of *Campylobacter* have been detected in the scald water overflow, feather picker drip water, carcass washer (Wempe *et al.* 1983), in air samples near processing machines, on hands of the workers (Oosterom *et al.* 1983b), and on different equipment (Izat *et al.* 1988). Thus, indirect measurements at the slaughter plant indicate that cross-contamination occurs, but to our knowledge no studies are available demonstrating the direct cross-contamination between broilers. In addition, it is not clear how much each of the different processing machines may contribute to the total spread of *Campylobacter* between broilers. We assume that in particular scalding, defeathering, and chilling have significant impact on cross-contamination.

Cross-contamination between flocks may occur when a *Campylobacter* negative flock enters the broiler processing plant immediately after a positive flock. At present no data available describes how many broilers in a negative flock that will be contaminated. In principle, the number could be anything between zero and the entire flock (or several flocks). Studies by Izat *et al.* (1988) have shown that in two out of three broiler plants the chiller water contained low amounts of *Campylobacter* after 4 hours of continued flow. In other words, although the level of *Campylobacter* will be lower on a cross-contaminated 'negative' bird than on a positive bird, it is possible, that broilers in a negative flock will be contaminated with *Campylobacter* up till 4 hours after the passage of a positive flock. This is of course dependent on the water dilution rate in the chiller as well as numerous other factors, which may vary considerably from plant to plant. Assuming a 4-hour delay before *Campylobacter* is diluted out and a slaughter rate of approximately 10,000 broilers per hour (a normal size slaughterhouse), up to 40,000 broilers in a negative flock may become contaminated if they are slaughtered immediately after a positive flock. Note, that the level of contamination will be higher for the first broilers slaughtered in the negative flock as compared to e.g. broiler number 40,000.

In the present model we have chosen to build cross-contamination into the model as a worst case scenario, assuming that the first carcass in a negative flock will obtain a concentration similar to the concentration of positive carcasses 'after washing and chilling'. We also assume that *Campylobacter* is diluted out of the slaughterhouse as a function of the number of broilers from the negative flock that is slaughtered after the positive flock. As the number of broilers that needs to be slaughtered before the *Campylobacter* concentration is reduced 50% (T_{half}) is currently unknown, we decided to run the model with four different values of T_{half}; 300, 1000, 3000 and 6000.

It was of interest to see the impact of cross-contamination on the prevalence and the concentration profile of *Campylobacter* on the slaughtered chicken carcasses. Using the values 300, 1000, 3000, and 6000 for T_{half}, it is seen that the slower the *Campylobacter* is diluted out of the slaughterhouse, the more carcasses in the negative flocks will become contaminated (Fig. 23).

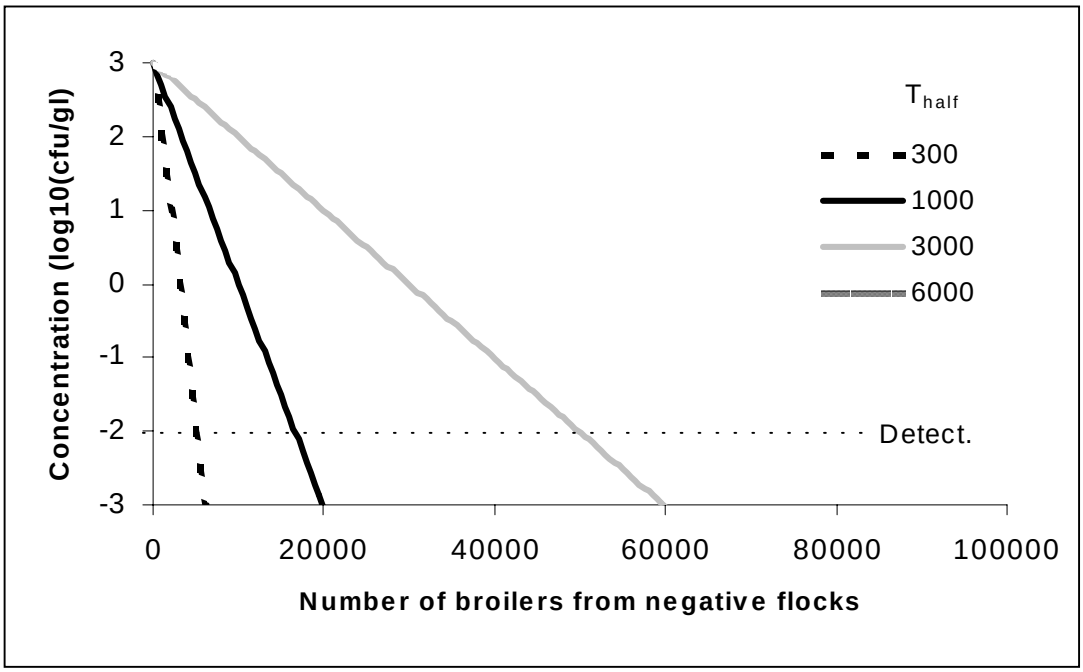

Figure 23. The number of cross-contaminated carcasses originating from *Campylobacter* negative flocks slaughtered after a positive flock at different values of T_{half} (= the number of broilers needed to be slaughtered, before the *Campylobacter* concentration is reduced to half the concentration) and the *Campylobacter* concentration on cross-contaminated carcasses. The concentration on the first broiler (originating from negative flocks) slaughtered after a positive flock is fixed to 10^3 cfu/g skin. The dotted line indicates the average minimum number of *Campylobacter*, which can be detected on a chicken carcass (approx. 1 per 100g skin).

Cross-contamination may also occur within positive flocks, i.e. there may be some cross-contamination from a broiler with a high concentration of *Campylobacter* to a broiler with a low concentration. Consequently, the broilers with a high concentration will obtain a lower concentration and those with low concentrations will get slightly higher concentrations through the processes. This means that the distribution of *Campylobacter* concentrations on broiler carcasses will end up being narrower at the end of the slaughter line than at the entrance to the slaughterhouse.

The cross-contamination within positive flocks is not accounted for in the present study. This homogeneous effect leads to a decrease of the variance estimate for the distribution of the concentration. It could possibly be built into the modelling of each of the processes together with the present estimates of the change in concentrations.

Slaughterhouse model - model building

Based on the input data described in the previous sections a computer program was developed, which allow us to model changes in the status of individual broilers with respect to *Campylobacter* concentration and prevalence throughout the different processing steps (= the slaughterhouse model).

The flow sheet (Fig. 24) gives a schematic view of the broiler processing-steps from the initial status of the broiler at the broiler house (immediately before slaughtering) to the status at retail level. In the Tables 27-28 the different parameters and distributions used to describe the different processing steps are presented. In the following, the different steps in the slaughterhouse model will be commented.

Summary of input data

Slaughterhouse

Based on the assumption that either all or none of the broilers in a flock are contaminated upon arrival to the slaughterhouse, the *Campylobacter* status of each individual broiler entering the slaughterhouse will be equal to the status of the flock which it belongs to. Therefore, the prevalence status of the flock (contaminated or not contaminated) is used as input data to describe the broiler prevalence ($P_{broiler}$). The concentration of *Campylobacter* on the broilers is not considered at this stage of the model.

In the model, the broilers are divided into two groups; those coming from negative flocks and those coming from positive flocks. With respect to *Campylobacter* positive flocks we do not expect any changes in *Campylobacter* concentration from the hanging station to the bleeding station. Therefore, the data from Mead *et al*. (1995) (based on neck skin samples after bleeding) are used as input data to describe the concentration, $C_{entrance}$, on *Campylobacter* positive broiler carcasses at the entrance to the slaughterhouse.

Changes throughout the different broiler plant processes are described by the data obtained from Izat *et al.* (1988), Oosterom *et al.* (1983), and Cason *et al.* (1997). These data have been converted into distributions as described in the previous sections. If the concentration changes to below 1 cfu per total weight (W_{skin}) of the chicken skin[2] the concentration is set to zero, and thus changing the *Campylobacter* status from positive to negative. Note that although a broiler carcass is negative in one process it may become re-contaminated through the following processes. Each process is described by two parameters, one for the prevalence and one for the concentration (see Table 27).

[2]*Minimum number of Campylobacter cells present on the chicken carcass is 1 per total weight of skin present on the chicken, because all measurements of the Campylobacter concentration are given by the number of Campylobacter per g skin (neck or pericloacal). The weight of skin was assumed to be proportional the total weight of the chicken. We determined the weight of chicken skin from 6 different 1000-gram chickens to approximately 100 g per chicken, i.e. 10% of the total chicken weight. Chickens vary in weight from approximately 850 g to 1250 g. We therefore introduced a uniform distribution with a minimum of 85 and a maximum of 125 g, which was used to describe the variation in chicken skin weight.*

Campylobacter negative flocks are divided into two groups. Those that are slaughtered first on a day (before a positive flock has entered the slaughterhouse) and those that are slaughtered after a positive flock. We do not assume any cross-contamination from one day to the next. Therefore, if a *Campylobacter* negative flock is slaughtered first on a slaughter day, all broilers in that flock will remain *Campylobacter* negative. If a negative flock is slaughtered after a positive flock, a certain degree of cross-contamination from the broilers in the positive flock to those in the negative flock is expected (see the section on page 59). The level of contamination of a particular broiler depends on

1) the number of negative broilers that have been slaughtered between the last slaughtered broiler in the positive flock and the broiler itself (N_{after_pos}) and
2) the distribution of the *Campylobacter* concentration after chilling ($C_{chiller}$) on the previously slaughtered *Campylobacter* positive flock.

This means that the first broiler in a negative flock, which is slaughtered after a positive flock, will obtain a *Campylobacter* concentration, which equals the concentration on the carcasses from the positive flock. As more and more *Campylobacter* negative broilers are slaughtered, the level of *Campylobacter* on the cross-contaminated broilers will decrease. The simulations are carried out with four different values of T_{half} 300, 1000, 3000 and 6000.
Although within flock cross-contamination may also occur in the slaughter process this has not been taken into account in the present model.

Retail

As previously described (see Table 3) the *Campylobacter* concentration is slightly reduced upon freezing of the chickens (approx. 0.5 –1.5 log units), whereas chilling not seems to affect the *Campylobacter* concentration considerably. Therefore, the retail step has been divided into chilled and frozen products and the ratio of chilled chickens relative to frozen chickens reaching the retail level has been included. In the model the reduction due to freezing of the chickens is described by a uniform distribution with a minimum of 0.5 and a maximum of 1.5. This is a very simple way to describe the reduction, but the available data are rather limited and do not allow production of a more exact estimate. A more general reduction in the level *Campylobacter* over time during storage has not been considered.

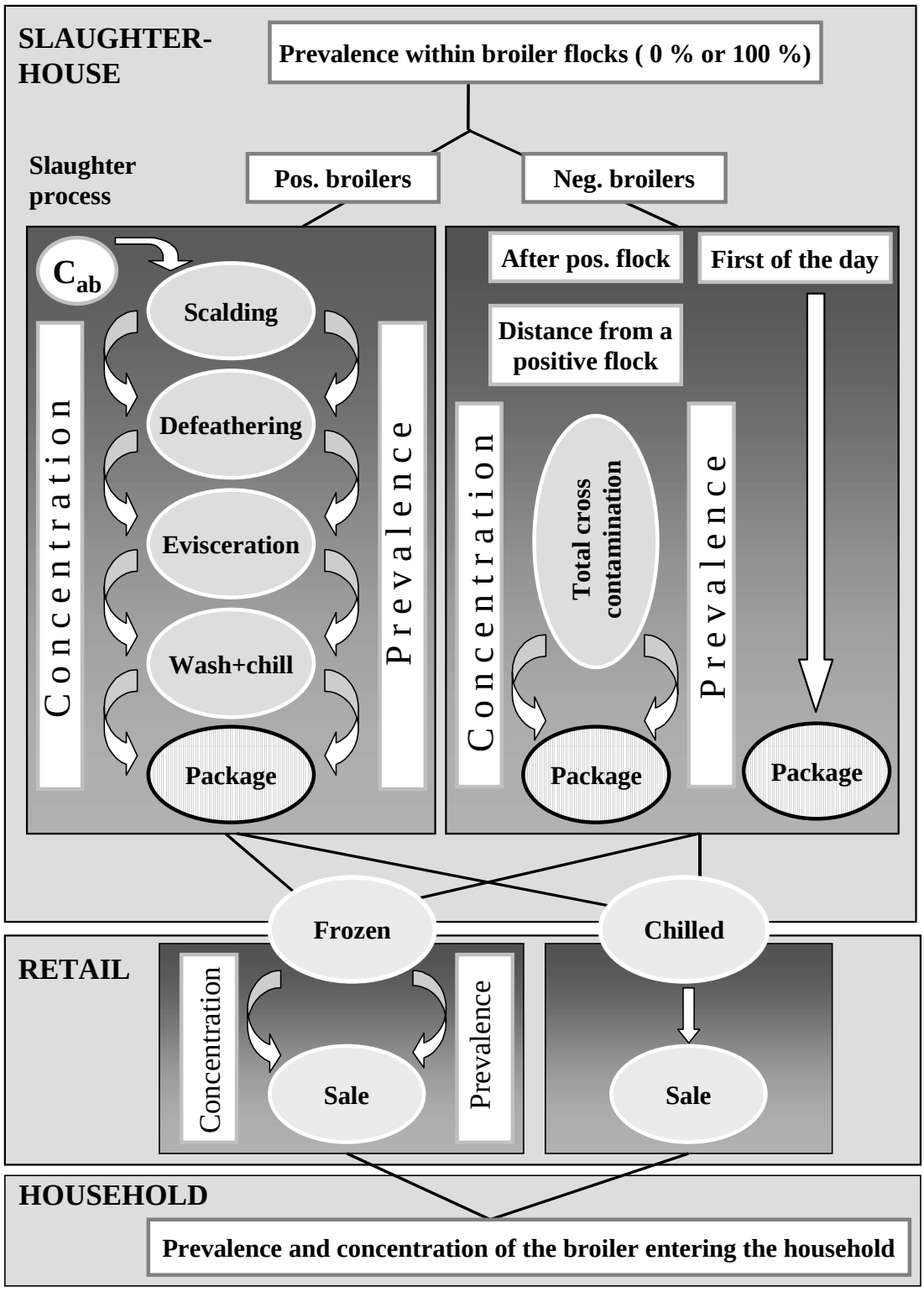

Figure 24. Schematic diagram of the slaughterhouse part of the QRA model

Table 27. Description of the steps in the slaughterhouse model.

Parameter	Description	Units	Distribution/expression
Positive flocks			
$P_{Broiler}$	Prevalence of broilers at the entrance to the slaughter house		Obtained from the slaughterprogram
W_{skin}	Weight of chicken skin	g	Uniform(85,125)
$C_{entrance}$	Concentration at entrance	Log_{10} cfu/g	Histogram from table 1
$P_{entrance}$	Prevalence at entrance		0 If $C_{entrance}<1/W_{skin}$
R_{scald}	Change through scalding		Histogram from table 2
C_{scald}	Concentration after scalding	Log_{10} cfu/g	$C_{entrance} - R_{scald}$
P_{scald}	Prevalence after scalding		0 If $C_{scald}<1/W_{skin}$
$R_{feather}$	Change through defeathering		Histogram from table 3
$C_{feather}$	Concentration after defeathering	Log_{10} cfu/g	$C_{scald} - R_{feather}$
$P_{feather}$	Prevalence after defeathering		0 If $C_{feather}<1/W_{skin}$
$R_{viscera}$	Change through evisceration		Histogram from table 4
$C_{viscera}$	Concentration after evisceration	Log_{10} cfu/g	$C_{feather} - R_{viscera}$
$P_{viscera}$	Prevalence after evisceration		0 If $C_{viscera}<1/W_{skin}$
$R_{chiller}$	Change through wash + chill		Histogram from table 5
$C_{chiller}$	Concentration after wash + chill	Log_{10} cfu/g	$C_{viscera} - R_{chiller}$
$P_{chiller}$	Prevalence after wash + chill		0 If $C_{chiller}<1/W_{skin}$
Negative flocks			
N_{after_pos}	Number of broilers slaughtered after positive flock	broilers	
T_{half}	Number of "negative" broilers needed to be slaughtered before the *Campylobacter* concentration is reduced to half	broilers	
$C_{crosscont.\ chiller}$	Concentration on carcasses which are contaminated during processing	Log_{10} cfu/g	$C_{chiller} \cdot \exp(-N_{after\_pos} \cdot \ln 2/T_{half})$
$P_{negative}$	Prevalence of contaminated carcasses		0 If $C_{crosscont.\ chiller}<1/W_{skin}$
All broiler flocks			
$C_{package}$	Concentration on contaminated chickens after package	Log_{10} cfu/g	$C_{chiller}$ if $P_{broiler} = 1$ $C_{crosscontchiller}$ if $P_{broiler} = 0$
$P_{package}$	Prevalence after package		0 If $C_{package} <1/W_{skin}$

Table 28. Description of the steps in the retail part of the model

Parameter	Description	Units	Distribution
R_{frozen}	Change by freezing		Uniform(-1.5;-0.5)
C_{frozen}	Concentration on frozen chickens	Log_{10} cfu/g	$C_{package}$- R_{frozen}
P_{frozen}	Prevalence in frozen chickens		0 If C_{frozen} <1/Wskin
F_{frozen}	Fraction of frozen broiler of total chickens		
R_{chill}	Change by chilling		0
C_{chill}	Concentration on chilled chickens	Log_{10} cfu/g	$C_{package}$- R_{chill}
P_{chill}	Prevalence in chilled chickens		0 If C_{chill} <1/Wskin

Development of the 'Slaughterhouse simulation program'

Programming tools and program structure

The programming tools for the development of a quantitative model for spread of *Campylobacter* through the slaughterhouse were developed on a Microsoft Excel spreadsheet platform. The Excel platform allows for administration of large input and output data sets. For simulation of the model the @RISK analysis software package from Palisade was used in combination with a macro produced in the Visual Basic programming language. The @RISK runs on the Excel platform as an additional tool package and allows introduction of distributions in the mathematical model instead of fixed parameter estimates. Thus, in the probabilistic approach each uncertain input parameter is determined by probability distributions rather than by single-point values. Accordingly, the outcome of a probabilistic model is a probability distribution. In order to calculate the outcome distribution @RISK uses Monte Carlo simulation. The model is simulated a number of times. Each time (iteration) the model is simulated, the values for each parameter are selected at random from the probability distribution defined for each parameter. The number of iterations is set sufficiently high to allow also rare combinations of parameter values to occur, or iterations are carried out until the outcome distribution is stable.

We were interested in producing a program, which could model the slaughter process as realistic as possible, i.e. on the basis of the 'actual' data from a slaughterhouse including slaughter order, flock sizes, and *Campylobacter* status. For each broiler the *Campylobacter* concentration would vary in accordance with the distributions given in Fig. 17-20. In order to handle this, the program was divided into two separate subroutines: 1) reads the data from the 'actual' slaughter program, 2) simulates on the individual broilers, and each iteration simulates the different changes in the *Campylobacter* concentration through the slaughter processes. The two subroutines where implemented as a single macro developed in the Visual Basic programming language. The Visual Basic also uses the Microsoft Excel platform for reading input and writing output data. In this way the reading and writing of the input and output data could be kept in a single Excel spreadsheet file. The output data are sent to the Excel spreadsheet and then collected by the @RISK program which then produces the final output distributions. A schematic view of the program is shown in Fig. 25. A more detailed description of the macro subroutines follows below.

Handling the 'actual' slaughter program (subroutine 1)

Subroutine 1 reads automatically from the 'actual' slaughter program (Fig. 25). During the simulations it reads the slaughter plan for one day at a time and uses that in the simulations (Table 29). For each slaughter day, information about flock sizes and *Campylobacter* status is obtained. When a negative flock is slaughtered after a positive flock a 'yes' flag is added, which indicates possible cross-contamination from the positive flock into the following negative flocks.

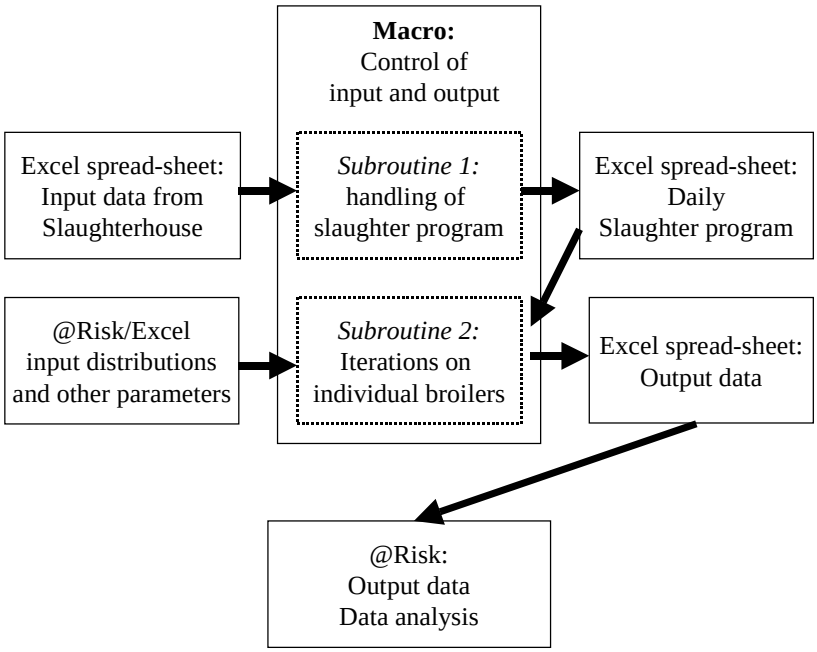

Figure 25. Schematic overview of the structure of the slaughterhouse simulation program.

Table 29. Example of a daily slaughter program which subroutine 1 reads from the input slaughter program and write in an Excel spreadsheet.

Date	Status of *Campylobacter* contamination	Broilers in flock	Possible cross-contamination
98-02-05	Negative	8100	No
	Negative	8100	No
	Positive	16100	No
	Negative	24000	Yes
	Negative	34000	Yes

Calculation of concentration and prevalence of Campylobacter (subroutine 2)

Subroutine 2 models the changes in the *Campylobacter* concentration on chickens during the selected processing steps in the slaughter plant.

Based on the information from the daily slaughter plan (as exemplified in Table 29) the broilers entering the slaughterhouse can be divided into three categories:

1. Broilers from a negative flock slaughtered first on a day.
2. Broilers contaminated (positive) at the arrival to the slaughterhouse.
3. Broilers from a negative flock slaughtered after a positive flock at the same day.

Subroutine 2 reads from the daily slaughter program (Table 29) whether a broiler belongs to group 1, 2 or 3. If the broilers belong to group 1, the concentration and prevalence will remain zero throughout the model. If the broilers are contaminated at the arrival (group 2), each carcass will be given a *Campylobacter* concentration from

the distribution in Fig. 15. As the carcass passes through the different slaughter processes, the *Campylobacter* concentration will decrease or increase in accordance with the distributions given in Fig. 17-20. The variability in concentrations on the carcasses is build into the model by using the @RISK program to generate different concentrations from the distributions. The *Campylobacter* concentration and prevalence is set to zero, if the concentration changes to below 1 cfu per total skin weight of the chicken. If the broilers belong to group 3, there is a chance that the carcasses will be cross-contaminated with *Campylobacter* from the positive flock slaughtered immediately before. Since relatively little is known about cross-contamination, we simply define that the concentration obtained due to cross-contamination is given by the concentration distribution representing positive carcasses 'after washing and chilling' multiplied with the *Campylobacter* reduction rate ($=\ln 2/T_{half}$).

Due to limited computer capacity the number of iterations per simulation could not exceed 260,000. The number of broilers slaughtered in one year is approximately 50 times as high. We therefore decided to simulate every 500 broiler in the 'actual' slaughter program, which is enough to obtain reproducible distributions of the concentration and prevalence of *Campylobacter* on the chickens.

Slaughterhouse model - results

The result of the risk modelling is dependent on the data and assumptions that form the basis of the model. It is though of interest to change the uncertainties and assumptions to see how much they affect the results. Therefore, a sensitivity analysis was carried out.

A sensitivity analysis provides information on how changes in the input data influence the outcome of the model. Input-changes could be changes in parameters such as the mean and/or the variance of a given input distribution or it could be changes in the choice of input distribution e.g. from a triangle to a normal distribution. In order to limit the number of analysis we did not examine the effect of different input distributions. The following changes in input parameters were examined:

- The effect of using input distributions describing the *Campylobacter* concentration either based on the data from Mead *et al.* (1995) or Oosterom *et al.* (1983b).
- The effect of using different values for the 'cross-contamination half time coefficient' (T_{half}).
- The effect of changing the flock prevalence.
- The effect of changing the mean values of the input distributions for the processes (scalding, defeathering, evisceration and washing + chilling).
- (The effect of freezing the chickens).

We carried out the sensitivity analysis by examining the parameters mentioned above separately and by running simulations for each change in the given parameter value. We assume that there is no correlation between the different parameters examined. The last point mentioned is given in brackets as only simple observations of the effect of freezing on the *Campylobacter* concentration were carried out. An analysis of the effect of the change in the concentration reduction was not performed.

The effect of changing the input distribution

As described in the section on page 46, the data used to describe the concentration on the broilers at the entrance to the slaughterhouse were obtained from two different studies and unfortunately these data were significantly different from each other. The distribution based on data from the study of Oosterom and co-workers was much broader and had a slightly lower mean than the distribution based on data from the study of Mead and co-workers (see Fig. 26a, which equals the distributions shown in Fig. 15). As previously described, several explanations may account for this difference. Therefore, instead of mixing the data from the two studies, independent simulations were carried out for each set of data. As shown in Fig. 26b, a broader distribution of the input concentration was seen for Oosterom *et al.* (1983b) as compared to Mead *et al.* (1995). This was, not surprisingly, also reflected in the output distributions. In addition, the mean reduction in the *Campylobacter* level was approximately 2 log cfu units, independently of the input distribution used. This is in agreement with the changes expected (see Fig. 21 and Fig. 22).

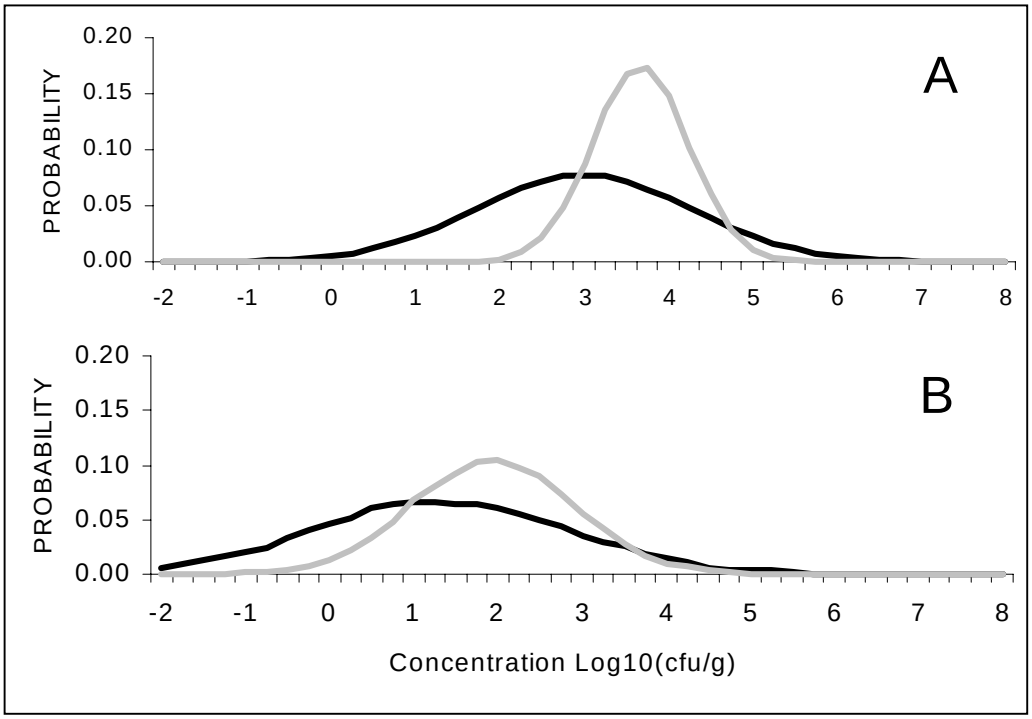

Figure 26. *Campylobacter* concentrations on chicken carcasses (originating from positive flocks) at the entrance to (A) and at the exit of (B) the slaughterhouse. Data are obtained from Oosterom *et al.* (1983b) (Black line) and Mead *et al.* (1995) (Grey line).

In the simulations, the output distribution represents the concentration of *Campylobacter* on chicken carcasses originating from *Campylobacter* positive broiler flocks, when the chickens leave the slaughterhouse. In both cases the output distributions were slightly broader than the corresponding input distributions (Table 30).

The variance of the output distributions is referred to as the total uncertainty and consists of both uncertainty and variability. The variances and the means of the output distributions from the exit of the slaughterhouse are simulated in Fig. 26b. However, these values for the positive flocks could as well have been calculated by the equations given in the section on page 58, where the means and the variances from the input distributions are summarised. Calculating an output distribution for the positive flocks plus the negative flocks, that are cross-contaminated, would have been quite complicated, though. Therefore – among others - the Monte Carlo simulation was implemented.

Table 30. Calculated means and variances of the output distributions describing the exit of the slaughterhouse. The distributions are based on the data from Oosterom *et al.* (1983b) and Mead *et al.* (1995).

	Input		Output			
	Mean	Variance	Mean	Variance	Lower conf.	Upper conf.
Mead *et al.*(1995)	3.79	0.34	1.85	1.06	-0.17	3.87
Oosterom *et al.*(1983b)	3.12	1.70	1.18	2.44	-1.88	4.24

The lower/upper conf. is the lower/upper 95% confidence interval around the mean of the output distribution.

The effect of changing the cross-contamination coefficient

The value of the 'cross-contamination half time coefficient' (T_{half}) is not based on 'real data' but on assumptions from considerations of the mechanism. The T_{half} coefficient is set to 0, 300, 1000, 3000, and 6000. The effect of using different values is examined for the broiler prevalence and the *Campylobacter* concentration at the exit of the slaughterhouse.

The relationship between the magnitude of cross-contamination and the broiler prevalence at the slaughterhouse exit is shown in Fig. 27. The percentage of positive broilers leaving the slaughterhouse will increase considerably, if cross-contamination occurs during the slaughterhouse processes. If, for example, we assume that the *Campylobacter* concentration is reduced to half the concentration for each thousand negative broilers slaughtered (T_{half} = 1000 broilers), the total number of positive chickens will increase almost 7%.

The prevalence of *Campylobacter* positive flocks varies over the season (Fig. 28) as also shown in Fig. 3. If no cross-contamination occurs, the prevalence varies from 60% in August and September to less than 15% in February and Marts. If cross-contamination occurs in the slaughterhouse, the number of *Campylobacter* positive chickens increases. The increase is slightly higher in May and June (Fig. 28) than the rest of the year. This fact is most likely 'just' due to the randomness in the order of slaughtering. A smaller proportion of the negative flocks (compared to the other months) was maybe slaughtered as the first of the day, and in this way relative more flocks were exposed to cross-contamination. In general, the seasonal variation seems to

be reflected in the number of positive broilers leaving the slaughterhouse independent on the value of T_{half} as seen in Fig. 28.

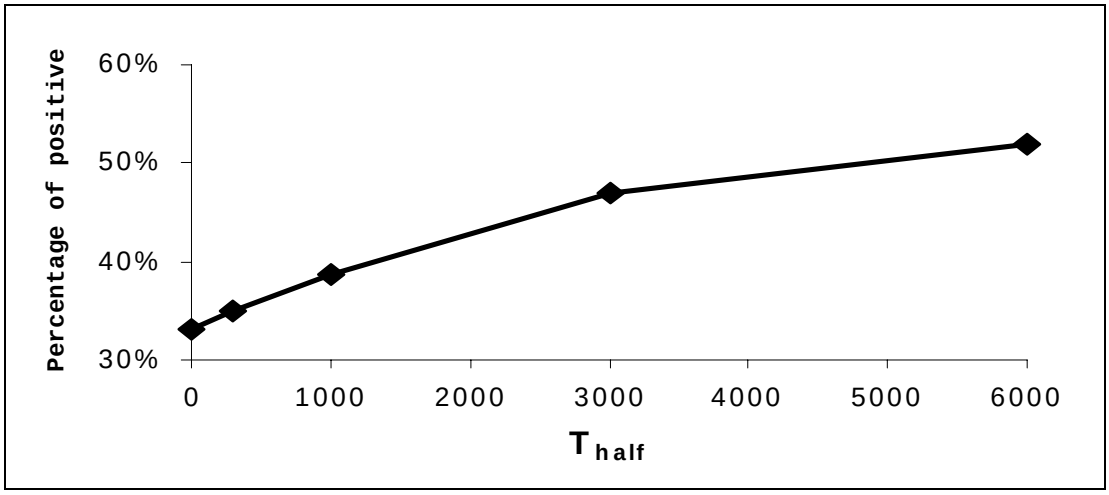

Figure 27. Increase in the percentage of *Campylobacter* positive chicken carcasses leaving the slaughterhouse given different levels of cross-contamination from positive to negative flocks. The level of cross-contamination is represented by T_{half} (= the number of *Campylobacter* 'negative' broilers needed to be slaughtered, before the *Campylobacter* concentration is reduced by 50%).

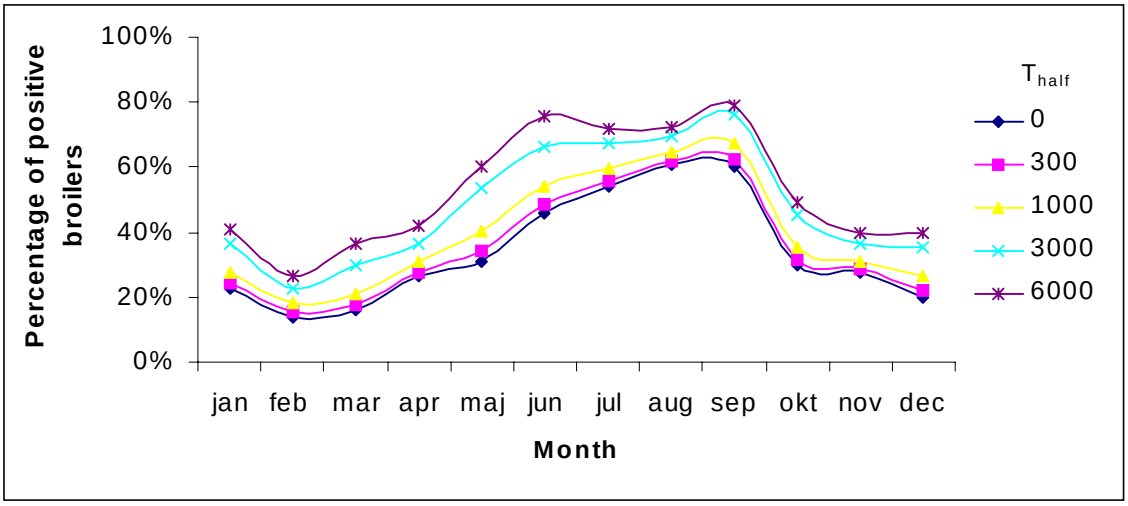

Figure 28. Seasonal variation in the number of *Campylobacter* positive chicken carcasses leaving the slaughterhouse at different levels of cross-contamination, represented by different values of T_{half} (= the number of *Campylobacter* 'negative' broilers needed to be slaughtered, before the *Campylobacter* concentration is reduced by 50 %).

The influence of cross-contamination on the distribution of *Campylobacter* concentration in positive chickens at the exit of the slaughterhouse is shown in Fig. 29.

The chickens getting cross-contaminated receive a low concentration of *Campylobacter* relative to the chickens that actually originated from positive broiler flocks. The resulting distribution of *Campylobacter* concentration has therefore a lower mean than if no cross-contamination occurs. Hence, the influence of cross-contamination on the *Campylobacter* level is relatively low, though it has a relatively high impact on the *Campylobacter* prevalence of slaughtered chickens (Fig. 28).

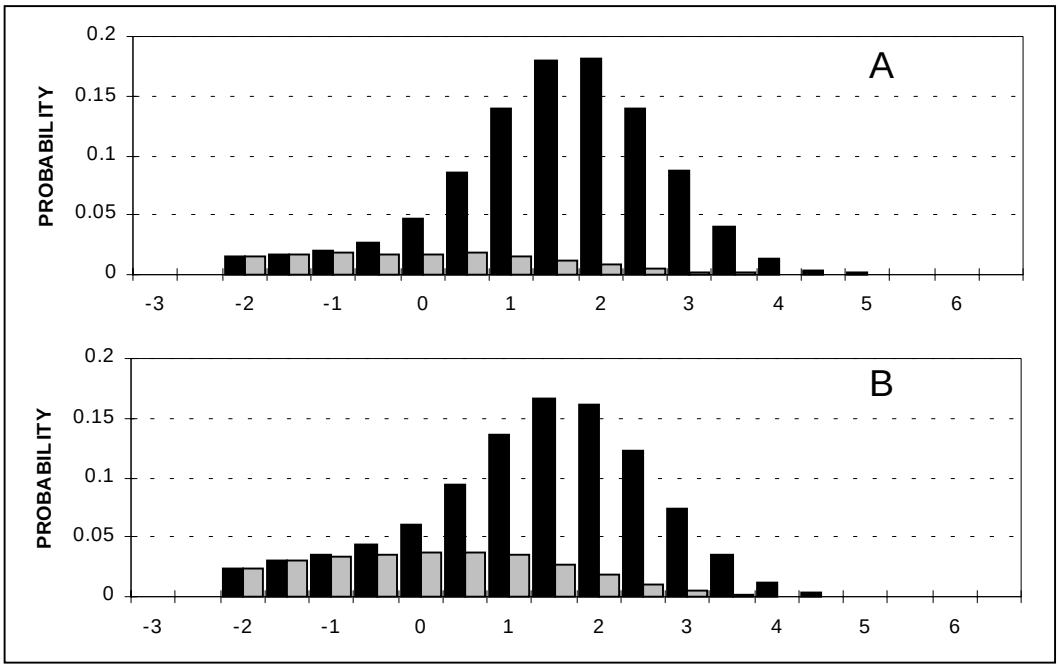

Figure 29. Distributions describing the *Campylobacter* concentrations in all contaminated chickens leaving the slaughterhouse (black bars). The grey bars indicate the concentration profile for the fraction of contaminated chickens that originated from negative flocks, but were cross-contaminated during the slaughter process. Distributions are based on T_{half} = 1000 (A) and T_{half} = 3000 (B).

The effect of changing the flock prevalence

As previously mentioned the input data concerning flock prevalence is fixed. This is a problem when we want to analyse, how the flock prevalence affects the fraction of positive chickens and the distribution of the *Campylobacter* concentration at the exit of the slaughterhouse. On the other hand the flock prevalence was found to vary considerably over the year. We therefore used this variation to analyse, how the variation in the flock prevalence was correlated to the fraction of positive chickens leaving the slaughterhouse (Fig. 30). The twelve months, each with different flock prevalence, were used as input.

A first order linear regression was fitted to the 12 data points and showed good agreement (R^2=0.958) (Fig. 30). A reduction of the flock prevalence of for example 0.1 results therefore in a reduction of 0.1 for the positive chickens leaving the slaughterhouse independent on the flock prevalence within the range.

Two factors may give a respectively higher or lower prevalence compared to the input prevalence. 1) Cross-contamination leads to that some of the non-contaminated chickens become contaminated, and 2) reduction of the *Campylobacter* concentration due to the different processes may lead to that some of the contaminated chickens become non-contaminated.

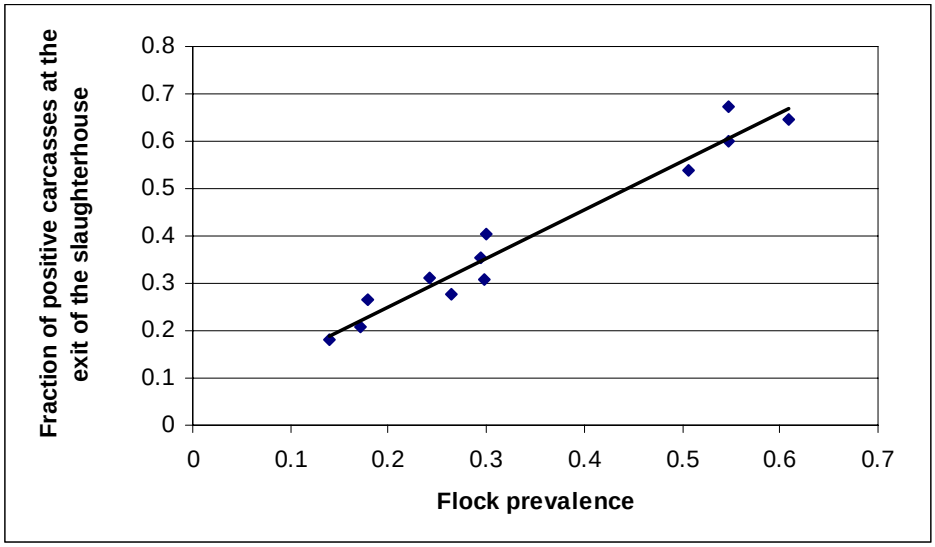

Figure 30. Relationship between flock prevalence and the fraction of *Campylobacter* positive chickens at the exit of the slaughterhouse. The relationship between flock prevalence and fraction of positive chickens was plotted for each of the 12 months. The line indicates a linear regression through the data points.

The relationship between the flock prevalence and the concentration on contaminated chickens at the exit of slaughterhouse is shown in Fig. 31. Low flock prevalence means that the probability of slaughtering a positive flock is little. When the flock prevalence is low there is also a low probability of slaughtering a positive flock after a positive flock compared to slaughtering a negative flock after a positive flock. In other words, it is more likely to slaughter a negative flock after a positive flock. Thus, in the case of low flock prevalence most of the positive flocks will course a cross-contamination to a negative flock. The cross-contaminated chickens will in average be contaminated with a lower concentration compared with the chickens from a positive flock. The mean value of the resulting distribution of the concentration (which consists of both chickens from positive flocks and cross-contaminated chickens) will therefore decrease the more cross-contaminated chickens that figure in the data. This means that when the flock prevalence is relatively low, the concentration is also relatively low, and when the flock prevalence is relatively high, the concentration is also relatively high.

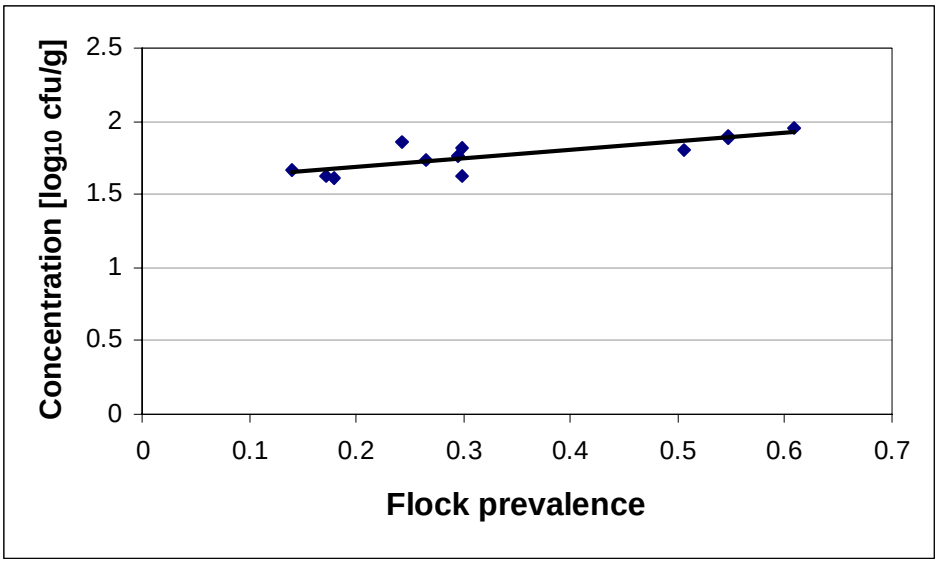

Figure 31. Mean concentration of *Campylobacter* at the exit of the slaughterhouse as a function of the flock prevalence.

The relationship between the flock prevalence and the concentration on contaminated chickens is also reflected in Fig. 32. As the flock prevalence varies over the year, the mean *Campylobacter* concentration of the contaminated chickens also varies over the year. The standard deviation is relatively high when the output concentration is low and visa versa. This is because the distributions for the low output mean concentrations consist of a relative large number of cross-contaminated chickens. The concentration distribution for the cross-contaminated chickens are namely wider compared to the distribution for the chickens in a positive flock (without cross-contaminated chickens).

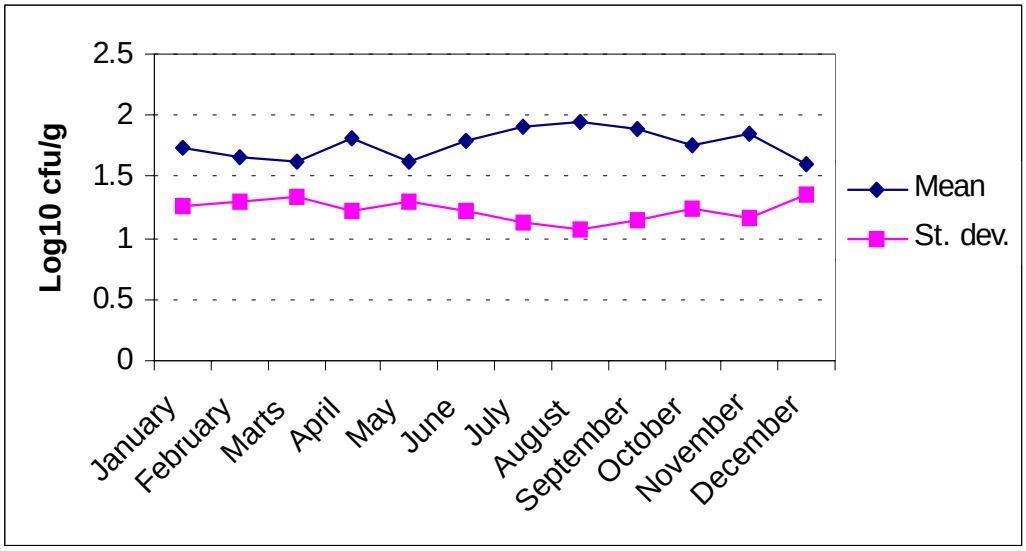

Figure 32. The seasonal variation in the mean concentration of *Campylobacter*. Data were obtained for a simulation where T_{half} was 1000 broilers.

The effect of changing the influence of different plant processes

The effect of changing the mean values of the input distributions for the four processes (scalding, defeathering, evisceration and washing + chilling) is almost the same. The only difference is that the fraction of chickens, that may become non contaminated (negative) during the last process (washing + chilling) - due to a reduction of the mean value for the change in concentration - will remain negative. Passing through the earlier processes there is a possibility that the negative chickens will become recontaminated. However, we do not believe that this difference has major importance and therefore, we do not model how changes in each individual process affect the net change in concentration at the exit of the slaughterhouse. We have limited the analysis to the effect of changing a single process. In the present model this was obtained by varying the level of the input distribution for the washing + chilling process.

The mean value was varied from -5 $\log_{10}$ cfu/g to +2 $\log_{10}$ cfu/g relative to the normal level of the input distribution for the washing + chilling process (of –1.46) (described by the distribution presented in Fig. 20). The change in percentage of *Campylobacter* positive chickens at the exit of the slaughterhouse (Fig. 33a) was found to be relatively insensitive to changes in the input distribution for the washing + chilling process, if these were small (up to 2 $\log_{10}$ cfu/g). A reduction of more than 3 $\log_{10}$ cfu/g relative to the normal level was needed to reduce the fraction of *Campylobacter* positive chickens at the exit of the slaughterhouse with a factor of 2. Thus, in order to eliminate *Campylobacter* from the chickens completely, the step introduced to reduce the *Campylobacter* concentration have to be extremely efficient (a more than 5 log cfu/g reduction is needed). Despite difficulties in reducing the exit prevalence, the average concentration on the chickens at the exit of the slaughterhouse is, not surprisingly, strongly reduced after introduction of a step in the slaughterhouse which reduces the *Campylobacter* concentration (Fig. 33b). The distributions describing the *Campylobacter* concentration on positive chickens at the exit of the slaughterhouse for changes of 0, -2 and -4 $\log_{10}$ cfu/g relative to the normal level is presented in Fig. 34. The increased reduction of *Campylobacter* during the washing + chilling process moves the distributions towards the lower limit, which is 1 cfu per skin weight (~ -2 $\log_{10}$ cfu/g skin). Consequently, more chickens will become *Campylobacter* negative and the distributions describing the concentration on the positive chickens will be more skewed.

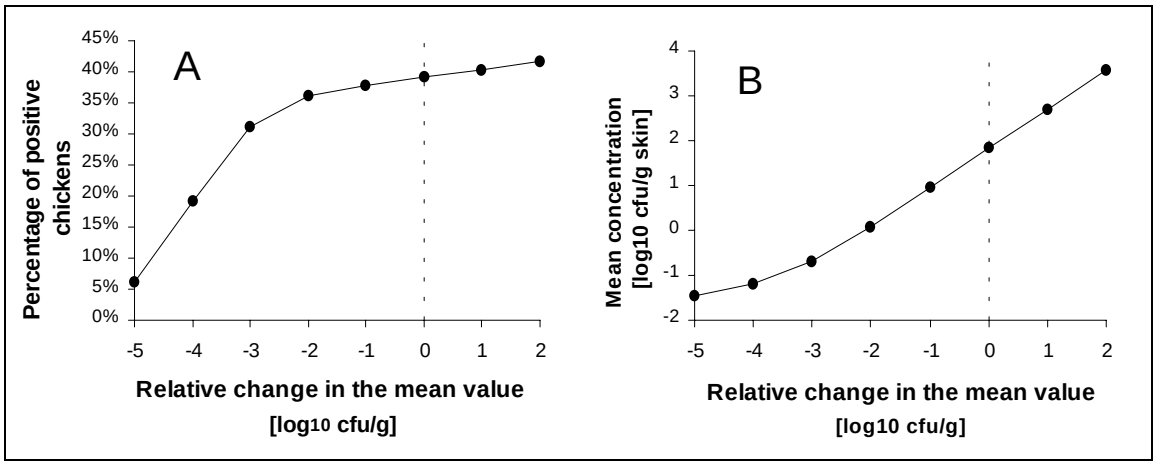

Figure 33. The effect of changing the mean value of the input distribution for the washing + chilling process on the fraction of *Campylobacter* positive chickens (A) or on the average concentration on positive chickens at the exit of the slaughterhouse (B). The $\log_{10}$ cfu/g change in the mean value for the washing + chilling process is presented relative to the normal level of –1.46 $\log_{10}$ cfu/g (dotted line).

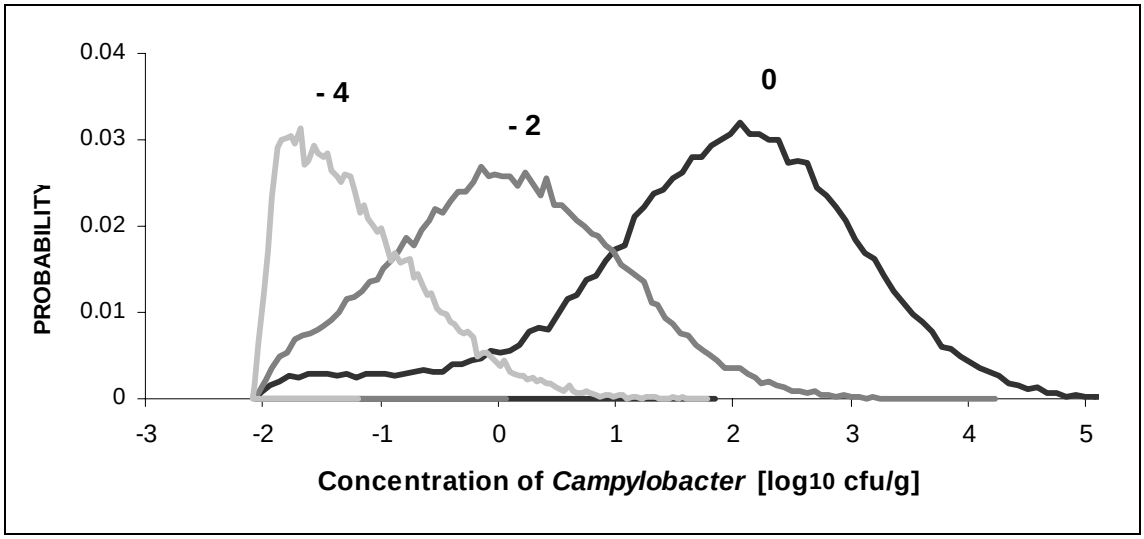

Figure 34. The distribution of the *Campylobacter* concentration on positive chickens at the exit of the slaughterhouse given different levels of changes of the mean value for the washing + chilling process. The changes ($\log_{10}$ cfu/g) are indicated on top of each distribution.

The effect of freezing the chickens

In the previous simulations, we looked at the concentration and prevalence of positive chickens at the exit of the slaughterhouse. However, from a consumer's point of view, the *Campylobacter* prevalence and concentration at retail level are probably more relevant in relation to the 'actual' exposure to *Campylobacter* originating from chickens. In the simulations we have included both chilled and frozen products (see also Table 28).

Simulations carried out to illustrate the *Campylobacter* concentration on retail products showed that the frozen chickens had a lower concentration than the chilled products (Fig. 35a). By taking the ratio of chilled relative to frozen chickens sold at the Danish market into account, the overall simulated concentration profile of Danish *Campylobacter* positive chickens looks as shown in Fig. 35b.

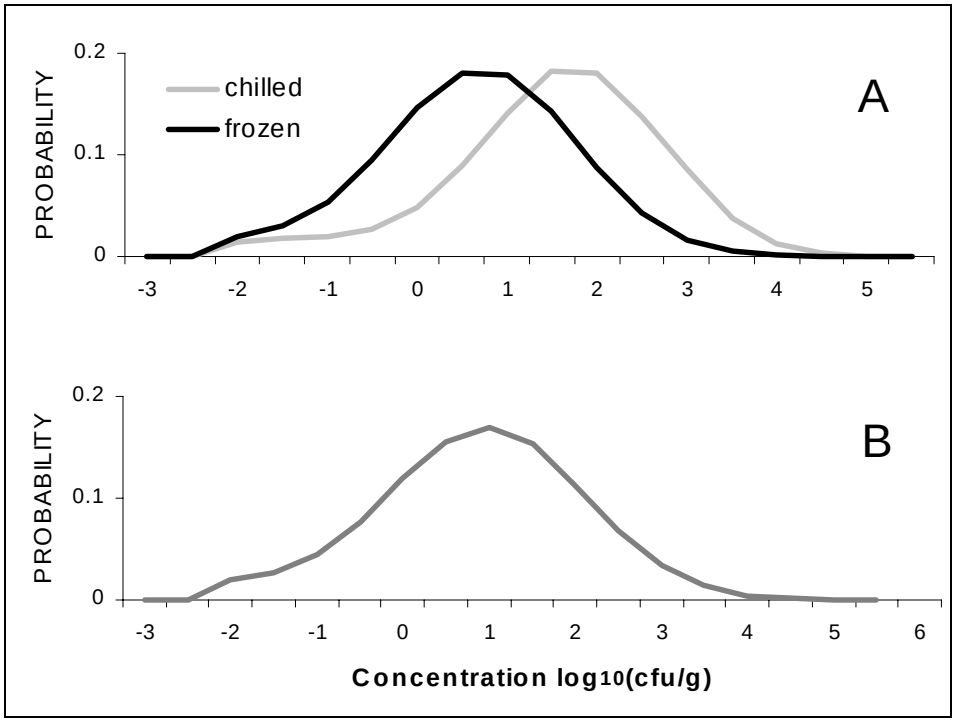

Figure 35. Simulated distributions describing the concentration on frozen and chilled *Campylobacter* positive chickens (A), and the total profile of all *Campylobacter* positive chickens sold in Denmark independent of cooling status (B). T_{half} = 1000 broiler in the simulations.

The simulations also showed that the prevalence of *Campylobacter* on chickens was reduced 4% due to freezing of the carcasses compared to chilled chickens. If for example the prevalence of the chilled chickens was 45% then the prevalence in the frozen products would be 43.2% (= 45% - (45% * 0.04)).

The simulated data for the prevalence and concentration for the frozen and chilled chickens were compared with data generated at retail level.
The simulations showed that the prevalence in frozen chickens was 4% lower than the prevalence in chilled chickens. At retail level (Fig.5), the difference between chilled and frozen chicken products was not significant in 1998 (28.5% (chilled), 25.4% (frozen), based on 367 samples), but in 1999 the difference was significant (32.5% (chilled), 15.8% (frozen), based on 314 samples). Hence, in 1999 the relative reduction in the prevalence between chilled and frozen products at retail level was 51% ((32.5% – 15.8%) / 32.5% = 0.51 => 51%). For comparison, the simulations showed a relative difference of 4%. Calculating the average reduction in the prevalence at retail for 1998 and 1999 the relative reduction due to freezing was 31%. Comparison of this value with

the simulated result (4%) shows that the 95% confidence interval [8.7 % - 55 %] belonging to the 31% does not contain 4%. Therefore, the simulated reduction is significantly different (lower) from the 'actual' difference between chilled and frozen products measured at retail level.

An explanation for the disagreement between the simulated and the measured reduction in prevalences due to freezing (4% versus 31%) could be that the concentration of *Campylobacter* is reduced considerably more upon freezing than the assumed level of 0.5 to 1.5 $\log_{10}$ cfu/g used in the simulations. If a broader variation in the effect of freezing was assumed (like a reduction of e.g. 1 to 5 $\log_{10}$ cfu/g), the simulated prevalence would be considerably more reduced (in this case the prevalence in the frozen products would be approximately 30% lower than the chilled). Another explanation for the difference could be the uncertainty associated with the microbial analysis of the retail chicken products.

The simulated concentration data for *Campylobacter* positive chickens were compared to measured semi-quantitative retail data (Fig. 36) i.e. the outcome of the slaughterhouse model was evaluated in relation to 'real' data. From Fig. 36 it is evident that the simulated concentrations of *Campylobacter* on the positive chickens seem to be in fairly good agreement with the data obtained from the retail level, although the distribution based on the simulated data seems to underestimate the high concentrations. This could be explained by the fact that the input data describing the changes in the *Campylobacter* concentration through the different slaughterhouse processes are based on foreign data which may be different from the actual Danish situation.

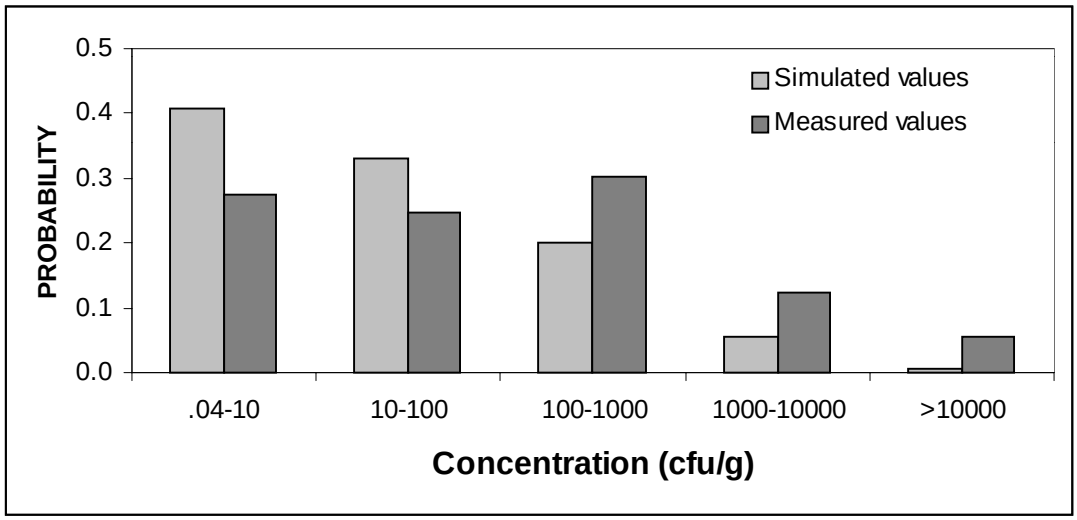

Figure 36. Comparison of the simulated *Campylobacter* concentration profile for positive chickens at retail level (T_{half} = 1000) with 'real' data measured on chickens sampled at retail level using a semi-quantitative method.

Slaughterhouse model - conclusion

A quantitative model for analysis of transfer and spread of *Campylobacter* through a chicken slaughterhouse has been developed.

In the model all broilers in a *Campylobacter* positive flock is assumed to be positive at the arrival to the slaughterhouse. The data used for the flock prevalence were obtained from a one years slaughter program from a Danish slaughterhouse. With respect to concentrations of *Campylobacter* on the chickens we only considered *Campylobacter* associated to the carcasses and not the faeces. A concentration profile on positive chicken carcasses was not considered until the chickens were hanged on the conveyer in the slaughterhouse. The changes in the *Campylobacter* concentrations through different slaughterhouse processes were modelled on the basis of data obtained from three foreign studies where changes in *Campylobacter* levels over different slaughterhouse processes were measured.

A simple sensitivity analysis of the model has been performed in order to examine certain assumptions and uncertainties in the model. The following were examined:

- The effect of using an input distribution describing the *Campylobacter* concentration after bleeding based on either data from Mead *et al.* (1995) or Oosterom *et al.* (1983b).
- The effect of using different values for the 'cross-contamination half time coefficient' (T_{half}).
- The effect of changing the flock prevalence.
- The effect of changing the mean values of the input distribution for the washing + chilling process.
- (The effect of freezing the chickens)

We have analysed how a possible cross-contamination from chickens in a positive flock to a negative flock may influence the *Campylobacter* status at packaging (at the exit of the slaughterhouse). The parameter T_{half} was introduced to describe the number of negative broilers slaughtered before the *Campylobacter* concentration on the contaminated chickens was reduced to half the concentrations. From the simulations we found that the fraction of positive chickens increased as the number of T_{half} increased. The mean concentration, however, on the cross-contaminated chickens was lower than those chickens originating from a *Campylobacter* positive flock.

The effect of changing the flock prevalence was also examined. Normally the mean of the input distribution for the flock prevalence could be changed (up and down). In our case this was not possible since we have used a fixed data set as input for the flock prevalence of the broilers at arrival to the slaughterhouse. Although a fixed data set was employed we could take advantage of the seasonal variation in the flock prevalence. The flock prevalence for each month were used as input to the model to determine the relation between flock prevalence (positive broilers entering the slaughterhouse) and the fraction of positive chickens at packaging /exit of the slaughterhouse. From the simulations we found a linear correlation between the fraction of positive chickens at

entrance and the fraction of positive chickens at the exit of the slaughterhouse, whereas the *Campylobacter* concentration on all the positive chickens remained relatively unaffected.

Furthermore, changes in the mean value for the input distribution describing the washing + chilling process was simulated. Interestingly, relatively large reduction levels (2-3 log cfu per gram) through the washing + chilling process was needed to reduce the fraction of positive chickens at packaging, significantly. The concentration on the positive chickens was reduced/increased in accordance with the changes in the mean value of the input distribution for the washing + chilling process. If the washing + chilling process decreased the mean concentration level by 1 log cfu/g relative to the normal level, the output concentration was likewise reduced by 1 log cfu/g.

Finally the semi-quantitative data sampled at retail level have been compared to the simulated data obtained from the slaughterhouse model. In the simulations we included the ratio between frozen and chilled chickens sold in Denmark. The simulated data turned out to be in relative good agreement with the data sampled at retail level, despite the fact that we have used data generated at slaughterhouses in foreign countries.

Consumer model - A QRA model for food handling in private kitchens

Cross-contamination during food preparation

The unsafe food handling procedures in private kitchens may be responsible for a large number of food-borne diseases. As shown in Fig. 4, 5, and 6 and as also demonstrated in the simulations the prevalence and the concentration of *Campylobacter* positive broilers is relatively high at the exit of the slaughterhouse and at retail level. We therefore expect that unsafe food handling plays a significant role in relation to the spread of *Campylobacter* in private kitchens and to the human exposure to *Campylobacter* from chickens.

Food handling procedures in private kitchens, for example the level of food safety habits and the types of utensils, cutting boards etc., probably varies as much as there are people in Denmark. Also the dose-response level and the virulence of different species of *Campylobacter* may vary considerably. The slaughterhouse model does not differentiate between different types of *Campylobacter jejuni*, which means that the model includes uncertainties about human individuals, the dose response relationship and the virulence of different *Campylobacter jejuni* types.

In the present work the 'farm to fork' approach has been implemented to describe the events involved in the spread of *Campylobacter* from slaughterhouse to consumer. Hence, to obtain a perfect risk estimate the model should include all possible pathways (including knowledge of the true parameter values) by which *Campylobacter* may be transferred from a contaminated raw chicken entering a private kitchen to the final exposure to humans. This area is not well studied. Therefore, there is a need for quantitative data describing all the possible pathways by which the pathogen is transferred from the raw chicken to humans. Including all possible transfer routes during food preparation in a quantitative model is not realistic, because of the large variability in the food-handling behavior of individual persons. Thus, using the 'farm to fork' approach in a QRA model for food handling in private kitchens is probably not the most optimal way to calculate a risk estimate. However, the 'farm to fork' approach allows us to obtain detailed knowledge about some of the important factors during food preparation that might contribute to the transfer of *Campylobacter* from a raw chicken to humans.

Although the model estimates the number of human *Campylobacter* cases caused by unsafe food handling in private kitchens, it is not the main purpose of the model. Instead the model should be used to analyse how changes in different kitchen processes or in the *Campylobacter* status of the chickens entering the kitchen would affect the probability of illness. In other words it is more important to focus on the relative changes in probability of illness caused by changes in the handling or input conditions instead of focusing on the actual risk estimates. Furthermore, the model might be used to analyse whether some parts of the population (different age and sex groups) are more exposed to *Campylobacter* transferred via chickens than others. Finally, the model can also be used to pinpoint our current state of knowledge of the different food handling

processes in private kitchens and maybe help us to improve the collection of relevant information in this field.

In the present work we have divided the food handling processes of a chicken into two major contamination routes (route 1 and route 2) (Fig. 37), which we believe will contribute most to the *Campylobacter* cases caused by unsafe food handling in kitchens. Not all steps mentioned under route 1and 2 are included in the model (see Fig. 37).

Route 1:
Hygiene level of the person who prepares the meal. The level of safety precautions taken by the person, who prepares the meal, will influence both the possibility of ***cross-contamination during preparation of the food*** and the possibility of ***insufficient heat treatment during cooking of the food***. With respect to cross-contamination, hands may be contaminated by touching the raw products, or utensils (knifes, fork, plates, cutting board etc.) which have been in contact with the raw chicken product. The person who prepares the meal may ingest the *Campylobacter* "***directly"*** by for example licking on the fingers or the person may contaminate the prepared meal "***indirectly"*** by transferring *Campylobacter* from the chicken to hands and utensils (e.g. cutting board) and via uncleaned utensils and hands to ready-to-eat chicken, salad, bread, etc. When *Campylobacter* is transferred to a prepared meal, the number of persons being exposed will depend on the number of persons eating the meal and the size of the meal ingested.

With respect to insufficient heat treatment of chickens, the level of rawness of the chicken served will depend on the temperature and the time the chicken has been heat-treated. As for the cross-contamination the number of persons being exposed will depend on the number of persons eating the meal and on the size of the meal ingested.

Route 2:
Cross-contamination to other food products via raw chicken liquids or direct contact. In some cases the liquid from, or direct contact with, the raw chicken may result in contamination of other food products or the person who touches the product. Contamination may occur for example during thawing of the chicken, when the chicken is stored in the refrigerator or in the supermarket where it may come in contact with other food items. Then, the risk is related to the other contaminated food products or the persons touching the product. The risk will depend on the ability of *Campylobacter* to survive in the 'new' environments. With respect to contamination of other food items, the probability of illness will depend on the amount of liquid (containing *Campylobacter*), that has been transferred to the product, and the amount of the contaminated product each individual in a household eats. With respect to contamination of a person, who has been in direct contact with the raw chicken, the probability of illness will depend on the survival of *Campylobacter* on for example the hands and the chance that *Campylobacter* actually is transferred to the mouth.

The present model is our first attempt to quantitatively describe the food-handling processes in private kitchens. Despite the lack of data we believe that cross-contamination related to unsafe food handling during preparation of a meal is one of the most important factors in relation to *Campylobacter* infections acquired in private

homes. Therefore, the model has been limited to focus only on the preparation processes where unsafe food handling procedures may lead to cross-contamination of *Campylobacter* from the raw chicken to the final meal. This means that insufficient heat treatment and cross-contamination from the raw chicken product to other food products during storage are not included in the model (see also Fig. 37). Also direct contamination of the person, who prepares the meal, has been left out. Thus, only the contamination routes indicated by bold lines and shaded boxes have been included in the model.

The model should not be considered as a final model, since several possible contamination routes have been left out. Instead, the model should be considered as the first building block of a consumer model, which should be extended with other contamination routes in a later version of the QRA model.

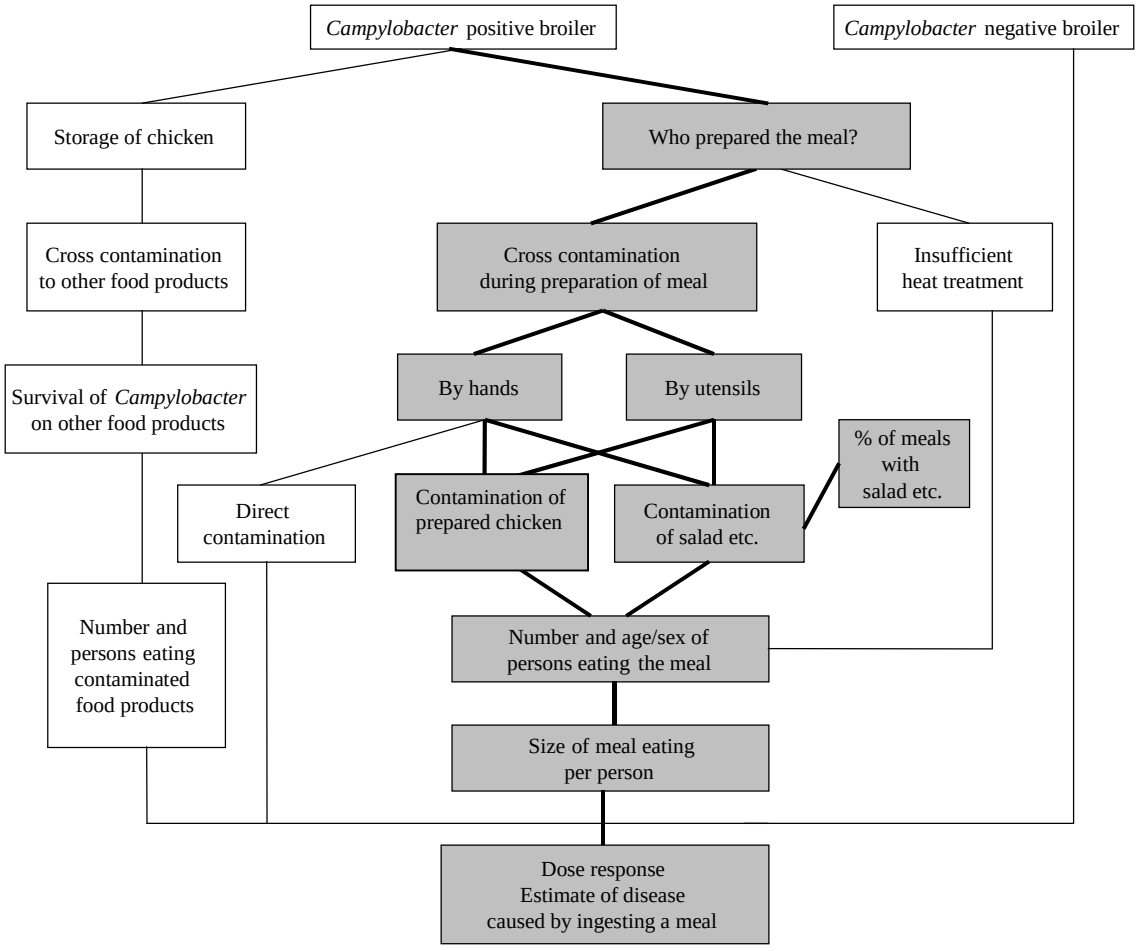

Figure 37. Illustration of possible *Campylobacter* transfer routes via raw chicken in private kitchens. Grey areas are included in the consumer model.

Consumer model - data input

The person preparing the meal

As shown in Fig. 37 one of the most important parameters in the model is the person who prepares the meal, since this person is responsible for the level of hygiene in the kitchen. We divided persons preparing the meal into 6 different groups, based on age and sex. The 6 groups are:

Women: 18-29 years, 30-65 years and above 65 years
Men: 18-29 years, 30-65 years and above 65 years

By combining the data from a Dietary Survey performed at the Institute of Food Research and Nutrition in 1995 (Andersen *et al.*, 1996) with data from Statistics Denmark the number of males and females in each age group, preparing meals was obtained.

With respect to preparation of meals the following assumptions were made:

1. The ratio (k_1) between consumption of meals prepared in private kitchens and food prepared in restaurants, fast food chains etc. is the same for all age and sex groups.
2. All age and sex groups have the same preference for preparing chicken, i.e. meals prepared with chicken is a constant fraction (k_2) of total meals prepared per day, independent of age and sex.
3. Only one meal is prepared per household per day.
4. In households with two adults one of each sex is represented.
5. Households consist of either one or two adults, households with more than one family are not included (for explanation, see later).
6. Two adults in the same household belong to the same age group.

Based on these assumptions the number of chicken meals $N_{CM|ASPM}$ prepared by each age and sex group can be written as:

$$N_{CM|ASPM} = k_1 \cdot k_2 \cdot N_{H|ASPM} \qquad \text{(eq. 1)}$$

where $N_{H|ASPM}$ indicate the number (N) of households (H) where adults from a certain age (A) and sex (S) group prepare the meals (PM) (=ASPM). CM stands for chicken meal and k_1 and k_2 are described above.

Thus, there is a direct correlation between the number of chicken meals prepared per day and the number of households in which a person with a certain age and sex prepares the meal.

From Statistics Denmark we have obtained information about the number of people living in households with either a single adult or two adults (Table 31). We divided the adults into the age and sex groups 18-29, 30-65, and above 65 years for the person preparing the meal. For each these groups, the number of adults, young adults and children living in households with a single or two adults, respectively, was listed. The

category “young adults” represents the adults in the age group 18-29 who still live at home with their parents. Although the young adults belong to the same age group as the adults in the age group 18-29 is it important to differentiate between these two groups, because the person normally preparing the food in these groups are different. In the category of young adults living at home it is normally the parent that makes the food, whereas for the adults aged 18-29 years it is either themselves or another person of approximately the same age that prepares the food.

In households with more than one family the population could not be divided into different age and sex groups, and is therefore left out of the study, although people living in such households constitute for approximately 12 % of the total Danish population. For people above 65 years of age only the fraction of households in which the people were expected to prepare food on their own was included. We included a guess, which say that 60 % of single men, 75 % of single women and 90 % of households with two adults prepare their own food.

Table 31. Relationship between person preparing the meal in a household and the number of adults, children and young adults (aged 18-29) in households with one or two adults.

	Age and sex of person preparing the meal (ASPM)						
	Age 18-29 years		Age 30-65 years		Age > 65 years		
	Men	Women	Men	Women	Men	Women	
	Households with single adult (SA)						
Number of households N_{HSA}	93430	92266	231570	264418	50782	192770	
Number of adults $N_{SA\|age18\text{-}29\|ASPM}$	93430	92266	0	0	0	0	
Number of adults $N_{SA\|age30\text{-}65\|ASPM}$	0	0	231570	264418			
Number of adults $N_{SA\|age>65\|ASPM}$	0	0	0	0	50782	192770	
Number of children < 18[a] $N_{CSA\|ASPM}$	447	24868	15957	120779	50	4	
Number of young adults 18-29[a,b] $N_{YASA\|ASPM}$	0	0	4385[c]	27207[c]	242[c]	1500[c]	
	Households with two adults (TA)						
Number of Households N_{HTA}	133677		978142		207361		
Number of adults $N_{TA\|ASPM}$	267354		0		0		
Number of adults $N_{TA\|ASPM}$	0		1956284		0		
Number of adults $N_{TA\|ASPM}$	0		0		458451		
Number of children < 18[a] $N_{CTA\|ASPM}$	49064		827221		756		
Number of young adults 18-29[a,b] $N_{YATA\|ASPM}$	0		159561[c]		8796[c]		
Fraction of meals prepared by males and females[d] $F_{meal\|ASPM}$	0.4011	0.5989	0.0966	0.9034	0.0509	0.9491	
	Total						**SUM[f] N_{TOT}**
Total number of people ingesting a meal[e] $N_{TOT\|ASPM}$	220800	306629	536276	3071106	74883	638467	**4848161**

[a]The percentage of young female adult and children relative to the males is considered to be 50% independent on the type of household (i.e. household with single male adult, single female adult or two adults) in which they live.

[b]The number of adults (aged 18-29) living at home with their parents.

[c] For calculations and assumptions see Appendix 9

[d] For calculations see Appendix 8

[e] Calculated as:

$$N_{TOT|ASPM} = N_{SA|adults,\,ASPM} + N_{CSA|ASPM} + N_{YASA|ASPM} + F_{meal|ASPM} \cdot (N_{SA|adults,\,ASPM} + N_{CSA|ASPM} + N_{YASA|ASPM})$$

[f] Total Danish population ingesting food prepared in private kitchens on a regular basis, except for those living in households with more than one family. $N_{TOT} = \sum_{all\_ASPM} N_{TOT|ASPM}$

The data from Statistics Denmark could not provide information about the fraction of men relative to women preparing meals in households with two adults. However, from the Dietary Survey (Andersen *et al.*, 1996) the number of males and females, preparing meals in each age group could be obtained. Unfortunately, in the current material from the Dietary Survey we have no information about the number of people living in households with a single adult and households with two adults. Therefore, this information was included using the data from Statistics Denmark (Table 31). Thus by merging the data from Statistics Denmark and the Dietary Survey the fraction of males and females preparing meals in households with two adults ($F_{meal|ASPM}$) could be calculated for each of the age and sex groups (Appendix 8).

Relationship between persons ingesting a meal and the person preparing the meal

If a person prepares a meal, which contains *Campylobacter*, not only the person who prepares the meal will be exposed to *Campylobacter*, but every person who ingests that meal (see Fig. 31). The age and sex of the person preparing the meal is important in relation to the average number of persons being exposed to a contaminated meal. If for example the person belongs to 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 age group of 30-65 years. 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 order to produce a relationship between the person preparing the meal and the person ingesting the meal a matrix (Table 32) was developed in which the percentage of persons from the different age and sex groups was calculated for each of the meal preparing age and sex groups. We denote this parameter ($P_{ASIS|ASPM}$).

To calculate this parameter it is assumed that in all cases where a meal is prepared, all the people in that household will ingest a serving of the meal. The number of chicken servings ($N_{CS|ASIS,\ ASPM}$) ingested by a certain age and sex group (ASIS) given the person preparing the meal (ASPM) is then determined by:

$$N_{CS|ASIS,\ ASPM} = \frac{N_{P|ASIS,\ ASPM}}{N_{H|ASPM}} \cdot N_{CM|ASPM}$$

which by insertion of (eq. 1) gives

$$N_{CS|ASIS,\ ASPM} = k_1 \cdot k_2 \cdot N_{P|ASIS,\ ASPM} \qquad \text{(eq. 2)}$$

$N_{P|ASIS,\ ASPM}$ is the number of people in a certain age and sex group, living in households where a person of a certain age and sex prepares the food.

The total number of chickens servings prepared per day (N_{CSTOT}) is given by:

$$N_{CSTOT} = \sum_{all\_ASIS}\left(\sum_{all\_ASPM} k_1 \cdot k_2 \cdot \left(N_{P|ASIS,\,ASPM}\right)\right) = k_1 \cdot k_2 \cdot N_{TOT} \qquad \text{(eq. 3)}$$

where N_{TOT} represent the total Danish population except for those living in households with more than one family and those that are assumed to ingest food prepared by catering companies on a daily basis. Thus, given the age and sex of the person preparing the meal, the percentage of chicken servings ingested by a certain group out of the total chickens servings ingested per day ($P_{ASIS|ASPM}$) can be determined as:

$$P_{ASIS|ASPM} = \frac{N_{CS|ASIS,\,ASPM}}{N_{CSTOT}} = \frac{k_1 \cdot k_2 \cdot \left(N_{P|ASIS,\,ASPM}\right)}{k_1 \cdot k_2 \cdot N_{TOT}} = \frac{N_{P|ASIS,\,ASPM}}{N_{TOT}} \qquad \text{(eq. 4)}$$

By determining the number of people for each age and sex ingesting a meal given age and sex of the person preparing the meal $N_{P|ASIS,\,ASPM}$, $P_{ASIS|ASPM}$ can be calculated for the different groups of people ingesting the meal.
Thus, for females under the age of 18 ingesting a meal (ASIS = females<18):

$$P_{female<18|ASPM} = \frac{0{,}5 \cdot \left(N_{CSA|ASPM} + F_{meal|ASPM} \cdot N_{CTA|ASPM}\right)}{N_{PTOT}}$$

for females aged 18-29 (ASIS=female18-29):

$$P_{female18\text{-}29|ASPM} = \frac{0{,}5 \cdot \left(N_{YASA|ASPM} + F_{meal|ASPM} \cdot N_{YATA|ASPM}\right) + N_{SA|age18-29|ASPM} + 0{,}5 \cdot N_{TA|age18-29} \cdot N_{meal|ASPM}}{N_{PTOT}}$$

for females aged 30-65 (ASIS=female30-65):

$$P_{female30\text{-}65|ASPM} = \frac{N_{SA|age30-65,ASPM} + 0{,}5 \cdot N_{TA|age30-65} \cdot N_{meal|ASPM}}{N_{PTOT}}$$

and finally for females above the age of 65 years (ASIS=female>65):

$$P_{female>65|ASPM} = \frac{N_{SA|age>65,ASPM} + 0{,}5 \cdot N_{TA|age>65} \cdot N_{meal|ASPM}}{N_{PTOT}}$$

The same calculations can also be obtained for the males in the same age groups. All parameters used in the calculations are presented in Table 31.

By inserting the data from Table 31, the percentage of people in each of the eight age and sex groups ingesting a given serving prepared by a person in one the six different age and sex groups was calculated (Table 32).

Table 32. Percentage of people ingesting a meal in each age and sex group divided into age and sex groups for the person who prepares the meal.

		Age and sex of person preparing the meal (ASPM)					
		Male 18-29	Male 30-65	Male > 65	Female 18-29	Female 30-65	Female 30-65
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%

Risk factors linked to the person who prepares the meal

Risk factors associated with the different processes in the private kitchens can be separated into two categories: i) those related to the persons who prepare the meal and ii) those related to the persons who consume the meal.

In the present section the risk factors related to category i) are presented and in the following section the risk factors related to category ii) is presented.

Cross-contamination via hands and utensils to the prepared meal

The prepared chicken as well as other food products, such as salad, bread etc. may become contaminated during preparation of the meal. In particular the cross-contamination from utensils to ready-to-eat food is considered to be important. This may occur when the same cutting board is used for the raw chicken and then for the prepared chicken or for cutting salad or bread, without cleaning it in between. The same lack in food hygiene may occur during barbecuing if the same plate or fork/knife is used for the raw chicken and afterwards for the final barbecued meal. In other words, the probability of serving a *Campylobacter* contaminated meal depends on whether the utensils have been washed before they are used for any ready-to-eat food product. In accordance with the telephone surveys (see section on page 23) surprisingly many people are not aware of (or do not care about) the risk of using the same utensils throughout preparation of a meal. In the model we used the data from an American telephone survey (Yang *et al.* 1998) comprising approx. 15,000 persons in 7 different states (Table 33). The survey was divided into the same age and sex groups as presented in Table 31 and 33. The interviewed people reported whether or not they usually washed the cutting boards with soap or bleach after contact with raw meat. The number of people who did not wash their cutting board can be described by a Binomial distribution, and from the Bayesian theory the percentage of people, who did not wash their cutting boards can be described by a Beta distribution. In the model we have chosen to describe the percentages by Beta distributions in order to include the uncertainty about the true unknown values.

Table 33. Percentage of respondents who reported that they usually did not wash the cutting board with soap or bleach after contact with raw meat. Categorised into sex and age groups. The data are based on an American telephone survey (Yang *et al.* 1998)

Age and sex group	Number of respondents (n)	Percentage of positive answers (p)	Probability Distribution
Male 18-29 years	963	36%	Beta(n·p +1,n·(1-p)+1)
Male 30-65 years	3065	27%	Beta(n·p +1,n·(1-p)+1)
Male >65 years	1477	18%	Beta(n·p +1,n·(1-p)+1)
Female 18-29 years	1363	19%	Beta(n·p +1,n·(1-p)+1)
Female 30-65 years	4339	15%	Beta(n·p +1,n·(1-p)+1)
Female >65 years	1477	9%	Beta(n·p +1,n·(1-p)+1)

Percentage of people returning the prepared chicken to the cutting board

In the model we have also included the fraction of persons, who put the prepared chicken back on the cutting board after heat treatment. At present we are not aware of any investigations describing this subject. We assume that the percentage is 20-60 % of the persons, who prepare the meal, independent on the age and sex of the person. The uncertainty related to this parameter is introduced with a Uniform distribution having a minimum of 20% and maximum of 60% and an equally likelihood for the values in between these values.

Risk factors linked to the person who ingest the meal

As there are risk factors linked to the person who prepares the meal, there are also risk factors associated with the person who eats the food. In the present model we have included a scenario where two types of food can become contaminated. One is the prepared chicken, which is returned to the cutting board after heat treatment, and the other is salad chopped on the cutting board and consumed together with the chicken. In relation to these factors it is important to know the fraction of people who eats salad with a chicken meal. We also need information about the size of the chicken and salad meals ingested, and finally, about the level of *Campylobacter* transferred from the raw chicken to the cutting board and then again from the cutting board to the salad and/or the prepared chicken.

Size of meal

The number of *Campylobacter* ingested will depend on the size of the meal. From the Dietary Survey we obtained data about the size of the meals ingested by the respondents. The data were categorised in accordance with the standard age and sex groups, including people below the age of 17. Analysis of the data showed that the sizes of meals were approximately log normal distributed for all groups (see Appendix 10) for an example). The data for each of the age groups were therefore fitted to a log normal distribution using the @RISK Bestfit 4.0 distribution fitting software package. The arithmetic means and the standard deviations were then calculated in order to transform the data to the original scale (Appendix 10). The input distributions used in the simulations are presented in Table 34. For the children (under the age of 18 years) the sizes of a meal were strongly correlated with the age of the child (Appendix 11). No significant difference between the sex groups was observed (data not shown). However,

in our model we do not distinguish between a child of 1 year and a child of 17 years. Therefore, we wanted to employ only one distribution that could represent the whole age group from 1-17 years. In this context it is important that the number of children in the subgroups (here defined as 1-5 years, 6-12 years, and 13-18 years) is represented in the same proportions as in the Danish population (data obtained from Statistics Denmark). Thus, a mean α and a standard deviation β were estimated for each of the 3 groups - for the logarithmically transformed data. The 3 set of α and β were then weighted in order to reflect the correct proportions of the three groups. A common set for α and β were then calculated (mean values) and transformed to the original scale.

The sizes of the salad portions ingested were obtained from the Dietary Survey (Table 35) and distributions for the salad sizes were developed in the same way as for the sizes of the chicken meals.

Table 34. Distribution of the sizes of chicken meals ingested divided in age and sex groups. Data are based on the data obtained from the Dietary Survey (Andersen *et al.*, 1996).

Age and sex group	Mean [a] (gram)	Standard-deviation [a]	Distribution
Female < 18 years	128.8	81.8	LogNormal(α,β)
Female 18-29 years	151.1	92.9	LogNormal(α,β)
Female 30-65 years	154.1	98.5	LogNormal(α,β)
Female >65 years	159.2	100.2	LogNormal(α,β)
Male < 18 years	128.8	81.8	LogNormal(α,β)
Male 18-29 years	256.4	197.4	LogNormal(α,β)
Male 30-65 years	189.0	126.9	LogNormal(α,β)
Male >65 years	178.4	129.0	LogNormal(α,β)

[a] Data generated with the @RISK BestFit 4.0 distribution fitting software and subsequently transformed to the original scale by the formulas given in Appendix 10.

Table 35. Distribution of the sizes of salad portions ingested by different age and sex groups. Data are based on the Dietary Survey (Andersen *et al.*, 1996).

Age and sex group	Mean [a] (gram)	Standard-deviation [a]	Distribution
Female < 18 years	54.6	36.7	LogNormal(α,β)
Female 18-29 years	66.5	30.5	LogNormal(α,β)
Female 30-65 years	67.6	47.5	LogNormal(α,β)
Female >65 years	67.1	46.3	LogNormal(α,β)
Male < 18 years	54.6	36.7	LogNormal(α,β)
Male 18-29 years	106.5	88.4	LogNormal(α,β)
Male 30-65 years	91.4	65.6	LogNormal(α,β)
Male >65 years	87.9	51.8	LogNormal(α,β)

[a] Data generated with the @RISK BestFit 4.0 distribution fitting software and subsequently transformed to the original scale by the formulas given in Appendix 10.

Percentage of people eating salad with the chicken meal

The percentage of people eating salad with their chicken meal was also acquired from the Dietary Survey (Table 36). The number of people who eats salad with their chicken meal can be described by a Binomial distribution, and with the Bayesian theory the percentage of people, who eat salad with their chicken meal, can be described as a Beta distribution. In the model we have chosen to describe the percentages by Beta distributions in order to include the uncertainty about the true unknown values. We did not see any significant differences between males and females in each age group (data not shown). Therefore, only one distribution was employed for both men and women in each age group.

Table 36. Fraction of people who eats salad together with their chicken meal. Data are based on Dietary Survey (Andersen *et al.*, 1996).

Age group	Number of chicken meals (n)	Number who eat salad with chicken(s)	Probability Distribution
< 18 years	736	110	Beta(s+1,n-s+1)
18-29 years	159	36	Beta(s+1,n-s+1)
30-65 years	494	85	Beta(s+1,n-s+1)
>65 years	180	24	Beta(s+1,n-s+1)

Level of cross-contamination from a *Campylobacter* positive chicken to salad and prepared chicken.

In a study by Zhao *et al.* (1998) the level of cross-contamination from a contaminated raw chicken to a cutting board and further from the cutting board to salad was reported. Although these data where based on another organism, *E. aerogenes*, the data have been used as a guide to produce distributions describing the level of cross-contamination from raw chicken to the cutting board and from the cutting board to the salad and/or back to the prepared chicken. Because the study was based on a different organism than *Campylobacter* and only included one way of transfer, more data are needed in the future to elucidate the transfer of *Campylobacter* in private kitchens during food handling.

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 resulted in transfer of approximately 1-2 log cfu per gram from the cutting board to the salad. Because of the extreme careful chopping of the chicken and the salad, the data seem to represent the optimal transfer of bacteria from the raw chicken to salad, rather than the average transfer of organisms in a random household. The distributions, which describe the transfer of *Campylobacter* from a contaminated raw chicken to the salad or a prepared chicken, should rather represent all levels of transfer that might occur during preparation of a random meal.

With respect to transfer of *Campylobacter* from a raw chicken, a certain fraction of the bacteria may be hidden in the feather follicles and not all parts of the skin may touch the cutting board. Consequently, we assume that the average transfer to the cutting board in a "real household" may be lower than shown by Zhao *et al.* (1998). In the present study we have therefore chosen to represent the fraction of *Campylobacter* transferred from the chicken to the cutting board by a Pert distribution with a minimum of 10^{-6}, a mode of 10^{-2} and a maximum of 10^{-1} (the maximum is given by Zhao *et al.*, 1998).

With respect to transfer from the unwashed cutting board to the salad and/or the prepared chicken, there may be some delay between the cutting of the raw chicken and the cutting of the salad, and certainly there will be a delay between the cutting of a raw chicken and the cutting of a prepared chicken. This delay may result in a reduction in the number of living organisms on the cutting board, e.g. because of drying of the surface of the cutting board. In some cases the delay may result in a large reduction in others not. Also the salad or the prepared chicken may be more or less carefully treated on the cutting board. Thus, we have chosen to employ a Pert distribution to describe the reduction in *Campylobacter* transferred from the cutting board to the salad and/or the prepared chicken, which in both cases has a minimum of 10^{-6}, a mode of 10^{-2} and a maximum of 10^{-1}.

Dose response

A dose response model estimates the probability of getting ill from ingesting a certain number of pathogenic organisms. The probability of illness after having ingested a dose of organisms depends on the ability of the organism to survive and colonise/infect the host, and once colonised the ability to cause illness.

In order to translate this process into a probability of getting ill, a mathematical formula is needed, which describes the different infection processes. Haas *et al.* (1983) used a stochastic model (Beta-Poisson) to describe the probability of infection (not illness) as a function of the ingested dose. In this model it is assumed that the micro-organisms in the ingested vehicle is randomly distributed (Poisson) and that each individual organism will have the same probability (*p*) of causing infection, where *p* is Beta(α,ß) distributed. The Beta distribution reflects the uncertainty and the variability between individual humans of the probability of an organism to cause infection.

A problem with dose response modelling is the lack of data needed to estimate the model parameters. There are three different ways of obtaining information about the dose response relationships: 1) feed trial experiments on animal models, 2) epidemiological data from outbreaks, and 3) feeding trial experiments on human volunteers. With respect to *Campylobacter* the information obtained from animal models and epidemiological studies is rather limited. At present all dose response models on *Campylobacter* infections have been based on a single feeding trial experiment on human volunteers, in which 111 young adult volunteers ingested *Campylobacter* doses ranging from $8x10^2$ - $2x10^9$ organisms (Black *et al.*, 1988). Based on data from 68 of the volunteers in the study (see Table 37) Medema *et al.*

(1996) calculated the maximum likelihood estimates for α and ß in the Beta-Poisson model (α = 0.145 and ß = 7.59).

Recently, Teunis and Havelaar (2000) have suggested an improved dose response model as an alternative to the Beta-Poisson model. However, at present we have not yet build this alternative model into the consumer model.

In the present model, the exact number of *Campylobacter* cells, which a person ingests, is known. By assuming that each individual organism will have the same probability (*p*) of infection in each individual person, the probability of infection can be calculated as:

$$P_{inf} = 1-(1-p)^n$$

where *n* is the dose of *Campylobacter* cells ingested. By including that our knowledge about the probability *p* is uncertain and may vary from person to person, the probability of infection is obtained as:

$$P_{inf} = 1-(1-\text{Beta}(\alpha, ß)^n$$

Where α and *ß* are the maximum likelihood estimates obtained by Medema *et al*. (1996).

From the studies presented by Black *et al.* (1988) it was evident that a *Campylobacter* infection is not always followed by symptoms of illness (Table 37). In only 11 of 50 infections the volunteers showed symptoms of illness. Interestingly, the highest number of people got ill from a relatively low dose ($9x10^4$ cfu). We do not believe that low doses should give a higher probability of illness, instead we take the result as an indication that the dose ingested and the probability of illness is two uncoupled processes. Thus, independently of the dose ingested, if a person becomes infected, there is a certain probability that the person will become ill. The uncertainty about the true value of this probability is described by a beta distribution, where α = 11 + 1 = 12 and ß = 50 – 11 + 1 = 40.

Table 37. Feeding trial data from Black *et al*. (1988)

	No of volunteers			Percentages of volunteers (%)	
Dose (cfu)	Total	With positive stool cultures	With symptoms	With positive stool cultures	With symptoms
$8x10^2$	10	5	1	50	10
$8x10^3$	10	6	1	60	10
$9x10^4$	13	11	6	85	46
$8x10^5$	11	8	1	73	9
$1x10^6$	19	15	2	79	11
$1x10^8$	5	5	0	100	0
Total	68	50	11	74	22

Consumer model – model building

A computer program for modelling food handling in private kitchens was developed on an Excel platform using the @RISK program to model distributions instead of means, using the same principals as for the slaughterhouse model (see the section on page 62). However, in contrast to the slaughterhouse model, a Visual Basic program was not needed. The model has been divided into the two categories described above: i) the persons who prepare the meal and ii) the persons who ingest the meal.

By initially assuming that the cutting board is unwashed and the prepared chicken is *Campylobacter* positive, the probability of exposure to *Campylobacter* and the probability of illness are calculated for three different types of servings ('behaviour parameters'):

C) The prepared chicken is put back on the **C**utting board after heat treatment and salad is not ingested with the chicken meal (only the prepared chicken may contain *Campylobacter*).

S) The prepared chicken is not put back on the cutting board after heat treatment and **S**alad (cut on the same cutting board) is ingested with the chicken meal (only the salad may contain *Campylobacter*).

C+S) The prepared chicken is put back on the **C**utting board after heat treatment and **S**alad (cut on the same cutting board) is ingested with the chicken meal (both products may contain *Campylobacter*).

The number of *Campylobacter* ($N_{C|ASIS}$, $N_{S|ASIS}$ and $N_{C+S|ASIS}$) in a salad or chicken serving is assumed to be Poisson distributed and is calculated for each of the three different types of servings as described in Table 38.
The probability of exposure (E) to *Campylobacter* ($P_{EC|ASIS}$, $P_{ES|ASIS}$ and $P_{EC+S|ASIS}$) are obtained as one minus the probability of not being exposed to any *Campylobacter* cells in a serving multiplied with the fraction of positive chickens (F_{POS}) that actually enters the kitchen (Table 38).
Given exposure, and the number of *Campylobacter* cells, the probability of infection for each individual person ($P_{infC|ASIS}$, $P_{infS|ASIS}$ and $P_{infC+S|ASIS}$) from one of the three types of servings is calculated.
In the present work we have not included the variability in the probability of getting infected from each individual serving containing *Campylobacter*. Instead, the average probability of getting infected was determined. For each iteration (*i*) in a simulation consisting of N iterations (one simulation consist of many iterations), the probability of infection ($P_{infC|ASIS,E}$, $P_{infS|ASIS,E}$ and $P_{infC+S|ASIS,E}$) was estimated by a Binomial distribution, and the average probability of illness (AP_{ill}) for each of the three servings was estimated as:

$$AP_{ill}(j) = \frac{1}{N} \cdot \left(\sum_{i=1}^{i=N} (Binomial(1, P_{inf\ |ASIS,E}(i, j))) \right)$$

where, j = C, S, C+S representing the three types of servings described above.

After having calculated the average probabilities in the three scenarios, the parameters (including the uncertainties on the parameters) describing the behaviour of the persons,

who prepare the meal ($P_{NWC|ASPM}$ and $P_{CC|ASPM}$) and the persons ingesting the meal ($P_{S|AIS}$) (Table 39), were included.

The probabilities for ingesting each of the three types of servings ($P_{C|ASPM}$, $P_{S|ASPM}$ and $P_{PC+S|ASPM}$) were determined as described in Table 40.

For each of the three types of servings the probability of exposure to *Campylobacter* was estimated by multiplying the probability of ingesting the serving with the probability of exposure from that serving. By further multiplying with the probability of infection given exposure we obtain the probability of infection from that type of serving.

Adding the probability of exposure, or infection, from each of the servings together and multiplying with the probability of not washing the cutting board ($P_{NWC|ASPM}$) and the percentage of persons ($P_{ASIS|ASPM}$, obtained from Table 32) for each age and sex group ingesting a meal, and for each age and sex group of person preparing the meal, the relative probability for exposure ($P_{E|ASIS, ASPM}$) and infection ($P_{inf|ASIS, ASPM}$), respectively, was obtained.
The probability of illness was calculated by multiplying the probability of infection ($P_{inf|ASIS, ASPM}$) with the probability of illness given the person has been infected ($P_{ill|inf}$).

Finally, by adding the relative probabilities of the 48 groups, the average probability of exposure ($\mathbf{P_{E\text{-}AV}}$), and illness ($\mathbf{P_{ill\text{-}AV}}$) were calculated. The probability for exposure ($P_{E\text{-}AV|ASIS}$) and illness ($P_{ill\text{-}AV|ASIS}$) per meal ingested for each age and sex group was determined by adding the relative probability for exposure ($P_{E|ASIS, ASPM}$) and illness ($P_{ill|ASIS, ASPM}$) and dividing with the number of meals out of the total number of meals ingested for each age and sex group.

Table 38. Risk of ingesting a chicken, salad or chicken + salad serving given that cutting board was not washed during preparation of the meal.

Parameter	Description	Units	Distribution/expression
F_{POS}	Fraction of *Campylobacter* positive chickens at retail level		Obtained from the slaughterhouse model
C_C	Concentration of *Campylobacter* on chicken	Log10 (cfu/g)	
R_{CCB}	*Campylobacter* log 'reduction' from the raw chicken to the cutting board		Pert(1,2,6)[a]
R_{CBC}	*Campylobacter* log 'reduction' from cutting board to the prepared chicken		Pert(1,2,6)[a]
R_{CBS}	*Campylobacter* log 'reduction' from cutting board to salad		Pert(1,2,6)[a]
$S_{C\mid ASIS}$	Size of chicken serving given ASIS[c]	g	See table 35
$S_{S\mid ASIS}$	Size of salad serving given ASIS[c]	g	See table 36
$N_{C\mid ASIS}$	Number of *Campylobacter* in a chicken serving given ASIS[c]		$Poisson(S_{C\mid ASIS} \cdot 10^{(C_C - R_{CCB} - R_{CBC})})$
$N_{S\mid ASIS}$	Number of *Campylobacter* in a salad serving given ASIS[c]		$Poisson(S_{S\mid ASIS} \cdot 10^{(C_C - R_{CCB} - R_{CBS})})$
$N_{C+S\mid ASIS}$	Number of *Campylobacter* in a chicken + salad serving given ASIS[c]		$N_{C\mid ASIS} + N_{S\mid ASIS}$
$P_{EC\mid ASIS}$	Probability of exposure to *Campylobacter* from chicken serving given ASIS[c]		$F_{POS} * (1 - Prob(N_{C\mid ASIS} = 0))$
$P_{ES\mid ASIS}$	Probability of exposure to *Campylobacter* from a salad serving given ASIS[c]		$F_{POS} * (1 - Prob(N_{S\mid ASIS} = 0))$
$P_{EC+S\mid ASIS}$	Probability of exposure to *Campylobacter* from chicken + salad serving given ASIS[c]		$F_{POS} * (1 - Prob(N_{C+S\mid ASIS} = 0))$
P_{1C}	Probability of illness from exposure to one *Campylobacter*		Beta(0.145,7.59)[b]
$P_{infC\mid ASIS,E}$	Probability of illness from a dose in a chicken serving given ASIS[c] and given exposure		$((1 - P_{1C})^{N_{C\mid ASIS}}, \quad N_{C\mid ASIS} > 0$
$P_{infS\mid ASIS,E}$	Probability of illness from a dose in a salad serving given ASIS[c] and given exposure		$((1 - P_{1C})^{N_{S\mid ASIS}}, \quad N_{S\mid ASIS} > 0$
$P_{infC+S\mid ASIS,E}$	Probability of illness from a dose in a chicken + salad serving given ASIS[c] and given exposure (E)		$((1 - P_{1C})^{N_{C+S\mid ASIS}}, N_{C+S\mid ASIS} > 0$
$AP_{infC\mid ASIS,E}$	Average probability of illness from a dose in a chicken serving given ASIS[c] and given exposure (E)		$\frac{1}{N} \cdot \sum_{i=1}^{i=N} (Binomial(1, P_{illC\mid ASIS,E}))$
$AP_{infS\mid ASIS,E}$	Average probability of illness from a dose in a salad serving given ASIS[c] and given exposure (E)		$\frac{1}{N} \cdot \sum_{i=1}^{i=N} (Binomial(1, P_{illS\mid ASIS,E}))$
$AP_{infC+S\mid ASIS,E}$	Average probability of illness from a dose in a chicken + salad serving given ASIS[c] and given exposure (E)		$\frac{1}{N} \cdot \sum_{i=1}^{i=N} (Binomial(1, P_{illC+S\mid ASIS,E}))$

[a] Pert(min, median, max)

[b] Beta(α,β)

[c] ASIS = Age and sex of person ingesting the serving

Table 39. Behaviour parameters

Parameter	Description	Distribution/expression
$P_{NWC\mid ASPM}$	Prevalence of people that do not wash the cutting board given ASPM[b]	See Table 33
$P_{PCC\mid ASPM}$	Prevalence of people putting prepared chicken back on the cutting board after heat treatment given ASPM[b]	Uniform(0.2,0.6)[a]
$P_{S\mid AIS}$	Percentage of people ingesting salad with their chicken meal given AIS[c]	See Table 36

[a]Uniform(min, max)
[b]ASPM = age and sex of person preparing the meal
[c]AIS = age of person ingesting the serving

Table 40. Overall risk calculations

Parameter	Description	Distribution/expression
$P_{ASIS\mid ASPM}$	Percent of people in each ASIS[a] for a given ASPM[b]	See Table 32
$P_{C\mid ASPM}$	Probability of ingesting a serving where only the heat treated chicken have been cut on the cutting board given ASPM[b]	$P_{PCC\mid ASPM} - P_{PCC\mid ASPM} \cdot P_{S\mid AIS}$
$P_{S\mid ASPM}$	Probability of ingesting a serving where only the salad have been cut on the cutting board given ASPM[b]	$P_{S\mid AIS} - P_{PCC\mid ASPM} \cdot P_{S\mid AIS}$
$P_{C+S\mid ASPM}$	Probability of ingesting a serving where the heat treated chicken and salad have been cut on the cutting board given ASPM[b]	$P_{PCC\mid ASPM} \cdot P_{S\mid AIS}$
$P_{E\mid ASIS,\ ASPM}$	Probability of exposure to *Campylobacter* in a serving given ASIS[a] and ASPM[b]	$(P_{C\mid ASPM} \cdot P_{EC\mid ASIS} + P_{S\mid ASPM} \cdot P_{ES\mid ASIS} + P_{C+S\mid ASPM} \cdot P_{EC+S\mid ASIS}) \cdot P_{NWC\mid ASPM} \cdot P_{ASIS\mid ASPM}$
$P_{inf\mid ASIS,\ ASPM}$	Probability of infection from a dose of *Campylobacter* in a serving given ASIS[a] and ASPM[b]	$(P_{C\mid ASPM} \cdot P_{ES\mid ASIS} \cdot AP_{illC\mid ASIS,\ E} + P_{S\mid ASPM} \cdot P_{EC\mid ASIS} \cdot AP_{illS\mid ASIS,\ E} + P_{C+S\mid ASPM} \cdot P_{EC+S\mid ASIS} \cdot AP_{illC+S\mid ASIS,\ E}) \cdot P_{NWC\mid ASPM} \cdot P_{ASIS\mid ASPM}$
$P_{ill\mid inf}$	Probability of illness given infection	Beta(12,40)[d]
$P_{ill\mid ASIS,\ ASPM}$	Probability of illness from a dose of *Campylobacter* in a serving given ASIS[a] and ASPM[b]	$P_{ill\mid inf} \cdot P_{inf\mid ASIS\mid ASPM}$
$P_{E\text{-}AV}$	Average probability of exposure to *Campylobacter* in a serving for all ASIS[a] and ASPM[b]	$\sum_{all\_ASPM}\left(\sum_{all\_ASPM} P_{E\mid ASIS,\ ASPM}\right)$
$P_{ill\text{-}AV}$	Average probability of illness from a dose of *Campylobacter* in a serving for all ASIS[a] and ASPM[b]	$\sum_{all\_ASPM}\left(\sum_{all\_ASPM} P_{ill\mid ASIS,\ ASPM}\right)$
$P_{E\text{-}AV\mid ASIS}$	Average probability of exposure to *Campylobacter* in a serving for each ASIS[a], taken into account the fraction of people in each age and sex group	$\frac{\sum_{all\_ASPM} P_{E\mid ASIS,\ ASPM}}{\sum_{all\_ASPM} P_{ASIS\mid ASPM}}$
$P_{ill\text{-}AV\mid ASIS}$	Average probability of illness from a dose of *Campylobacter* in a serving for each ASIS[a], taken into account the fraction of people in each age and sex group	$\frac{\sum_{all\_ASPM} P_{ill\mid ASIS,\ ASPM}}{\sum_{all\_ASPM} P_{ASIS\mid ASPM}}$

[a]ASIS = Age and sex of person ingesting the serving; [b]ASPM = age and sex of person preparing the meal;
[c]AIS = age of person ingesting the serving; [d]Beta(α,β)

Consumer model - results

Estimation of the probability of illness

Best guess of the probability of illness including all age and sex groups ($P_{ill\text{-}AV}$)

It was of interest to analyse whether the probability of illness ($P_{ill\text{-}AV}$) would be affected by ingesting a frozen chicken or a chilled chicken. Therefore simulations were made with two different input distributions for the concentration of *Campylobacter* (C_C), one for chilled chickens and another for frozen chickens (Fig. 35a). As a result of the simulations, the probability of illness by ingesting a chicken sold as chilled or frozen was estimated to 1 of 6300 servings or 1 of 26600 servings, respectively (Fig. 38, grey bars). The probability of illness was about 4 times higher for a chilled compared to a frozen chicken. Thus, a moderate reduction of 0.5 to 1.5 $\log_{10}$ cfu/g of the *Campylobacter* concentration due to freezing seems to have a relatively large effect on the probability of getting ill. According to preliminary data from the Danish retail surveillance, the difference in the mean concentration between chilled and frozen chickens seems to be 1.5 log cfu/g rather than 1 log cfu/g as assumed in the model. This reduction in concentration results in a reduction in the probability of getting ill by approximately a factor 10. In accordance with the model a reduction in the *Campylobacter* concentration by a factor 100 (2 log cfu/g) will reduce the probability of getting ill by approximately a factor 25.

By taking the fraction of chilled relative to frozen chickens sold in Denmark into account we have estimated the mean probability of illness to 1 out of 14300 chicken servings (Fig. 38, black bar). From Table 36 we could estimate that approximately 10% of all servings ingested per day included chicken. This results in approximately 201 mill. servings with chicken per year in Denmark. If the mean estimate of 1 ill out of 14300 servings is true, the expected number of *Campylobacter* cases caused by cross contamination from contaminated chickens in private kitchen would be approximately 14,000 per year. Compared to the 4,164 registered human cases in 1999 and taking into account that the actual number of cases may be from 30,000 to 400,000 the estimated number of *Campylobacter* cases arising from eating chicken seems to be a realistic result. However, one should also keep in mind that there may be other routes of infection via chicken (Fig. 31) as well as other sources of infection than chickens which may contribute to a fraction of the *Campylobacter* cases in Denmark.

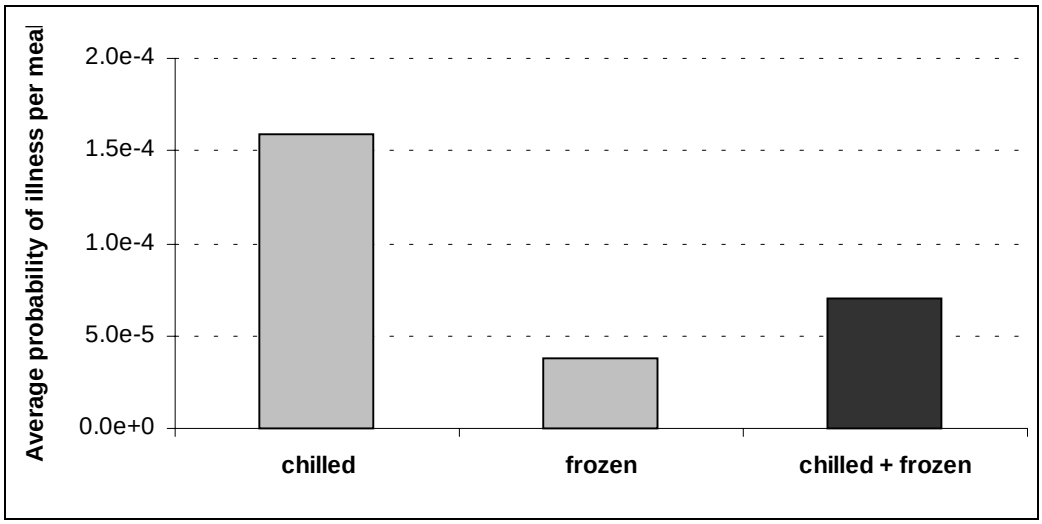

Figure 38. Average probability of illness from a chicken serving prepared either from a frozen or a chilled chicken (grey bars). For the frozen chickens a uniform distribution with min. of 0.5 and max of 1.5 was subtracted C_C. For the chilled chickens we did not assume any reduction in the *Campylobacter* level. The black bar represents the probability of getting ill from a chicken meal in Denmark, assuming that 26.5% of chickens are sold as chilled and 73.5% as frozen (see Table 17).

Probability of illness for different age and sex groups

The consumer model allows us to divide the risk of ingesting a chicken meal into age and sex groups. Taken into account the fraction of meals ingested in each of the age and sex groups, the average probabilities of illness ($P_{ill\text{-}AV|ASIS}$) for each group were estimated (Fig. 39a). In the calculations the ratio between frozen and chilled products sold in Denmark was included. Especially, young adult men in the age of 18-29 years seem to have a higher risk of getting ill, whereas people above the age of 65 seems to have a lower probability of illness. The simulated age distribution seems to be in reasonable good agreement with the actual age and sex distribution of *Campylobacter* cases registered in Denmark (Fig. 39b), though there are some differences. The simulations resulted in a higher probability of getting ill among young males than young females. The registered cases show the opposite, namely a higher incidence rate among young females as compared to the young males. Further on, the probability of illness among children seems to be underestimated in the model. A possible explanation for the differences could be that the dose response levels are lower for children and young females as compared to other age groups. The dose response model used is based on data from a study comprising young males (see section on page 92).

The fact that young men generally ingest more per serving and also have a lower hygiene level in the kitchen are the two main factors responsible for the higher simulated probability of infection in this group. Especially the hygiene level is important. This indicates that if the relative hygiene level could be improved, it would be a possible way to reduce the probability of infection (see later). An interesting observation is the relatively high probability of illness for women in the age of 18-29 years compared to men of 30-65 years, as the hygiene level of women is higher as compared to men (see Table 33). An explanation for this could be that women (18-29

years) often ingest food prepared by men in the age of 18-29 (having a lower hygiene level), whereas men (30-65 years) on the other hand most often ingest meals prepared by women (30-65 years), who have a higher level of hygiene.

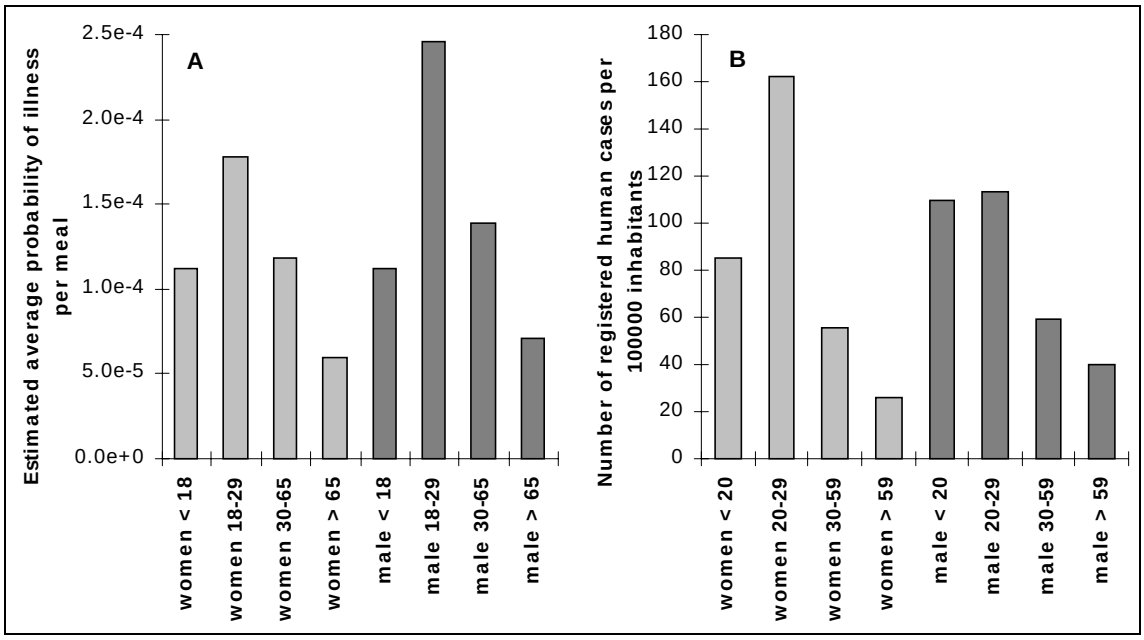

Figure 39. Simulated probability of illness per meal (A) and number of human cases in 1999 in Denmark as registered by Statens Seruminstitut (B) divided into age and sex groups.

Including uncertainty

In the model simulations we have included uncertainty related to the behaviour parameters (Table 39) and the uncertainty/variability in the probability of getting ill once infected ($P_{ill \mid inf}$). The simulations showed that the uncertainty related to the average probability of illness from ingesting a chilled chicken was within the range $0.9 \cdot 10^{-4} – 2.5 \cdot 10^{-4}$ (= within a factor of 3) (Fig. 40a). This seems to be within a satisfactory range. However, it is important to note that at present there are several of the model parameters to which uncertainty has not been included. These are: i) uncertainty related to distributions describing the transfer of *Campylobacter* from raw chicken to cutting board (R_{CCB}) and from cutting board to salad (R_{CBS}) and prepared chicken (R_{CBC}), ii) uncertainty in relation to the way people have been divided into the different age and sex groups, iii) uncertainty in relation to the dose response model used in the simulations. In principle, we do not know how uncertain we are on these parameters, but in particular the distributions describing the transfer processes of the *Campylobacter* from the raw chicken to the final meal are based on vague assumptions. Therefore, if it were possible to include the uncertainty on these parameters, the distribution for the true probability of illness would become considerably broader.

The distributions for the probability of illness in the different age and sex groups were compared. In Fig. 40b the two age and sex groups with the highest and the lowest mean probability of illness (see Fig. 39) are compared to the probability distribution for all age and sex groups. Even when the uncertainty related to the behaviour parameters is included the probability of getting ill is significantly higher for young men than woman above 65 years.

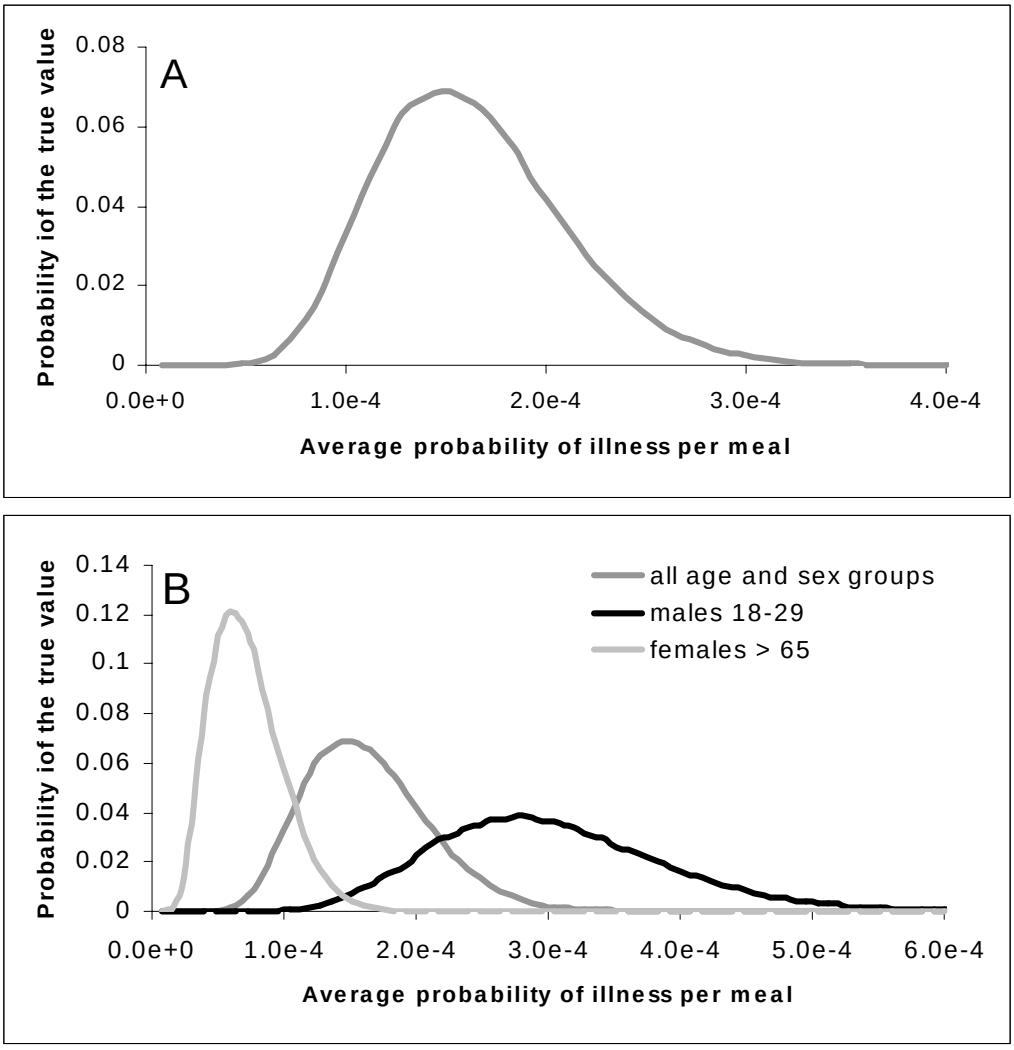

Figure 40. Uncertainty distributions related to the true value of the average probability of illness from ingesting chilled chickens. (A) The total uncertainty distribution for the estimated value of the probability of getting ill including all sex and age groups. (B) The total uncertainty distributions for the average probability of getting ill for females > 65 years and young men aged 18-29 years. For comparison the overall distribution for all age and sex groups is also plotted.

Relationship between *Campylobacter* status of chickens and the average probability of exposure and illness.

In the previous section it was mentioned that that freezing of chickens seems to reduce the probability of illness due to a reduction in the *Campylobacter* concentration on the chickens. However, the probability of illness may also be reduced by introducing changes at farm and slaughterhouse level, that reduce the occurrence of *Campylobacter* at the chickens, which are sold at retail level.

In accordance with the slaughterhouse model there are three distinct factors that may have influence on the *Campylobacter* status (concentration and prevalence) of the chickens leaving the slaughterhouse and thereby also the chickens sold at retail:

i) The prevalence of *Campylobacter* positive broiler flocks that enter the slaughterhouse.
ii) The level of cross-contamination during the slaughterhouse processes.
iii) The change in the *Campylobacter* concentration on carcasses during the slaughterhouse processes.

In the following, the risk of exposure from a meal with *Campylobacter* and the subsequent probability of illness is discussed in relation to these three factors. In the simulations we have not focused on the exact estimate of the probability of illness per meal, but we have concentrated on the relative changes in e.g. probability of illness upon changing the fraction of positive chickens and the *Campylobacter* concentration during slaughter. For simplicity we have used the distributions for the *Campylobacter* concentration and fraction of positive chickens at packaging in the slaughterhouse as input (F_{POS} and C_C) to the consumer model, which means that only chilled chickens are considered.

Relationship between the fraction of positive chickens entering the kitchen and the probability of illness

In order to analyse how changes in the fraction of positive chickens prepared in private kitchens affect the probability of illness ($P_{ill\text{-}AV}$), the fraction of positive chickens (F_{POS}) entering the kitchen was varied over the range from 0 to 1. A linear correlation was found between the fraction of positive chickens, F_{POS} and the probability of illness (Fig. 41).

From the simulations in the slaughterhouse model we observed an almost linear correlation between the fraction of *Campylobacter* positive chickens entering the slaughterhouse and the fraction of *Campylobacter* positive chickens leaving the slaughterhouse (Fig. 30). In contrast, the concentration of *Campylobacter* on the positive chickens remained almost unaffected (Fig. 31). Only a small positive linear correlation was seen. The concentration was only reduced by 0.35 $\log_{10}$ cfu/g from a flock prevalence of 70% to a flock prevalence of 15%. Consequently, the relationship between the fraction of *Campylobacter* positive chickens entering the slaughterhouse and the probability of illness is also linear. Thus, considering that the fraction of *Campylobacter* positive broilers in the broiler houses could be reduced by for example a

factor of 2 (e.g. from 60% to 30%) a corresponding factor 2 reduction would be expected for the probability of getting ill – according to the model.

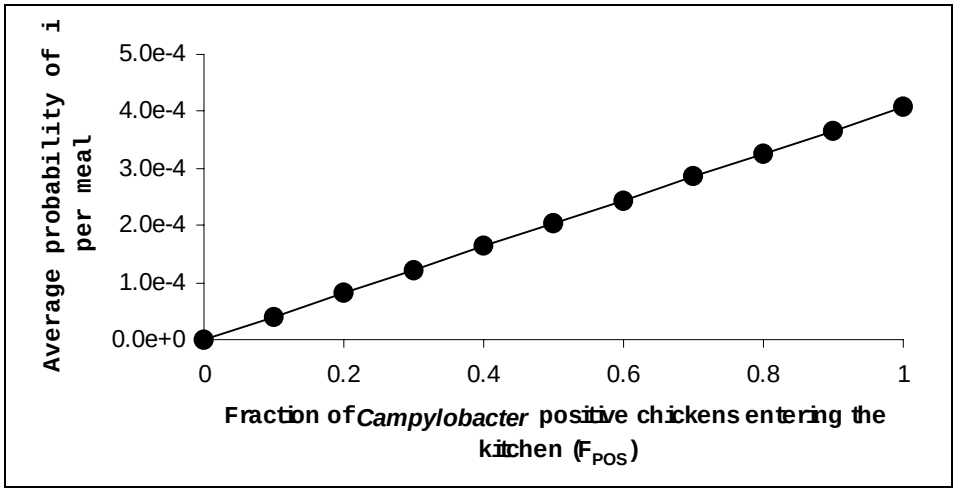

Figure 41. Relationship between the fraction of *Campylobacter* positive chickens prepared in the kitchen and the average probability of getting ill per meal.

Relationship between cross-contamination in the slaughterhouse and the probability of illness

One of the results of the slaughterhouse model showed that an increase in the degree of cross-contamination between *Campylobacter* positive flocks and *Campylobacter* negative flocks would result in a considerable increase in the fraction of positive chickens leaving the slaughterhouse (Fig. 27). It was therefore of interest to analyse, whether an increase in the fraction of positive chickens leaving the slaughterhouse caused by cross-contamination also affected the probability of illness. Fig. 42 shows the relationship between the degree of cross-contamination (T_{half}) and the probability of exposure and illness. From the figure it is obvious that cross-contamination will affect the probability of illness, but certainly not to the same degree as the increase in the fraction of *Campylobacter* positive chickens leaving the slaughterhouse could indicate. The explanation for this is that the cross-contaminated chickens causing the increase in the fraction of positive chickens generally have a lower concentration than the positive chickens originating from positive flocks. The lower concentration results in a lower probability of getting exposed to *Campylobacter*, and therefore also a reduced probability of illness. This is due to the 'reduction' and dilution during the different kitchen processes (given by the parameters R_{CCB}, R_{CBC} and R_{CBS}).

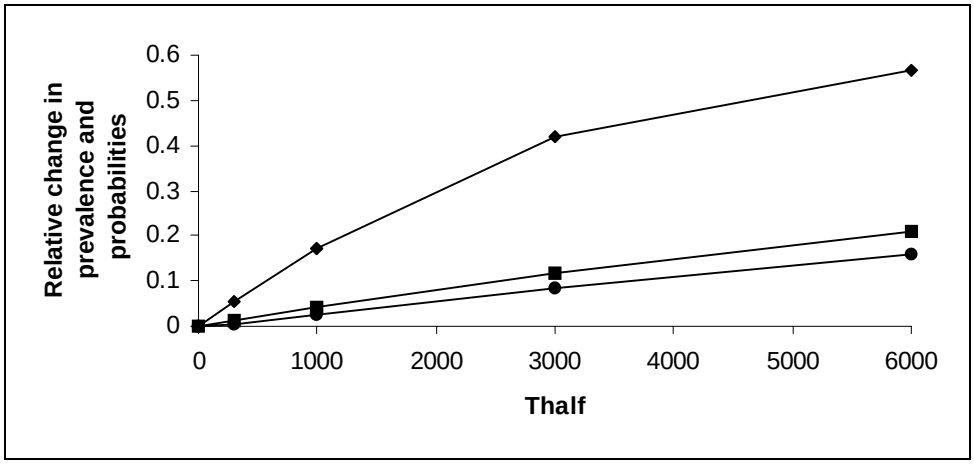

Figure 42. Comparison of relative changes in the fraction of positive chickens (F_{POS}) (♦), the probability of exposure to *Campylobacter* in a chicken meal ($P_{E\text{-}AV}$) (■), and the probability of illness from a chicken meal ($P_{ill\text{-}AV}$) (●) for different levels of cross-contamination in the slaughterhouse (T_{half}). The changes were taken relative to the simulation where no cross-contamination was assumed ($T_{half} = 0$).

Relationship between changes in the *Campylobacter* status on chickens during the slaughter process and the probability of illness.

In the slaughterhouse model we have analysed how changes in a single slaughter process could affect the overall prevalence of *Campylobacter* positive chickens and the concentration on these chickens at packaging. The simulations (Fig. 33) showed that the fraction of positive chickens leaving the slaughterhouse was only slightly affected by a reduction in the concentration below 3 $\log_{10}$ cfu/g. The concentration on the chickens, however, was reduced nearly 3 $\log_{10}$ cfu/g. In contrast to changing the prevalence of positive chickens at farm level, changing a process in the slaughterhouse has a large impact on the concentration, but relatively little effect on the fraction of positive chickens leaving the slaughterhouse. Simulations were carried out to analyse how changes in the *Campylobacter* status of chickens leaving the slaughterhouse affect the probability of illness. The output distributions describing the *Campylobacter* concentration and prevalence of positive chickens for different reduction levels in the washing + chilling process (Fig. 33) were used as input data (F_{POS} and C_C).

The simulations showed that the probability of exposure to *Campylobacter* as well as the probability of illness could be reduced considerably by reducing the concentration of *Campylobacter* on carcasses during the slaughter process (Fig. 43). A reduction in the *Campylobacter* concentration of 1 $\log_{10}$ cfu/g, obtained for example during the washing + chilling process, reduced the probability of illness by a factor 4; a reduction of 2 $\log_{10}$ cfu/g reduced the probability of illness by a factor 25; a reduction of 3 $\log_{10}$ cfu/g reduced the probability of illness by a factor 200, etc. Thus, if it is possible to introduce a step in the slaughterhouse, which reduces the *Campylobacter* concentration by a factor 10 (1 $\log_{10}$ cfu/gram) or more, this would have a significant impact on the probability of illness after having ingested a chicken meal. The main reason for this is that the concentration of and hence the exposure to *Campylobacter* is reduced upon the transfer

of *Campylobacter* during food handling to final meal (R_{CCB}, R_{CBC} and R_{CBS}). In most cases the concentration will be too low to allow transfer of any *Campylobacter* all the way from the raw chicken to the prepared meal (salad or chicken).

Note that, even though the *Campylobacter* concentration on the chickens is reduced by 1-3 $\log_{10}$ cfu/g, the fraction of positive chickens that enters the kitchen will remain almost unaffected (Fig. 33). Therefore, the traditional qualitative methods for detection of *Campylobacter* are not detailed enough to determine whether a change in a slaughterhouse process has an effect on the *Campylobacter* risk after having ingested a chicken meal. In relation to the probability of illness, the *Campylobacter* concentration on the chicken is important.

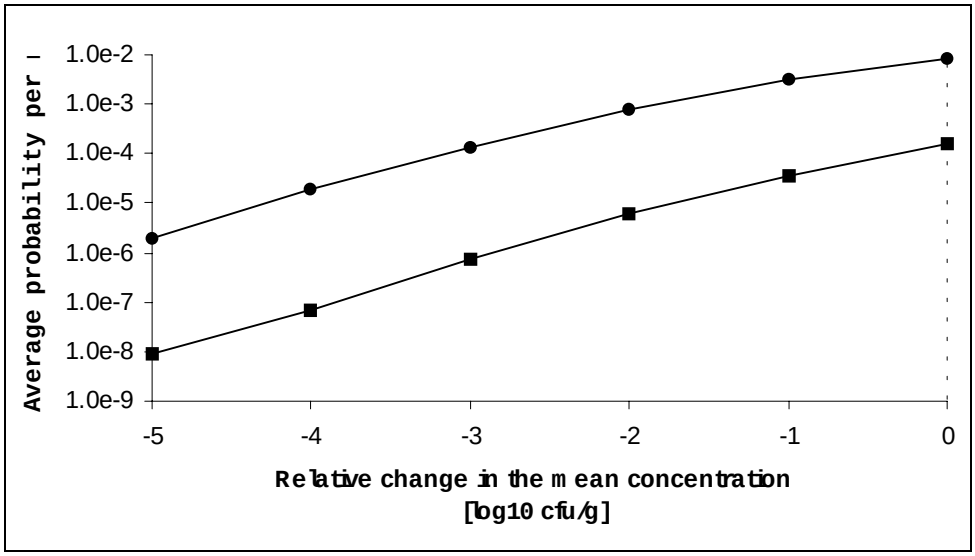

Figure 43. Relationship between a change in the mean value of the input distribution for the washing + chilling process (changing the *Campylobacter* concentration (C_C) on the chickens leaving the slaughterhouse, see Fig. 33) and the average probability of exposure (●) and illness (■) from ingesting a chicken meal. The $\log_{10}$ cfu/g change in the mean value for the washing + chilling process is presented relative to the normal level of –1.46 $\log_{10}$ cfu/g (dotted line).

Relationship between hygiene level and probability of illness

In the previous section the simulation showed how changes in the *Campylobacter* status (concentration and prevalence) of chickens entering kitchen might affect the probability of illness. However, changes in the food safety habits within the kitchen might also have influence on the probability of getting ill. In the present work we have focussed on the washing of the cutting board after having prepared a raw chicken. It was of interest to analyse how the probability of illness would be affected by a change in the hygiene level, modelled by changing the fraction of people who washes the cutting board. This was done by making equal relative changes in the "percentage of positive answers" (see Table 33) for all age and sex groups.

The simulated data in Fig. 44 shows that there is a linear relationship between the fraction of people washing the cutting board and the probability of illness. Thus, it might be possible to reduce the probability of getting ill by improving the kitchen habits of the people preparing the meal. By doubling the number of people washing the cutting board (from a relative change in hygiene level of 1 to 0.5 in Fig.44), the probability of illness is reduced by a factor 2.

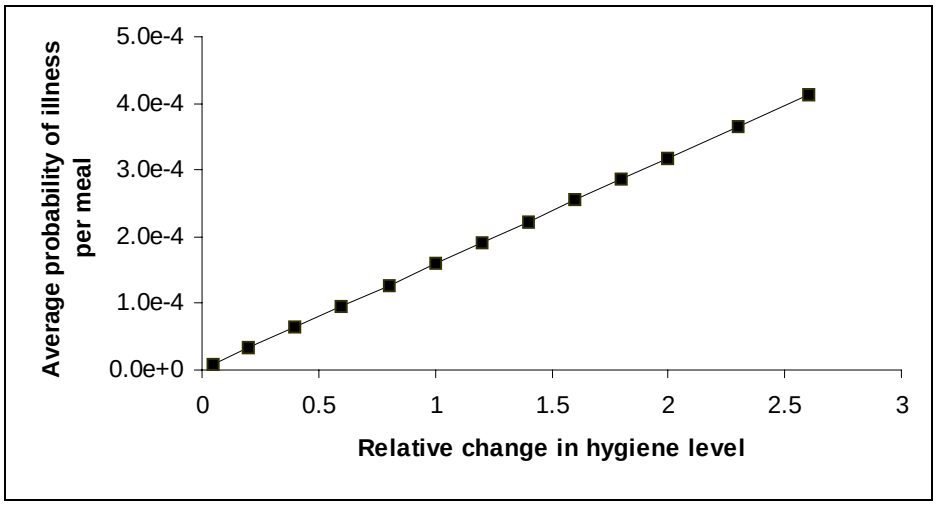

Figure 44. Relationship between the fraction of people who do not wash the cutting board and the probability of illness. The changes in the fraction of people who does not wash the cutting board are presented as relative changes in the "percentage of positive answers" for all age and sex groups in Table 33. The relative change in hygiene level = 1 indicates the standard "percentage of positive answers" used in all other simulations.

Consumer model - conclusions

A model has been developed which allow us to simulate the relationship between *Campylobacter* contaminated chickens and the probability of illness.

The critical part of the model is the uncertainty about the true estimates of the exposure levels and probabilities of illness and the relationship between dose and response. We have included uncertainty related to the behaviour of the person who prepares the meal. However, uncertainty related to the distribution describing the transfer of *Campylobacter* from the raw chicken to the cutting board and from the cutting board to the salad or the prepared chicken has not been included. Nor have we included the uncertainty about the dose response relationship for different sub-populations. Certainly, our knowledge about the food handling procedures in private kitchens is limited and the true distribution describing these procedures might be different from the distributions used in the present model. Consequently, the risk estimates obtained in the present study could be quite different from the true values. As a best guess we have estimated that the probability of getting ill is approximately 1 out of 14300 chicken servings. If this estimate is true, the expected number of *Campylobacter* cases caused by cross contamination from contaminated chickens in private kitchen would be approximately 14,000 per year. Compared to the 4,164 registered human cases in 1999

or an assumed actual number of cases of 30,000 - 400,000 the estimated number of *Campylobacter* cases arising from eating chicken seems to be a realistic result. Despite the fact that there may be other routes of infection via chicken as well as other sources of infection than chickens, the estimate indicates that cross-contamination in private kitchens from *Campylobacter* infected chickens seems to be an important source of infection.

The simulations showed that especially young men (aged 18-29 years) and to some extend women (aged 18-29 years) were at risk. This is in agreement with the actual figures observed by the Statens Seruminstitut (see Fig. 39) and supports the theory that cross-contamination in private kitchens is an important factor for illness, since especially young men have a poor kitchen hygiene.

In addition, low broiler flock prevalence was found to give low probability of illness, which is also in agreement with the correlation that is seen between seasonal variation in the flock prevalence and the probability of illness (Fig. 9).

Assuming that *Campylobacter* contaminated chickens are an important source of human campylobacteriosis, we can ask whether it is possible to reduce the probability of illness. In order to do that we have looked at relative changes for certain parameters.

In the simulations we have focussed on three distinct ways in which the probability of illness may be reduced:

i) by reducing the prevalence of positive broilers at farm level,
ii) by changing the *Campylobacter* concentration of the chickens sold at retail level,
iii) by improving the hygiene habits in private kitchen.

With reference to the prevalence in broiler flocks, the simulations showed a linear correlation between the flock prevalence and the probability of illness. Thus, if it is possible to create a significant reduction in the flock prevalence it should be possible to observe an effect on the probability of illness.

As regards a change in the *Campylobacter* concentration on the chickens sold at retail level, the simulations showed that it seems possible to reduce the probability of illness if a processing step, which reduces the average concentration on the slaughtered chickens, is introduced in the slaughterhouses. Even though such a process would not have a significant influence on the fraction of positive chickens leaving the slaughterhouse, the reduction in the concentration on the positive chickens by for example a factor 100, would result in a reduction in the probability of illness by approximately a factor 25. For comparison, a reduction of a factor 25 in the flock prevalence is needed to obtain the same reduction in the probability of illness. It might also be possible to reduce the probability of illness by taking advantage of the reduced *Campylobacter* levels in frozen compared to chilled chickens. In practise, it could be speculated to slaughter chickens from *Campylobacter* negative flocks in one slaughterhouse and sell these as chilled products and slaughter chickens from *Campylobacter* positive flocks on another slaughterhouse and sell these as frozen products.

In relation to the hygiene habits in private kitchens we have only focused on the effect of not washing the cutting board. From the simulations it was obvious that it would be possible to reduce the probability of illness by improving the hygiene level in private kitchens (by washing the cutting board). We found a linear relationship between the prevalence of not washing the cutting board and the probability of illness, which means that efforts, directed into improving the kitchen hygiene, would be fruitful with regard to minimise the transfer of *Campylobacter* from food to humans.

In conclusion, the presented model provide us with some good indications of which chicken processing steps in the 'slaughterhouse to consumer' chain that are important in relation to a reduction of the transmission of *Campylobacter* to humans. However, it should be kept in mind that the consumer model needs to be further developed. Taken into account the exclusion of several possible contamination routes in the model as well as the uncertainty about some of the parameters, it should be considered whether other modelling approaches than the 'farm to fork' approach could be useful. At present new research is ongoing in order to produce new methods, which might allow us to produce a better risk estimate. However, the methods will probably not allow us to obtain the same level of insight in the actual food handling processes, which occur in the kitchen.

DISCUSSION

RISK MANAGEMENT OPTIONS

Several sources of *Campylobacter* infections in humans have been suggested on the basis of epidemiological data and case control studies. From these studies it was evident that poultry - and in particular chickens - may constitute one of the major sources of human campylobacteriosis. Also handling raw poultry and ingestion of undercooked poultry have been described as important risk factors. As previously mentioned, the relatively high prevalence of *Campylobacter* in chicken products at retail, the epidemiological data, and the amount of available data on *Campylobacter* in the chicken production have formed the basis of the risk management decision to initiate the present risk assessment of *Campylobacter jejuni* in chicken products. However, other possible sources of human campylobacteriosis should not be ignored. To point out all the major sources of exposure to *Campylobacter* in the Danish population, ongoing surveillance and research should be continued in order to list the most cost-effective preventive options on the basis of scientifically elaborated risk assessments.

Due to the widespread distribution of *Campylobacter* in the wild fauna, a wide range of production animals, pets and environmental reservoirs, a total elimination of this hazard from the food chain is not considered as a realistic goal for the time being. Initiatives implemented for the control of *Campylobacter* in the food chain should in general be focused on options that will reduce the *Campylobacter* concentration and prevent cross-contamination at all steps from slaughter to the consumer.

Flock level

As the present quantitative risk assessment only covers events taking place during processing at the slaughterhouse and food handling in private kitchens, the factors related to the introduction, spread and colonisation of *Campylobacter* within the broiler flocks are not considered. Risk assessment related to *Campylobacter* in broiler flocks is subject to ongoing research at the Danish Veterinary Laboratory and other institutions.

Even though the dynamics of *Campylobacter* in broiler flocks are not fully understood several options have been discussed for the prevention or reduction of contamination of live birds. In order to validate proposed tools like vaccination and competitive exclusion further research is needed since no conclusive results have been published so far (Stern 1994; Widders *et al.* 1996). Until now establishment of ”strict hygienic barriers” or “biosecurity zones” at each poultry house seems to be the only preventive option shown to work in practice (Kapperud *et al.* 1993; Humphrey *et al.* 1993; Berndtson *et al.* 1996). Biosecurity zones should as a minimum include strict hygienic routines when the farm workers enter the rearing room, avoiding partly slaughter of flocks, active pest control, avoiding contact with other animals and non authorised personnel and disinfection of drinking water if necessary. Related to the introduction and spread of *Campylobacter* in broiler flocks, the possible benefits of restricted contact with the

environment in the intensive broiler production, could pose a paradox to the raising demands by the consumers on increased animal welfare including admittance to free areas.

Slaughterhouse level

Because a large proportion of the broiler flocks delivered to the slaughterhouses are *Campylobacter* positive, the preventive measures at this level of production should mainly focus on reduction of the *Campylobacter* concentration on the broiler carcasses and prevention of cross-contamination during processing. The use of disinfectants has been investigated in order to reduce the *Campylobacter* concentration in scalding and chilling water and on broiler carcasses (Okrend *et al.*, 1986; Hudson & Mead, 1987). Apparently, this technique have not shown successful results, possibly because of the heavy organic load in the process water and the residence of *Campylobacter* in the deeper layer of the skin e.g. in the feather follicles and in the peritoneal cavity (Berndtson *et al.*, 1992). Ongoing field trials indicate that replacement of the spin-chilling process by forced air cooling could reduce the level of i.e. cross-contamination (Tornøe, personal communication, 1999).

In general, preventive measures against pathogenic microorganisms in poultry at slaughter should be based on implementation of the Hazard Analysis Critical Control Point-system (HACCP), as this concept now is recognised as the most efficient way of controlling food-borne pathogens, including *Campylobacter*, in the production line (ICMSF, 1988).

Management options based on the present model

In the present risk assessment the effect of changing the prevalence of *Campylobacter* contaminated flocks, and the effect of the different processing steps – including the effect of freezing - has been investigated. Based on the results the following management options should be considered:

- A linear correlation seems to exist between the prevalence of *Campylobacter* contaminated flocks and the prevalence of the products leaving the slaughterhouse (see the section on page 72). As an example, a reduction in the prevalence of *Campylobacter* positive flocks by a factor 25 is estimated to lower the risk of getting ill from chicken products by a factor 25. This indicates that the flock prevalence at farm level will have some effect on the number of contaminated chickens leaving the slaughterhouse.

- Cross-contamination seems to have a relative large impact on the prevalence of *Campylobacter* positive chickens leaving the slaughterhouse while the concentration level seems relatively less affected unless the degree of cross-contamination is much larger than we have assumed in this report. Assuming that cross-contamination between *Campylobacter* positive and negative flocks occurs, slaughtering of flocks with a known *Campylobacter* negative status at the start of the day could reduce the number of positive carcasses leaving the slaughterhouse, and thus, also the probability of getting ill. However, the effect is rather limited, because the

concentration on the cross-contaminated chickens is relatively low as compared to the chickens originating from positive flocks.

- The simulations indicates that implementation of processes at any step of the slaughter line, that reduce the concentration of *Campylobacter* on contaminated carcasses leaving the slaughterhouse, will also reduce the risk of getting ill from chicken-products (see the section on page 104). As an example, a reduction in the concentration of *Campylobacter* on the chicken carcasses by a factor of 100 (2 log cfu pr g) is estimated to lower the risk of illness by a factor 25.

- If freezing reduce the *Campylobacter* concentration on positive carcasses by approximately 1 log cfu/g, as assumed in the model, the simulations show that the prevalence of the positive chickens will be reduced by approximately 4%. However, the probability of getting ill will be reduced 4 times. Data obtained from investigations at retail level indicate that the actual reduction could be in the range of 10–20% or more. This means that the reduction in the *Campylobacter* concentration on the carcasses due to freezing could be higher than the assumed 1 log cfu/g. If that is the case, the effect on the probability of getting ill from frozen chickens could be significantly more than the 4 times reduction obtained in the simulations.

Secondary production, commercial caterers, transport and retail

For all kinds of foods, the main preventive measures at this level of production and distribution should be based on implementation of procedures to avoid cross-contamination and temperature abuse. Also, procedures tended to secure sufficient heat treatment in relevant food items should be implemented (ICMSF, 1988; Bryan, 1990). The safety and quality of foods at this stage of production and distribution should be ensured and documented by implementation of a HACCP based quality assurance system (Schlundt, 1999).

Consumer level - including vulnerable groups

At the consumer level preventive measures should mainly be based on risk communication such as education and information (Foegeding & Roberts, 1996, Lammerding, 1997; Schlundt, 1999). Education and information should focus on correct handling and storage of foods and the risks associated with cross-contamination and temperature abuse. Further, risks associated with ingestion of raw and undercooked foods and contaminated drinking water should be stressed out.

Management options based on the present model

From case-control studies and epidemiological data it seems that unsafe food handling procedures may have a major impact on the number of human cases of campylobacteriosis. In particular among the younger age groups there seems to be a need for information regarding safe food handling procedures. The results from the present model indicate a linear correlation between the fraction of people who do not

wash the cutting board and the probability of getting ill from a meal. Therefore, education on food hygiene of younger people should be intensified, preferably at primary school level.

Monitoring

The effectiveness of implemented risk management tools should be validated through monitoring and surveillance programmes (WHO, 1997; Schlundt, 1999). Both the prevalence and the concentration of the pathogen as well as the impact on the number of human cases caused by the pathogen should be included. Programmes for monitoring the effect should be established at all relevant stages in the production of foods where a certain factor for the control of *Campylobacter* contamination has been implemented. Relevant sites for monitoring could be the flock prevalence at farm level and the prevalence and concentration in products at slaughterhouses and in retail foods. Changes in the number of human cases of *Campylobacter* infections should be monitored by establishing surveillance-programmes based on data generated by medical staff in both general practices and hospitals.

Comparable data and methods of analysis

Comparable data regarding the prevalence and concentration of *Campylobacter* in foods within and between countries greatly depends on validated and harmonised methods for analysis. Additionally, the 'options of choice' in risk management should be based on quantitative risk assessment, which is based on quantitative methods of analysis. Therefore, the authorities should take action to ensure that such well-documented quantitative methods for analysis are developed and implemented.

Further on, it is important to develop and implement a sufficiently discriminatory and validated method for typing *Campylobacter* species in order to point out the most important sources of human *Campylobacter* infections – and thereby be able to make the right choice within the proposed risk management options.

DATA GENERATION IN THE FUTURE

Going through the available data describing *Campylobacter* in chickens from through the slaughterhouse process, retail and consumer, it has become evident that the material is rather limited. We therefore recommend that additional Danish data are generated in the future. Special attention should be given to the data listed below.

- Data describing the prevalence within the slaughtered broiler flocks.
- Data describing the concentration of *Campylobacter* during the slaughter processes from entrance to final packed chilled and frozen product.
- Data describing the relationship between the concentration of neck skin samples and the concentration of the whole chicken in order to estimate a conversion factor.
- Data describing the effect of different scalding temperatures.
- Data describing the effect of chilling method (water chilling contra air chilling).

- Data describing the effect of different packaging methods (e.g. packaging in modified atmosphere).
- Data describing the actual cross-contamination between positive and negative flocks and within positive flocks during the different slaughter processes.
- Data describing the *Campylobacter* prevalence and concentration in different retail chicken products, both chilled and frozen and whole and cut products.
- Data describing the spread and the concentration of *Campylobacter* on utensils and cross-contaminated ready to eat food (bread, salad, heat-treated chicken) during food handling in private kitchens and in catering companies.
- More recent and more detailed data describing the dietary habits of Danish consumers.

In addition, data describing the prevalence and concentration of *Campylobacter* in pigs, cattle and turkeys during the processes from farm to fork need to be further investigated. Also the concentration of *Campylobacter* in ready-to-eat vegetables and fruits and in seafood and drinking water needs to be investigated.

To obtain trustworthy data on the prevalence and concentration of *Campylobacter* more effort has to be directed into developing reliable detection methods that reflects the true occurrence as closely as possibly.

Routinely typing of isolates from all thinkable reservoirs and humans should also be conducted to elucidate the epidemiology of *Campylobacter* infections.

SUBJECTS TO BE CONSIDERED IN NEXT VERSION OF THE RISK ASSESSMENT

In the next version of the risk assessment of *Campylobacter jejuni* in chicken products further subjects should be considered and eventually included in the QRA model. These subjects are

- imported chicken products
- partly slaughter of flocks
- different scalding temperatures
- different cooling systems at the slaughterhouse (water chilling contra air chilling)
- different package methods (e.g. packaging in modified atmosphere)
- more detailed information of cross-contamination during the slaughter processes
- food handling in catering companies
- a re-evaluation of the consumer model. We intend to rebuild the consumer model by linking the knowledge about the number of people getting ill from campylobacteriosis to the fraction of *Campylobacter* positive chickens leaving the slaughterhouse. By doing this, a single mathematical equation replaces the consumer model and the dose response relationship.

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APPENDICES

1. TESTS AND ESTIMATES RELATED TO 'AFTER BLEEDING', BARTLETT'S TEST PLUS ANALYSIS OF VARIANCE

Data which form the basis of the analyses.

Reference	Sample type	Number of samples	Flock number	plant	Log_{10} cfu/unit	Standard deviation	Unit
Mead et al. (1995)	Neck skin	10	M.1	A (UK)	3.7	0.60	g
Mead et al. (1995)	Neck skin	10	M.2	A (UK)	4	0.30	g
Mead et al. (1995)	Neck skin	15	M.3	A (UK)	3.9	0.39	g
Mead et al. (1995)	Neck skin	15	M.4	A (UK)	3.8	0.39	g
Mead et al. (1995)	Neck skin	15	M.5	A (UK)	3.4	0.39	g
Mead et al. (1995)	Neck skin	15	M.6	A (UK)	3.9	0.39	g
Mead et al. (1995)	Neck skin	15	M.7	A (UK)	3.6	0.39	g
Mead et al. (1995)	Neck skin	15	M.11	A (UK)	3.5	0.39	g
Mead et al. (1995)	Neck skin	15	M.12	A (UK)	4.3	0.39	g
Mead et al. (1995)	Neck skin	15	M.13	A (UK)	3.9	0.80	g
Mead et al. (1995)	Neck skin	14	M.15	A (UK)	3.7	1.10	g
Oosterom et al. (1983)	Pericloacal skin	4	O.1	A (NL)	2.39	1.08	g
Oosterom et al. (1983)	Pericloacal skin	4	O.2	A (NL)	3.42	1.65	g
Oosterom et al. (1983)	Pericloacal skin	4	O.3	A (NL)	3.44	1.92	g
Oosterom et al. (1983)	Pericloacal skin	4	O.4	B (NL)	3.99	1.00	g
Oosterom et al. (1983)	Pericloacal skin	4	O.5	B (NL)	3.3	0.92	g
Oosterom et al. (1983)	Pericloacal skin	4	O.6	B (NL)	2.18	0.67	g

Bartlett's test for equality of variances (see formula described in Technique I, the section on page 40) was carried out for the data listed above. The test gave the following results:

Bartlett's test

	Mead et al. (1995) and Oosterom et al. (1983b)	Mead et al. (1995)	Oosterom et al. (1983b)
Test value	83.6	16 - 42	6.4
$\chi^2(k-1)_{0.95}$	26.3	18.3	11.1
No. of groups (k)	17	11	6
Significant different variances?	Yes	No / Yes	No

The variances in the whole group with a total of 17 variance estimates cannot be said to be of the same magnitude. The data were divided into two groups, one for the data published by Oosterom et al. (1983b) and one for the data published by Mead et al. (1995). The variances related to the data of Oosterom and co-workers could be said to be homogeneous. As regards the data of Mead and co-workers the test value was within the range of approximately 16-42 depending on the magnitude of the "true value" related to the rounded data given in the paper. For example flock number M.4 with 15

samples was reported to give a mean of 3.8 and a standard deviation of the mean (SEM) of 0.1. If the "true value" of SEM instead was 0.14 the standard deviation (SE) would have been 0.54 instead of 0.39. These relative small changes result in a large change in the test value, which again results in significant or not significant different variances. In the further analysis we assume that the variances reported by Mead et al. (1995) are not significant different from each other.

Mead et al. (1995): one-way variance analysis plus estimates of the variances

Variance source	Sum of Squares	Degrees of Freedom	Mean Square	Test Value	$F(df_1, df_2)_{0.95}$
Between flocks (bf)	4.70	10	0.47	1.61	1.83
Within flocks (wf)	41.98	144	0.29		
Total	46.68	154			

	Formula	Value
Variance component	$\sigma_0^2 = (MS_{bf} - MS_{wf})/n_0$	0.013
Variance of residual	$\sigma_\varepsilon^2 = MS_{wf}$	0.29
Weighted group average	$n_0 = (N - \frac{\sum_i n_i^2}{N})/(k-1)$	14
Variance of a random sample	$\sigma_{total}^2 = \sigma_\varepsilon^2 + \sigma_0^2$	0.33

⇒ RESULT: Distribution for the data of Mead et al. (1995): N(3.79 , 0.33)

Oosterom et al. (1983b): One-way analysis of variance plus estimates of the variances

Variance source	Sum of Squares	Degrees of Freedom	Mean Square	Test Value	$F(df_1, df_2)_{0.95}$
Between flocks (bf)	9.59	5	1.92	1.17	2.77
Within flocks (wf)	29.61	18	1.65		
Total	39.20	23			

	Formula	Value
Variance component	$\sigma_0^2 = (MS_{bf} - MS_{wf})/n_0$	0.068
Variance of residual	$\sigma_\varepsilon^2 = MS_{wf}$	1.65
Weighted group average	$n_0 = (N - \frac{\sum_i n_i^2}{N})/(k-1)$	4
Variance of a random sample	$\sigma_{total}^2 = \sigma_\varepsilon^2 + \sigma_0^2$	1.71

⇒ RESULT: Distribution for the data of Oosterom et al. (1983b): N(3.12 , 1.71)

2. TESTS AND ESTIMATES RELATED TO 'AFTER BLEEDING', F-TEST AND MODIFIED T-TEST

Test for equality of variances for the data published by Mead et al. (1995) and Oosterom et al. (1983b) was carried out with an F-test.
Mead et al. (1995): N(3.79; 0.33) (normal distributed with a mean and a variance)
Oosterom et al. (1983b): N(3.12; 1.71) - do –
The variances for each of these two distributions are estimated as the sum of the variance component plus the variance of the residual (see Appendix 1).

Hypothesis: $H_0 : \sigma_1^2 = \sigma_2^2$ against $H_1 : \sigma_1^2 \neq \sigma_2^2$

Test value: $z = \frac{s_1^2}{s_2^2}$

Critical area: $z < F(n-1, m-1)_{1-\alpha/2} \vee z > F(n-1, m-1)_{\alpha/2}$

	Variances	Number of obs. (n)	Z – test value	$F(154,23)_{0.975}$	$F(154,23)_{0.025}$
Mead et al. (1995)	0.34	155	0.193	0.58	2.00
Oosterom et al. (1983b)	1.70	24			

$\Rightarrow$ RESULT: z is outside the critical area, meaning that the two variances are significantly different from each other.

The t-test for equality of the means is slightly modified, since the two variances are significant different:

Hypothesis: $H_0 : \mu_1 = \mu_2$ against $H_1 : \mu_1 \neq \mu_2$

Test value: $z = \frac{\overline{X} - \overline{Y}}{\sqrt{\frac{s_1^2}{n} + \frac{s_2^2}{m}}}$

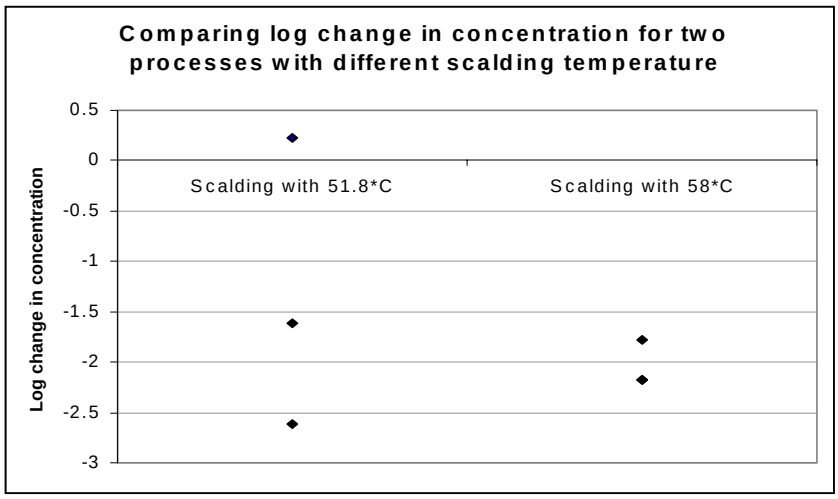
Critical area: $z < t(r)_{\alpha/2} \vee z > t(r)_{1-\alpha/2}$

where r is given by $\frac{1}{r} = \frac{c^2}{n-1} + \frac{(1-c)^2}{m-1}$, $c = \frac{\frac{1}{n}s_1^2}{\frac{1}{n}s_1^2 + \frac{1}{m}s_2^2}$

	Mean values	Variances	Number of obs. (n)	S^2/n	Z – test value	$t[24]_{0.025}$
Mead et al. (1995)	3.79	0.34	155	0.0022	2.48	2.06
Oosterom et al. (1983b)	3.12	1.70	24	0.0708		

$\Rightarrow$ RESULT: z is outside the critical area, meaning that the two means are significantly different from each other

3. TESTS AND ESTIMATES RELATED TO THE SCALDING PROCESS, INFLUENCE OF SCALDING TEMPERATURE



Data published by Oosterom et al. (1983b)

The following tests were carried out on the scalding data:

1. Bartlett's test for equality of variances for the group with temperature of 51.8°C and 58°C, respectively.
2. One-way analysis of variance plus estimates of variance components if Bartlett's test showed equality (or close to)
3. t-test to determine if there was a significant difference between the log change in concentration due to the different scalding temperatures.

1) Bartlett's test

	Scalding with 51.8°C	Scalding with 58°C
Test value	1.71	10.54
$\chi^2(k-1)_{0.95}$	7.38	7.38
No. of groups (k)	3	3
Significant different variances?	No	(Yes) almost Not

2) Variance analysis + estimate of variance component for 51.8°C

Variance source	Sum of Squares	Degrees of Freedom	Mean Square	Test Value	$F(df_1, df_2)_{0.95}$
Between flocks (bf)	16.60	2	8.3	4.78	4.26
Within flocks (wf)	15.61	9	1.73		
Total	32.21	11			

	Formula	Value
Variance component	$\sigma_0^2 = (MS_{bf} - MS_{wf})/n_0$	1.64
Variance of residual	$\sigma_\varepsilon^2 = MS_{wf}$	1.73
Weighted group average	$n_0 = (N - \frac{\sum_i n_i^2}{N})/(k-1)$	4
Variance of a random sample	$\sigma_{total}^2 = \sigma_\varepsilon^2 + \sigma_0^2$	3.38

2) Variance analysis + estimate of variance component for 58°C

Variance source	Sum of Squares	Degrees of Freedom	Mean Square	Test Value	$F(df_1, df_2)_{0.95}$
Between flocks (bf)	0.42	2	0.21	0.080	4.26
Within flocks (wf)	23.47	9	2.61		
Total	23.89	11			

	Formula	Value
Variance component	$\sigma_0^2 = (MS_{bf} - MS_{wf})/n_0$	0.00
Variance of residual	$\sigma_\varepsilon^2 = MS_{wf}$	2.61
Weighted group average	$n_0 = (N - \frac{\sum_i n_i^2}{N})/(k-1)$	4
Variance of a random sample	$\sigma_{total}^2 = \sigma_\varepsilon^2 + \sigma_0^2$	2.61

3) Test for difference between groups

	Mean change	Variance	S^2/n	Test value	r ; c	t(r)-distribution
Scalding with 51.8°C	-1.340	3.38	0.281	0.996	21.6	2.83
Scalding with 58°C	-2.04	2.61	0.217		0.56	

⇒ RESULT: the test value is not outside the critical area, meaning that the two means are not significantly different from each other. In other words, the change in concentration for the two different scalding temperatures is not significantly different.

4. TEST AND ESTIMATES RELATED TO THE SCALDING PROCESS, BARTLETT'S TEST, ANALYSIS OF VARIANCE AND T-TEST

One-way variance analysis

Variance source	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	Test Value (z)	$F(df_1, df_2)_{0.95}$
Between flocks (bf)	19.99	5	4.00	1.84	2.77
Within flocks (wf)	39.08	18	2.17		
Total	59.07	23			

Estimation of the variances

	Values
Variance component, σ_0^2	0.46
Variance of residual, σ_ε^2	2.17
Weighted group average	4
Variance of a random sample	2.63

Test for if the estimated change in concentration is significant

Mean μ_{ch}	Variance $\sigma^2_{ch,total}$	Number of samples (n)	Test value $\mu_{ch} / (\sigma_{ch,total} / \sqrt{n})$	Table values $t(n-1)_{0.025}$
-1.86	2.63	24	5.62	2.07

⇒ RESULT: The mean change in concentration is significant different from zero.
Input distribution for the scalding process: $N(\mu_{ch}, \sigma^2_{ch,0})$ = N(-1.86 ; 0.46)

5. TEST AND ESTIMATES RELATED TO THE DEFEATHERING PROCESS

Bartlett's test

	Izat et al. (1988) Oosterom et al. (1983b)
Test value	4.18
$\chi^2(k-1)_{0.95}$	11.07
No. of groups (k)	6
Significant different variances?	No

One-way variance analysis

Variance source	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	Test Value (z)	$F(df_1, df_2)_{0.95}$
Between flocks (bf)	7.73	5	1.55	1.62	2.77
Within flocks (wf)	17.19	18	0.95		
Total	24.91	23	1.08		

Estimation of the variances

	Values
Variance component, σ_0^2	0.15
Variance of residual, σ_ε^2	0.95
Weighted group average	4
Variance of a random sample	1.10

Test for if the estimated change in concentration is significant

Mean μ_{ch}	Variance $\sigma^2_{ch,total}$	Number of samples (n)	Test value $\mu_{ch} / (\sigma_{ch,total} / \sqrt{n})$	Table values $t(n-1)_{0.025}$
1.03	1.10	24	4.81	2.07

⇒ RESULT: The mean change in concentration is significant different from zero. Input distribution for the defeathering process: N(μ_{ch}, σ_0^2) = N(1.03 ; 0.15)

6. TEST AND ESTIMATES RELATED TO THE EVISCERATION PROCESS

Bartlett's test

	Izat et al. (1988) + Oosterom et al. (1983b)
Test value	2.9
$\chi^2(k-1)_{0.95}$	11.07
No. of groups (k)	6
Significant different variances?	No

One-way variance analysis

Variance source	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	Test Value (z)	$F(df_1, df_2)_{0.95}$
Between flocks (bf)	8.93	5	1.79	1.18	2.77
Within flocks (wf)	27.23	18	1.51		
Total	36.16	23	1.57		

Estimation of the variances

	Values
Variance component, σ_0^2	0.07
Variance of residual, σ_ε^2	1.51
Weighted group average	4
Variance of a random sample	1.58

Test for if the estimated change in concentration is significant

Mean μ_{ch}	Variance $\sigma^2_{ch,total}$	Number of samples (n)	Test value $\mu_{ch} / (\sigma_{ch,total} / \sqrt{n})$	Table values $t(n-1)_{0.025}$
0.35	1.58	24	1.36	2.07

⇒ RESULT: The mean change in concentration is not significant different from zero. Input distribution for the evisceration process: N(μ_{ch}, σ_0^2) = N(0.35 ; 0.07)

7. TEST AND ESTIMATES RELATED TO THE WASHING + CHILLING PROCESS

The analysis is carried out as though the number of samples in the study of Cason et al. (1997) was 20 and not 90 samples. This was done in order to obtain less weight relatively on the data of Cason et al. (1997).

Bartlett's test

	Cason et al. (1997) + Oosterom et al. (1983b)
Test value	4.70
$\chi^2(k-1)_{0.95}$	7.81
No. of groups (k)	4
Significant different variances?	No

One-way variance analysis

Variance source	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	Test Value (z)	$F(df_1, df_2)_{0.95}$
Between flocks (bf)	2.15	3	0.72	0.74	2.95
Within flocks (wf)	26.92	28	0.96		
Total	29.06	31			

Estimation of the variances

	Values
Variance component, σ_0^2	0.00
Variance of residual, σ_ε^2	0.96
Weighted group average	6.00
Variance of a random sample	0.96

Test for if the estimated change in concentration is significant

Mean μ_{ch}	Variance $\sigma^2_{ch,total}$	Number of samples (n)	Test value $\mu_{ch} / (\sigma_{ch,total} / \sqrt{n})$	Table values $t(n-1)_{0.025}$
-1.46	0.96	32	8.43	2.06

⇒ RESULT: The mean change in concentration is significant different from zero. Input distribution for the washing and chilling process: N(μ_{ch}, σ_0^2*) = N(-1.46 ;0.05)

σ_0^2* : Since the estimated variance is zero, we give the variance used in the input distribution a new but small value.

8. CALCULATION OF $F_{MEAL|ASPM}$

For the males in the age groups 30-65 years (ASPM = male 30-65) $F_{meal|male}$ 30-65 is calculated as:

$$F_{meal|male30-65} = \frac{\left(F_{survey|male30-65} \cdot N_{TOT|age30-65} - N_{SA|male30-65}\right)}{N_{TA|age30-65}}$$

$F_{survey|male30-65}$ indicates the fraction of males (aged 30-65) preparing food. This parameter is obtained from the dietary survey. $N_{TOT|age30-65}$ is the total number of adults in the age group, $N_{SA|age30-65}$ is the number of adult men in households with a single adult and $N_{TA|age30-65}$ is the number of adults in households with two adults.
Similar calculations can be made for females aged 30-65 and for males and females above 65 years of age. In households with adults in the age group of 18-29 years it is slightly more complicated to calculate $F_{meal|ASPM}$ because this group also includes young adults which live at home with their parents. For this group $F_{meal|male18-29}$ is calculated as:

$$F_{meal|male18-29} = \frac{\left(F_{survey|male18-29} \cdot \left(N_{age18-29} +\right) - N_{SA|male18-29} - F_{meal|male30-65} \cdot N_{YA|age30-65} - F_{meal|male>65} \cdot N_{YA|age>65}\right)}{N_{TA|age18-29}}$$

Where, $N_{age18-29} = N_{TOT|age18-29} + \sum_{all\_ASPM} (N_{SA|ASPM} + N_{TA|ASPM})$

A similar calculation can be made for females aged 18-29.

9. CALCULATIONS IN RELATION TO YOUNG ADULTS LIVING AT HOME WITH THEIR PARENTS

For the households with young adults living at home we could only obtain information about the total number of households and the total number of people living in these households for each adult age group. In order to divide the these households, for each adult age group, into the same categories as for the households with children under the age of 18 (i.e. single adult male, single adult female and two adults), we assumed that the mutual relationship between the number of households with young adults in the 3 categories was the same as the mutual relationships for the households with children under the age of 18.

In order to calculate the number of young adults living with adults in the age group of 30-65 in the different categories of households, the calculations and assumptions shown below were made.

From Statistics Denmark we obtained the total number of households with young adults ($NH_{YATOT|age30\text{-}65}$).
Assuming that in 90% of these households only one young adult live at home and in 10% two young adults live at home, the total number of adults in these households could be determined as:

$$N_{YATOT\ |age30\ -65} = 0.9 \cdot NH_{YATOT\ |age30\ -65} + 2 \cdot 0.1 \cdot NH_{YATOT\ |age30\ -65}$$

The total number of young adults in household with a single adult parent ($N_{YASA|age30\text{-}65}$) was determined as:

$$N_{YASA|age30-65} = N_{YATOT|age30\text{-}65} \cdot \frac{NH_{CSA|age30-65}}{NH_{CSA|age30-65} + NH_{CTA|age30-65}}$$

Where $NH_{CSA|age30\text{-}65}$ and $NH_{CTA|age30\text{-}65}$ denote the total number of households with a single and two adults, respectively, which have children under the age of 18 yearsliving at home. These parameters were obtained from statistics Denmark.
The total number of young adults in households with a two adult parents ($N_{YATA|age30\text{-}65}$) was determined as:

$$N_{YATA\ |age30\ -65} = N_{YATOT\ |age30\ -65} - N_{YASA\ |age30\ -65}$$

The total number of young adults in households with a single adult male parent ($N_{YASA|male30\text{-}65}$) was determined as:

$$N_{YASA|male30-65} = N_{YASA|male30-65} \cdot \frac{NH_{CSA|male30-65}}{NH_{CSA|male30-65} + NH_{CSA|female30-65}}$$

Where $NH_{CSA|male30-65}$ and $NH_{CTA|female30-65}$ denote the total number of households with a single male or a single female adult, respectively, which have children under the age of 18 years living at home. These parameters were also obtained from Statistics Denmark.

The households with a single adult female $N_{YASA|female30-65}$ was determined as:

$$N_{YASA|female30-65} = N_{YASA|age30-65} - N_{YASA|female30-65}$$

For households in which the adults were above the age of 65 with young adults living at home, the same calculations as described above were made. However, in these households only one young adult was assumed to live at home. In addition, the relationship between the number of young adults living in the different types of households was assumed to be similar to the relationships seen for households with children in the adult group aged 30-65. In other words, the relationship was not expected to be similar to the relationship seen for the household with children in the adult group of ages above 65 years. The reason for this is that adults above the age of 65 only in extremely rare cases have children under the age of 18 living at home. Thus,

$$N_{YATOT|age>65} = NH_{YATOT|age>65}$$

$$N_{YASA|age>65} = N_{YATOT|age>65} \cdot \frac{NH_{CSA|age30-65}}{NH_{CSA|age30-65} + NH_{CTA|age30-65}}$$

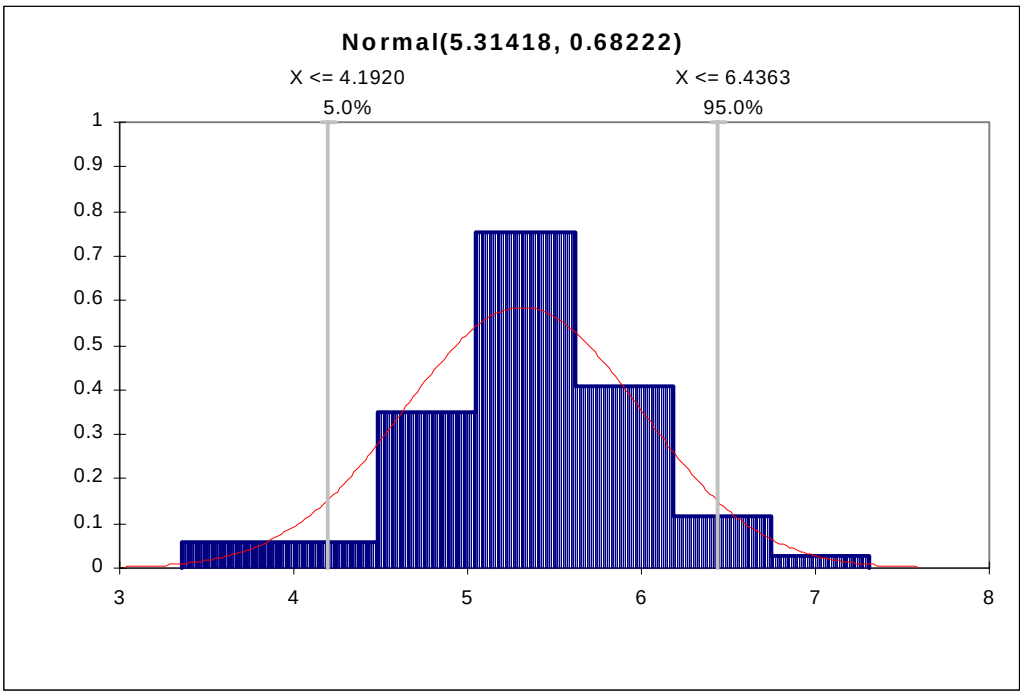
$$N_{YATA|age>65} = N_{YATOT|age>65} - N_{YASA|age>65}$$

$$N_{YASA|male>65} = N_{YASA|male>65} \cdot \frac{NH_{CSA|male30-65}}{NH_{CSA|male30-65} + NH_{CSA|female30-65}}$$

$$N_{YASA|female>65} = N_{YASA|age>65} - N_{YASA|female>65}$$

10. FITTING THE DATA FROM THE DIETARY SURVEY

The data describing for example the size of chicken meal ingested for a certain age and sex group are logarithmically distributed and when log-transformed the data are well described by a normal distribution. Fitting a normal distribution to the logarithmically transformed data or fitting a LogNormal distribution to the original data give the same result.
An example is shown below, which describe the size of chicken meals ingested by men aged 18-29 years.



Normal distribution fitted to LN-transformed data (the same as fitting a $\log_e$-normal distribution to the original data).

The mean and standard deviation belonging to this normal distribution (5.31448 and 0.68222) is transformed back to the original scale by calculating the arithmetic mean and the arithmetic standard deviationgiven: Mean = 256.4 and Standard Deviation (SD) = 197.4

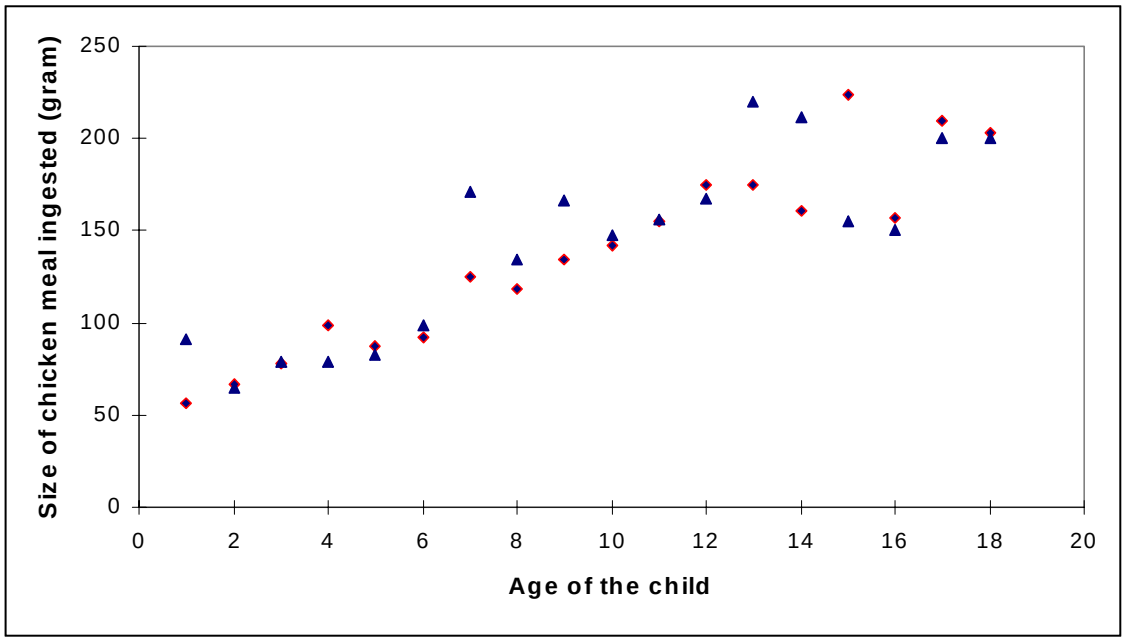
Mean $E(X) = e^{\alpha + \frac{1}{2}\beta^2}$

SD $V(X) = e^{2\alpha+\beta^2}(e^{\beta^2} - 1)$

where α and β are the mean and standard deviations belonging to the normal distribution for logarithm transformed data.

11. RELATIONSHIP BETWEEN AGE OF A CHILD AND SIZE OF CHICKEN MEAL

The relationship between the age of a child and the size of an ingested chicken meal was examined.



Relationship between age of a child and size of an ingested chicken meal. ▲, the average size of the chicken meal for a boy at a given age. ◆, the average size of a chicken meal for a girl at a given age.

The relation between the age of a child and the size of a chicken meal ingested seems to be linear until the age of 12 years, then the curve breaks off and only a small increase is seen for the rest of the curve.